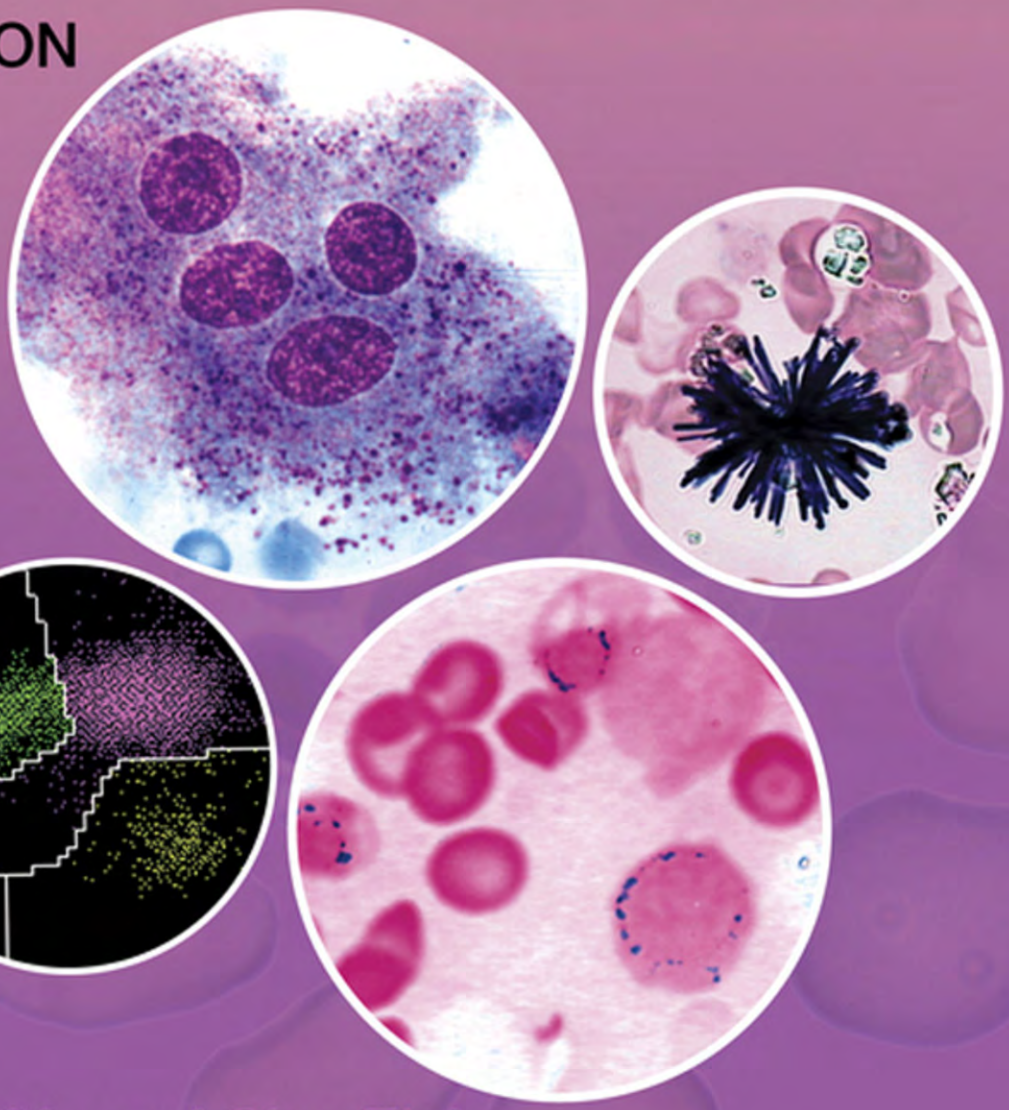
# Morphology of Blood Disorders

SECOND EDITION



**Giuseppe d'Onofrio and Gina Zini**
Translated by **Barbara J Bain**

WILEY Blackwell

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## Second Edition


**Giuseppe d'Onofrio**

Professor in Haematology and Clinical Pathology
Centre for Research into Automated Methods in Haematology (ReCAMH)
Catholic University of the Sacred Heart
Rome, Italy

**Gina Zini**

Professor in Haematology
Centre for Research into Automated Methods in Haematology (ReCAMH)
Catholic University of the Sacred Heart
Rome, Italy


***Translated by***


**Barbara J. Bain, Dip ELIC IoL, Dip Trans IoL, AIL**

Professor in Diagnostic Haematology
St Mary's Hospital Campus
Imperial College London
and
Consultant Haematologist
St Mary's Hospital
London
UK



WILEY Blackwell

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111 River Street, Hoboken, NJ 07030-5774, USA

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# Preface

This is a translation from the Italian of the second edition of a textbook on the morphology of the blood and bone marrow in blood diseases. Morphological details are supplemented by detailed descriptions of the output and role of automated instruments in disorders of the blood. Despite the enormous advances made in immunophenotyping and in cytogenetic and molecular aspects of haematology, morphology of the blood and marrow remains fundamental in haematological diagnosis. The authors are renowned in this field. I have translated the text into English so that their expertise can be shared with a wider audience.

Barbara J. Bain
*London, January 2014*

CHAPTER 1

# Blood and Bone Marrow Cells

## Biology of haemopoiesis

In the second half of the 19th century, developments in microscopy and in staining techniques permitted the observation of various types of blood cell, leading to a developing understanding of their heterogeneity, morphological characteristics and manner of proliferating and differentiating. The German pathologist, Neumann, recognised that the formation of blood cells from a single progenitor cell took place in the bone marrow. From this recognition, together with the work of the Russian scientist, Maximov, evolved the monophyletic or unitary theory of haemopoiesis, of which Adolfo Ferrata became one of the most convinced supporters (Fig. 1.1).[1] It was the studies of McCulloch and Till, at the beginning of the 1960s, that provided the necessary experimental evidence with their demonstration of the capacity of bone marrow cells to form multilineage myeloid colonies in the spleen when injected into animals rendered aplastic by irradiation.[2] This experiment was repeated in man with the first transplants of bone marrow between twins and into workers accidentally exposed to nuclear irradiation.[3]

The blood, understood as both the haemopoietic matrix and the circulating cells, is a complicated tissue in which numerous elements are selected, organized and regulated like the instruments of a symphonic orchestra, to provide a harmonious, stable and effective result. The formation of the blood depends on the existence of a multipotent stem cell, recognised by its capacity to establish long-term cultures *in vitro*. This cell is at the top of a tree with increasing numbers of branches towards the base. The initial division is into two progenitors that differ in their potential, restricted respectively to lymphopoiesis and myelopoiesis. Successive branches give rise to other progenitors, organised in a hierarchy, with the capacity for differentiation becoming more limited and specific. This process culminates in the production of the population of morphologically recognisable haemopoietic cells of the bone marrow and ultimately of the mature elements that circulate in the peripheral blood.

### Stem cells

Haemopoietic stem cells are multipotent and it is from them that all the circulating blood cells derive. Haemopoiesis is the well-organised and carefully controlled process by which this fundamental capacity is realised. The adjective 'stem' derives from the Latin *stamen*, meaning thread, to which, figuratively speaking, is bound the life of every man: the thread in classical mythology is spun and then cut off by the Fates.[4] The stamen in botany, moreover, is the flower's male organ. While this concept was used in the 19th century in Darwin's evolutionary theory to indicate the unicellular ancestor of all living creatures, it was only at the beginning of the 20th century that the term 'stem cell' was attributed to the common precursor of all the cells of the blood.[5]

The haemopoietic stem cells have two essential properties (Fig. 1.2):

- They are multipotent, that is capable of differentiating into all the various types of blood cell; this differentiation is associated with the loss of multilineage potential and the formation of committed haemopoietic progenitors;
- They are capable of indefinite self-renewal, maintaining themselves through many generations as a small constant percentage of daughter cells with their multilineage potential conserved.

The maintenance of stem cell numbers is necessary to maintain effective multilineage haemopoiesis for the life of an individual. In stable conditions (homeostasis), the majority of the rare mitoses seen in stem cells are symmetrical, producing two identical daughter cells, which are destined to give rise to differentiated progeny. The maintenance of a stable number of stem cells is achieved by the simultaneous occurrence of a more limited number of asymmetric divisions, in which a single stem cell produces two non-identical daughter cells, one of which maintains the original multipotent capacity while the other takes the path of differentiation. This asymmetric mitosis could be predetermined at the moment of mitosis, as is suggested by the polarized distribution of regulatory molecules,[6] messenger RNA and individual proteins, which are not distributed equally between the daughter cells. In other instances, the factors determining that one of the daughter cells takes the pathway of differentiation could reside in the microenvironment.[7]

The multipotent long-term culture initiating haemopoietic stem cells (LT-HSC) can be identified and enriched in the

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**Fig. 1.1** Morphogenesis of blood cells, according to the monophyletic theory, illustrated in the original table in Adolfo Ferrata's text, *Le Emopatie*, published in 1933 by the *Società Editrice Libraria*. From the morphologically undifferentiated haemopoietic progenitors, grouped together at bottom left within an incomplete circle, originate the various lineages of bone marrow precursors, which progressively develop their distinctive morphological characteristics, finally giving rise to the mature cells of the peripheral blood. From left to right the lineages are basophil granulocytes, eosinophil granulocytes, neutrophil granulocytes, erythroid series and, bottom right, megakaryocytes. The illustration is incomplete, lacking the lymphocytes and monocytes; it nevertheless shows the high level of intuition and knowledge achieved by morphological observation alone.

mouse thanks to their surface membrane immunophenotype, characterised by positivity for c-Kit, Thy-1.1 (weak) and Sca-1 (stem cell antigen 1), and negativity for all the lineage-specific markers expressed by committed progenitors, differentiated precursors and mature cells. There is neither a single marker, nor a combination of markers, that is specific for haemopoietic stem cells in man. The immunophenotypic markers most often used, particularly for counting stem cells in relation to transplantation, are CD34, CD133, CD90 and CD117. The functional capacity of stem cells can be demonstrated by culture, i.e. they can give rise to long-term cultures *in vitro*. The morphology of these forms is similar to that of small blast cells, with a very high nucleocytoplasmic (N:C) ratio, scanty weakly basophilic cytoplasm and homogeneous nuclear chromatin, which is fairly compact (Figs 1.3, 1.4 and 1.5). In some cells the cytoplasm is more abundant and basophilic. The nucleus can have visible nucleoli of variable prominence.

## The haemopoietic niche and the microenvironment

Stem cells and haemopoietic progenitors, from birth onwards, are located in the bone marrow where, in stable conditions, they divide very slowly (less than 10% enter cell cycle each day), with a delicate balance maintained between self-renewal, differentiation and quiescence.[8] Alterations in the regulation of this carefully balanced system underlie the majority of haemopoietic neoplasms.

Stem cells self-renew and differentiate into progenitors and precursors at specific sites in the medullary cavity. These sites are known as haemopoietic niches. It is possible that there are different types of haemopoietic niche. They can be located close to the endosteum, which covers the trabecular surface of the bones, where the major influence on them is that of the osteoblasts, derived from mesenchymal stem cells, or alternatively in a perisinusoidal position, in the centre of the intertrabecular space, where the major influence is that of the endothelium.[7]

The stem cell niche can be defined as a localised microenvironment with optimal conditions for self-renewal. They are sites where largely quiescent stem cells accumulate and are maintained in a dormant state, i.e. differentiation is inhibited, by being anchored to the osteoblasts that surround the niche by adhesion molecules (N-cadherin, integrin). In the presence of external stimuli, such as those produced by blood loss or growth factor therapy, the interaction with signalling molecules and the resultant modification of transcription factors and other cytoplasmic proteins favour the re-awakening of the dormant cells and their entry into the cell cycle. The ultimate result is the reconstitution of the integrity of the body's supply of mature blood cells.[9]

The stem cell niche has a heterogeneous cellular composition, including not only differentiating haemopoietic cells and macrophages, the latter also derived from haemopoietic stem cells, but also numerous stromal elements. The osteoblastic niches contain osteoblasts, fibroblasts, osteoclasts, neurons of the sympathetic nervous system, and even mesenchymal stem cells, which give origin to cells of the bone and connective tissue and are implicated in maintaining the migration of the haemopoietic stem cells.[10] The perisinusoidal niches contain primarily endothelial cells and reticulum cells, in addition to numerous megakaryocytes.[9,11]

Stem cells are characterised by striking mobility: they are capable of repeatedly entering, exiting and re-entering the haemopoietic niches in response to specific signals. This phenom-

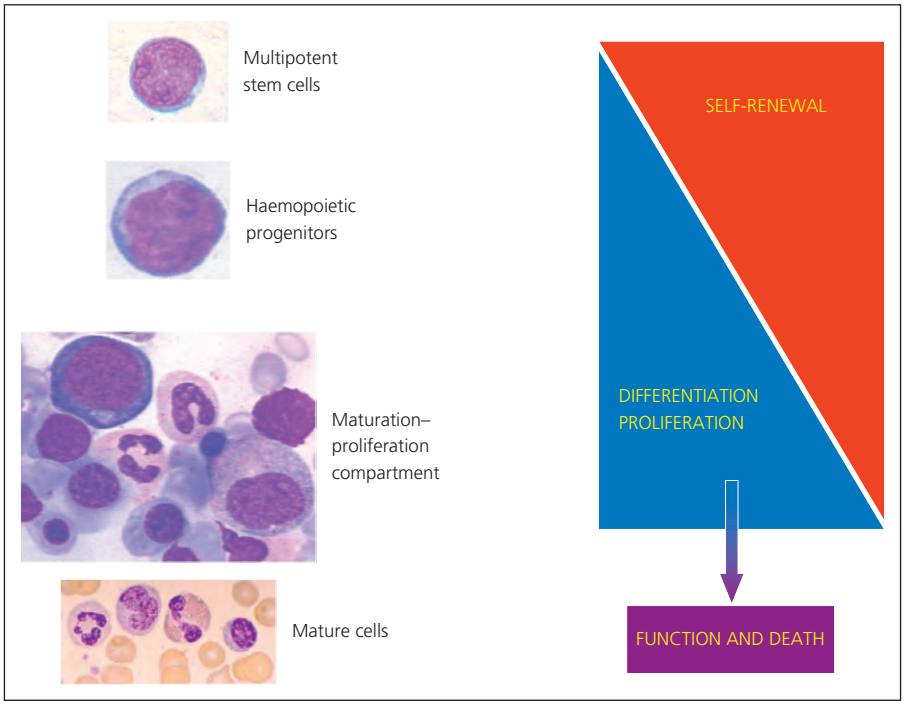



**Fig. 1.2** Schematic diagram of the haemopoietic compartments and their principal functional characteristics. Cells of the multipotent stem cell compartment have the maximum self-renewal capacity, proliferate quite slowly and only show differentiation to a minimal extent. Cells of the compartment of haemopoietic progenitors, while maintaining the morphological uniformity of the undifferentiated cells, are more heterogeneous and organised in a hierarchical manner (see Fig. 1.6): the capacity for self-renewal is reduced, while the cells proliferate and differentiate. The cells of the maturing-proliferating compartment, which correspond to the morphologically recognisable multilineage haemopoietic cells of the bone marrow, have lost the capacity for self-renewal. From this compartment originate the mature cells that pass into the peripheral blood: these are destined to carry out their functions without dividing again (with the exception of the lymphocytes).

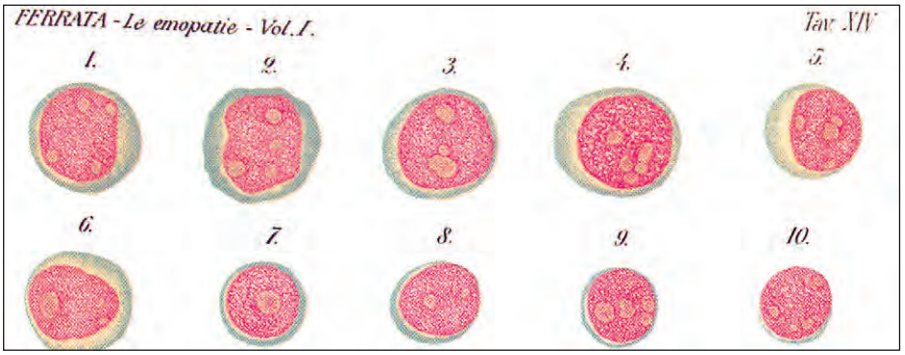



**Fig. 1.3** The haemocytoblasts of Ferrata (original illustration from Ferrata A, *Le emopatie*, 1933). The cells numbered from 8 to 10 particularly evoke the morphology and size of stem cells and haemopoietic progenitors as one recognises them today (see Figs 1.4 and 1.5). The other cells are more similar to myeloblasts, with an angulated nuclear outline (e.g. 1 and 2), or lymphoblasts.

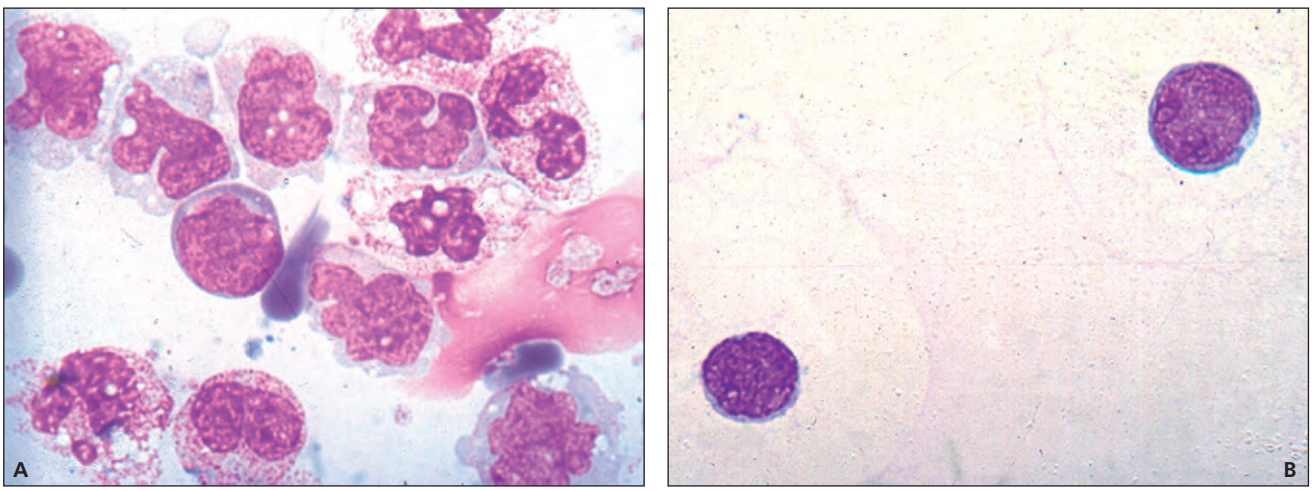



**Fig. 1.4** Morphology of haemopoietic stem cells. (A) Cytocentrifuge preparation of mononuclear cells harvested for peripheral blood stem cell transplantation on a cell separator (stem cell apheresis) from a donor treated with granulocyte colony-stimulating factor (G-CSF). There are numerous monocytes, some partly vacuolated neutrophils, a metamyelocyte on the lower edge and, just to the left of the centre, an undifferentiated haemopoietic progenitor, which has a high nucleocytoplasmic (N:C) ratio and the appearance of a small blast cell; it has dispersed chromatin, small nucleoli and a thin rim of basophilic agranular cytoplasm, which has a pale paranuclear area. (B) Haemopoietic stem cells, CD34 positive, harvested by stem cell apheresis and purified by an immunomagnetic method. The nucleus occupies nine tenths of the cell and has dispersed homogeneous chromatin; the top right cell has a visible nucleolus. The cytoplasm shows no features of differentiation.

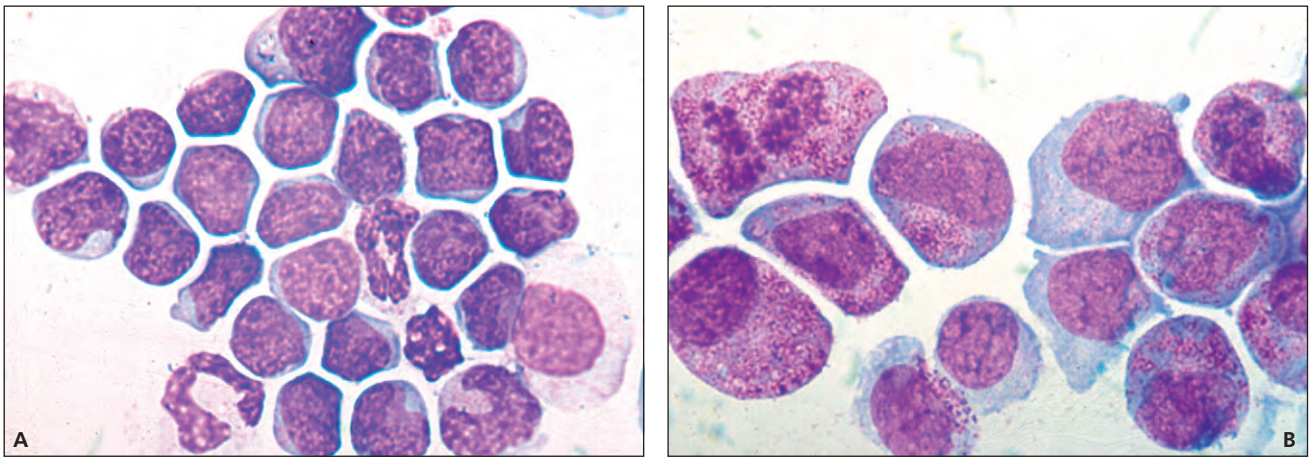



**Fig. 1.5** Haemopoietic stem cells and progenitors from cord blood. (A) Cytocentrifuge preparation of cord blood stem cells after enrichment by magnetic immunoselection on a column using anti-CD34. The morphological features are fairly uniform: all the cells are small to medium sized and lack any morphological signs of differentiation. (B) Morphological differentiation of the same cord blood stem cells after two weeks in semiliquid culture in the presence of G-CSF. The majority of the cells now have cytoplasmic granules, indicating neutrophilic differentiation. Top left, a cell in anaphase. The most heavily granulated cell also shows chromatin condensation, with the morphological features of a promyelocyte or neutrophil myelocyte.

enon is used in the field of transplantation to gather stem cells from the peripheral blood by cytapheresis. As well as controlling the division and differentiation of stem cells and haemopoietic progenitors, the haemopoietic niche, by virtue of its structural organisation, fulfils essential functions in the control of this traffic. An extensive network of signals passes to and fro between the stem cells and the cells of the niche; the intercellular contacts and the contacts with the intercellular matrix control, by means of a large number of molecules (agrin, cadherins, integrins, chemokines, cytokines, signalling molecules and receptors), the spatial distribution and the biological behaviour of cells within the niche. Equally important are the effects of molecules produced at sites remote from the niche, such as erythropoietin, and the effect of the central nervous system, particularly the secretion of noradrenalin by sympathetic fibres within the niche, which has significant effects on osteoblasts.[12]

Long-term continuous morphological analysis of single living cells (single-cell imaging) has been used in recent studies to analyse and measure the dynamics of haemopoiesis, the behaviour and destiny of the stem cells in the haemopoietic niche and the cellular response to regulatory molecules. This has involved advanced optical technology and computerisation (confocal laser scanning, multiphotonic microscopy, optical projection tomography and optoacoustic tomography).[13,14]

## Haemopoietic progenitors

The term 'progenitor' refers to cells derived directly from haemopoietic stem cells and morphologically similar to them, but which have taken the first steps in differentiation (commitment) and are thus directed towards the production of an increasingly more limited range of mature haemopoietic cells (Fig. 1.6).

The haemopoietic progenitors were initially given the generic designation 'colony-forming units' (e.g. CFU-S, the cells first described by McCulloch that give rise to spleen colonies[2]). It is now known that each progenitor cell stage corresponds to an individual cell type, which has specific immunophenotypic and cultural characteristics, and can give rise to a well-defined limited repertoire of mature cells. At each stage, each cell undergoes multiple cellular divisions in the course of differentiation, thus amplifying the number of progenitors and, in turn, the number of haemopoietic precursors. Furthermore, with each transition from one stage to the next, a complex series of molecular events further restricts the capacity for differentiation. Thus, the first progenitors still have multilineage potential, albeit confined to the lymphoid (common B and T progenitor) or myeloid (CFU-GEMM; granulocyte, erythroid, macrophage, megakaryocyte) lineage. The CFU-GM, in turn, gives rise to progenitors with bilineage potential (CFU-GM for granulocytes and monocytes/macrophages; CFU-ME for megakaryocytes and erythroid cells). In the final stage, unilineage progenitors develop from the CFU-GM and CFU-ME; in appropriate cultural conditions, these can produce colonies composed of morphologically recognisable haemopoietic cells of a specific lineage. Each type of progenitor is controlled by glycoprotein growth factors binding to surface membrane receptors.

## Erythroid progenitors

Cells that form pure erythroid colonies arise from the common myeloid progenitor, the CFU-GEMM, by means of an intermediate stage with bilineage erythroid/megakaryocyte potential (the CFU-ME; also known as the megakaryocyte-erythroid progenitor [MEP]). These erythroid-committed progenitors have

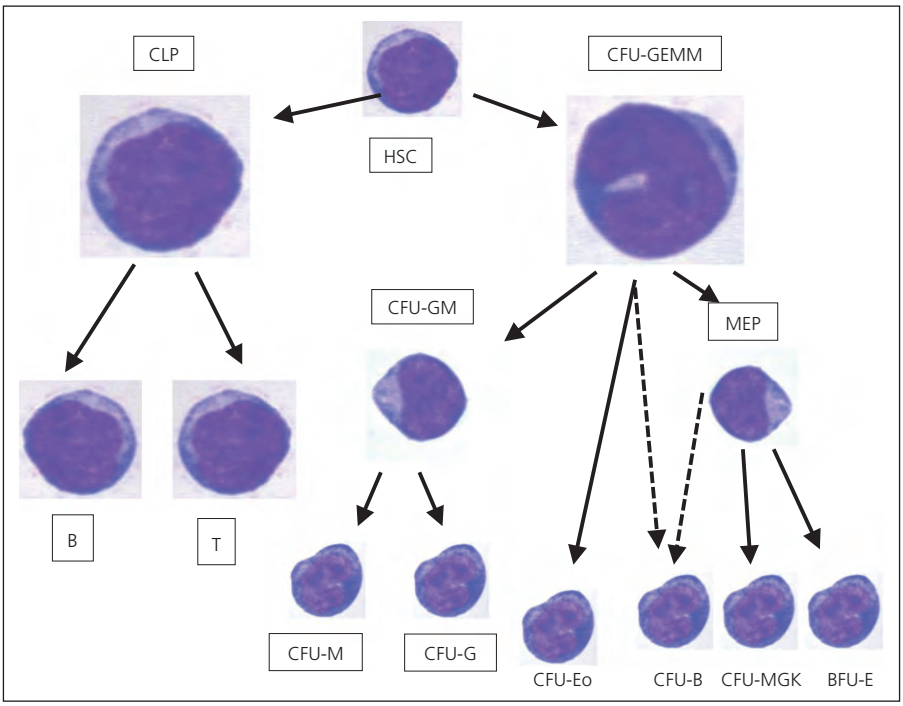



**Fig. 1.6** Schematic representation of the hierarchy of haemopoietic stem cells (dendrogram). In the absence of morphological signs of differentiation, the characterisation of these stages of maturation is by immunophenotyping and by *in vitro* culture techniques. HSC, haemopoietic stem cell; CLP, common lymphoid progenitor; CFU-GEMM, multipotent myeloid stem cell, colony-forming unit granulocyte/erythroid/macrophage/megakaryocyte; CFU-GM, colony-forming unit granulocyte/macrophage, MEP, megakaryocyte/erythroid progenitor.

not been identified morphologically, but by their capacity to produce, *in vitro*, in the presence of the growth factor erythropoietin, clonal colonies composed of erythroblasts capable of synthesising haemoglobin (see Chapter 2). Two different types of erythroid progenitor can be identified, representing successive maturation stages:

- Burst-forming unit erythroid (BFU-E): these give rise in 14–16 days to large 'explosive' colonies, which have small numbers of erythropoietin receptors on their surface membrane; the morphology of these cells resembles that of very immature blast cells, with moderately basophilic cytoplasm, sometimes with pseudopods, and very delicate nuclear chromatin with visible nucleoli[15];
- Colony-forming unit erythroid (CFU-E): in about a week these give rise to small colonies of 16 or 32 haemoglobinised erythroblasts; these cells are rich in erythropoietin receptors and are now close to becoming proerythroblasts, even if their morphology is still that of an immature cell with delicate nuclear chromatin, a high N:C ratio and basophilic cytoplasm with pseudopods. On electron microscopy these cells can be seen to have many mitochondria and pinocytotic vesicles.[16]

## Megakaryocyte progenitors

These originate from the common megakaryocyte-erythroid progenitor (MEP) which, like the stem cells, has no defining morphological features, has receptors for growth factors of both lineages and is starting to show weak expression of CD41, CD61 and CD42. Two types of progenitor have also been identified for the megakaryocyte series, both of which have unilineage differentiation potential:[17]

- BFU-MK, which in 21 days form colonies of hundreds of cells.
- CFU-MK, which in 10–12 days form colonies of 3–50 mature megakaryocytes.

These cells are CD34 negative and have specific markers of the megakaryocyte lineage. The main growth factor for megakaryocytes is thrombopoietin, which binds the receptor encoded by the *MPL* gene. MPL is present and active on megakaryocyte progenitors: it is a 70-kDa glycoprotein that is similar to erythropoietin. Numerous other cytokines have a positive effect on megakaryocytopoiesis (interleukin [IL]-3, IL-6, IL-11, granulocyte-macrophage colony-stimulating factor [GM-CSF], stem cell factor [SCF], leukaemia inhibitory factor [LIF]), while transforming growth factor (TGF) beta, interferon alpha and the proteins of platelet granules (PF4, beta-thromboglobulin) have, conversely, inhibitory effects.

## Granulocyte and monocyte/macrophage progenitors

The differentiated progenitors of neutrophil granulocytes (CFU-G) and monocytes/macrophages (CFU-M) originate from a common precursor (CFU-GM), which in its turn has differentiated from a CD34-positive haemopoietic stem cell. The growth factors for these lineages are glycoproteins, which interact with specific receptors on the various committed progenitors: GM-CSF stimulates the production of mixed colonies;

G-CSF leads to the production of granulocyte colonies containing myeloblasts, promyelocytes and more mature granulocytes; M-CSF induces the formation of macrophage colonies. These glycoproteins are widely used in therapy.

The principle growth factor for eosinophil progenitors is IL-5. Progenitors of basophil granulocytes and mast cells also originate from a CD34-positive haemopoietic stem cell. They differentiate along two distinct maturation pathways, influenced by multiple and different growth factors (SCF, IL-3, IL-6, GM-CSF). According to recent investigations, there may also exist a common basophil-megakaryocyte progenitor; a possible hybrid eosinophil-basophil progenitor has also been described.[18]

### Lymphocyte progenitors

The production of diverse populations of T and B lymphocytes is a complex phenomenon, which starts with the first lymphocyte-committed progenitor and continues in the primary and secondary lymphoid organs. The differentiation of stem cells and lymphocyte progenitors is directed by their interaction with stromal cells and with appropriate growth and differentiation factors. Development into T lymphocytes occurs in the microenvironment of the thymus and into B lymphocytes in the microenvironment of the bone marrow. The differentiation of B lymphocytes occurs in two phases. The first phase is antigen independent and involves the differentiation of stem cells and progenitors into pro-B progenitors and then into immature B cells, which express immunoglobulin M. The second phase is antigen dependent and involves the transformation of an immature B cell into a plasma cell. The production of T lymphocytes starts with the migration of a subpopulation of common lymphoid progenitors into the thymus: there they differentiate into multipotent T-cell progenitors from which in turn develop CD4-postive and CD8-positive subpopulations. One of the functions of the Notch receptors, which belong to a family of surface membrane proteins with an intracellular domain, is a key role in the development of T and B lymphocytes, acting directly at the level of transcription of specific genes. Their interactions with various specific molecules have a determining effect, particularly in the early phase of T-cell maturation and in the regulation of the T-cell immune response.[19]

The study of surface markers is fundamental in following the stages of maturation of the lymphocyte subclasses.

## Technical aspects of haematological cytology

The biological and morphological study of blood cells is facilitated by the ease with which a blood sample can be obtained by venepuncture. In addition, the aspiration of haemopoietic tissue, with its complex and fascinating heterogeneity, from the bone marrow cavity is a minimally invasive biopsy procedure.

Morphological analysis is based on microscopy of stained films of blood and bone marrow. The techniques for making these haematological preparations are widely known and the reader is referred to texts on haematological techniques for the technical details.[20,21] The interpretation of morphology, however, requires knowledge of certain basic methods, including the correct manner of preparing a film, suitable staining methods to demonstrate cellular structures optimally and the most efficient methods of microscopic examination.

### Peripheral blood film

#### Preparation of the film

For making a blood film the most widely practised technique is that of making a film on a glass slide using either a drop of capillary or venous blood without anticoagulant or a drop of EDTA-anticoagulated venous blood from a specimen tube: the latter method has the advantage of avoiding platelet aggregation, while blood films of non-anticoagulated blood are optimal for the observation of erythrocyte morphology. The film can be made using a cover-slip that is narrower than the glass slide itself[22] (all photographs of peripheral blood films that appear in this book have been prepared using this simple method). Automated instruments are available to make blood films using the double slide technique: in general, these provide good quality films in a reproducible manner. Alternatively the spreading of a blood film can be automated using an instrument based on centrifugation: the films thus obtained are more homogeneous with regard to thickness and the distribution of cells, but the technical problems related to the dispersion of aerosols means that they have not been widely employed.

For blood specimens from patients with leucopenia, and also when searching for pathological cells present in low numbers, it is useful to make a film from a drop of blood taken directly from the white cell layer formed after centrifugation, i.e. from the buffy coat. In these samples the haematocrit is very high and can be reduced by diluting the sample in a physiological solution or, better, in compatible plasma of blood group AB.[20] Another method of leucocyte enrichment is cytocentrifugation of the mononuclear cell fraction that has been separated on a Ficoll-Hypaque density gradient: this method can increase the sensitivity of a search for pathological erythroblasts, lymphoma cells and blast cells of various types.[23]

The films must be left to dry in air. Subsequently, they can be fixed in methanol or stained directly with an alcohol-based stain.

#### Staining

The staining that is practised in haematology laboratories represents the perfecting of the mixture of acid and basic dyes that Paul Ehrlich first developed and which enabled him, in 1879, to publish the first detailed description of the different types of circulating leucocytes and their precursors in the bone marrow. Ten years later, the Russian protozoologist, Romanowsky, whose work was aimed principally at visualising intra-erythrocytic malaria parasites, mixed methylene blue and eosin to obtain the

chromatic results that still carry his name (the Romanowsky effect). These two dyes, which are respectively a deep blue and an intense orange, when applied to a biological substrate produce a typical chromatic spectrum with these two colours at the extremes as well as a range of blues, reds and subtle intermediate colours, depending on the composition and the physicochemical characteristics of the cellular constituents.[24] The dyes used most widely today in Europe (May-Grünwald-Giemsa) and in the United States of America (Wright) are an aqueous or alcoholic mixture of two stains or groups of stains: polychromed methylene blue and eosin Y. The first, which is a mixture of various oxidation derivatives of methylene blue, is basic, cationic; its fundamental active component is azure B, the colour of which is seen in almost pure form in the strongly basophilic cytoplasm of plasma cells or immature erythroblasts. Eosin Y is acidic, anionic, and its intense orange colour is seen in almost pure form in the granules of eosinophils.

The staining of cellular structures is a physicochemical phenomenon that depends on the interaction of the cationic molecule of azure blue with acidic basophilic substances such as cytoplasmic RNA, and on the interaction of the anionic eosin molecule with basic acidophilic substances such as the major basic protein of the eosinophils granules or the haemoglobin of the erythrocytes. The Romanowsky effect, that is, the typical colour, neither blue nor orange, of individual cellular components, such as the nucleus, depends on the combined complex chemical interaction of the two stains with cellular structures. In the nucleus, for example, the azure B molecule binds to the phosphoric groups of DNA, while the eosin molecule binds to cationic sites on nuclear proteins, together giving the reddish purple colour characteristic of chromatin. Basophil granules, however, show the phenomenon of metachromasia, in which binding to a blue dye, such as azure B or toluidine blue, conveys a different colour to the structure with which it has affinity, such as a very dark red, almost black, with azure blue B staining or a vivid red with toluidine blue. The primary granules of the neutrophil series show a typical metachromatic affinity for azure B, which gives them a very intense vivid red colour: they are therefore designated azurophilic.

The results with various manual staining techniques are poorly reproducible, particularly because of the variable composition of different batches of commercially available stains. The International Council for Standardization in Haematology (ICSH) has proposed a reference staining method based on the use of purified forms of azure B and eosin Y.[25] This method provides excellent results both with regard to quality and reproducibility.

Nowadays numerous film staining instruments are available, which in general produce good quality staining, even if the heterogeneity of the results makes it imperative for the user to carry out a preliminary evaluation of the chromatic range and the appearance of fine cytological details (chromatin network, cytoplasmic granules) in comparison with the manual method previously in use.

### Microscopic examination

Naked eye analysis of a stained blood film permits the detection of technical imperfections and also irregular distribution of cells, such as that due to erythrocyte agglutination. Staining anomalies, such as increased blue staining attributable to a marked increase in the concentration of basophilic plasma proteins or the reddish colouration of a specimen that has been taken into heparin, can also be detected. The presence of small particles resembling small bone marrow particles in the tail or the body of the film may be the result of cryoglobulin precipitates, gross red cell agglutination, clumps of white cells and platelets or, exceptionally, the presence of large masses of neoplastic cells.[20]

Blood films are generally observed at medium power (×20, ×25, ×40 or ×50 objective combined with ×8 or ×10 eye pieces). A quick but careful scan of the total film is particularly useful for choosing the optimal area to carry out a white cell differential count, this being the zone where the red cells touch each other without being superimposed, where morphology is optimal (Fig. 1.7). In well-made films, the distribution of white cells is uniform, with crowding confined to the tail and the edges, which is the area where neutrophils and monocytes tend to be most numerous. Observation at low or medium power also permits the detection of artefacts and anomalies of a generalised nature (Fig. 1.8), such as increased background staining and the presence of precipitates linked to hyperproteinaemia or the presence of a paraprotein (Figs 1.9; see also Figs 2.7, 9.1, 9.2 and 9.3) or cryoglobulin (Figs 1.10 and 1.11);[26–28] platelet agglutination (Fig. 1.12; see also Fig. 1.27), leucocyte aggregation (Fig. 1.13) or red cell agglutination (see Fig. 2.54); a tendency to red cell stacking (rouleaux formation) (see Figs 2.7, 2.55 and 9.1); or the presence of fibrin strands (Fig. 1.14).

The peripheral blood film and automated instrument analysis of blood cells should be seen as complementary investigations (see Report 1.1).

## Bone marrow aspirate

Morphological examination of a bone marrow aspirate remains even today the most important means of evaluating the function of the bone marrow and anomalies of haemopoiesis. According to a classical, but still valid, affirmation, it takes second place only to examination of the peripheral blood.[29]

The bone marrow of an adult constitutes 4.7 ± 1.3% of total body weight (equivalent to 1.5–3.7 kg). The red or haemopoietic marrow, which represent about a quarter of this volume (500–700 g), is principally located within the central axial skeleton, in the flat bones and in the epiphyses of the long bones. Its structure is a network of trabecular bone lined by endosteum (formed from a single layer of flattened cells, associated with osteoblasts and osteoclasts), which crosses and delimits a cavity that is rich in sinusoids, reticular stoma, fat cells and small islands and cords of haemopoietic cells. There are around $10^{12}$ haemopoietic cells throughout the body ($0.4 \times 10^{12}$ erythroblasts; $0.5 \times 10^{12}$ granulocytic series; $1 \times 10^{9}$ megakaryocytes). The reticular cells

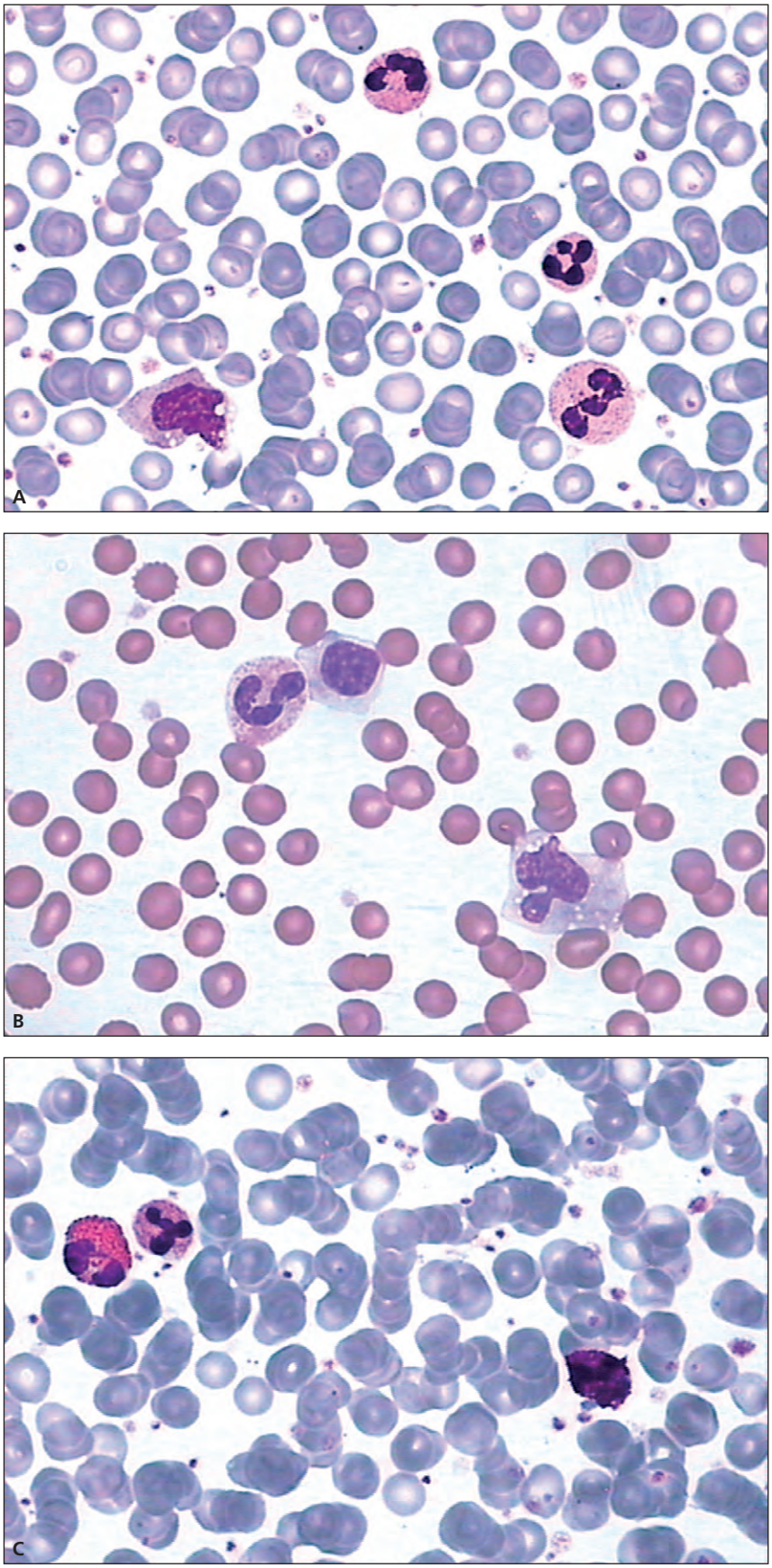



**Fig. 1.7** Morphology of peripheral blood films according to the area of the film selected for examination. (A) Optimal distribution for observation of cytological details. The red cells show minimal superimposition and their morphology appears well preserved. The white cell characteristics are clearly displayed: the nuclei of the three neutrophils on the right, for example, do not show any superimposition of lobes and their granules are clearly seen; the monocyte at bottom left also does not show any distortion. The platelets are separated from each other and well distributed. (B) A thinner area in which the morphology of the erythrocytes is partly altered (there are some echinocytes). The white cells (from the left, a neutrophil with a small nuclear appendage, a lymphocyte with plentiful cytoplasm and a monocyte with a number of small vacuoles) appear well spread and their characteristics are recognisable. The platelets, which appear hypochromatic, are clearly seen. (C) A thicker area of the film. The red cells are stacked and their morphological characteristics cannot be readily identified. The platelets are numerous and hyperchromatic. The three granulocytes appear compressed, but are still readily identifiable because of the characteristics of their granules: from the left, an eosinophil, a neutrophil and a basophil.

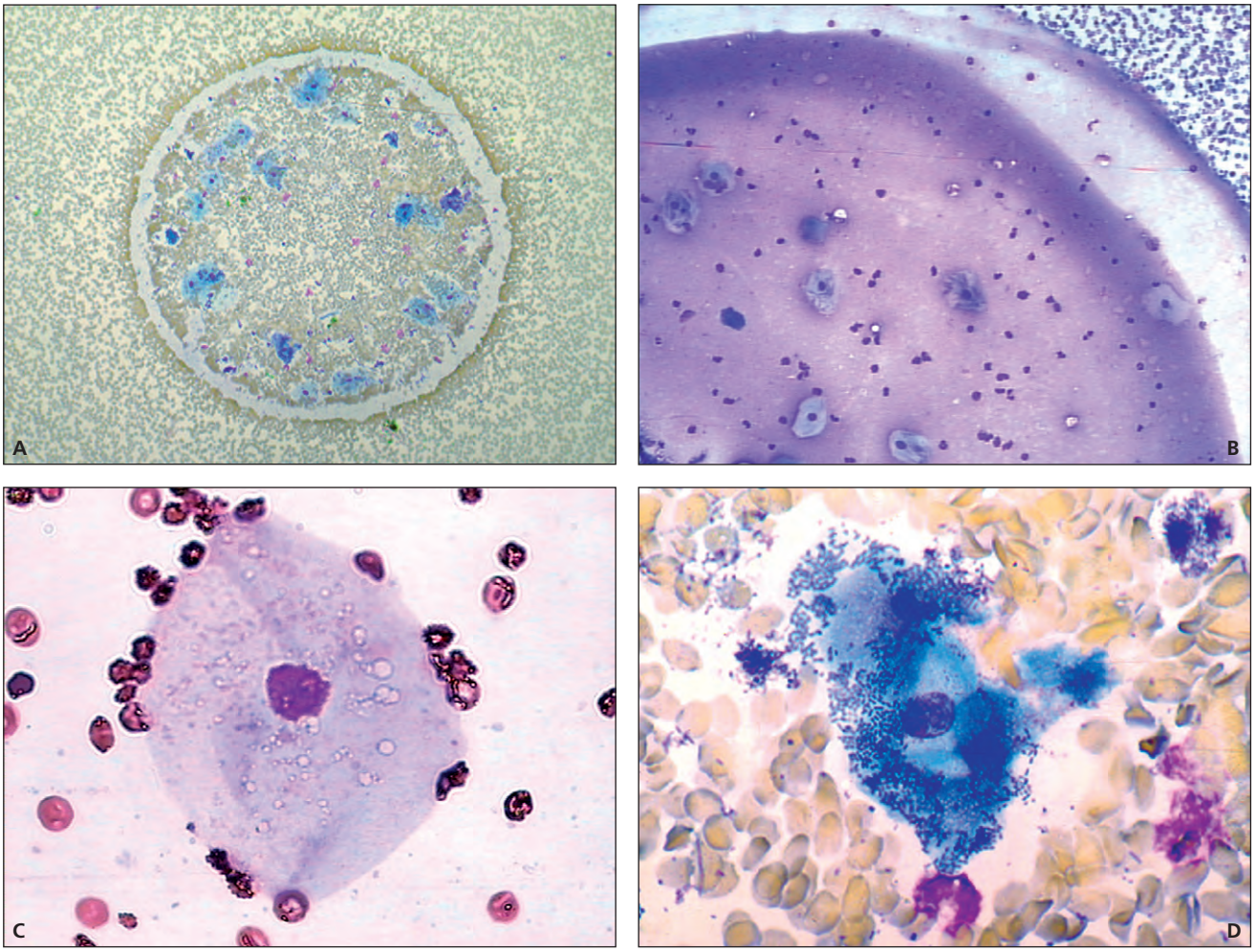



**Fig. 1.8** Salivary material in a peripheral blood film. This artefact is caused by a cough, a sneeze or some other vigorous respiratory activity on the part of the operator during the spreading of the film. (A) Low power: there is the appearance of a pseudocyst, with a pale circumference and within this, superimposed on the red cells, which are still visible because the overlying cells are transparent, are numerous large cells with dark nuclei and plentiful pale thin cytoplasm. (B) In this case, at higher power, proteinaceous material representing mucus is visible within the pale circle; the background red cells are not visible. There are numerous lymphocytes, visible as hyperchromatic nuclei, and large epithelial cells of oral mucosal origin; the latter are polygonal with a central dark round nucleus. (C) The epithelial cells desquamated from the oral mucosa of the operator making the film may be seen as isolated cells, as in this case. (D) This epithelial cell from the operator's oral mucosa, which has contaminated the peripheral blood film, has its cytoplasm invaded by numerous cocciform bacteria, sometimes forming short chains.

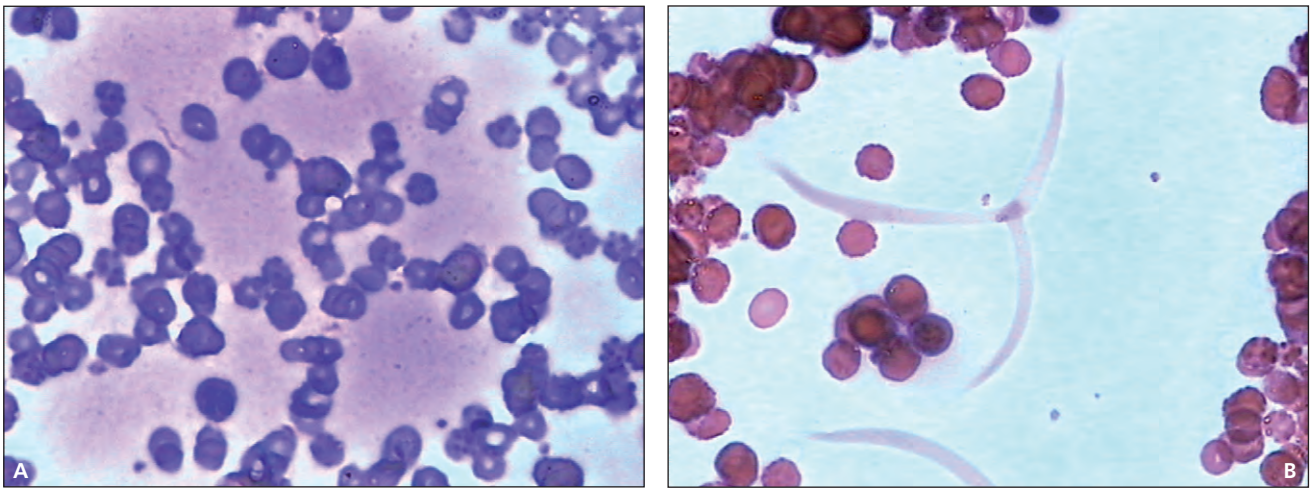



**Fig. 1.9** Artefact due to precipitation of protein in the peripheral blood film. (A) Cloudy violaceous masses with indistinct margins in a patient with polyclonal hypergammaglobulinaemia. (B) Bow- or comma-shaped protein deposits in a patient with hepatic cirrhosis and dysproteinaemia.

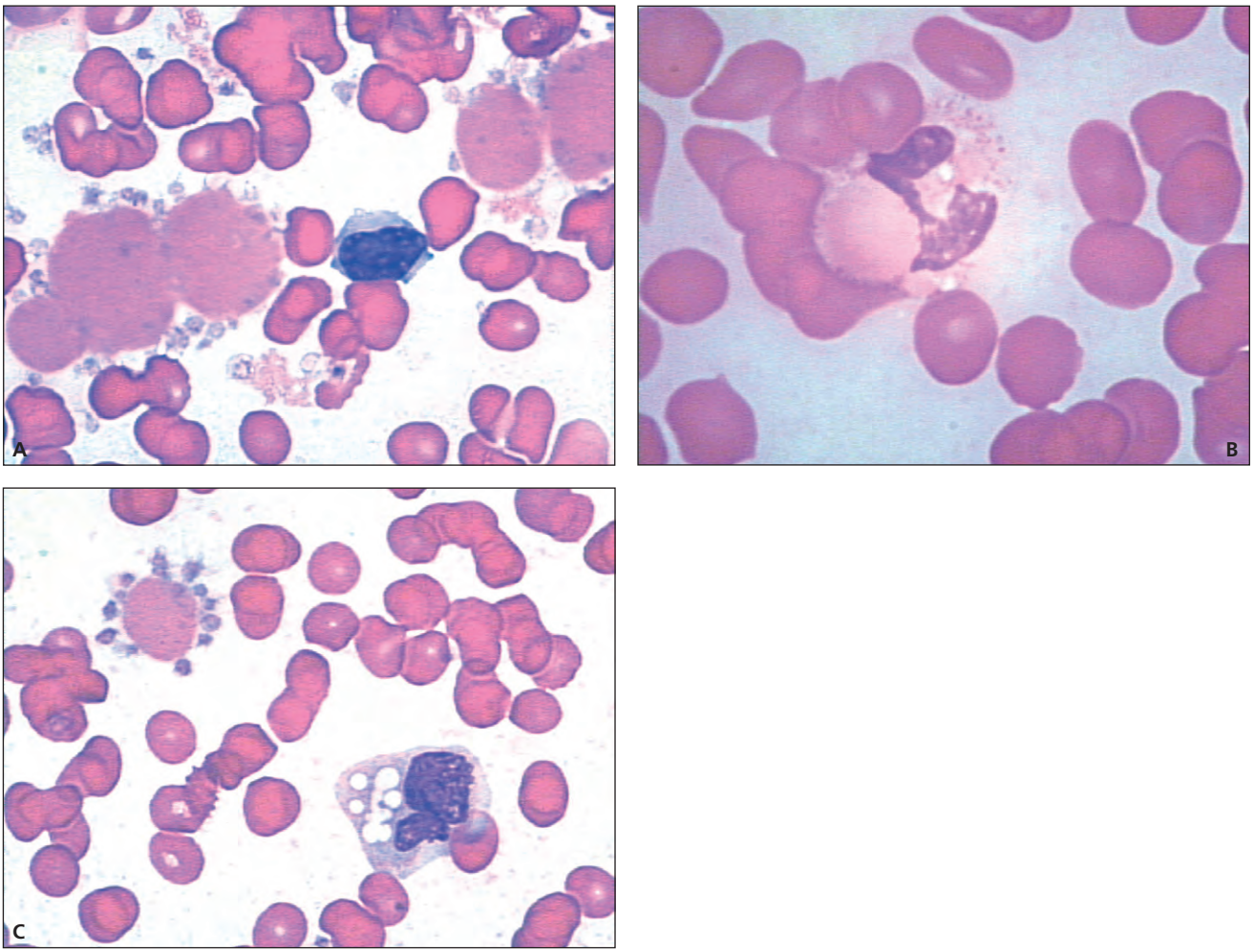



**Fig. 1.10** Amorphous cryoglobulin deposits in a peripheral blood film. (A) Coalescent globular masses of amorphous pink material, with partly adherent peripheral platelets. There is a normal appearing lymphocyte. (B) A neutrophil granulocyte has phagocytosed a spherule of precipitated cryoglobulin. The cytoplasm is heavily vacuolated. (C) Top left, satellitism of platelets around an amorphous deposit of cryoglobulin. Bottom right, a monocyte with plentiful heavily vacuolated cytoplasm.

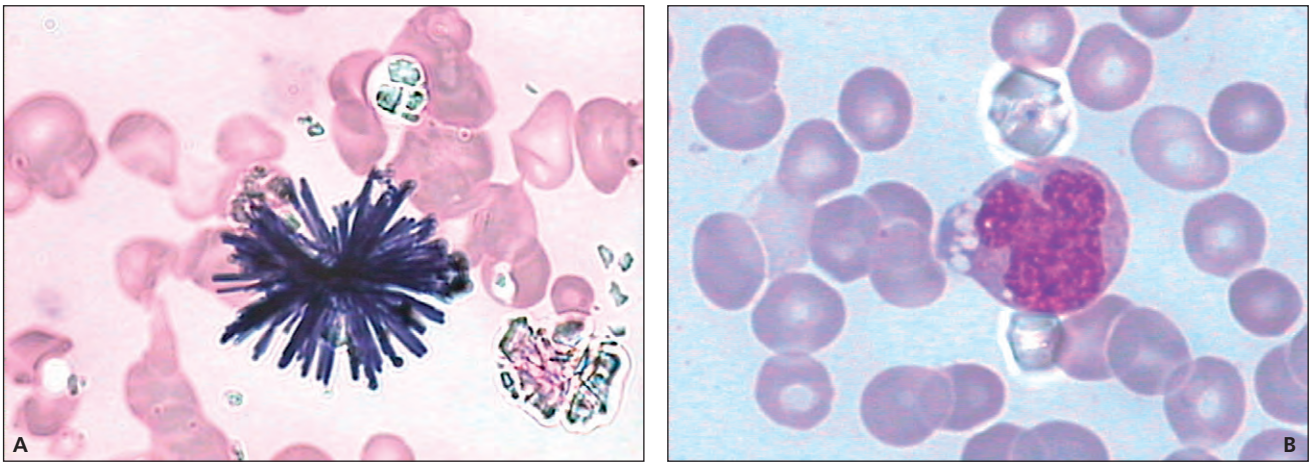



**Fig. 1.11** Crystalline cryoglobulin deposits in peripheral blood films. (A) Crystals of various shapes, some refractile. (B) Small crystalline precipitates above and below a monocyte in the blood film of a different patient.

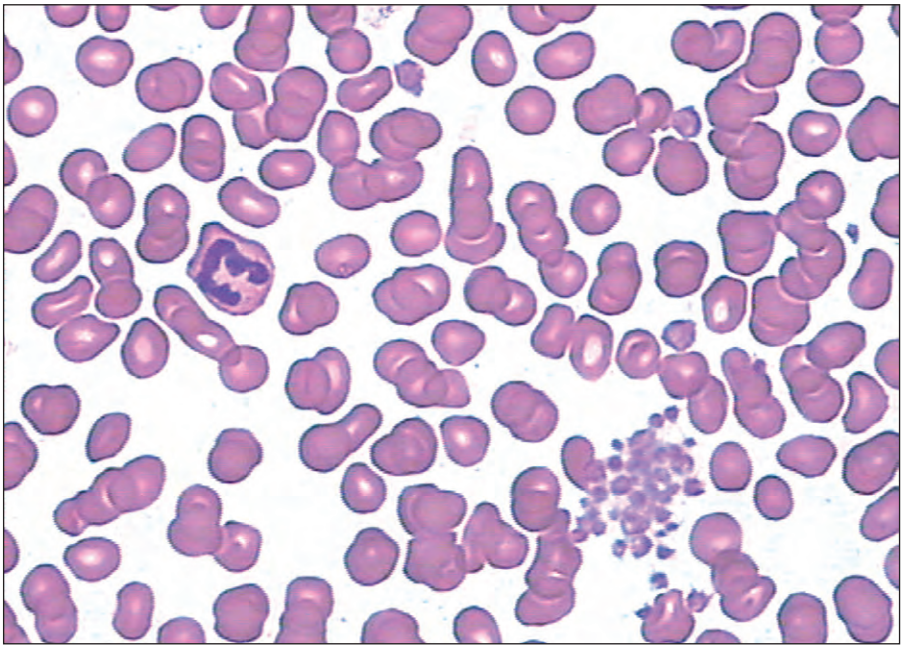


**Fig. 1.12** Platelet agglutination. Bottom right, a small mass of platelets, which appear to have adhered to each other, although the individual platelets are still easily discerned. This appearance, which can be associated with a factitiously low platelet count and a 'flag' on automated instruments, is typical of pseudothrombocytopenia caused by EDTA-dependent platelet antibodies. On the left, a neutrophil band form.

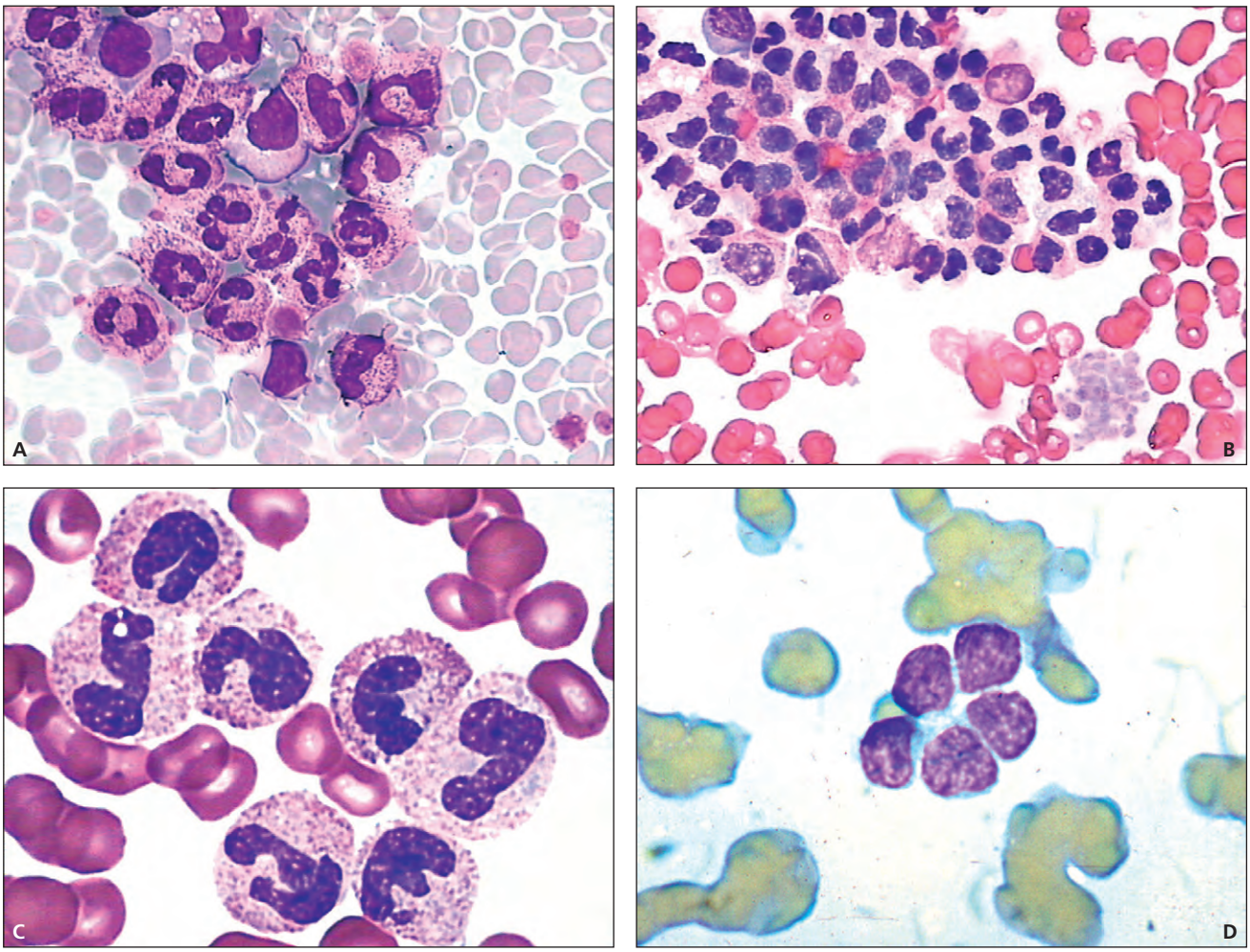



**Fig. 1.13** White cell agglutination in peripheral blood films. (A) This large agglutinate includes two monocytes, in addition to numerous very granular neutrophils. The platelets, clearly visible on the right, appear hyperchromic and heterogeneous in size: one giant platelet is visible towards the lower pole of the agglutinate, immediately above a small lymphocyte. (B) A large agglutinate of neutrophils, top left. A smaller agglutinate of platelets is seen near the bottom right corner. (C) Two small agglutinates of very granular neutrophil band forms. The individual cells seem to have adhered over a limited part of the cell circumference, which is straight in the area of contact. Cell morphology is well preserved. (D) Small flower-like aggregate of lymphocytes in an HIV-positive patient, in the absence of a lymphoproliferative disorder. The cells appear to have adhered at their poles.

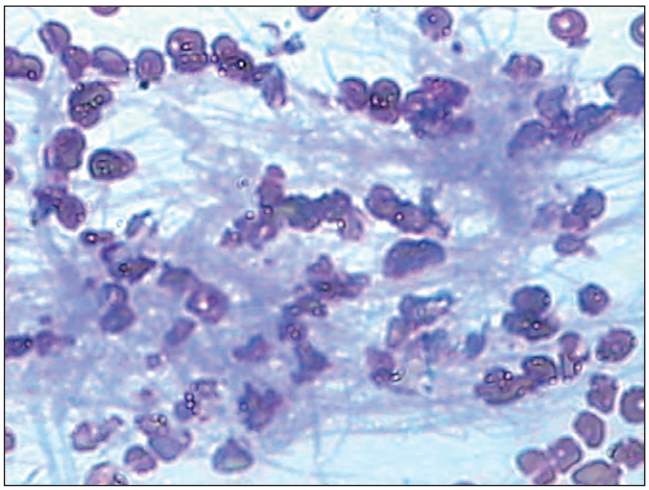


**Fig. 1.14** Fibrin strands in a peripheral blood film.

spread their long cytoplasmic processes over the internal surfaces of the trabeculae and over the adventitial tissue that invests the sinusoids.[30]

### Obtaining a bone marrow aspirate and preparing films

The aspiration of bone marrow is usually performed, with informed consent, using an appropriate single-use needle, from the posterior superior iliac spine.[31] This route of access to the marrow cavity allows a second needle to be immediately inserted to obtain a core biopsy of bone and bone marrow with a low frequency of complications and minimal inconvenience to the patient. Other sites can be used in particular cases, such as the sternum in obese patients and in those who have had pelvic irradiation or who are immobile, or the vertebral spinous processes or the proximal epiphysis of the tibia in children.

A high quality bone marrow aspirate requires that dilution of the sample by peripheral blood be kept to a minimum; this dilution increases in proportion to the volume of the aspirate.[32] For this reason the maximum volume of fluid that should be aspirated must not be more than 0.2–0.3 ml. If it is necessary to have more material for further investigation, such as immunophenotyping or cytogenetic analysis, a second sample should be taken into a separate syringe.

There are numerous techniques described for dealing with aspirated bone marrow. The most widely employed, and the one used in the laboratory of the authors, is that described in the classic manual, *Dacie and Lewis Practical Hematology*',[21] which is based on a film being made from a drop of particulate aspirate, after having disposed of surplus fluid by inclining the slide. The fragments of bone marrow are dragged along the glass slide, depending on their dimensions and density, to the tail of the preparation, leaving behind them cell-rich trails, which are optimal for microscopic examination. The quantity and quality of cells suitable for examination in these preparations can be increased by the delicate apposition and spreading of isolated particles, gathered with forceps or with the angle of a cover-slip, and placed on another slide. An alternative equally valid technique in expert hands and recommended for the quality of the cytological detail and for the absence of peripheral blood dilution, is based on the crushing of fragments between a slide and a cover-slip.[33]

The methods used for staining bone marrow films are not dissimilar to those used for staining blood films. The stained films can be protected by permanent mounting of a suitable cover-slip. In addition to panoptic staining such as May-Grünwald-Giemsa, it is useful to routinely perform an iron stain using the Perls' method. Cytochemical stains such as peroxidase, esterases, toluidine blue and periodic acid-Schiff (PAS) can be reserved for specific indications in selected cases.

### Pre-microscopic and microscopic examination

Evaluation of a bone marrow aspirate must follow a systematic approach, which permits an assessment of the quality of the preparation and helps to validate the conclusions that are reached.[34,35] Evaluation involves a pre-microscopic phase, which includes the acquisition of clinical information, noting the diagnostic question that is being asked, an assessment of potentially relevant therapeutic information (chemotherapy, iron, vitamins, growth factors, corticosteroids), examination of the full blood count and a peripheral blood film, consideration of information gained at the time of obtaining the sample (such as reduced or increased hardness of the bone), and the results of physical examination (such as the presence of splenomegaly or cutaneous lesions).

Examination of bone marrow films must first be done at low power (×10 objective). These important preliminary procedures guide the subsequent morphological assessment, which uses an approach that is both rigorous and systematic (Fig. 1.15):

1. Evaluation of whether or not the sample is representative: a bone marrow film can be considered representative of the actual cells in the marrow if fragments are present, if the films have been prepared in the correct manner and are suitable for cytological assessment, and if the majority of cells appear intact (Fig. 1.16).
2. Semiquantitative evaluation of cellularity, in relation to the stroma and fat cells in the particles (Figs 1.17 and 1.18). A correct estimate of cellularity is dependent on careful consideration of many factors (the quality of the material, adequacy of the aspirate, suitability of the films and age of the patient). This initial assessment is fundamental for reaching the desired objective, a valid microscopic assessment. The evaluation is subjective and difficult, but is effective. It does not provide a numerical estimate of cellularity (which is achieved by histological sections), but nevertheless can be expressed in seven semiquantitative descriptive terms (normal; slightly reduced, moderately or markedly reduced; slightly increased, moderately or markedly increased).

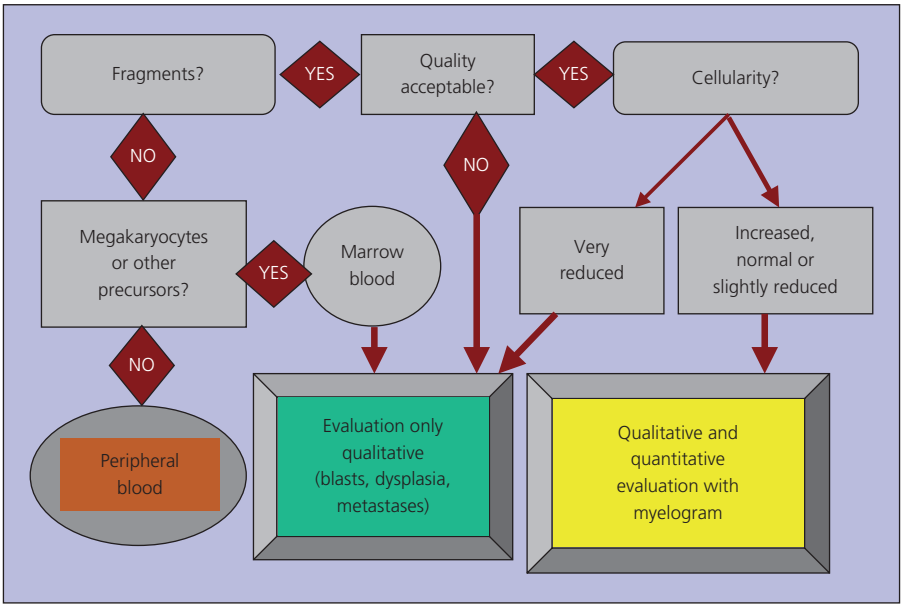
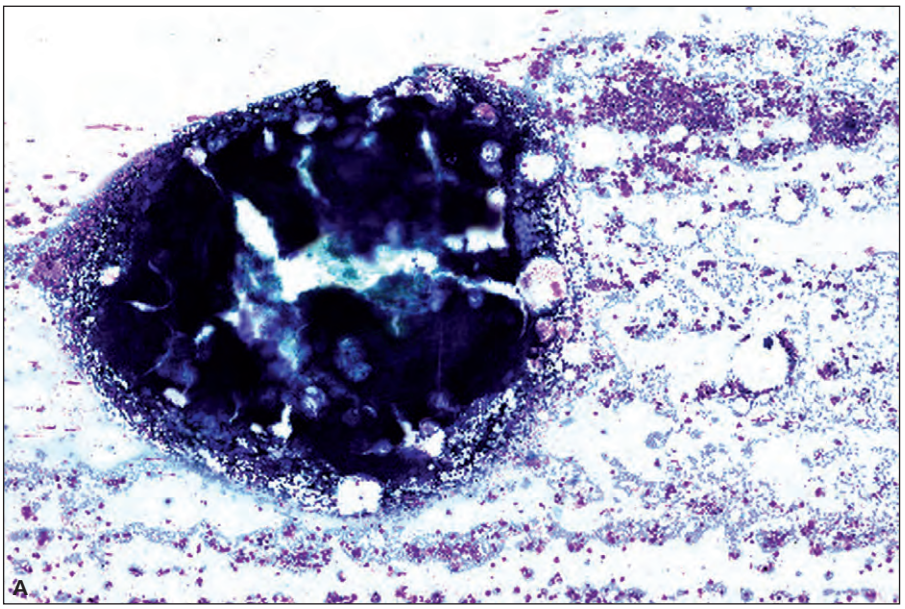



**Fig. 1.15** Schematic representation of the process of evaluating bone marrow aspirate films. The performance of a differential count (a myelogram) is obligatory only when particles are present, when the quality is acceptable and when the number of cells is sufficient. In other cases, if the presence of megakaryocytes and haemopoietic precursors indicates that the material is bone marrow, it is possible to make a qualitative evaluation.



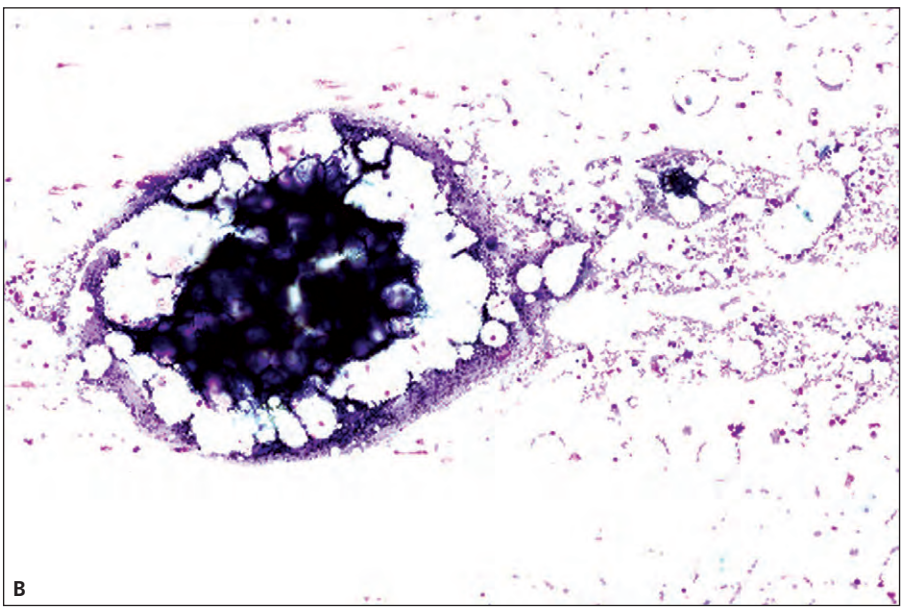




**Fig. 1.16** Fragments or particles of marrow in bone marrow aspirate films, low power. (A) The fragment has been dragged along the slide, using a spreader at an angle of 45°, to the distal extremity or tail of the film. The particle visualised here is compact and very cellular. The area of choice for cytological evaluation at higher power is where cells have been left behind the fragment as the film is spread: in this case, top right. (B) In this small fragment, fat cells predominate and surround a dense mass of unidentifiable material. The cellularity of the preparation, judging from the trail behind the fragment, appears low.

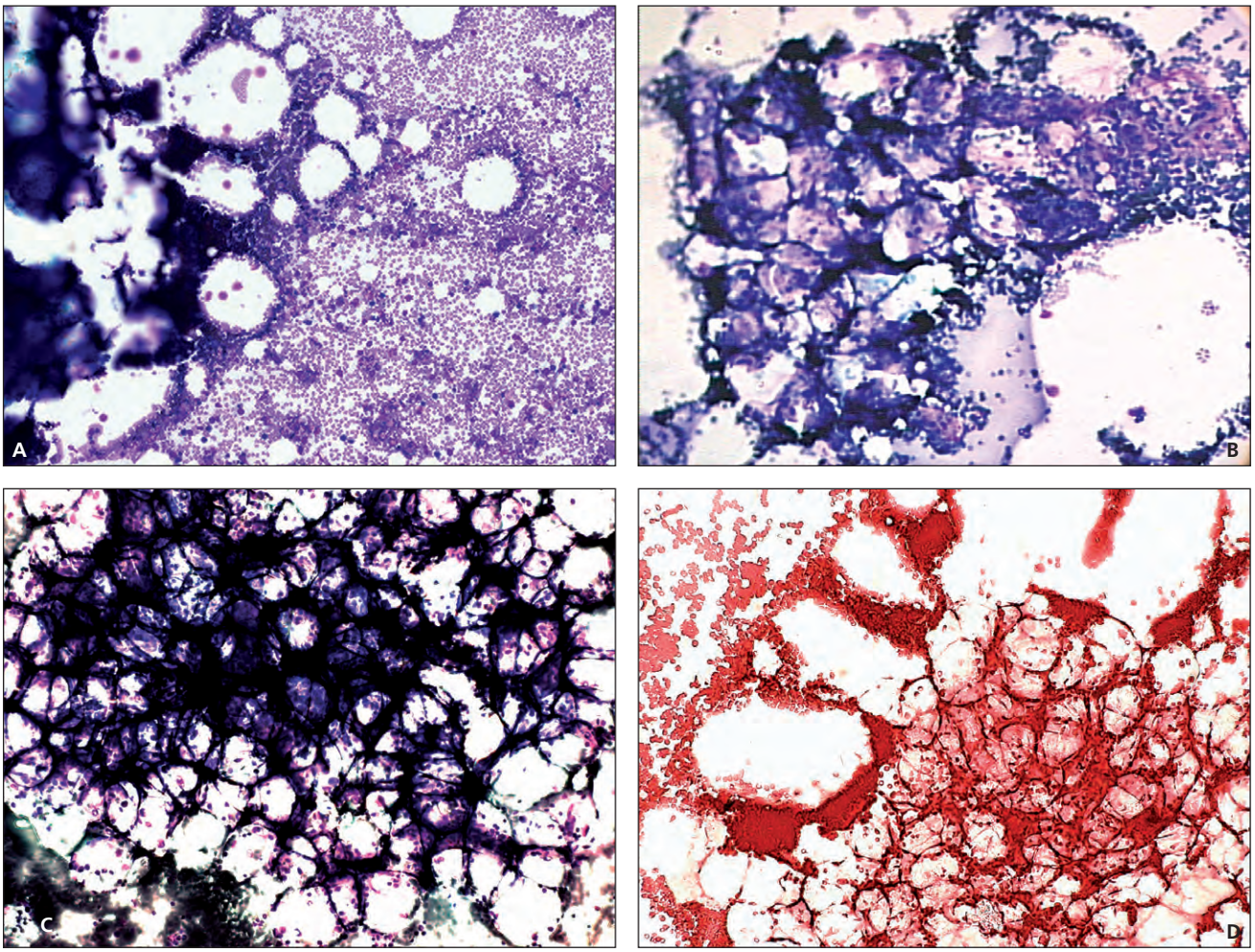
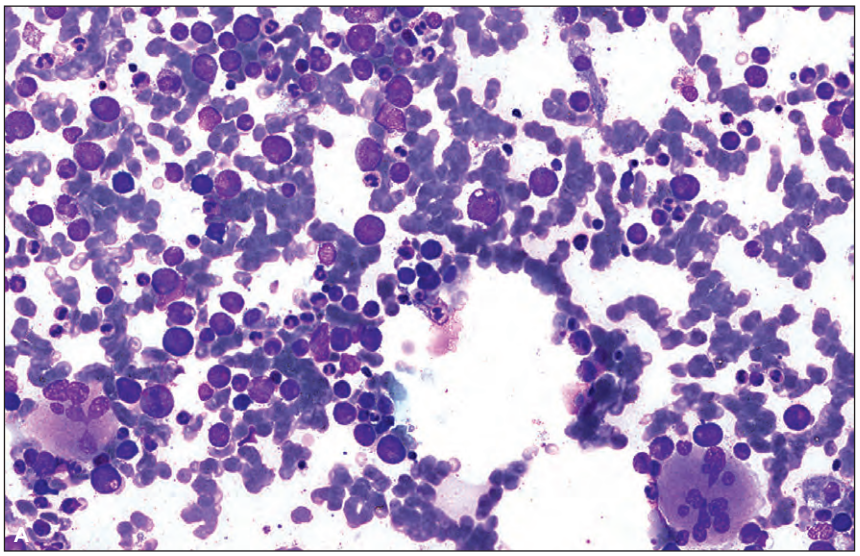
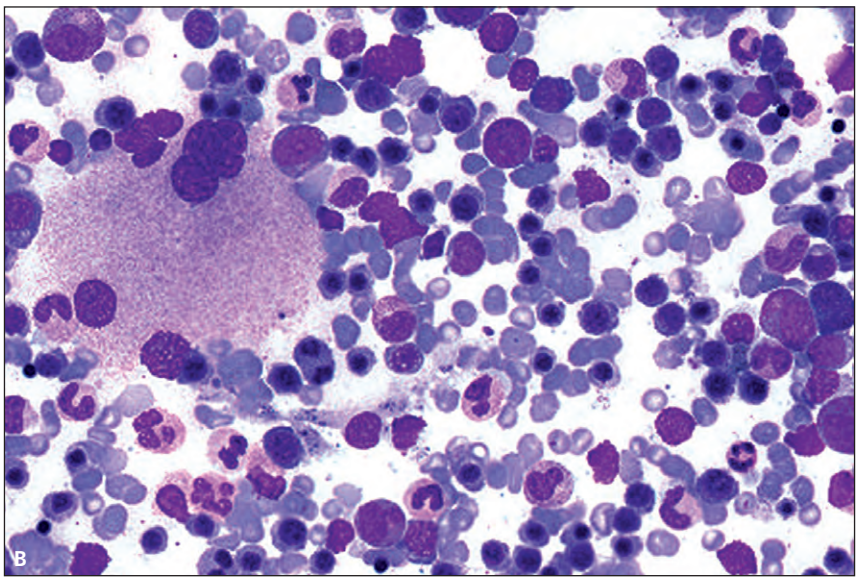



**Fig. 1.17** Bone marrow fragments in aspirates: variation in cellularity. (A) This particle has left on the slide a good number of well-preserved cells that appear adequate for morphological analysis. (B) Hypocellular particle composed mainly of fat cells, the cytoplasm of which has been transformed by fixation in alcohol into large globular optically empty spaces. Top right, a clump of fibronecrotic material. A preparation like this, at least judging from the area seen here, is not ideal for cytomorphological evaluation. (C) Hypocellular fragment with numerous fat cells and a thick network of fibres supporting the few cells in its interstices. (D) Hypocellular particle, Perls' stain. Storage iron is not visible and the cellularity seems greatly reduced, with no nuclei of haemopoietic cells being apparent.

3. Cytological assessment of large cells present in small numbers: presence, number, disposition and morphological characteristics of megakaryocytes (Fig. 1.18); identification of other large cells that are easily recognisable, which can be normal marrow constituents, such as osteoblasts and osteoclasts, plasma cells or mast cells, or groups or clumps of cells, which in their turn may be normal, reactive or neoplastic (Fig. 1.19).
4. Selection of the best and most representative area for high power examination: the choice is necessarily usually the trails of cells left behind a particle. The fundamental principle is that the area selected must be where the cells are regularly distributed, close together without being superimposed, with optimal preservation and visibility of cellular structures, and without an excess of damaged cells. Diagnostic errors, sometimes serious, can result from an inadequate cytological evaluation of a non-ideal preparation or a non-ideal area of the film, in which it is impossible to appreciate fine details such as chromatin structure, nucleoli or the characteristics of granules (Fig. 1.20).

Diagnostic cytohaematology is based essentially on observation at high power with an oil immersion objective (×50 and ×100). A qualitative morphological analysis of the cellular characteristics is normally accompanied by a quantitative estimate of the proportion of cells belonging to the various haemopoietic lineages plus an assessment of the proportion showing specific



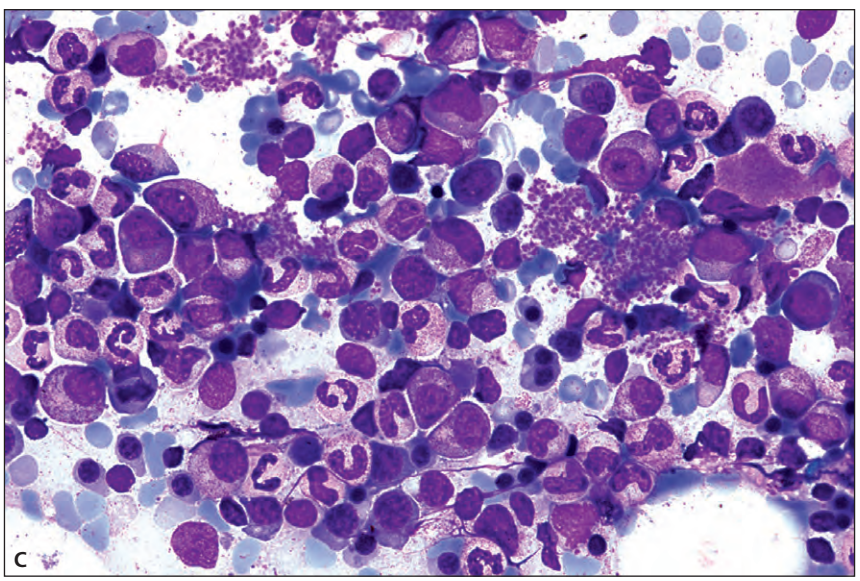




**Fig. 1.18** General evaluation of cellularity and identification of large cells in an optimal area of bone marrow films; low to medium power. (A) Preparation with optimal cellularity, distinguished by heterogeneous differentiation with all stages of maturation being present. One observes, in particular, the predominance of granulocyte precursors, with a reduced proportion of mature neutrophils (a useful observation for excluding dilution by peripheral blood, which here is minimal). At bottom right and bottom left are mature megakaryocytes with well-lobated nuclei; in the case of the cell on the right, the nucleus is probably hyperlobated: this is potentially a dysplastic feature which requires verification by examining a larger number of megakaryocytes, as discussed in Chapter 3. (B) Hypercellular film in which the cytomorphological feature are well preserved. In this case erythroid cells predominate in all areas of the field; they show normal maturation with good representation of all maturation phases. Mature neutrophils are relatively less numerous, guaranteeing that dilution by peripheral blood is minimal. The large megakaryocyte shows plentiful, fully mature cytoplasm which is ready to form platelets, the granules being dense and numerous demarcation zones becoming apparent. The nucleus is multilobated and hyperdiploid, as is usual, with a rounded detached lobe in the lower left part of the cytoplasm: slight suggestions of dysmorphism of this type are observed in a reasonable proportion of normal subjects. (C) A very cellular and heterogeneous marrow, with granulocytic and erythroid precursors showing well-preserved maturation. The violaceous filaments visible at the top and at the bottom are composed of nuclear material derived from cells that were damaged during the spreading of the film. No megakaryocytes are seen but there are numerous masses of well-formed platelets.

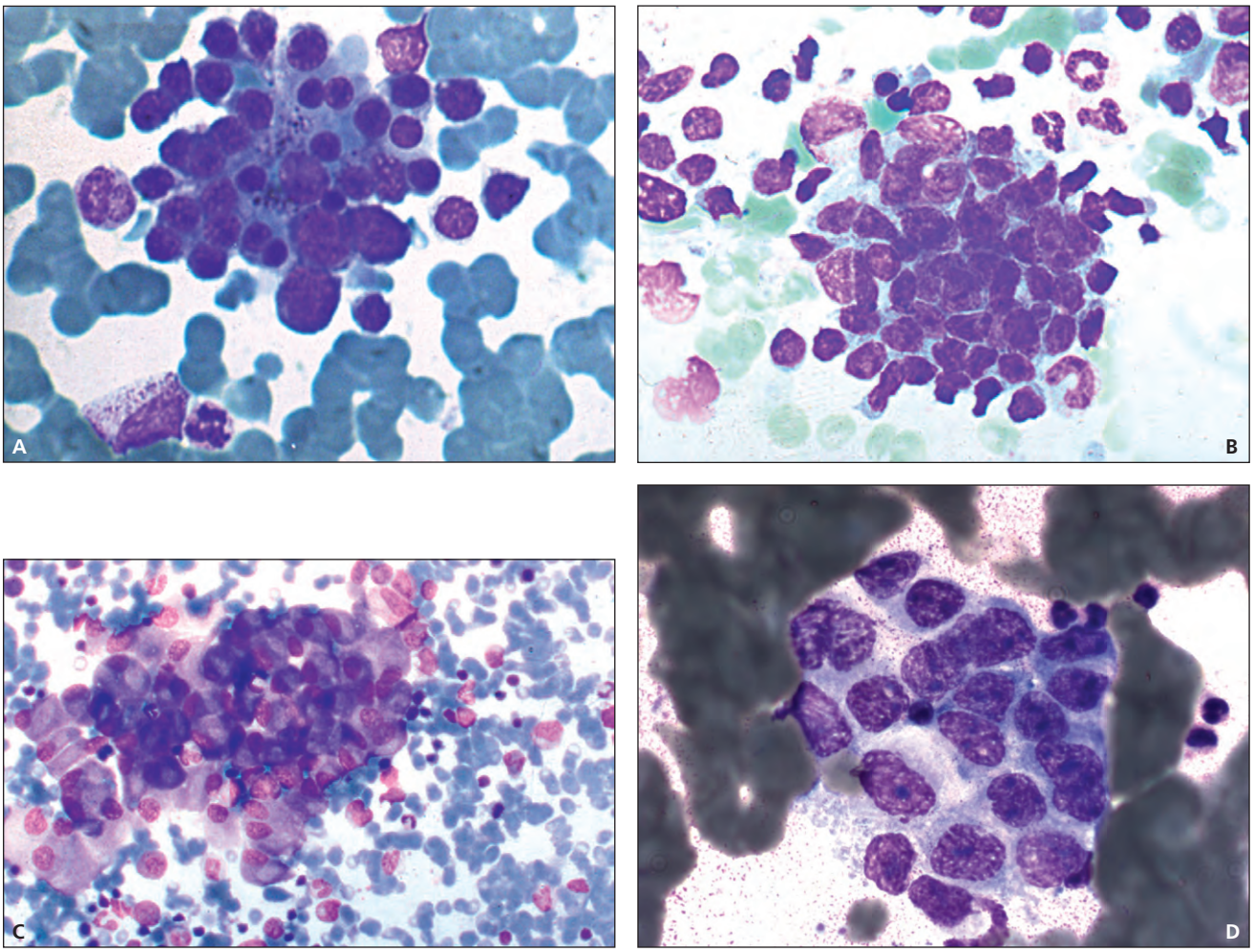



**Fig. 1.19** Clusters of cells detectable at low power in bone marrow films. (A) An erythroid island with erythroblasts at various stages of maturation crowded around a macrophage; the pale nucleus of the macrophage and its cytoplasm laden with blackish granules can be seen. (B) Lymphoid nodule from a patient with Waldenström macroglobulinaemia. The nuclei, which appear mature, are superimposed on each other in a disorderly manner; lymphoid cells are also present outside this nodule. (C) Clump of osteoblasts, without any specific pathological significance. The recognition of cytological details is easier when these cells are partly detached at the periphery of the clump: for example, the eccentric nucleus and the presence of a large Golgi zone detached from the nucleus is clearly seen in the cell at the bottom and in those to the left. (D) Bone marrow metastasis of undifferentiated carcinoma. The cells, not dissimilar to lymphoid blasts, have scanty cytoplasm and appear crowded together with a clear outline suggesting a glandular origin.

anomalies, such as dysplastic features. A differential count – a myelogram (Tables 1.1 and 1.2) – can be performed in various ways. Assuming that there is a good quality preparation (presence of fragments and adequate cellularity), the percentage of cells in various categories, including mature cells, such as segmented neutrophils, can be calculated: this procedure avoids arbitrary modifications of the evaluation and permits an assessment, based on the percentage of mature neutrophils (which generally is not greater than 10–15%), of the representative nature of the aspirate. It is necessary to count at least 500 cells, using two different films. On this basis the relationship between total cells belonging to the granulocytic series and total erythroblasts (the myeloid:erythroid or M:E ratio) varies in normal subjects from 1.5 to 4.5: lower values suggest erythroid hyperplasia or granulocytic hypoplasia, while higher values have the opposite significance. In addition to determining the percentage of cells of various haemopoietic lineages and the M:E ratio, classical evaluation of the marrow required assessment of various quantitative parameters which are now little used. The execution of the so-called maturation curve can occasionally give useful information: it permits an evaluation, with the help of a graphical representation, of the various maturation phases of a specific haemopoietic lineage, with a shift to the left indicating the presence of an increased proportion of less differentiated

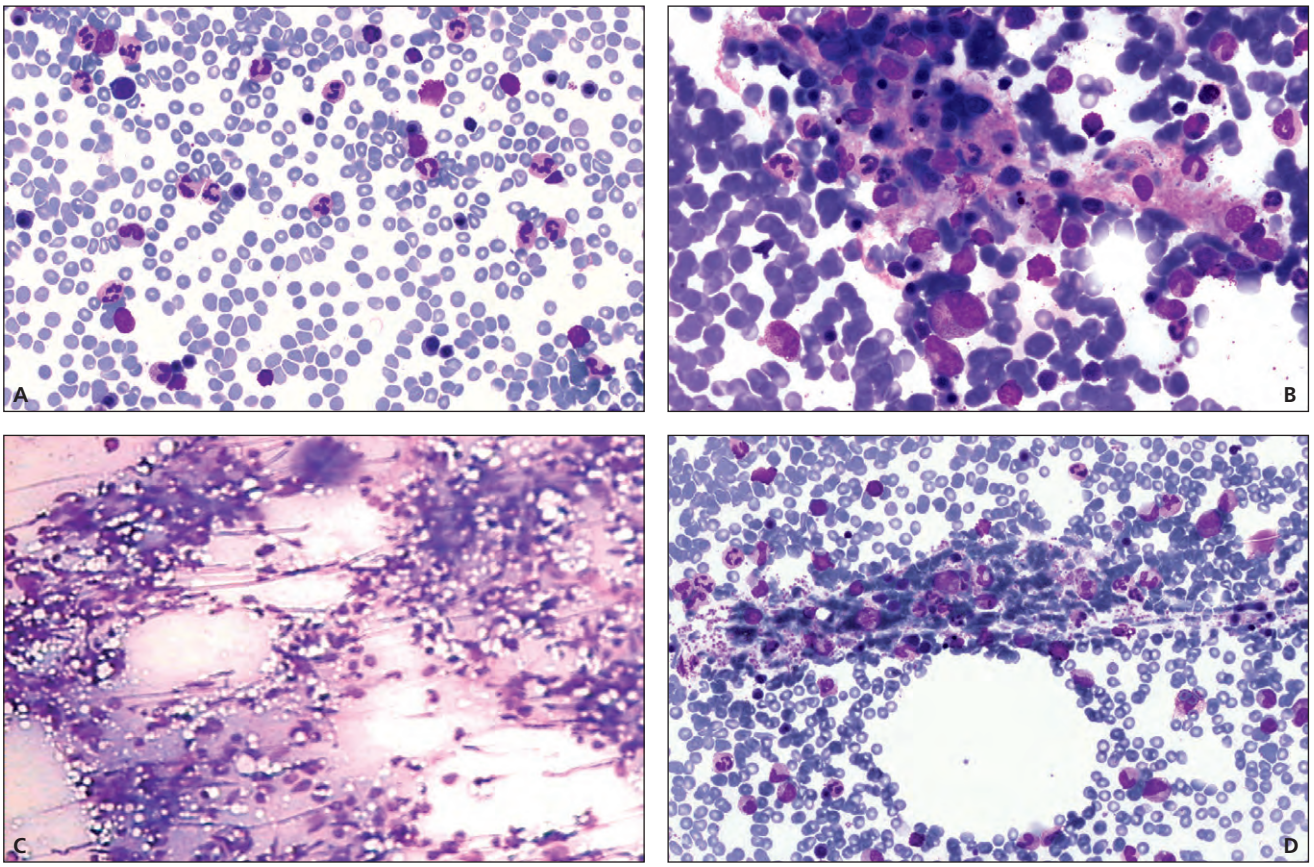



**Fig. 1.20** Bone marrow aspirates that cannot be assessed quantitatively. (A) Low cellularity with predominance of segmented neutrophils, which indicates considerable dilution by peripheral blood. There are some erythroblasts which can be assessed qualitatively, but the performance of a differential count on a preparation such as this would give misleading results. (B) Haemopoietic and stromal cells forming a fibronecrotic mass, in which context a quantitative evaluation would be inadequate. In the centre at the bottom, a promyelocyte and a neutrophil metamyelocyte can be recognised. (C) Massive cell damage in a bone marrow film. No evaluation of a field such as this is possible. At the top in the centre, however, it is still possible to recognise a large megakaryocyte. (D) Hypocellular film with an abundance of mature neutrophils and cells caught in a mass of fibrin and platelets; the preparation cannot be evaluated quantitatively.

forms, or to the right, of an increased proportion of cells at a more advanced stage of maturation. Determining the percentage of mitotic figures can be of interest (on average 0.5% in the granulocytic series and 1–2% in the erythroid series, with higher values in women than in men), although the karyological curve, which shows graphically the proportions of cells in various phases of the cell cycle, is no longer performed.[36] Measuring the percentage of megakaryocytes is not useful. It is generally preferable to make a semiquantitative assessment in terms of the number of megakaryocytes seen in a low power microscopic field; now almost forgotten, but perhaps worth re-evaluating in the context of thrombocytopenia or thrombocytosis, is the differential count of megakaryocytes in different stages of maturation.

Determining the percentage of blast cells is of considerable importance as is the detection of dysplasia in any lineage; the World Health Organization (WHO) classification of acute myeloid leukaemia and the myelodysplastic syndromes is based on these assessments. All pathological cells (blast cells, lymphoid cells, plasma cells) and also parasites must be analysed and if possible quantified, with their morphological characteristics being described.

The report (Table 1.3) must contain information on the quality of the preparation and the type of evaluation that has been possible. The myelogram must be reported for all samples with adequate and representative cellularity (according to the algorithm shown in Fig. 1.15). The conclusions can be confined to an accurate description of what has been observed or, when the morphological picture makes it possible, can include a definitive diagnosis, which in the majority of cases will be based on an integration of immunophenotypic, cytogenetic, molecular and histological data.

**Table 1.1** Myelogram and maturation curve

| | Myelogram (%) (of all cells) | Maturation curve (%) (with regard to the relevant lineage) |
|---|---|---|
| Blasts (myeloblasts) | 1–3 | 1–4 |
| Promyelocytes | 1–7 | 5–10 |
| Neutrophil myelocytes | 5–20 | 20–40 |
| Neutrophil metamyelocytes | 10–30 | 20–40 |
| Segmented neutrophils and band forms | 5–25 | 10–35 |
| Eosinophil series | 1–4 | – |
| Total granulocytic series | 35–65 | – |
| Proerythroblasts | 1–5 | 2–5 |
| Basophilic erythroblasts | 2–8 | 10–20 |
| Polychromatic erythroblasts | 2–20 | 30–60 |
| Orthochromatic erythroblasts | 2–10 | 20–40 |
| Total erythroid series | 15–35 | – |
| Monocytes | 0–3 | – |
| Lymphocytes | 3–15 | – |
| Plasma cells | 0–3 | – |
| Macrophages, reticular cells | <2 | – |
| Mast cells | <1 | |
| Megakaryocyte series | 1–2 per field** | – |
| Myeloid:erythroid ratio* | 1.5–4.5 | – |

*Myeloid:erythroid or granulocyte:erythroid ratio: calculated as the ratio between total cells of the granulocytic series, including the mature granulocytes and monocytes, and total cells of the erythroid series.
**Low power (×10 objective).
NB. The values reported were obtained by counting all nucleated cells, mature and immature, in cellular bone marrow films.

**Table 1.2** Myelogram: median values obtained in 50 healthy volunteers between the ages of 21 and 50 years[50]

| | Reference interval (%) (central 95%) | Median value (%) |
|---|---|---|
| Blasts (myeloblasts) | 0–3.0 | 1.4 |
| Promyelocytes | 3.2–12.4 | 7.8 |
| Neutrophil myelocytes | 3.7–10.0 | 7.6 |
| Neutrophil metamyelocytes | 2.3–5.9 | 4.1 |
| Segmented neutrophils and band forms | | |
| Men | 21.9–42.3 | 32.1 |
| Women | 28.8–45.9 | 37.4 |
| Eosinophil series | 0.7–6.3 | 3.5 |
| Basophil series | 0–0.4 | 0.1 |
| Erythroblasts (total) | | |
| Men | 16.2–40.1 | 28.1 |
| Women | 13.0–32.0 | 22.5 |
| Monocytes | 0–2.6 | 1.3 |
| Lymphocytes | 6.0–20.0 | 13.1 |
| Plasma cells | 0–1.2 | 0.6 |
| Macrophages | 0–1.3 | 0.4 |
| M:E ratio | | |
| Men | 1.1–4.1 | 2.1 |
| Women | 1.6–5.2 | 2.8 |

NB. The volume of aspirate was 0.1–0.2 ml and the films were made using the standard wedge-spread technique. Small difference between men and women were recognised in mature neutrophils, erythroblasts and the myeloid:erythroid (M:E) ratio. With regard to Table 1.1, one notes particularly the higher values for promyelocytes, neutrophils and lymphocytes.

### Histological examination

Bone marrow core biopsies are nowadays obtained with a suitable needle as part of a single procedure with the bone marrow aspirate. The biopsy specimens are fixed, sectioned and stained with haematoxylin and eosin (H&E), by the Giemsa technique and with a stain for reticulin fibres (e.g. a Gomori stain). The sections can also be used for immunohistochemistry, a fundamental procedure for investigating many pathological processes that cannot be recognised by morphology alone (Fig. 1.21).

The indication for histological examination depends on patient characteristics, but often it is necessitated by the suspicion of bone marrow fibrosis, hypocellularity of irregularly distributed focal lesions. In practice, the pathological processes in which a core biopsy is necessary, and indeed is quite often more useful than the aspirate, are lymphomas and lymphoproliferative disorders (to evaluate the type of infiltration, which is often nodular or focal), myeloproliferative neoplasms (to grade fibrosis and identify the characteristics of the megakaryocytes), multiple myeloma and other plasma cell disorders, solid tumours with the suspicion of bone marrow invasion and granulomatous conditions such as sarcoidosis.[37,38] Above all, in patients in whom the preceding bone marrow aspiration has been a 'dry tap' or has yielded only a few drops of blood or diluted marrow without particles, it is very useful to utilise a core biopsy to make imprints for cytological examination.

## Principles of haematological cytomorphology

The quantitative and qualitative characteristics that blood cells manifest when blood and bone marrow films are examined microscopically are the result of the simultaneous action of three biological forces, which act synergistically to produce adequate numbers of functioning circulating cells to meet the biological needs of the body. The first force is the reproductive force, manifest as proliferation of haemopoietic cells: these

**Table 1.3** Example of a report on a bone marrow aspirate

| | |
|---|---|
| **Report:** Haematology | **Date of sample:** 3rd April 2012 |
| **Procedure:** Dr. G. d'Onofrio | **Site:** posterior iliac crest |
| **Name and surname:** John Smith | **Informed consent:** Yes |
| **Age:** 58 years | |
| **Clinical problem:** leucocytosis, thrombocytopenia, suspicion of acute leukaemia | |

| **Description** | **Comment** |
|---|---|
| Quality: good<br>Fragments: present<br>Cellularity: good<br>Iron stain: storage iron well represented | Marked infiltration by blast cells, some of which are granulated and some of which contain Auer rods; peroxidase positive; dyserythropoiesis <10%. Dysplasia of mature granulocytes |
| **Myelogram** | **Cytomorphological diagnosis** |
| Neutrophil series: 10%<br>Erythroid series: 14%<br>Lymphocytes: 13%<br>Plasma cells: 3%<br>Blast cells: 60%<br>M:E ratio: not evaluable<br>Megakaryocytes: some present | Acute myeloid leukaemia with maturation, of the type associated with t(8;21), to be confirmed by molecular studies |
| Signature: Dr. G. Zini | |

increase in number as a result of mitotic divisions co-ordinated by the combined action of growth factors. The second force, tightly linked to the first, is that of evolution and differentiation: this induces the cell, at the same time as it is proliferating, to acquire certain functional abilities together with corresponding morphological changes, becoming increasingly close to the mature end cell. These first two processes, in the healthy adult, occur in the haemopoietic tissue of the marrow and in lymphoid organs. The third force is that driving migration, which leads to the entry of mature cells into the circulation and also, in the case of granulocytes, lymphocytes and monocytes, to their exit from the circulating blood into extravascular tissues. The subtle hormonal equilibrium that co-ordinates this orderly process is now in large part well known.[39]

The proliferation and maturation occur in a somewhat similar manner in all haemopoietic lineages: in each cell lineage, for example, the most immature stages, in which proliferative forces dominate, show functional and volumetric hypertrophy of the nucleus and its structures and immature chromatin with nucleoli, all aimed at division into two cells. The drive to differentiation, on the other hand, is seen throughout the entire sequence of maturation, there being two simultaneous processes. The first process is the reduction in function, size and structure of the nucleus, in which the nucleoli become less evident and finally disappear and the chromatin becomes dense and compact; this process is maximally expressed in erythroblasts, thanks to the final asynchronous division of the orthochromatic erythroblast, which liberates the nucleus. The second process, at the level of the cytoplasm, is the acquisition of the cells' specific functions, which leads to the progressive loss of basophilia and the appearance of new structures that profoundly alter the original staining characteristics and appearance, such as the appearance of haemoglobin and of the primary and secondary granules.

The morphological study of blood cells, bearing in mind the increasingly numerous biotechnological methods which are being developed and put at the service of diagnostic medicine, unites the great advantages of simplicity and relatively low costs with the indispensable need for a high level of competence and experience. Despite its apparent methodological poverty, morphological study has a complex multifaceted aim, no less than the analysis of the three forces described above and the results of their complex interactions on the quantitative and qualitative characteristics of the bone marrow. To the initial indispensible quantitative assessment, including the myelogram, morphological evaluation adds careful and accurate assessment of single cells, including the structural details and staining characteristics of their visible constituents. This qualitative assessment permits the recognition of minimal deviations from normal, such as asynchrony in maturation, or major deviations from normal, such as the replacement of the balanced heterogeneity of the normal blood and bone marrow by a monomorphic population of more or less atypical immature or abnormally maturing cells. Morphological abnormalities must be used to throw light on functional or pathological processes which involve the ultrastructure, biochemistry and genetics of the cell. Some morphological-functional correlations are simple and immediately apparent, such as the relationship between cytoplasmic basophilia and the presence of RNA or the recognition of the clear juxtanuclear zone as the negative image of the Golgi apparatus. In other areas, increasing knowledge has enhanced the ability to interpret morphological features, so that their description is more precise and informative, making it possible to use morphology to predict molecular abnormalities.

The description of morphological features involves not insignificant semiological difficulties, i.e. putting into words what is seen. Morphological interpretation is based on sensory perceptions of the observer, which determine the manner, in part subjective, of the approach. Communication of experience in this field is based on descriptions that are mainly visual (shape and colour), but also tactile (density and the impression of a smooth or rough surface). For this reason, the description in words of such individual and specific sensations may be more or less effective depending on individual ways of appreciating what is seen; this verbal communication must be accompanied, moreover, by a rich collection of images, which serve to decodify the verbal message, and thus permit the construction of a personal code for cytological identification. One must also mention the numerous semantic traps in this field: the term

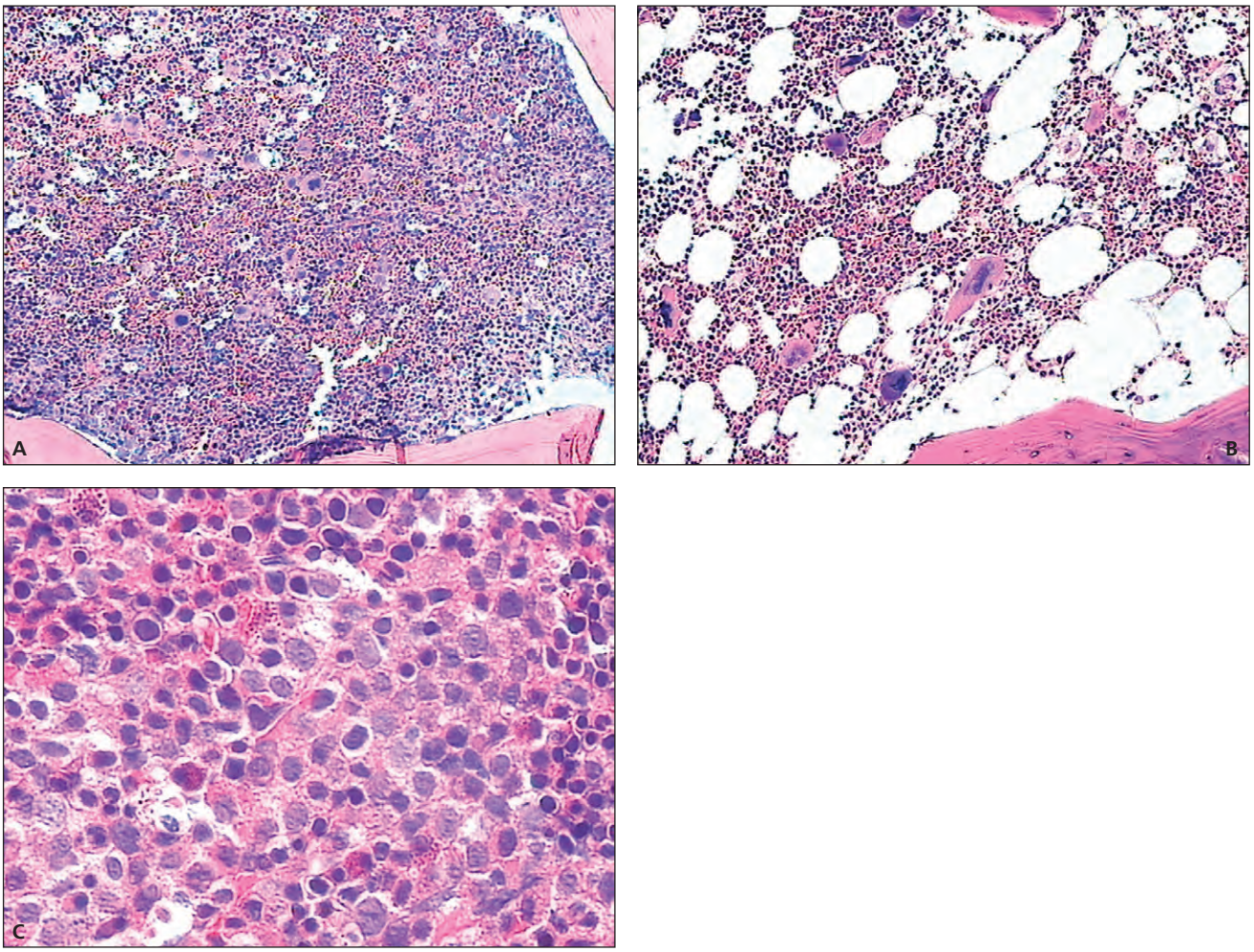



**Fig. 1.21** Examples of histological sections of bone marrow trephine biopsies (haematoxylin and eosin stain). (A) Hypercellular marrow with trilineage hyperplasia, virtual absence of fat cells and very numerous megakaryocytes (from a patient with a myeloproliferative neoplasm at presentation). At the bottom, a bone trabecula. (B) A cellular marrow with haemopoetic tissue admixed with fat cells. The megakaryocytes are relatively numerous, with features of gigantism (from a patient with essential thrombocythaemia). (C) At higher power, a hypercellular field with abundant granulocyte precursors in the central area and with erythroblasts at the top and to the right (a feature that recalls the abnormal localisation of immature precursors [ALIP] that is typical of myelodysplastic syndromes). There are also some eosinophils.

'myeloid', for example, is used by some as a synonym for 'medullary', thus including the three haemopoietic (non-lymphoid) lineages (and this is the usage in the WHO 2008 classification), and by others is used in contrast to 'erythroid', thus limiting it to the granulocytic lineages.

Another terminological difficulty is the naming and interpretation of single cell types. This is linked in large part to different scientific and didactic traditions which have become established during the last two centuries in various parts of the world. Today, this can be overcome by international harmonisation both of teaching and of laboratory reporting, aided by information technology that permits the exchange of images and makes possible an interpretive exercise: in a recent multicentric European study, in which 28 expert morphologists participated, consensus terminology was established and a glossary divided according to cell lineage was drawn up (Table 1.4).[40] A selection of various examples of individual cell types, drawn from the collection of images used for this cooperative study, and the tabulated definitions of these cell types, were established by consensus and can be consulted on the website of the European LeukemiaNet[41] and that of the European Hematology Association.[42] On the site of the Società Italiana di Ematologia (SIE), in the section 'Attività formative' there is also an online test for self-validation in cytomorphology, based on the identification of normal and pathological cells, and also a selection of clinical cases based on cytomorphology.[43]

**Table 1.4** Consensus advice from the morphology working group under the auspices of the LeukemiaNet European group[40]

1. Erythroid precursors can be designated by two groups of parallel names, according to the usual practice in different countries:
   - Proerythroblasts
   - Basophilic or early erythroblasts
   - Polychromatic or intermediate erythroblasts
   - Orthochromatic or late erythroblasts

The same rules are applicable to megaloblasts.

2. A neutrophil can be considered hypersegmented only when it has at least six lobes. Hypersegmentation can, however, also be represented by the presence of an increased proportion of neutrophils with five lobes
3. The term 'dysplasia' indicates morphologically abnormal maturation and is not synonymous with 'myelodysplastic syndrome'. The term 'dysplastic' is applicable only to cells of the three myeloid lineages. For cells of other lineages, such as lymphocytes, plasma cells and mast cells (notwithstanding the myeloid origin of the latter), it is preferable to use the term 'atypical'
4. Dysplastic haemopoiesis can lead to the production of abnormal erythrocytes and platelets recognisable on cytological examination (e.g. poikilocytes or red cell dimorphism; hypogranular platelets or platelets with abnormal granules). However, the term 'dysplastic' should be reserved for nucleated cells
5. Dysplasia cannot be evaluated in patients receiving growth factors. Furthermore, the presence of hypergranular neutrophils, often observed in patients with sepsis, should not be included among the criteria for quantifying dysplasia (applying the WHO classification) in patients with a suspected myelodysplastic syndrome or acute myeloid leukaemia. In addition, subtle morphological abnormalities are not sufficient to categorise a cell as dysplastic
6. The abnormal cells of acute promyelocytic leukaemia should be designated, in conformity with the WHO classification, as atypical or abnormal promyelocytes, hypergranular or microgranular (hypogranular), according to the individual case. Although both these types of cells are, in fact, dysplastic, the term 'dysplastic' is not useful in this context. Hypogranular promyelocytes, other than in acute hypogranular promyelocytic leukaemia, can be included in the general category of 'dysplastic promyelocytes'
7. Use of the definition of blast cells proposed by the International Working Group on Morphology of MDS is recommended.[56] Classification of blast cells as agranular or granular replaces the proposals of the FAB group. Blasts that cannot be recognised as belonging to a particular lineage should be designated 'blast cells, not otherwise specified'. The assigning of blast cells to a specific lineage is more straightforward if the cells are related to other cells, rather than being viewed in isolation
8. Monoblasts and promonocytes should be defined in conformity with the 2008 WHO classification. The monoblast has a round or oval nucleus. The promonocyte has a convoluted, folded or indented nucleus. Given that a promonocyte is regarded as a 'blast equivalent', the term 'promonocyte' must only be applied to cells that have a delicate dispersed chromatin structure, analogous to that of a monoblast. The distinction between monoblasts and promonocytes does not have any practical importance, since both are included in the blast cell count. Conversely, the distinction between promonocytes and atypical/abnormal/immature monocytes can be difficult, but is very important for the correct differential diagnosis between chronic myelomonocytic leukaemia and acute monocytic leukaemia: a careful evaluation of the delicate network of immature chromatin is of crucial value in recognising a cell as a promonocyte and thus a 'blast equivalent'
9. Based only on morphology, atypical lymphocytes can be further subdivided into:
   - Atypical lymphocytes, suspect reactive
   - Atypical lymphocytes, suspect neoplastic
   - Atypical lymphocytes, uncertain nature
10. It is generally preferable to avoid eponyms for designating specific cells or particular morphological features. However, certain designations are well established and have a totally clear meaning (e.g. Auer rods) and one can continue to use them
11. Dutcher bodies, single and multiple Russell bodies and the cytoplasmic inclusions of Mott cells represent the morphological evidence of a single type of cytoplasmic inclusion, which in the case of Dutcher bodies is entirely invaginated into the nucleus. The terms proposed for plasma cells that contain these inclusions are 'plasma cell, atypical, with nuclear inclusions' or 'plasma cells, atypical, with cytoplasmic inclusions/vacuoles'
12. The term 'macrophage' is used for the phagocytic cell derived from a monocyte. The term 'histiocyte' has a broader meaning, which includes macrophages and dendritic cells, such a Langerhans cells
13. Gaucher cells and pseudo-Gaucher cells cannot be distinguished from each other by cytology alone: they can be identified as 'Gaucher cells' explaining, when possible, the context (e.g. in Gaucher disease or in chronic myeloid leukaemia)
14. In general, bone marrow aspirates are not adequate for the diagnosis of cancer. However, the presence of syncytial masses of immature cells is suggestive of metastasis of a solid tumour which should be reported as 'cytologically not classifiable', to be confirmed by a trephine biopsy supplemented by immunohistochemistry

As an introduction to the description of circulating cells and also of bone marrow cells, at different stages of maturation, and also as an introduction to all the pathological conditions described in the following chapters, it is opportune to give at this stage some general characteristics that should be assessed (Table 1.5). These form the basis of the knowledge and skills of the morphologist and can be applied in an almost automatic manner to normal morphology, which consists of classifying the normal marrow cells and the mature cells derived from them, and pathological morphology, that is the recognition of the manner in which there is deviation from the normal process of maturation.

## Size and shape of cells

In general, the size of haemopoietic precursors diminishes from the most immature to the most mature. This happens in an ordered and regular manner in the erythroid series, whereas in the granulocytic series there is often a slight increase in size

**Table 1.5** General cytomorphological features for evaluating single cells in morphological preparations

**General characteristics of the cell**
- Size
- Shape
- Nucleocytoplasmic ratio

**Nuclear characteristics**
- Shape
- Position
- Outline:
  - Regular:
  - Angular, notched or cleft
  - Thickness of nuclear membrane
- Chromatin:
  - Density of the chromatin network
  - Number and shape of areas of condensation
  - Pale fissures and spaces
- Nucleoli:
  - Prominence
  - Number
  - Accentuated by chromatin condensation

**Characteristics of the cytoplasm**
- Staining:
  - Colour
  - Homogeneity or heterogeneity
- Golgi zone:
  - Apparent
  - Shape
  - Location relative to the nucleus
  - Relationship to granules
- Granules:
  - Staining characteristics
  - Number
  - Size
  - Distribution
- Cell membrane:
  - Regularity
  - Extensions
  - Well defined or not
- Inclusions, vacuoles

between the myeloblast and the promyelocyte, followed by a slight decrease in size in the following stages. Monocytes and lymphocytes maintain, in general, a fairly constant size in the course of maturation. In the megakaryocyte series, the opposite occurs: maturation is in fact accompanied by a marked increase in cellular dimensions. An assessment of size is one of the least reliable criteria for lineage assignment: the classical example is that of the beginner who bases distinction solely on size and who finds himself in difficulty with large atypical lymphocytes, which are sometimes larger than monocytes. In the same way, myeloblasts are generally larger than lymphoblasts, but this criterion is not very useful in the diagnosis of acute leukaemia.

Erythroid precursors have a regular round or oval shape (Fig. 1.22), while granulocytes can have a somewhat irregular outline. Some cells, e.g. plasma cells and osteoblasts, are oval but with one pole being broader so that they can be somewhat pyriform. Shape also is a morphological criterion generally of secondary importance. Characteristic of certain cells, such as the atypical lymphocytes of infectious mononucleosis, is the easy deformability of the cell margins, so that their shape is easily altered when they contact an adjoining cell.

## Nucleocytoplasmic ratio

This is high in blast cells and in the earliest stages of maturation, in which the nucleus dominates the cytoplasm; it reduces progressively in the course of maturation, as a result of the progressive expansion of the cytoplasm and the simultaneous contraction of the nucleus. An exception to this general rule is seen in the lymphoid series, in which there is a very high N:C ratio in circulating mature cells.

## Shape and position of the nucleus

Nuclear morphology is a very important criterion, particularly in the granulocytic series, in which it constitutes a distinctive

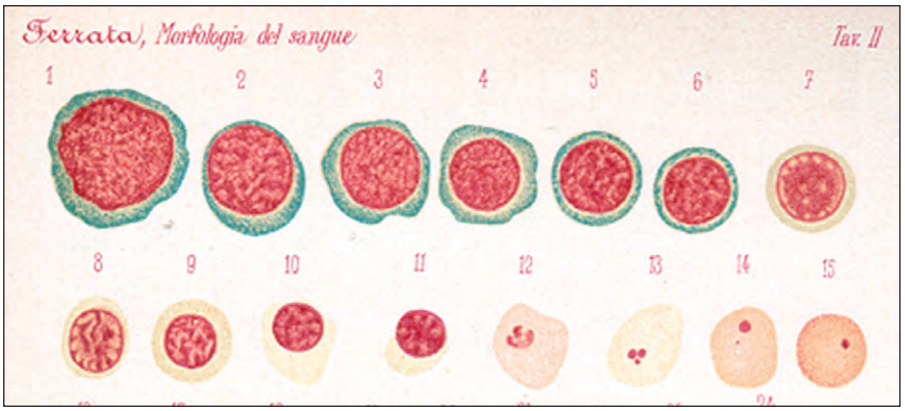



**Fig. 1.22** Morphology of erythroid precursors reproduced from the first text of Adolfo Ferrata.[1] This illustration shows the changes in morphological features during the course of erythroid maturation: there is a reduction in size from the proerythroblast (1) onwards; there is a modification of the chromatin structure with progressive condensation, first into clumps in the basophilic erythroblasts (2–6) and then into larger masses; there is a transition of the staining characteristics of the cytoplasm towards polychromasia (7–9) and then orthochromasia (10 and 11). Cells 12–15 are erythrocytes with nuclear remnants, typical of dyserythropoiesis.

characteristic in intermediate and advanced stages of maturation, up to the stage of segmentation of the nucleus of the mature granulocyte. In normal haemopoiesis the nuclei of erythroid progenitors are always round and their circumference is sharp, as if drawn with a compass; only in proerythroblasts can the nuclear outline be slightly oval. Anomalies in the nuclear shape provide clear evidence of dysplasia. An erythroblast nucleus is generally central, surrounded on all sides by a significant amount of cytoplasm, so that the nucleus does not touch the cytoplasmic membrane. The nuclei of granulocyte precursors are in general less regularly round and there can be straight edges and angles in the myeloblast outline, with indentations increasingly evident in the later stages of maturation, in which the nucleus is found to be slightly eccentric. In the more immature precursors of the monocyte lineage, as in monoblasts of acute monoblastic leukaemia, the nucleus is paradoxically round and central; only on maturation does it become irregular, developing the characteristic complex outline that one observes in circulating monocytes. In other cells, such as plasma cells, osteoblasts and macrophages, the markedly eccentric nucleus is a distinctive characteristic that helps with the immediate recognition of the lineage.

## Chromatin

The structure of chromatin, i.e. the twisted skeins of long thin filaments that constitute the unwound chromosomes, is a very important morphological detail, but also one that can be difficult to appreciate: here words are often inadequate to describe a range of sensations that seem to be both visual and tactile. The morphology of the chromatin reveals the structure of the chromosomal mass: the filaments of which they are composed form a loose tangle, which is less dense in the more immature cells that are still involved in the synthesis of nucleic acid which precedes the division of the nucleus. When nuclear function diminishes, increasingly long sections of the chromosomes, known as chromocentres, remain condensed. In early cells, then, immature chromatin is described as delicate, pale, loose, open, an open network, reticular, tenuous, dispersed or homogeneous; it is also described as euchromatin, to indicate that this state corresponds to metabolically and synthetically functional chromatin; the nuclei in which this form predominates are referred to as leptochromatic. The cells that show maximal nuclear immaturity are myeloid blast cells, particularly those in acute monoblastic leukaemia, in which the chromatin is filamentous, reticular, very pale and very delicate, and also the promegaloblasts of pernicious anaemia with their typical granular appearing chromatin that is dust-like and finely stippled. In mature cells, on the other hand, in which the process of proliferation has stopped, the chromatin has become a more homogeneous compact mass, preventing the internal structure from being seen, a dense net of alternating light and dark zones. This appearance is also seen in orthochromatic erythroblasts with their pyknotic nuclei, prior to expulsion, and in the nuclear lobes of segmented neutrophils. This dense chromatin is called heterochromatin. In the interval between these two extremes one finds the majority of bone marrow cells, in which proliferation and differentiation proceed simultaneously: the chromatin network of the cells in interphase shows a heterogeneous structure with small darker clumps of heterochromatin alternating with clear spaces, called parachromatin, arranged in a more or less regular manner, depending on the cell type; the condensation becomes gradually more gross as maturation proceeds. The separation between light and dark zones can be very distinct, as in basophilic and early polychromatic erythroblasts, or indistinct, as in myelocytes and metamyelocytes.

In some cells of females it may be possible to detect a mass of triangular or ovoid condensed chromatin, often situated close to the nuclear membrane: this is the sex chromatin or Barr body, which is the morphological correlate of the inactive X chromosome, heteropyknotic, which duplicates last in the course of mitosis. In mature neutrophils with segmented nuclei, the sex chromatin can have the appearance of a drumstick-shaped nuclear appendage, formed of a spherical or ovoid head composed of very dense homogeneous chromatin that is joined to one of the nuclear lobes by a thin filament; the same significance attaches to the deeply staining sessile nodules that protrude from the perimeter of a lobe by more than half of their diameter. This is a relatively infrequent observation (one neutrophil in 50 has a drumstick appearance, three out of 50 have sessile nodules), which can be confused with less specific nodules, sessile or pedunculated; the latter can be distinguished by the thickness of the filament or by the heterogeneity of the chromatin network; they often have a paler centre (a racquet form). The significance of nuclear appendages in the diagnosis of the myelodysplastic syndromes is still under debate.

## Nucleoli

Nucleoli are the site of assembly of ribosomes and of transcription of RNA; by electron microscopy they appear to be formed of a filamentous argentophilic structure, called the nucleolonema, embedded in a diffuse amorphous matrix. They are mainly composed of RNA: this explains their weakly basophilic characteristics with a Romanowsky stain. In cytomorphological preparations, nucleoli are visible in normal immature cells (proerythroblasts, myeloblasts, promyelocytes), in some stromal cells and in blast cells of acute leukaemia. In lymphoid blasts they are less numerous, with a pale area at the perimeter, which is often incomplete and not well delineated so that the nucleolus can merge in part with the heterogeneous structure of the nuclear chromatin; they are often not evident at all in lymphoid blasts. A single pale nucleolus on a background of dense chromatin is the principal characteristic of the prolymphocyte. In monoblasts, proerythroblasts and some myeloblasts, the nucleoli are in general clearly visible, as if stamped out in the delicate chromatin network by virtue of the border or circle of condensed, dark, perinucleolar chromatin that surrounds them. The number of nucleoli is not an infallible morphological criterion: they are, however, less numerous, usually only one or two,

in lymphoid blasts, whereas in myeloid blasts there may be four or even more.

## Cytoplasm

The cytoplasm is the site of cellular functions. In immature cells it is often not very abundant and is characterised by basophilia, the variable intensity of which reflects the abundance of ribonucleoprotein and, thus, the activity of protein synthesis. One can easily recognise, from the deep ultramarine colour of the cytoplasm of the most immature erythroid cells, that the cell is ready for the imminent synthesis of haemoglobin. Similarly in the plasma cell, the end cell of the B-lymphoid lineage, one can recognise its role in the synthesis of large quantities of immunoglobulin from the deep blue colour of its cytoplasm. The basophilia of more immature cells sometimes becomes accentuated in the earliest stages of differentiation, as a result of the synthesis of ribonucleoprotein preparatory to the production of specific cytoplasmic structures: the paradoxical occurrence, described for the first time by Ferrata, is most evident in erythroid maturation, but occurs also in other lineages, e.g. in the megakaryocyte lineage. With maturation, cytoplasm in general loses its basophilia: this can happen gradually, as with the polychromatic cytoplasm and amphoteric reaction of intermediate erythroblasts, in which the basic residues of the haemoglobin molecule merge with the ribonucleoprotein, leading to a progressive modification of the tinctorial qualities, from blue to grey to pink; alternatively, it can occur in a non-homogeneous manner, as often happens in the granulocytic lineage, in which fairly large pale areas that are weakly basophilic can persist, even after the initiation of granule production. In mature cells, the background cytoplasm is weakly acidophilic, pale pink or colourless, particularly in neutrophils; it remains however weakly basophilic in lymphocytes, while in monocytes it has a typical grainy appearance, greyish, dirty, like frosted glass, because of the presence of very fine granules, individually invisible, on a background of very weak basophilia.

The Golgi zone appears as a pale area, weakly stained or colourless, without granules, sited at the centre of the cell in contact with the nucleus. At an ultrastructural level it corresponds to the vesicles and membranes of the Golgi apparatus. This structure, fundamental both in the assembly of complex molecules, such as lysosomal enzymes or immunoglobulins, and in granulopoiesis, appears to hypertrophy in the intermediate stage of maturation in the granulocytic lineage, from the promyelocyte to the metamyelocyte. In these cells the nucleus can appear to mold itself around this consistent clear zone in the centre of the cell, developing a concavity in which the Golgi apparatus locates itself; even in mature neutrophils the nuclear lobes often tend to arrange themselves as an incomplete circle beneath the cytoplasmic membrane, leaving the central area free for these organelles, albeit now hypotrophic. The clear area of the Golgi zone is also very evident in plasma cells, in which it is found very close to the nucleus, sometimes deforming it slightly, sometimes curving into a semi-lunar shape to partially surround it. The Golgi zone is equally evident in osteoblasts but in contrast to the plasma cell, it is separated from the nucleus by a band of basophilic cytoplasm. In the proerythroblasts and, to a minor degree, in basophilic erythroblasts the Golgi zone takes the definite form of arcoplasm, this term indicating a semilunar or crescent moon shape, around the nucleus.

In the most intensely basophilic cytoplasm, one sometimes observes, in addition to the large clear area of the Golgi zone, some lack of tinctorial homogeneity, attributable to the presence of an areola of pale round or oval clear areas, sometimes particularly numerous (resembling a bees' hive): these correspond to the negative images of mitochondria or, more rarely, secretory granules or pinocytotic microvesicles.

## Granules

Cytoplasmic granules, which in general are the morphological expression of hypertrophic lysosomes, are typical of phagocytic cells and are present, by definition, in granulocytes, but also, with important morphological and functional differences, in monocytes and lymphocytes. In the three granulocytic series the tintorial characteristics of the granules provide the basis for the morphological subdivision into the three classes: neutrophils, eosinophils and basophils. In the neutrophil series there are two principle types of granule. The primary or azurophilic granules, containing myeloperoxidase, neutral proteinase and acid hydrolase, are produced in small numbers in myeloblasts but are particularly abundant in promyelocytes; it is these granules, production of which starts in the mature promyelocyte and continues as far as the metamyelocyte stage, which contain, among other things, lactoferrin and the vitamin $B_{12}$ transport protein. The term 'azurophilic' indicates the affinity of the primary granules for azure B (or its homologues) which, since staining is metachromatic, produces a light red, very vivid colour that stands out from the weakly basophilic background of the promyelocyte cytoplasm: it is this appearance that often helps to identify nascent granulopoiesis in these cells, in which the pale juxtanuclear Golgi zone looks like a solar disc from which dozens of azurophilic granules radiate in all directions. The term 'neutrophil', on the other hand, lacks its original functional significance: the term was coined by Ehrlich to indicate the fact that the granules of segmented leucocytes in the circulating blood were the only cellular structures that had an affinity for his triacid stain, composed of a neutral reaction mixture of methyl green, orange G and acid fucsin. Classical morphology taught that the primary granules correspond morphologically to the azurophilic granules, the tinctorial qualities of which were progressively lost in the course of differentiation: these would be present, but invisible, in mature cells, as indicated by cytochemical positivity for peroxidase. They were replaced by specific granules, the neutrophilic granules, which had a darker brick-red colour and were observed from the promyelocyte onwards. Electron microscopy and ultrastructural

biochemistry have recently indicated that this ancient dogma is wrong. What changes with maturation, in fact, is only the chromatic characteristics, or rather the metachromatic characteristics, from light red to dark red, of the primary azurophilic granules. Only these granules, because of their dimensions between 500 and 800 nm, are visible with the light microscope. The tinctorial change depends on biochemical alterations in the granules, in particular an increase in acid mucosubstance which forms complexes with basic proteins. The specific granules, which are smaller (200–300 nm), are below the level of resolution of the light microscope, but because of their large numbers they convey a pink tinge to the cytoplasmic background of the mature granulocytes. Anomalies of neutrophil granulation are common in acute and chronic inflammatory conditions: these so-called 'toxic' granules are larger and more numerous than normal and are intensely stained – a dark purplish red: they are large, hypertrophic primary granules, which maintain their azurophilic characteristics even in mature cells.

The cytoplasmic granules of monocytes are azurophilic; they have a low peroxidase content and, with a diameter of less than 200 nm, are so small that individually they are not visible. Their presence, however, modifies the appearance of the cytoplasm, which appears to contain minute grains of sand. Their presence influences both the structure, which appears granular and not homogeneous, and the staining characteristics, which range from weakly basophilic to ash grey with a pinkish tinge; this latter feature is more evident when the azurophilic granules are more numerous. This ground-glass appearance of the cytoplasm is one of the most useful morphological criteria, along with the typical nuclear morphology, for distinguishing monocytes from the largest lymphocytes; the latter have more abundant, pale, weakly basophilic cytoplasm that is limpid – 'like water in a rocky mountain pool'. Some lymphocytes, usually larger because of their more abundant cytoplasm, have a small number, from five to 15, of large azurophilic granules, of a vivid red colour, such that they stand out strongly from the weakly basophilic background cytoplasm. These large granular lymphocytes (LGL) are immunophenotypically either cytotoxic T cells (CD3 positive) or natural killer (NK) cells. Their lysosomal granules are large (1000–2000 nm) and rich in acid hydrolase, this correlating with their cytotoxic function.

The granules of eosinophils are very characteristic: they are larger than those of neutrophils, of an intense orange colour, identical to that of eosin in solution, spherical, usually densely packing the cytoplasm. With polarized light they are refractile. These specific granules start to appear at the promyelocyte stage; at this stage particularly and in the next stage of the eosinophil myelocyte some of the granules have affinity for basic stains, with a typical dark violet metachromasia. These particular pro-eosinophilic granules acquire, with further maturation, the characteristic affinity for eosin as a result of the synthesis of increasing amounts of basic proteins.

Finally, basophil granules are large and either round, oval or angulated. Their characteristic colour is a very dark wine-red, almost blackish; this typical metachromasia is attributable to the high content of acid mucopolysaccharides and is even more evident when staining with toluidine blue or alcian blue, both of which produce a bright red colour. These granules are less numerous than those of eosinophils, but differ in that they cover the nucleus, obscuring some of its structure. Basophil granules are water soluble: if the content of water in the fixative is too high, they tend to dissolve, leaving vacuoles that in part appear empty, in part occupied by residues of metachromatic material. The granules of tissue mast cells are very similar to those of circulating basophils, but they are smaller and more numerous, to the extent that they pack the cytoplasm, although they do not usually obscure the nucleus.

## Other cytoplasmic inclusions

Various cells may contain clearly outlined, apparently empty vacuoles, which vary greatly in size and number. The cytoplasm of circulating monocytes typically contains vacuoles, particularly if the blood specimen was anticoagulated with EDTA. Highly characteristic also is the lacy appearance of the cytoplasm of the lymphoblasts with a mature B immunophenotype that are found in Burkitt leukaemia/lymphoma, resulting from the presence of numerous vacuoles of very variable size, which stand out from the intensely basophilic cytoplasm. The presence of nuclear vacuoles is a sign of nuclear degeneration or dysplasia.

In erythroblasts and mature erythrocytes one can observe various anomalous cytoplasmic inclusions, which will be described in Chapter 2: Howell-Jolly bodies represent residual nuclear material detached from single chromosomes on the mitotic spindle in the course of abnormal mitosis; basophilic stippling or punctate basophilia is attributable to anomalous aggregation and persistence of polyribosomes; Cabot's rings correspond to persistence of the filamentous structure of the mitotic spindle; Pappenheimer bodies are granules of ferritin bound to large protein molecules; Heinz bodies are composed of denatured haemoglobin and can be visualised by staining with vital dyes such as Nile blue or methyl violet.

In mature neutrophils it is sometimes possible to observe round inclusions of nuclear material, very similar to Howell-Jolly bodies and actually having an analogous origin; they are indicative of dysplasia. Döhle bodies are elongated basophilic inclusions that actually correspond to a zone of neutrophil cytoplasm, often in contact with the nuclear membrane, which appears to lack granules and is weakly basophilic; this can be considered as an example of asynchronous cytoplasmic maturation in which the persistence of basophilic material corresponds at an ultrastructural level to the presence of numbers of single ribosomes or, more often, to parallel arrays of sacs of rough endoplasmic reticulum, associated with glycogen deposits. These are very frequent in the May-Hegglin anomaly, but can also be observed in many inflammatory and infective conditions and in the myelodysplastic syndromes.

## Cytomorphology of normal blood and bone marrow

Stem cells and haemopoietic progenitors can be isolated from the peripheral blood by means of selection using monoclonal antibodies to CD34. These cells are small with a thin rim of weakly or intensely basophilic cytoplasm. They have no granules or Golgi zone, and have a round nucleus with dense reticular chromatin composed of closely intertwined fine filaments (see Figs 1.4 and 1.5)[44]; the N:C ratio is usually very high. Starting with these very immature cells, differentiation of haemopoietic cells is initially manifest by hypertrophy of the nucleus with the chromatin becoming open, immature and active, and by increasing cytoplasmic basophilia, attributable to the appearance of the large numbers of ribosomes that are required for protein synthesis. In the next stage of maturation, differentiation continues with the progressive reduction in function, size and structure of the nucleus, and with the reduction of basophilia and the acquisition of specific cytoplasmic characteristics. This change is manifest differently in different cell lineages, making possible the recognition of precursor cells starting with the proerythroblast, the myeloblast, the monoblast and the megakaryoblast, and continuing through the intermediate stages of maturation, up to the mature cells that are liberated into the lumen of the sinusoids and circulate in the blood stream.

### Peripheral blood

In the adult the volume of circulating blood on average is little less than 5 l (50 ml/kg), of which almost half comprises the erythrocytes, leucocytes and platelets that are suspended in plasma. The total number of these cells is no less than 30 000 billion. Some of these cells – the granulocytes – circulate for only a number of hours, others – the platelets – for a week; yet others are long-lived cells – red cells circulate on average for 120 days and some lymphocytes – memory T cells – for many years.

Red cells are anucleate with a diameter of 7–8 μm, of a pinkish colour with a pale area corresponding to the physiological central indentation. Their morphology will be described in Chapter 2.

The platelets have the appearance of small cytoplasmic fragments without a nucleus (diameter 2–5 μm). They are round or oval in blood films made from EDTA-anticoagulated blood; in films made from capillary blood, one sees activated star-shaped forms and aggregates. Platelets are produced in the bone marrow by fragmentation of megakaryocyte cytoplasm, and typically have a central granulomere, in which small bright red granules are concentrated, and a peripheral hyalomere, composed of colourless or weakly basophilic cytoplasm (Fig. 1.23); in other instances, the granules are dispersed through the cytoplasm or are arranged in a ring around a small vacuole. When there is accelerated thrombopoiesis, young, large platelets appear in the circulation (Fig. 1.24); these are richer in RNA to the extent that they can be recognised in automated blood cell analysers as the 'immature platelet fraction'.[45–47] The morphology of platelets varies in various congenital and acquired disorders (Figs 1.25 and 1.26): giant platelets are seen in the myelodysplastic syndromes, in Bernard-Soulier syndrome and in various hereditary macrothrombocytopenias, such as the May-Hegglin anomaly,

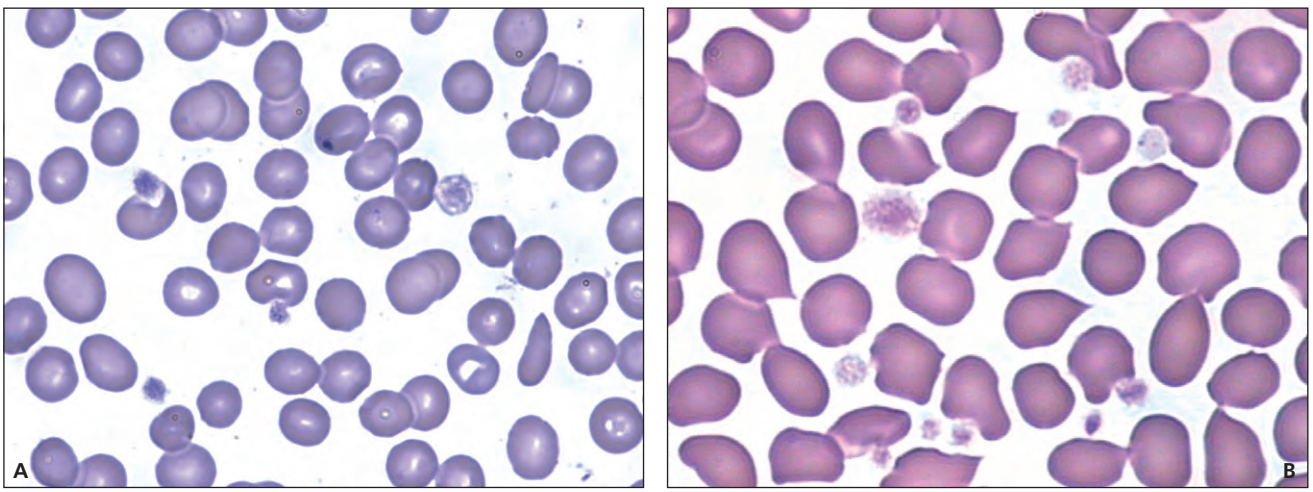



**Fig. 1.23** Platelets in peripheral blood films. (A) Platelet morphology, as in this healthy subject, is distinguished by a significant variation in size, with a proportionately greater variation in diameter than is seen in erythroblasts. The presence and compactness of the granulomere, the central area where granules are grouped together, is also heterogeneous from one platelet to another. (B) In this field, from the film of a patient with essential thrombocythaemia, there is increased variation is platelet size, i.e. platelet anisocytosis, and also in staining, platelet anisochromasia: one platelet is abnormally large; another, on the right, appears almost agranular. The presence of a number of teardrop poikilocytes (dacrocytes) suggests the presence of bone marrow fibrosis.

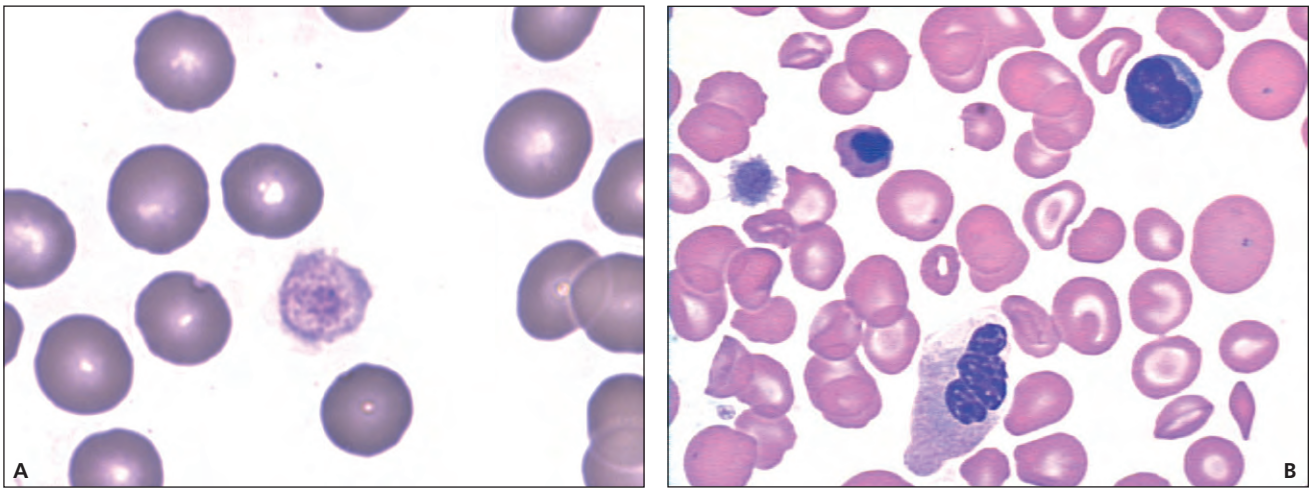



**Fig. 1.24** Moderate platelet dysmorphism. (A) Large immature platelet in a patient with autoimmune thrombocytopenic purpura. The appearance, almost like a target, is the result of a dense granulomere surrounded by a pale hyalomere with a strongly basophilic peripheral tinge. (B) Peripheral blood film of a premature neonate. On the left, a giant platelet with a hypertrophic granulomere beside a small orthochromatic erythroblast with a pyknotic nucleus and an irregular outline. At the bottom, a dysmorphic neutrophil; top right, a normal lymphocyte. The red cells show marked anisopoikilocytosis with macrocytosis, target cells and macrocytes with a polychromatic tinge; bottom left, a helmet cell; lower right, a hyperchromic rhomboidal schistocyte.

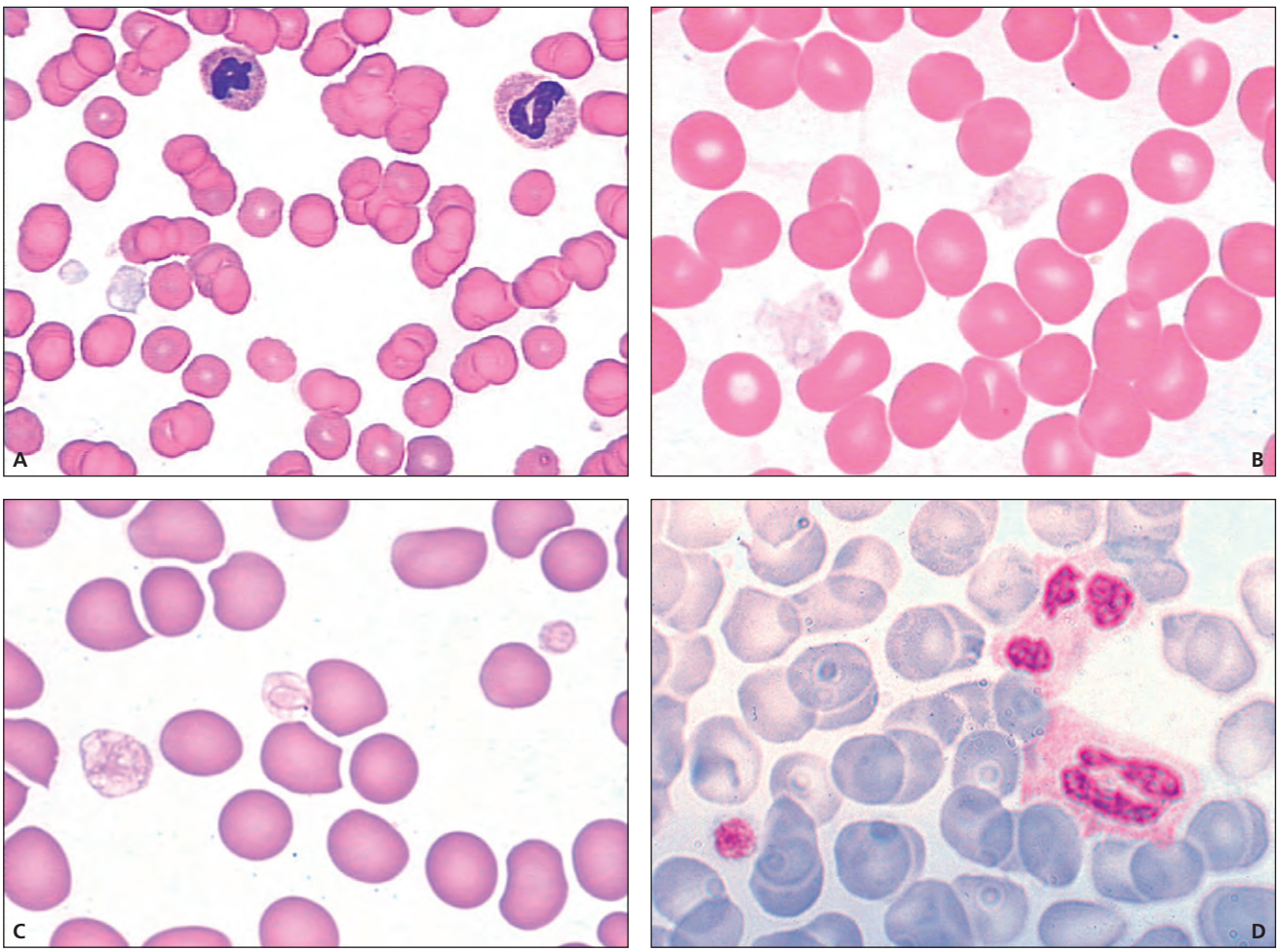



**Fig. 1.25** Platelet dysmorphism in hereditary thrombocytopenia. (A) Absence of granules in a patient with the grey platelet syndrome; moderate stacking of the red cells; at the top, two normally granulated neutrophils. (B and C) Platelets without a granulomere from patients with the same condition. (D) Sebastian syndrome. On the left, a large hyperchromic platelet. On the right, two mature neutrophils with weakly basophilic peripheral cytoplasmic inclusions that resemble Döhle bodies.

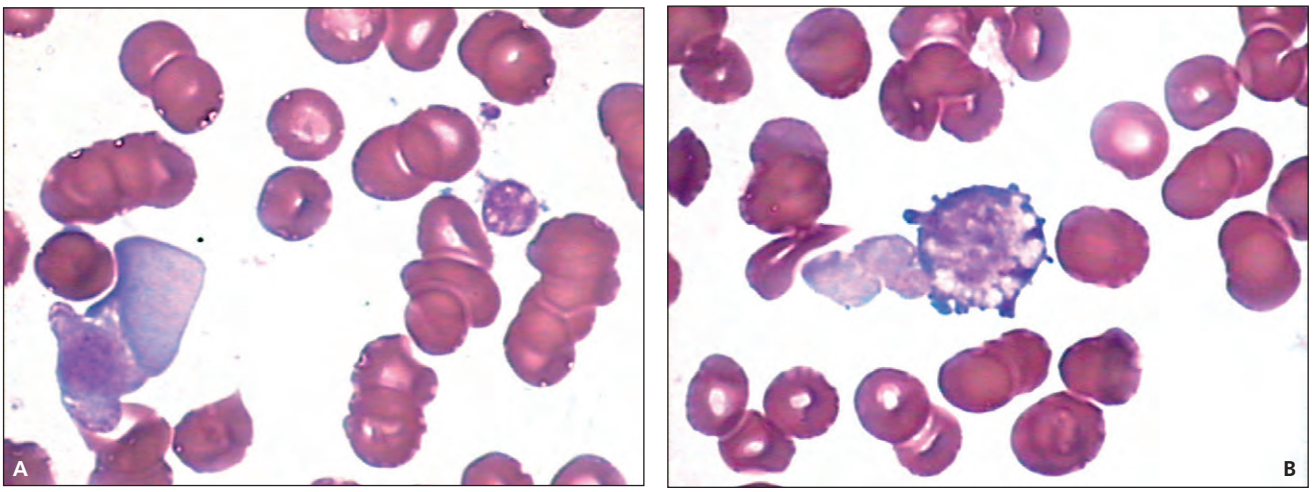



**Fig. 1.26** Marked platelet dysmorphism in the peripheral blood of a patient with a myelodysplastic syndrome, refractory anaemia with excess of blasts. (A) On the left, a giant platelet with a large granulomere and cytoplasmic blebs, one of which is very large. On the right, a large platelet with hardly any hyalomere. (B) Giant comet-shaped platelet: the diameter is more than double that of a red cell; the granulomere is gigantic and the intensely basophilic hyalomere is vacuolated and seems to have numerous small rays around its circumference, in addition to the long tail.

in which inclusions that resemble Döhle bodies are also present in leucocytes, or Fechtner syndrome or Sebastian syndrome, in which the platelets and the leucocyte inclusions are smaller; hypogranular or agranular platelets are present in myelodysplastic syndromes and in storage pool disorders, such as the grey platelet syndrome.[20,48] Observation of platelets in blood films also permits a semiquantitative evaluation of their numbers in relation to other cells and the recognition of pseudothrombocytopenia due to agglutination, aggregation or satellitism (Figs 1.12, 1.27 and 1.28).

The leucocytes in the peripheral blood represent the mature elements that result from differentiation and proliferation of the progenitors and precursors in the bone marrow and, with regard to the lymphocytes, in the primary and secondary lymphoid organs. In normal circumstances, one can recognise three principal groups: the granulocytes, designated polymorphonuclear because of the variable nuclear form, divided into three groups on the basis of the tinctorial, biochemical and functional difference between their granules; the monocytes and lymphocytes, which together constitute the group of leucocytes designated 'mononuclear' (Figs 1.29 and 1.30).

The neutrophil granulocytes (Figs 1.29 and 1.30; see also Fig. 1.37) have a rounded form and are intermediate in size (diameter 12–15 μm). The nucleus is typically divided into lobes, joined by thin filaments of chromatin; in fixed and stained films the lobes are arranged in a fairly random manner, while in cells examined *in vivo* the nucleus forms a segment of a circle around the centrosome, which occupies the geometric centre of the cell. The number of lobes increases with the degree of maturation: Arneth's scheme, now little used, classifies granulocytes on the basis of the number of nuclear lobes (two in 10–30% of circulating neutrophils, three in 40–50%, four in 10–20%, five in <5%); the average lobe count determined from 100 neutrophils varies between 2.5 and 3.3. Mature neutrophils with a non-segmented nucleus (band or stab forms) account for less than 5% of cells. Nuclear chromatin is markedly condensed into large, very dark masses, with a typical violaceous tinge, separated by thin zones of pale chromatin. The cytoplasm is pale, pink and weakly acidophilic; it contains numerous fine opaque brick-red granules, usually evenly spread through the cytoplasm. Counting band neutrophils is not diagnostically useful, both because of imprecision in their recognition attributable to the variability of nuclear folding and the subjectivity of interpretation. Moderate dysmorphism of the neutrophils is not rare in serious inflammatory conditions, in sepsis and during treatment with growth factors (Figs 1.31, 1.32 and 1.33); congenital anomalies that are not rare are hereditary deficiency of myeloperoxidase (Fig. 1.34) and the inherited Pelger-Huët anomaly.

Eosinophil granulocytes (Figs 1.35 and 1.36) are slightly larger than neutrophils (diameter 12–18 μm) and have a regularly rounded shape. The nucleus is bilobed, like a pair of spectacles, in 75% of cells (non-lobed 5%, three lobes 20%; average lobe count on 100 eosinophils: 2.3); the chromatin is dark, mottled and violaceous. The granules are larger than those of neutrophils and have an intense orange colour; they are numerous, packing the cytoplasm, although they are less dense in the area of the Golgi zone, between the nuclear lobes. Cytoplasm is apparent mainly in hypogranular cells, which are occasionally seen in eosinophilia and in the myelodysplastic syndromes, and is then seen to be slightly more basophilic than that of the neutrophil.

Basophil granulocytes (Figs 1.36 and 1.37) are the smallest circulating granulocytes (diameter 10–14 μm). The nucleus is barely visible, obscured by metachromatic reddish-black gran-

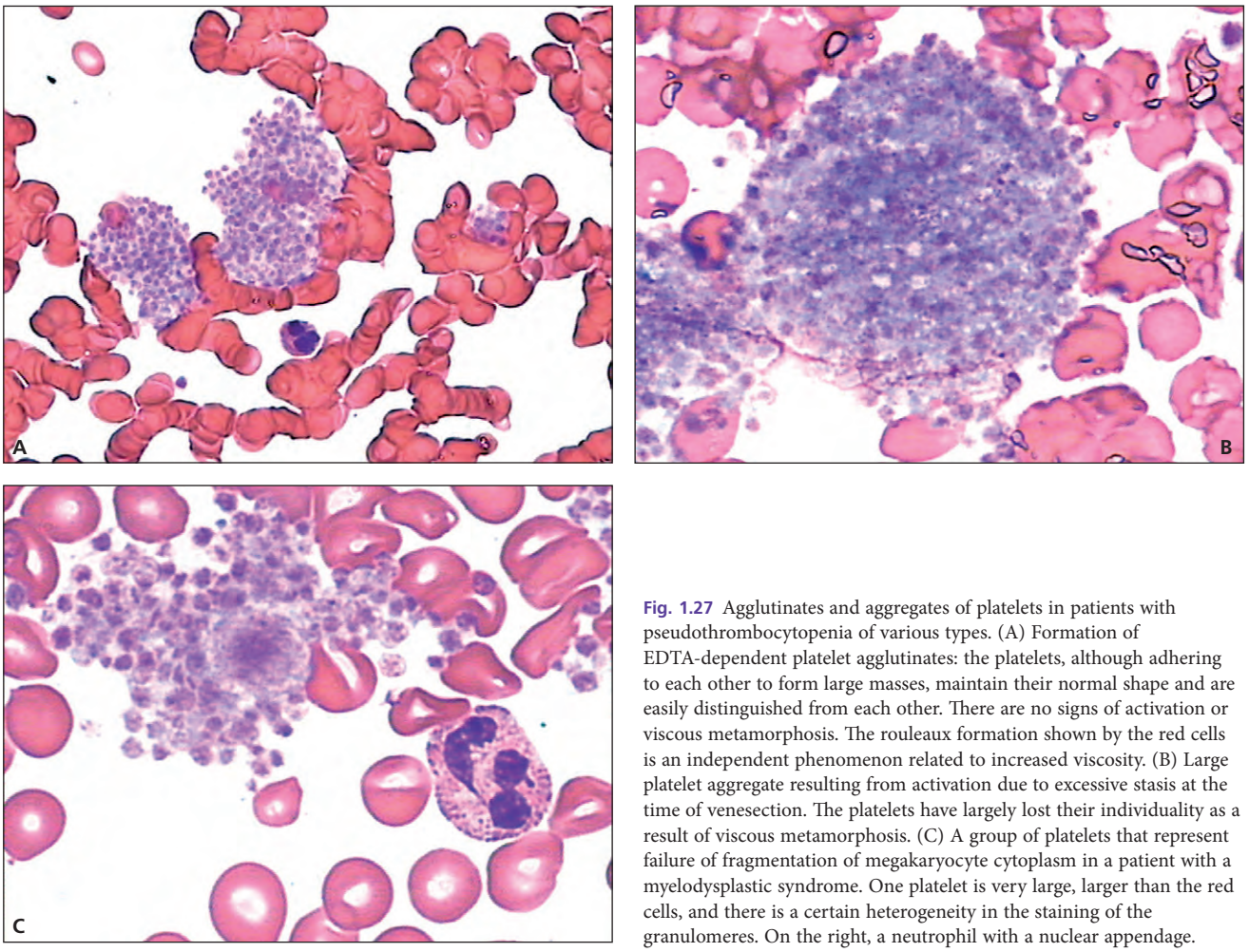



**Fig. 1.27** Agglutinates and aggregates of platelets in patients with pseudothrombocytopenia of various types. (A) Formation of EDTA-dependent platelet agglutinates: the platelets, although adhering to each other to form large masses, maintain their normal shape and are easily distinguished from each other. There are no signs of activation or viscous metamorphosis. The rouleaux formation shown by the red cells is an independent phenomenon related to increased viscosity. (B) Large platelet aggregate resulting from activation due to excessive stasis at the time of venesection. The platelets have largely lost their individuality as a result of viscous metamorphosis. (C) A group of platelets that represent failure of fragmentation of megakaryocyte cytoplasm in a patient with a myelodysplastic syndrome. One platelet is very large, larger than the red cells, and there is a certain heterogeneity in the staining of the granulomeres. On the right, a neutrophil with a nuclear appendage.

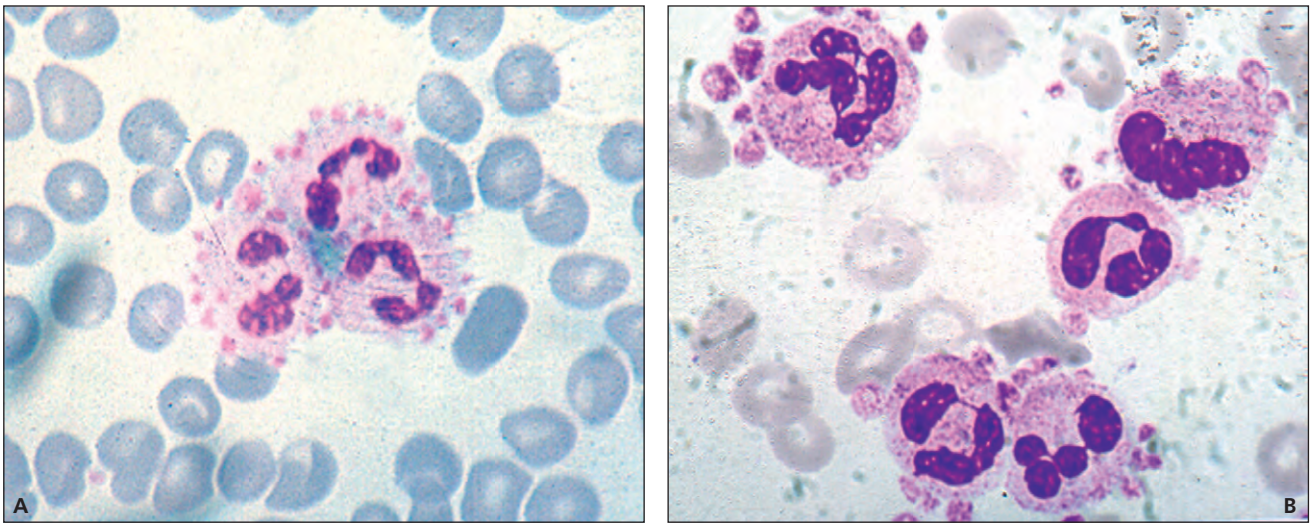



**Fig. 1.28** Pseudothrombocytopenia and pseudoleucopenia in two patients with platelet satellitism; peripheral blood film of $K_2$EDTA-anticoagulated blood. This phenomenon results from the presence of EDTA-dependent antibodies and does not have any pathological significance. (A) Small agglutinate of three neutrophils with a dense halo of normally formed platelets that have adhered to the plasma membrane. (B) Platelet satellitism in a patient with a myelodysplastic syndrome; film from buffy coat of peripheral blood. The neutrophils show dysmorphic nuclear segmentation and one cell is hypogranular.

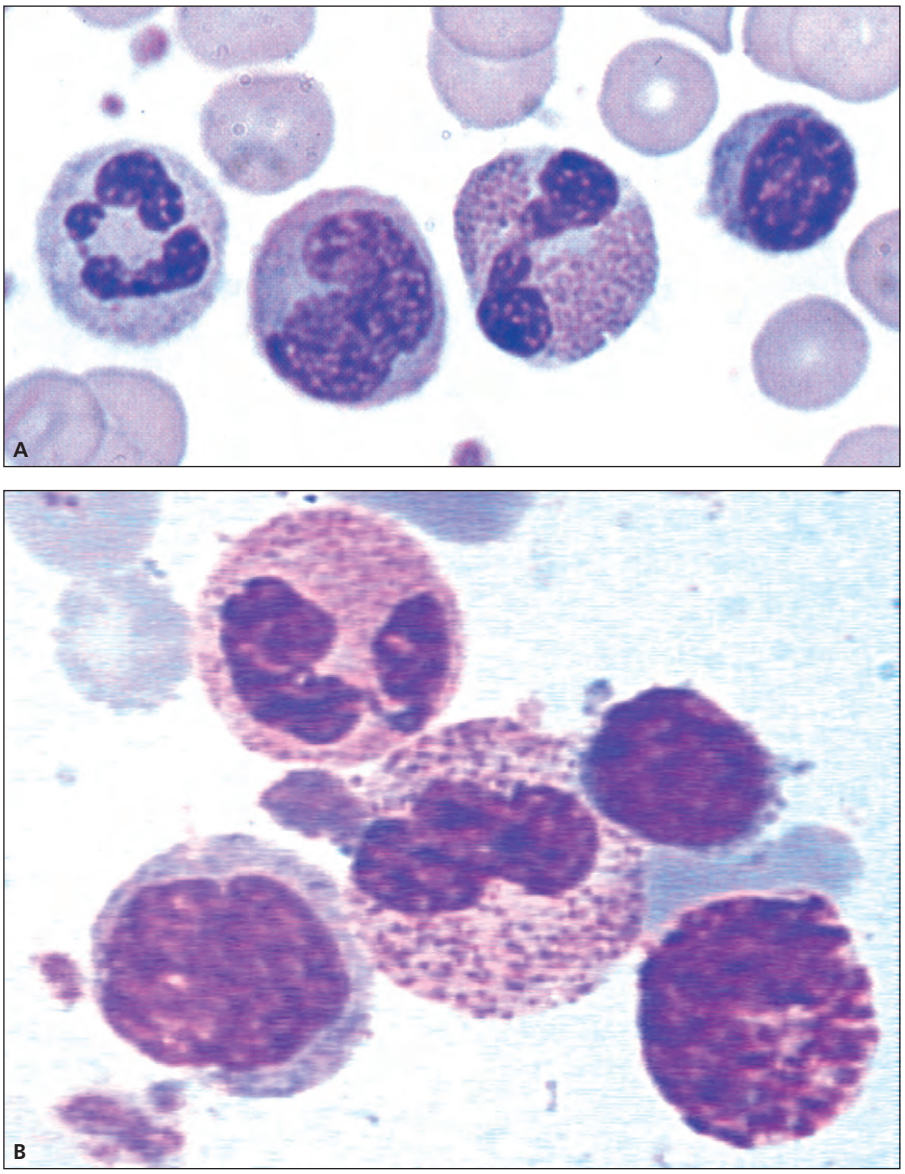



**Fig. 1.29** Leucocytes in the blood films of healthy subjects. (A) From left to right: a neutrophil granulocyte, a monocyte, an eosinophil granulocyte and a lymphocyte. (B) In the centre, a band neutrophil with dark red cytoplasmic granules standing out clearly from the background pinkish cytoplasm. From the left, clockwise: a fairly compact monocyte with blue-grey cytoplasm and an incised nucleus with heterogeneous chromatin; a segmented neutrophil with less distinct granules; a lymphocyte; a basophil granulocyte with a bilobed nucleus that is almost obscured by the large metachromatic aubergine-coloured granules.

ules: the chromatin is very dark, almost pyknotic, and lobulation is incomplete so that the nucleus is leaf shaped. The granules are less numerous than those of the neutrophil and less densely packed than those of the eosinophil, but in contrast to the latter, they generally cover the nucleus. If the alcoholic fixative is not pure, the water solubility of these granules leads to their dissolution so that the cell appears degranulated.

Lymphocytes are small (diameter 8–12 μm) with a high N:C ratio (Fig. 1.38). The cytoplasm is scanty and weakly basophilic (sky blue), homogeneous and clear, without inclusions. The nucleus is round or slightly indented, in a slightly eccentric position; it occupies 90% of the cell. The chromatin is intensely stained and distributed in dense masses or clumps, alternating with irregular paler zones, without a clear demarcation between them. Sometimes there is a small pale paranuclear zone. This usual morphology of the circulating lymphocyte is, however, subject to considerable variability (Figs 1.39 and 1.40) as it changes, in the absence of haematological or lymphoprolifera-

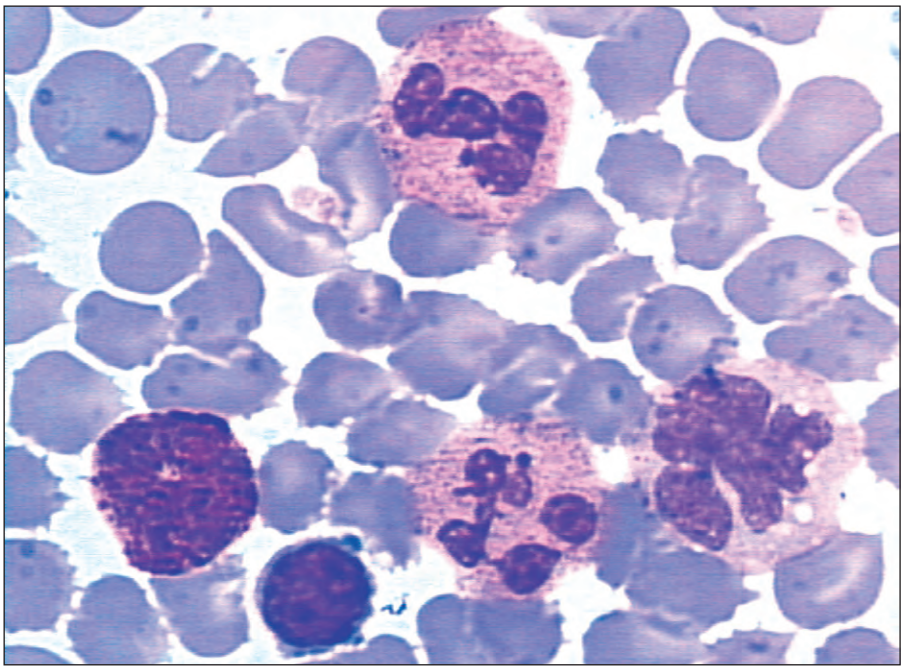


**Fig. 1.30** Blood film of a healthy woman. At the bottom, from the left: a basophil granulocyte, a lymphocyte, a neutrophil with two small filiform appendices and a drumstick (corresponding to a Barr body) projecting from the smallest nuclear lobe, a well spread out monocyte with greyish vacuolated cytoplasm and a convoluted nucleus with pale chromatin. At the top, another neutrophil with a Barr body.

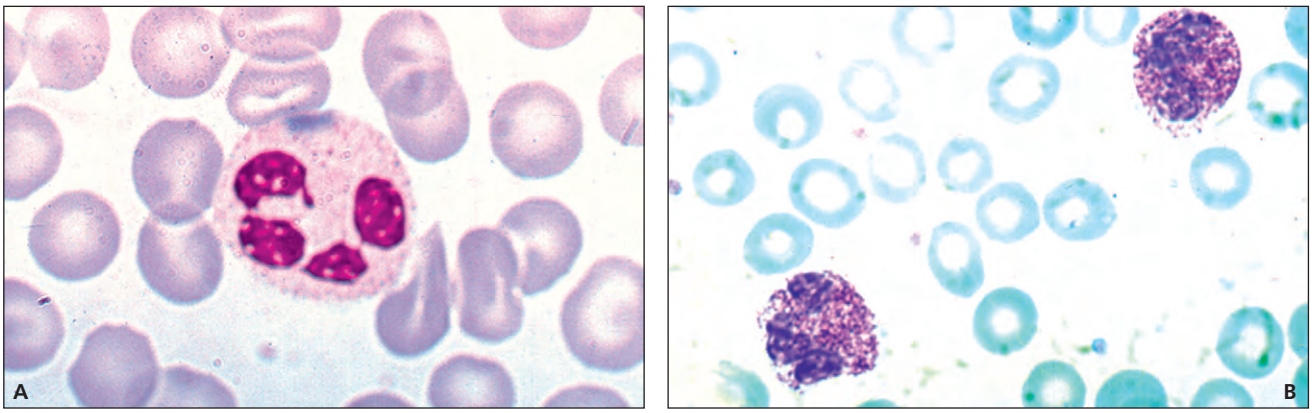



**Fig. 1.31** Alterations in neutrophil granulocytes in inflammatory and infective conditions. (A) The cytoplasm of this neutrophil contains, at the top in contact with the internal layer of the cytoplasmic membrane, a basophilic inclusion: it is a Döhle body, representing an assembly of membranes of the endoplasmic reticulum. (B) Two neutrophils with so-called 'toxic' granulation: the cytoplasm is packed with numerous granules that are more brightly staining than those of normal neutrophils.

tive pathology, following various immunological stimuli: in inflammatory conditions and infections, particularly viral infections (exanthematous disorders, cytomegalovirus, hepatitis), toxoplasmosis, pertussis, and in allergic reactions and autoimmune disorders (Tables 1.6 and 1.7). Following an antigenic stimulus, activated B cells from germinal centres enter the blood stream via the thoracic duct and thus reach other organs, including the bone marrow. From the morphological point of view, activated B cells can have the appearance of large lymphocytes, or plasmacytoid lymphocytes or immunoblasts. The latter are large round or oval cells, with a diameter of the order of 20–30 μm. The cytoplasm is intensely basophilic because of the high content of RNA and sometimes there are a few vacuoles; the nucleus is voluminous, round or oval with delicately

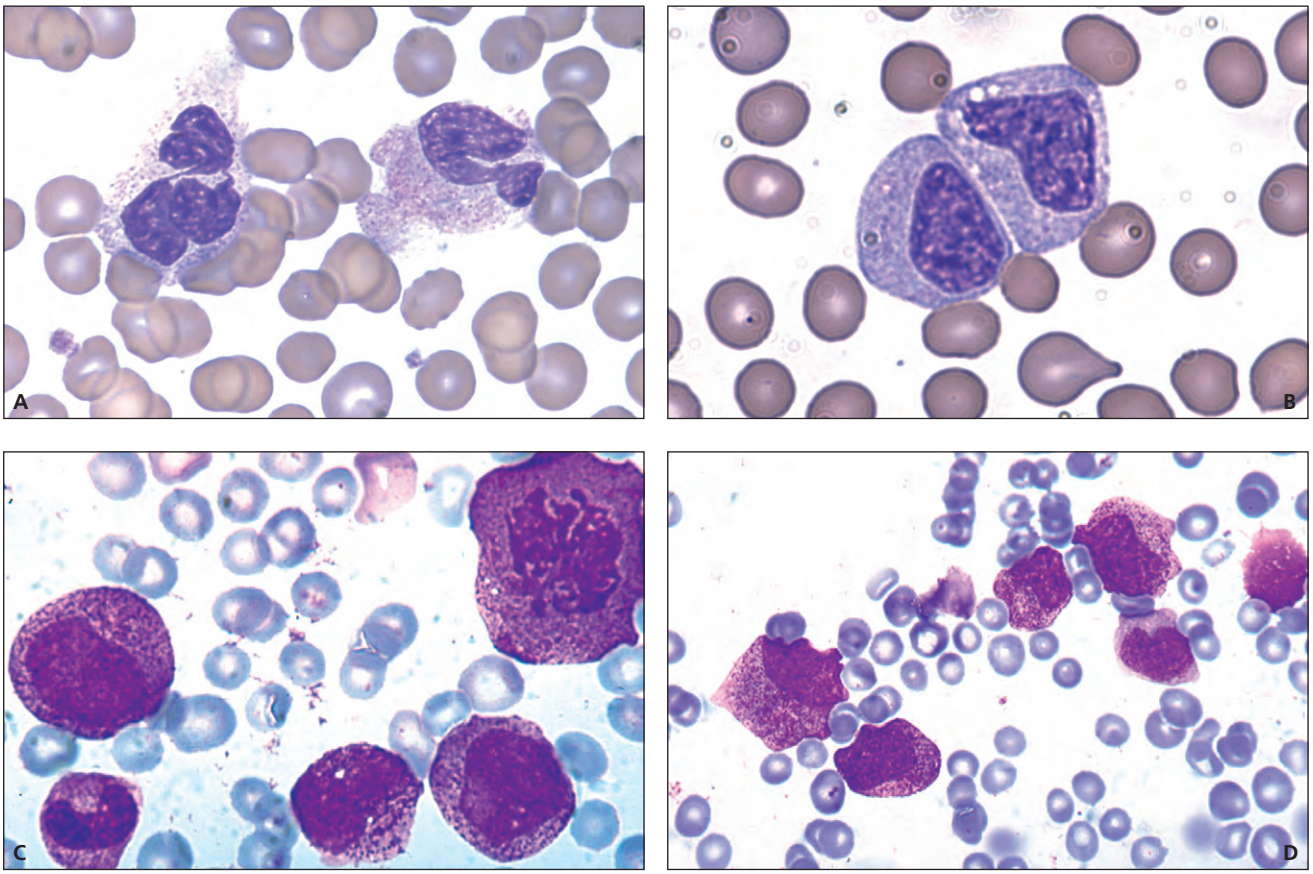



**Fig. 1.32** Variable degrees of dysmorphism in neutrophil granulocytes during the course of growth factor (G-CSF) treatment; peripheral blood film. (A) On the left, a neutrophil with slightly hypogranular cytoplasm and a segmented nucleus with lobes joined by filaments. On the right, a band neutrophil with numerous fine granules and persistence of areas of cytoplasmic basophilia. (B) Two small neutrophil myelocytes present in the circulation; the cytoplasm appears more basophilic than normal and the granules are fine and numerous. (C) Circulating immature granulocyte precursors with violaceous cytoplasm laden with granules; at the top, to the right, a mitotic figure. (D) Marked increase in circulating granulocyte precursors in a healthy subject receiving G-CSF prior to the harvesting of haemopoietic stem cells for allogeneic transplantation. A monocyte, on the right, also appears dysmorphic.

interwoven chromatin, in which there are one or more prominent pale blue nucleoli. At this stage of maturation the ribosomes are not yet associated with the endoplasmic reticulum and the cell is thus incapable of antibody synthesis: an incomplete line of arcoplasm often surrounds two thirds of the nucleus. Once they reach peripheral sites, the B immunoblasts divide and mature into plasma cells, which are end-stage cells normally incapable of further division, destined to synthesise a single type of antibody and expire in 5–14 days. About 5% of immunoblasts from the thoracic duct differentiate into long-lived small memory B cells. The intermediate cells between the B immunoblasts and the mature plasma cells are plasmacytoid lymphocytes, cells that have the capacity to secrete immunoglobulin, particularly IgM. In other conditions, such as infectious mononucleosis (Figs 1.41 through to 1.45), morphologically atypical reactive lymphocytes are of the T lineage.

Finally, monocytes are the largest cells normally present in the peripheral blood (diameter 15–20 μm) (Figs 1.10C, 1.29, 1.30, 1.43D, 1.46 and 1.47). The nucleus occupies about half of the cell; it is often slightly eccentric, variable in shape (round, oval, indented, renifom, lobulated, folded, bean or fetus-shaped); the chromatin is spongy, reticular, pale, composed of a network of filaments that interweave in a very characteristic loose network (which provides one of the most useful criteria for differentiating them from large lymphocytes). The cytoplasm is voluminous, from blue to greyish with a pink tinge that is more or less prominent depending on the quantity of numerous, minute, irregular azurophilic granules: these hardly ever individually reach the threshold of visibility, but give the cytoplasm a sandy, ground-glass, dusty appearance. Vacuoles are often present and may be numerous, particularly after a prolonged period in EDTA.

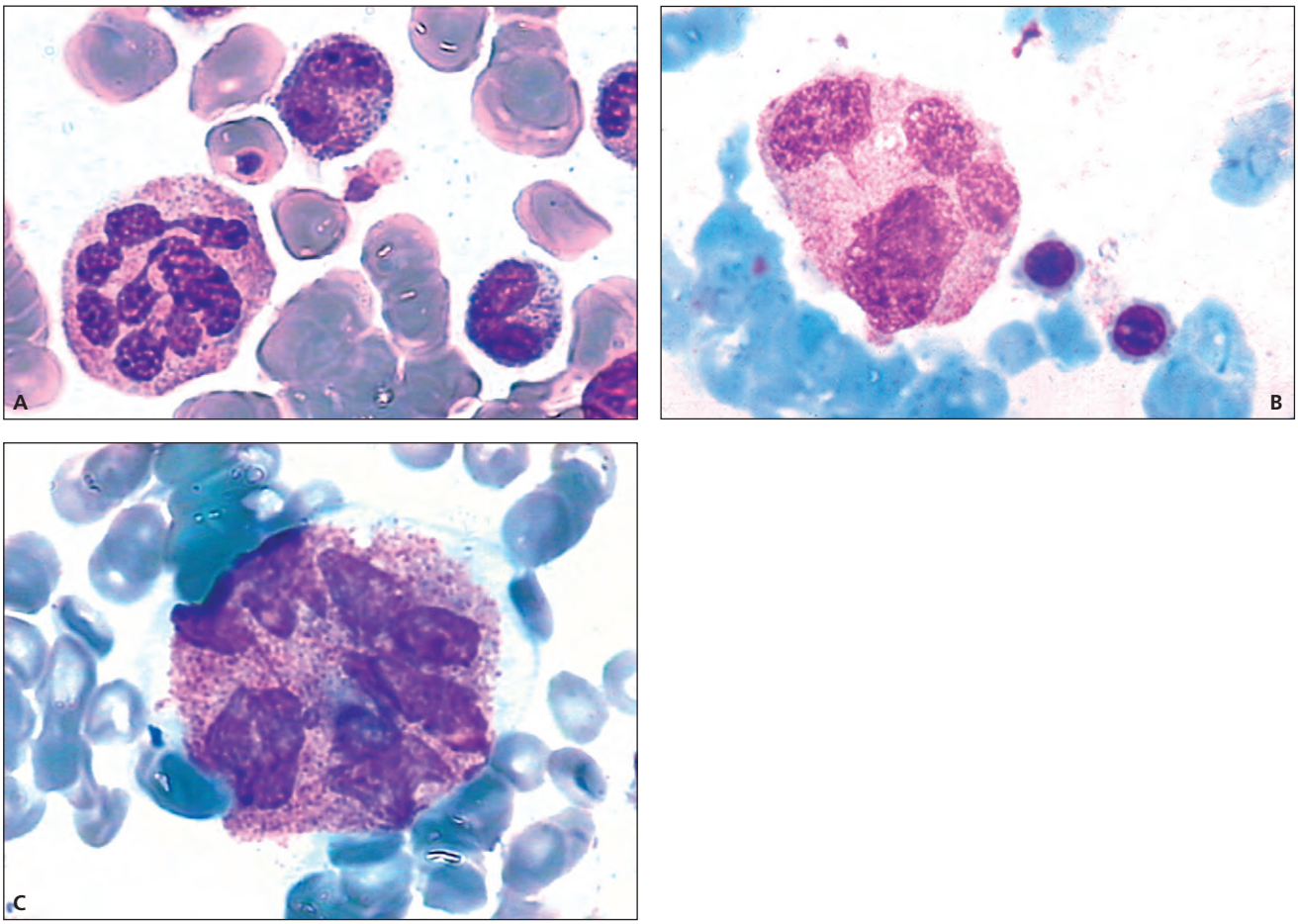



**Fig. 1.33** Other examples of dysmorphism of the granulocyte series during growth factor (G-CSF) treatment; bone marrow aspirate. (A) Giant neutrophil with a hyperdiploid, hypersegmented nucleus. (B) Monstrous neutrophil granulocyte, with hyperdiploidy, hypersegmentation and giant lobes, in a severely neutropenic patient two months after an autologous bone marrow transplant. (C) Another giant neutrophil with a hyperdiploid and hypersegmented nucleus; the heavily granulated cytoplasm shows several areas of residual basophilia and has a veil-like agranular area on the right.

Bizarre forms are sometimes seen in a blood film, their appearance altered by the phenomena of apoptosis and degeneration, by unusual physiologicopathological responses or by artefactual changes (Fig. 1.48): these cells must not be confused with pathological cells,which would lead to an incorrect interpretation. A cytomorphological diagnosis must be always based on an awareness of the possible anomalies and how they can be positively identified.

## Bone marrow aspirate

**The erythroid series** (Table 1.8; Figs 1.49 through to 1.54)

The first morphologically identifiable erythroid precursors, the direct progeny of the progenitors known as CFU-E, are the proerythroblasts. These are very large cells (diameter 20–25 μm), with a regular round or oval shape. The nucleus is large (eight tenths of the size of the cell), round or oval, central and separated from the nuclear membrane by an ample area of cytoplasm (as occurs always in the erythroid series). The nuclear outline is well defined, as if drawn by a compass. The chromatin is very characteristic; a delicate network, regular and homogeneous, dotted in an orderly manner by small zones of condensation. The nucleoli are easily visible, encircled by a perinucleolar condensation of chromatin. The cytoplasm is intensely basophilic with a certain lack of homogeneity attributable to small pale areas corresponding to numerous mitochondria and a pale elongated juxtanuclear zone, sometimes like a cowl or half moon around a nuclear pole (arcoplasm).

In basophilic erythroblasts there is a reduction in cellular dimensions and in the N:C ratio. The nucleus is round, central and without nucleoli. The chromatin is condensed into dark masses or blocks, polygonal and separated by pale elongated fissures arranged in rays throughout the nucleus; the number of

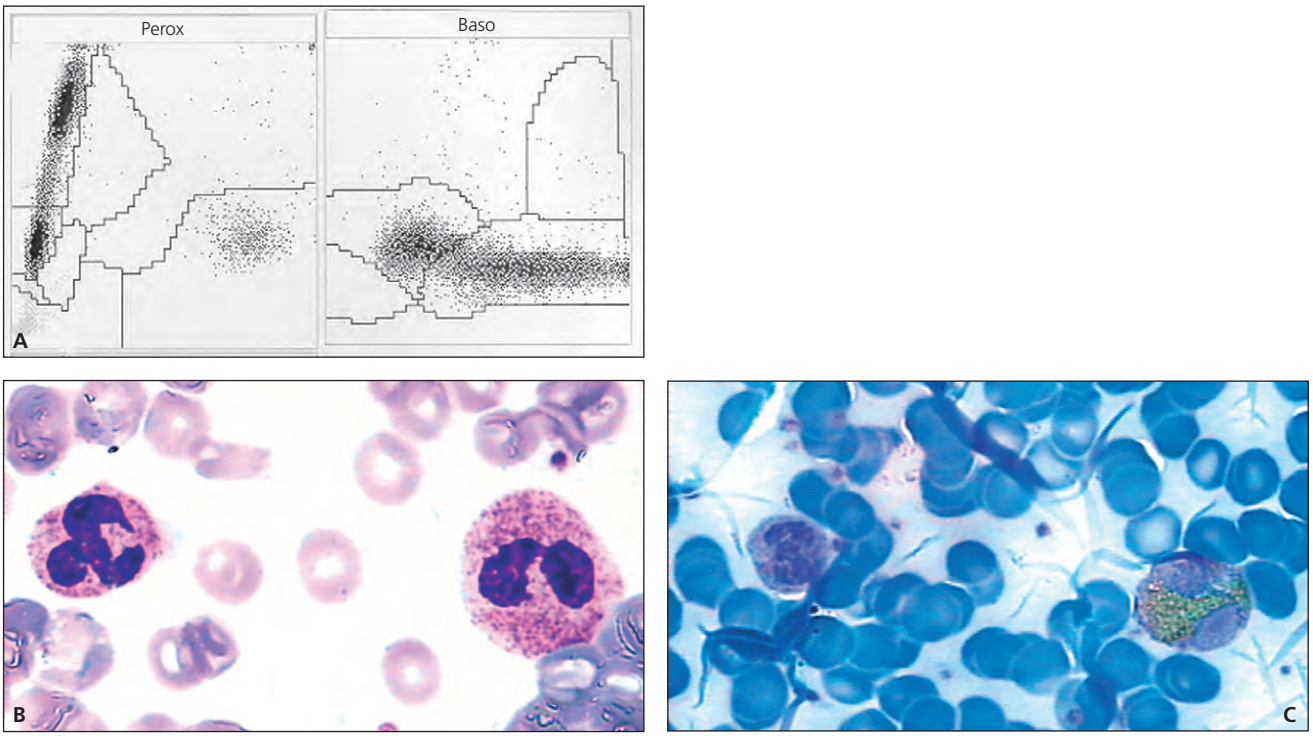



**Fig. 1.34** Hereditary myeloperoxidase deficiency. (A) This anomaly, which is not associated with any specific pathological conditions or with any increased susceptibility to infection, is usually recognised from printouts of the ADVIA system. On the left, the Perox cytogram shows the complete absence of a cluster in the area usually occupied by neutrophils; however, an obvious elongated cluster of large unstained cells (LUC) (49.4%) is present, these large peroxidase-negative cells representing enzyme-deficient neutrophils; a group of peroxidase-positive cells is well represented in the eosinophil area (6.5%), these appearing to show normal enzyme activity. In the Baso cytogram there is a normal tail of polymorphonuclear cells (PMN 57.1%) corresponding to the sum of the mature eosinophils and neutrophils: the latter appear totally normal in this analytical channel. (B) The morphology of the neutrophils, after conventional May-Grünwald-Giemsa staining of a peripheral blood film, does not show any deviation from normality. (C) Cytochemical reaction for peroxidase, peripheral blood film. A mature neutrophil on the left appears completely negative. The peroxidase reaction is however, as would be expected, strongly positive in the bilobed eosinophil granulocyte on the right.

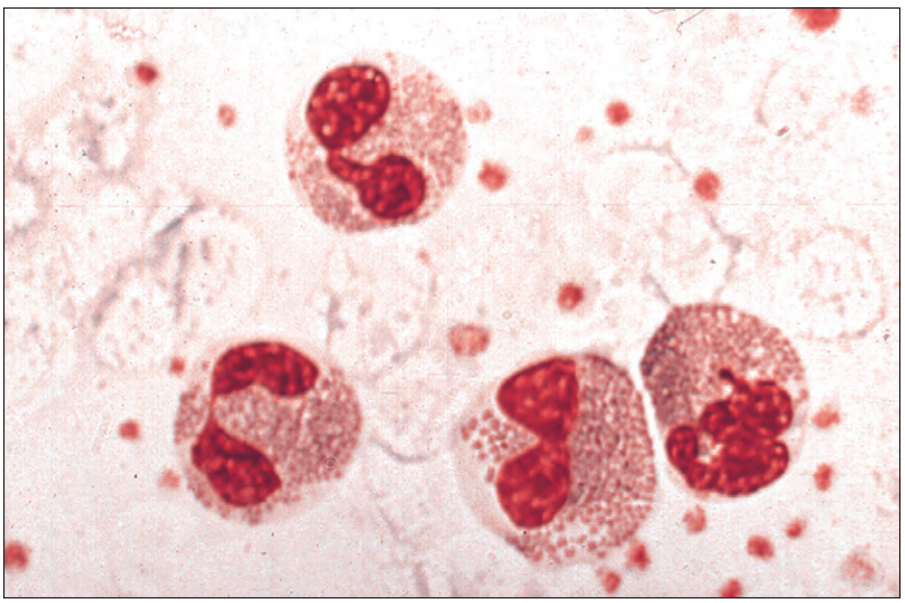


**Fig. 1.35** Peripheral blood. Four mature eosinophil granulocytes with normal morphology.

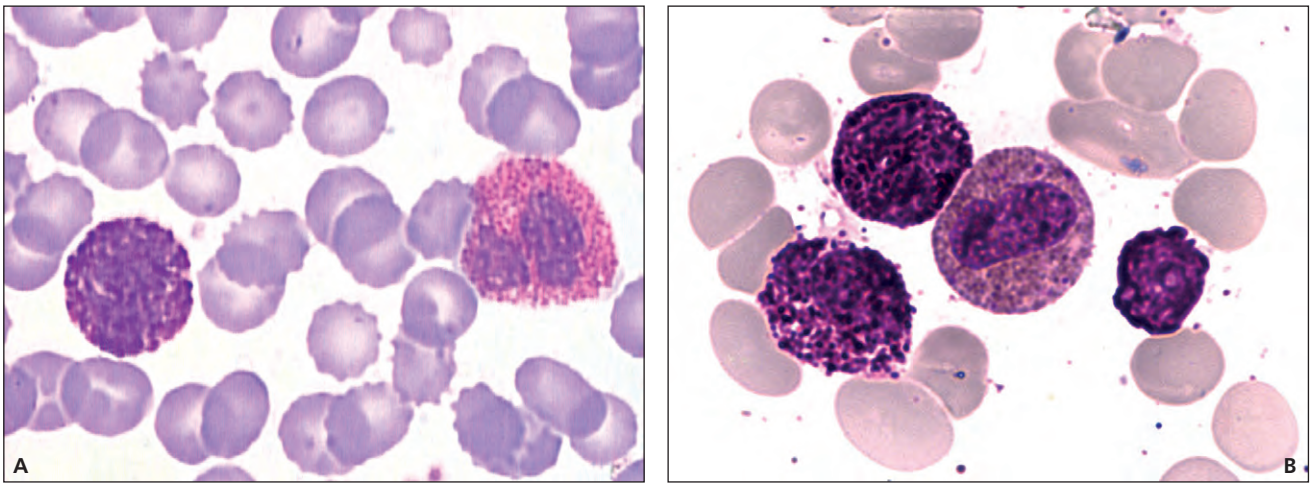



**Fig. 1.36** Eosinophil and basophil granulocytes in peripheral blood films. (A) On the left, a basophil granulocyte with the nucleus completely obscured by granules. On the right, an eosinophil granulocyte in which the granules are not superimposed on the nuclear lobes. (B) Two basophil granulocytes and a band eosinophil.

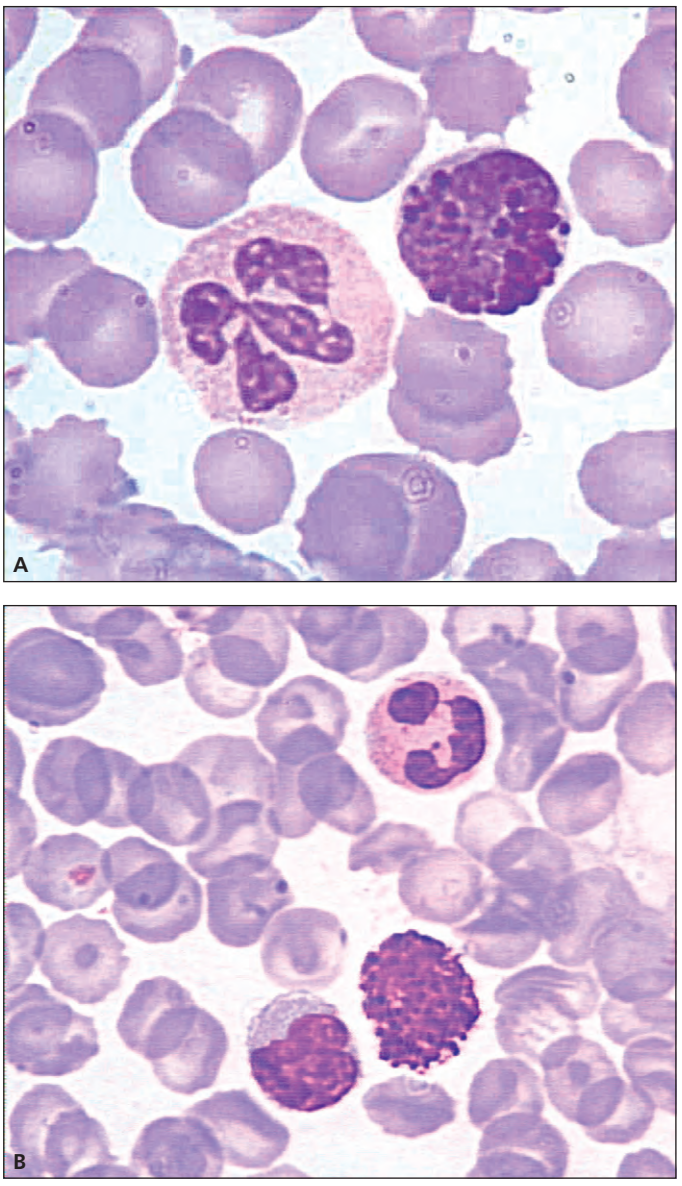



**Fig. 1.37** Mature basophil granulocytes in peripheral blood films. (A) A neutrophil with four nuclear lobes and a well-formed basophil with numerous large granules. (B) At the top, a neutrophil with a nuclear appendage. At the bottom, a lymphocyte and a basophil.

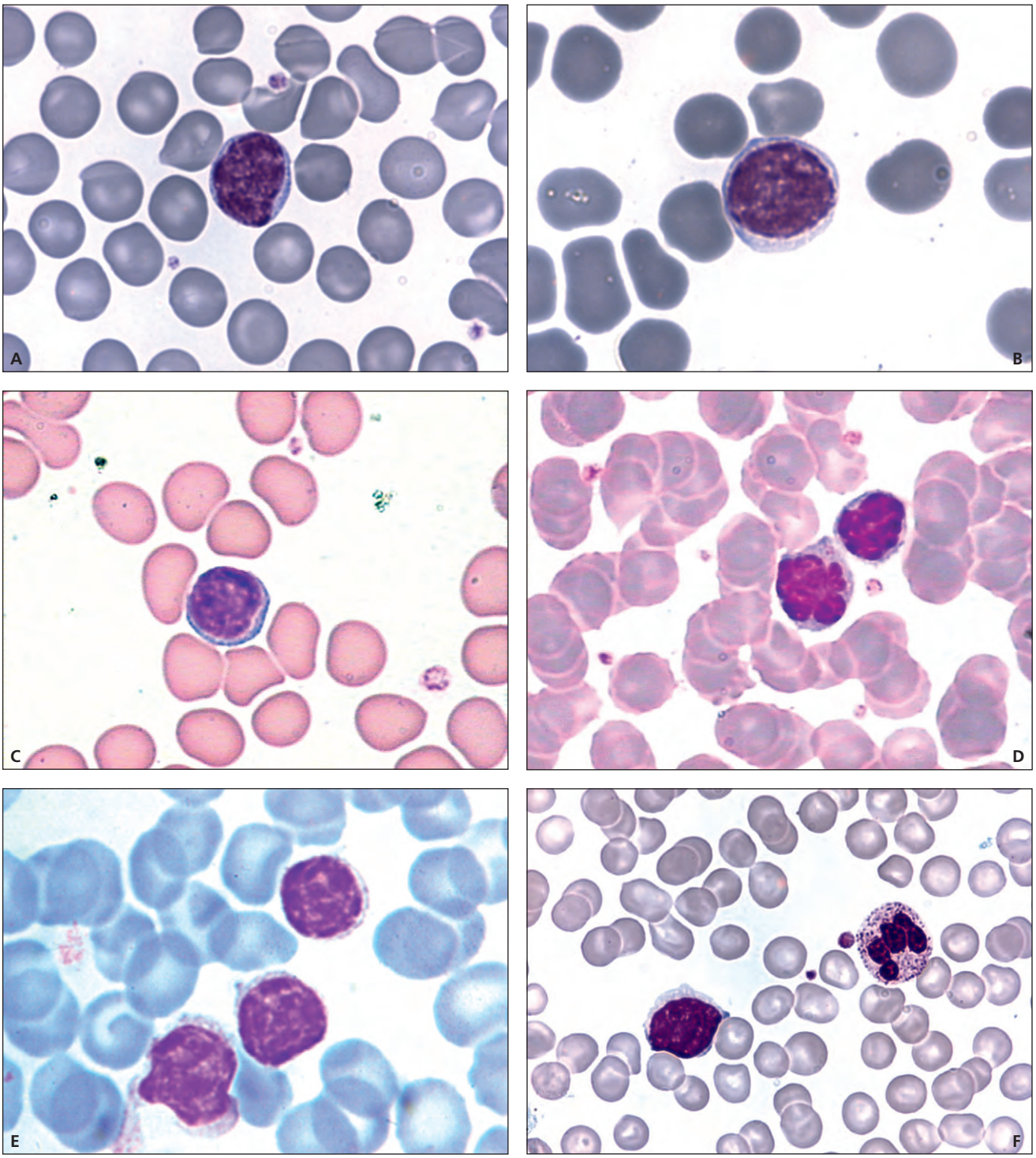



**Fig. 1.38** (A–F) Morphology of small mature lymphocytes in peripheral blood films. The nuclei, round or slightly indented, are usually eccentric in scanty weakly basophilic cytoplasm. The chromatin is dark and condensed, usually into large clumps of very variable shape with small paler spaces between them. In (D), a large granular lymphocyte (LGL) with a lobated nucleus.

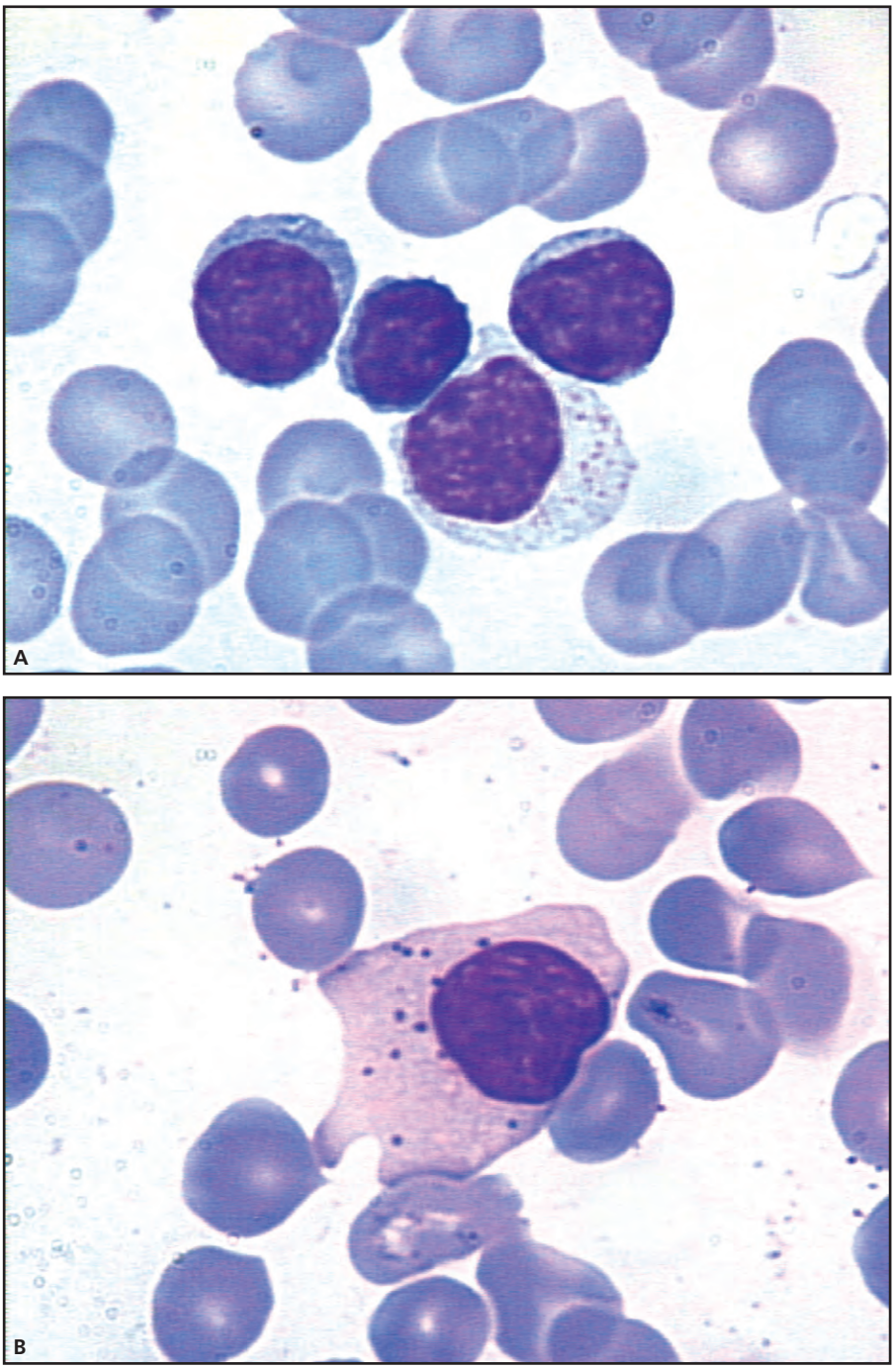



**Fig. 1.39** Morphological variability of lymphocytes in peripheral blood films: large granular lymphocytes. (A) From the left, at the top: a plasmacytoid lymphocyte with an eccentric nucleus and intensely basophilic cytoplasm, which is slightly more plentiful than normal; a small lymphocyte; a larger normal lymphocyte. At the bottom: a large granular lymphocyte with very plentiful pale cytoplasm, blue-grey because of very weak basophilia, in which stand out about 20 large, dark red azurophilic granules, which are clearly separated from each other. The nuclear chromatin is paler than in the other lymphocytes. (B) Large granular lymphocyte: the plentiful pale cytoplasm has a violet tinge; there are 13 very distinct large granules.

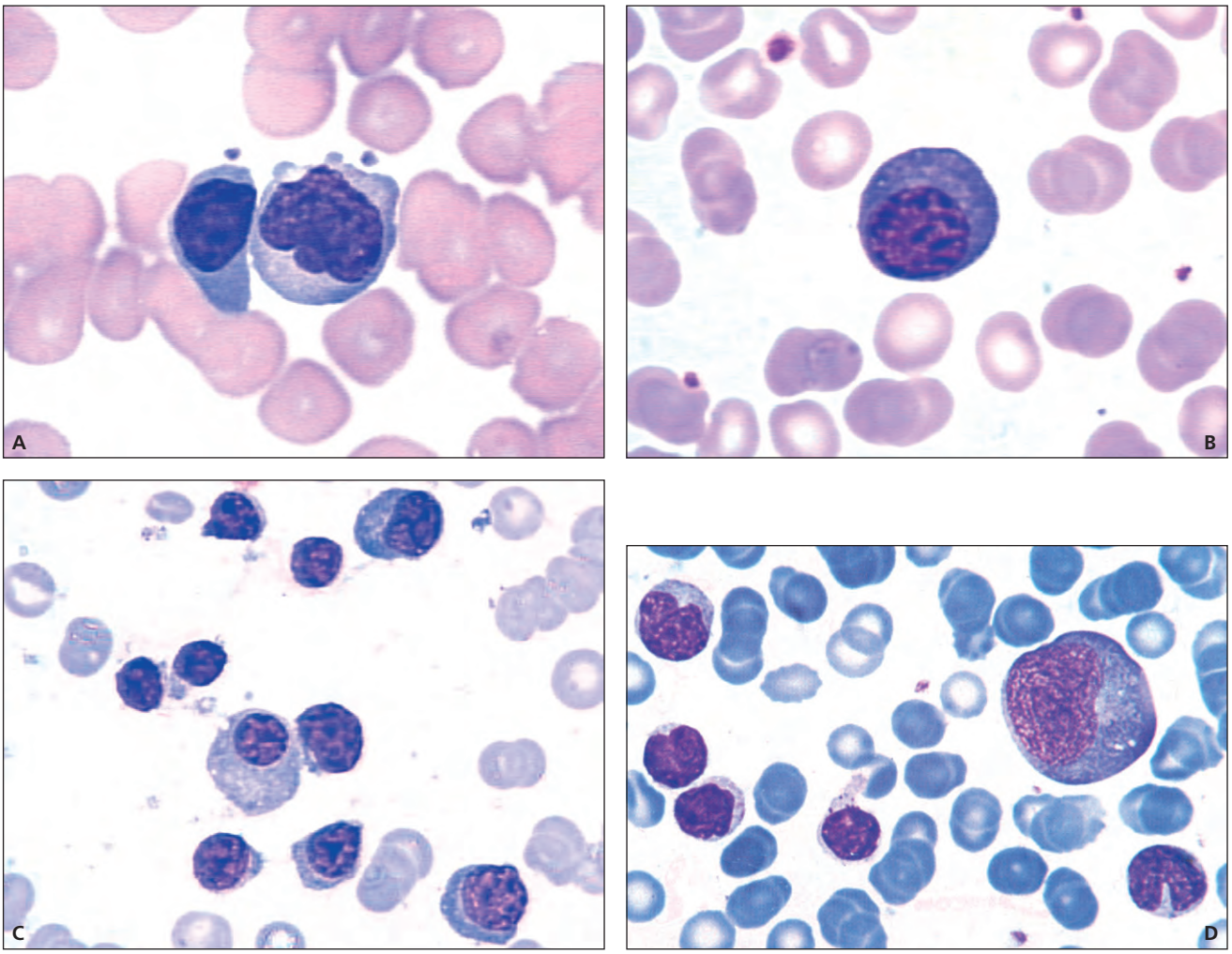



**Fig. 1.40** Morphological variation of lymphocytes during the course of various infections; peripheral blood film. (A) From a patient with *Leishmania donovani* infection: on the left, a small plasmacytoid lymphocyte; on the right, a monocyte. The distinction between atypical or reactive lymphocytes and monocytes is sometimes difficult for less expert observers: in general, the morphology of monocytes shows less variability than that of lymphocytes and can therefore serve as a reference point. (B) Plasmacytoid lymphocyte with a morphology very close to that of a mature plasma cell, in the peripheral blood of a patient with influenza. The N:C ratio is still, however, sufficiently high for the cell to be regarded as a lymphocyte. (C) Marked lymphocytosis with numerous plasmacytoid lymphocytes and a plasma cell in a child with pertussis. Infection with *Bordetella pertussis* is one of the rare bacterial infections associated with lymphocytosis, with the lymphocytes showing reactive features. (D) From a patient with hepatitis B infection. The large cell on the right is a blast cell with a nucleus that has an irregular outline, a nucleolus and chromatin condensed into small clumps and bizarre elongated filaments; the cytoplasm is very plentiful and strongly basophilic with a visible Golgi zone and some small vacuoles. This cell can be regarded as an immunoblast (or as a Downey type III cell). In the centre, towards the bottom, a small lymphocyte with elongated cytoplasm, a hand-mirror cell, in which there are a small number of large violaceous granules.

blocks is between ten and 20, exceeding the number in the nuclei of plasma cells, which is always less than ten. The chromatin tends also to be peripheral, adjacent to the inner surface of the nuclear membrane. The cytoplasm is intensely blue and appears more homogenous because of the reduction in size of the arcoplasm. There are no traces of haemoglobin at this stage.

The next phase of erythroblast maturation is characterised by chromatic variation of the cytoplasm, which ranges from basophilic to acidophilic because of the progressive reduction in ribosomes, replaced gradually by the newly synthesised haemoglobin molecules. Reduction in size continues in the polychromatic erythroblasts (diameter 8–12 μm), as does the reduction in the N:C ratio. The nucleus is smaller and darker but still round, with chromatin clumped into large, well-defined, very dark masses, regularly distributed and separated by very pale spaces (a chess-board appearance). In the more mature cells the chromatin masses tend to coalesce; the nuclear membrane is very distinct and nucleoli are not seen. The cytoplasm

is no longer blue, because the superimposition on the basophilia of a certain acidophilia imparts a violet colouration, then reddish and finally greyish, depending on the respective amounts of ribosomes and haemoglobin; a small clear perinuclear halo can persist. In orthochromatic erythroblasts the nucleus is still round but is further reduced by pyknosis: the chromatin clumps and condenses into a single dark, violaceous, amorphous mass, without any structure. In the cytoplasm acidophilia now dominates: the colouration is, however, slightly different from that of mature erythrocytes, because of the persistence of a polychromatic greyish tinge: for this reason some authors prefer to use the term 'pyknotic erythroblast' or 'late polychromatic erythroblast' for this stage of maturation. For the same reason, others prefer to use the terms 'early erythroblast', 'intermediate erythroblast' and 'late erythroblast' for the three stages of maturation.

The expulsion of the nucleus, which happens by an asymmetric cell division, determines the final transformation of the erythroblast into a reticulocyte (see Chapter 2). This cell still contains ribosomes, mitochondria and residues of the

**Table 1.6** Activated or reactive atypical lymphocytes: the most common cytological alterations

- Increased quantity of cytoplasm
- Increased nucleocytoplasmic ratio
- Increased cytoplasmic basophilia
- Lack of homogeneity of cytoplasmic basophilia
- Cytoplasmic vacuoles
- Appearance of a pale Golgi zone
- Presence of large azurophilic granules
- Reduced chromatin condensation
- Increased size of nucleus
- Appearance of nucleoli

**Table 1.7** Reactive large granular lymphocytes and atypical lymphocytes: the most common morphological variants (Figs 1.39 through to 1.44)

- Large lymphocytes. The nucleus is identical to that of a small lymphocyte, but the cytoplasm, which preserves its pale clear appearance and is weakly basophilic, is more abundant so that it completely surrounds the nucleus, which does not touch the cell membrane; the nucleocytoplasmic ratio is reduced
- Large granular lymphocytes (LGL). These are larger because of the presence of an increased amount of cytoplasm, in which there are large azurophilic granules, of a vivid red colour, which stand out clearly from the weakly basophilic cytoplasm
- Large hyperbasophilic lymphocytes (immunoblasts). These are similar to lymphocytes stimulated ***in vivo*** with phytohaemagglutinin. They are large, with plentiful ultramarine, intensely basophilic cytoplasm, and a large central nucleus with reticular granular chromatin and one to three nucleoli. Sometimes the chromatin is denser and the nucleoli are not visible
- Plasmacytoid lymphocytes (intermediate forms, Turk cells). These are larger than small lymphocytes with increased cytoplasm and cytoplasmic basophilia; the pale Golgi zone is absent or indistinct. The nucleus is only slightly eccentric with irregularly clumped chromatin. The morphology is intermediate between that of a small lymphocyte and a plasma cell
- Lymphocytes of infectious mononucleosis (Epstein-Barr virus infection). These are reactive T lymphocytes, the particular morphological features of which were described by Downey. He defined them as type I – plasmacytoid lymphocytes only slightly larger than small lymphocytes, with a round or oval nucleus and chromatin condensed into small indistinct masses not easily distinguished from the pale zones of parachromatin, and basophilic cytoplasm that is often foamy because of the presence of small vacuoles; type II – larger with more plentiful cytoplasm, blue-grey with a characteristic peripheral enhancement of the basophilia, sometimes containing a few azurophilic granules, easily deformed by the surrounding erythrocytes; nuclear chromatin is less dense; type III – with even more plentiful cytoplasm, similar to an immunoblast with analogous staining characteristics, a more immature irregular nucleus, sometimes trapezoidal or notched, with reticular chromatin and visible nucleoli

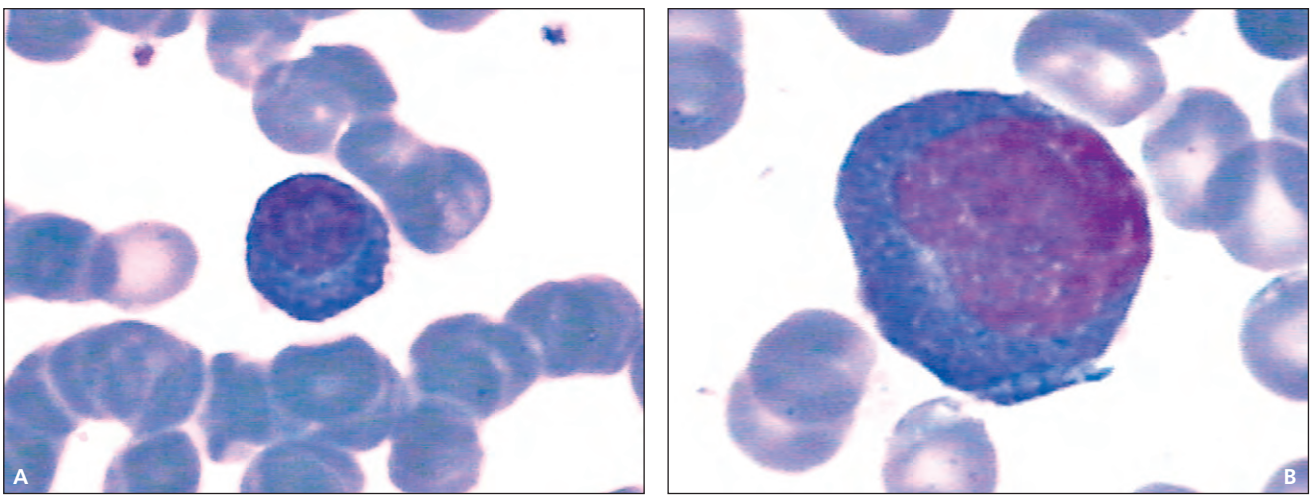



**Fig. 1.41** Lymphocytes with hyperbasophilic cytoplasm in cases of infectious mononucleosis. (A) Small plasmacytoid lymphocyte with an eccentric nucleus (Downey type I). (B) Large lymphocyte with the features of an immunoblast (Downey type III); the nuclear chromatin is condensed into large clumps.

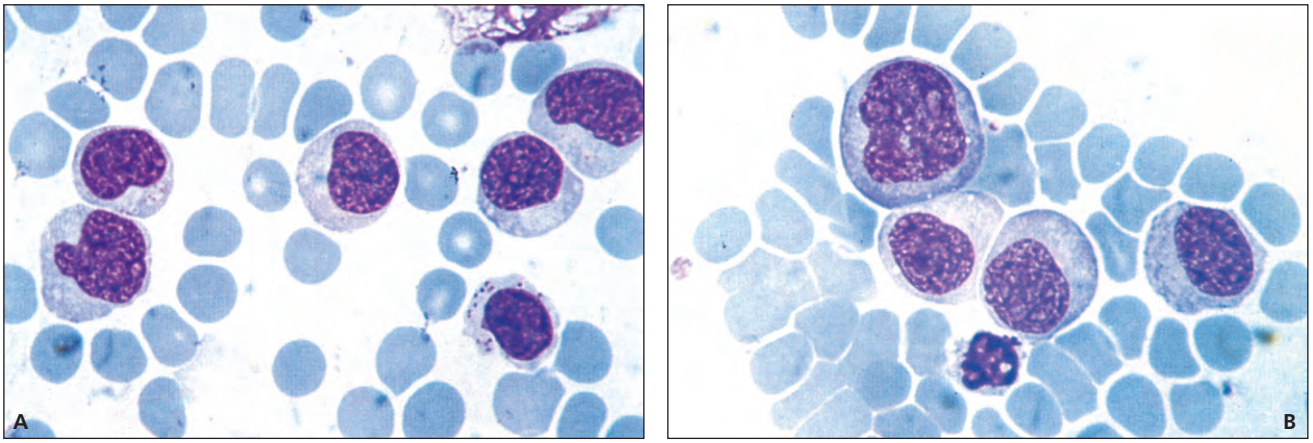



**Fig. 1.42** Variability of lymphocyte morphology in infectious mononucleosis; peripheral blood film. (A) Large lymphocytes (Downey type II) with plentiful greyish, very weakly basophilic cytoplasm, with fairly obvious Golgi zones; the nuclei are pale and spongy with a greater degree of alternation of pale and dark areas than in the small lymphocytes. In the larger cells the nuclei are irregular and distinguishing these cells from monocytes is more difficult; the cell at top left has very fine azurophilic granules, while that at bottom right is a large granular lymphocyte with a smaller number of very distinct large reddish granules. (B) In addition to three large lymphocytes with pale nuclei, the cell at the top is very typical of Epstein-Barr virus infection because of the greater degree of cytoplasmic basophilia, enhanced at the periphery, and the greater immaturity of the nucleus, in which are apparent two nucleoli surrounded by a thick circle of condensed chromatin (Downey type III).

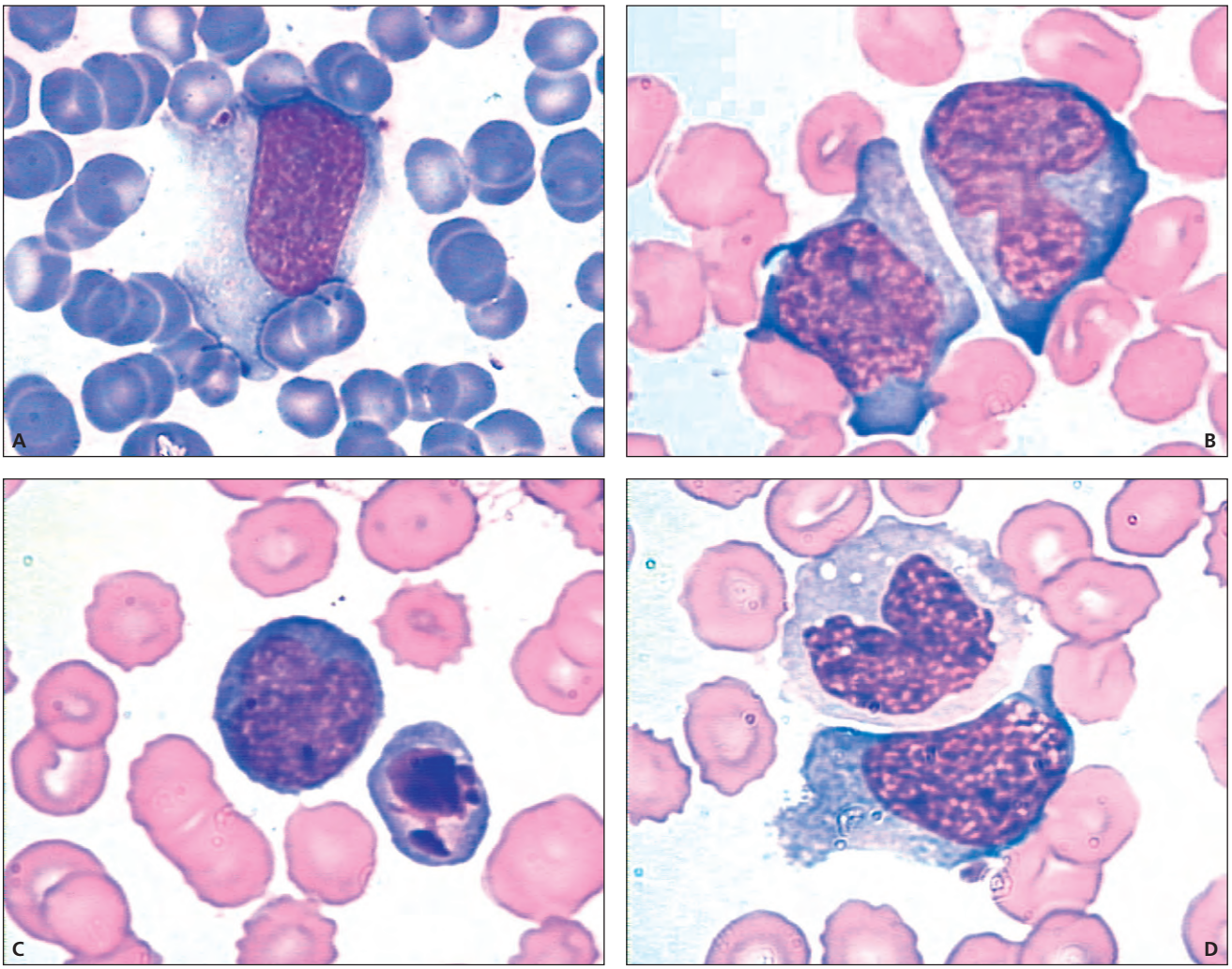



**Fig. 1.43** Typical variation of lymphocyte morphology in infectious mononucleosis. (A) Large lymphocyte with vermicular (worm-like) chromatin, with small pale spaces similar to nucleoli; the cytoplasm is very plentiful with a typical blue colour which is intensified in some areas and fades away in others (Downey intermediate type II/III). (B) Large reactive lymphocytes with cytoplasm that is deep blue with a greyish tinge and peripheral enhancement of the basophilia. The nuclear chromatin is open; the nuclear outline of the cell on the right is tending to convolution, resembling that of monocytes (which, however, never have such blue cytoplasm). (C) Large hyperbasophilic lymphocyte and a lymphocyte with an apoptotic nucleus. Apoptotic cells are very frequent in the films of patients with infectious mononucleosis. (D) At the top, a monocyte, easily recognisable from its nuclear morphology (a deep incision) and from the characteristic grey colouration of the vacuolated cytoplasm. At the bottom, a hyperbasophilic immunoblast (Downey type III).

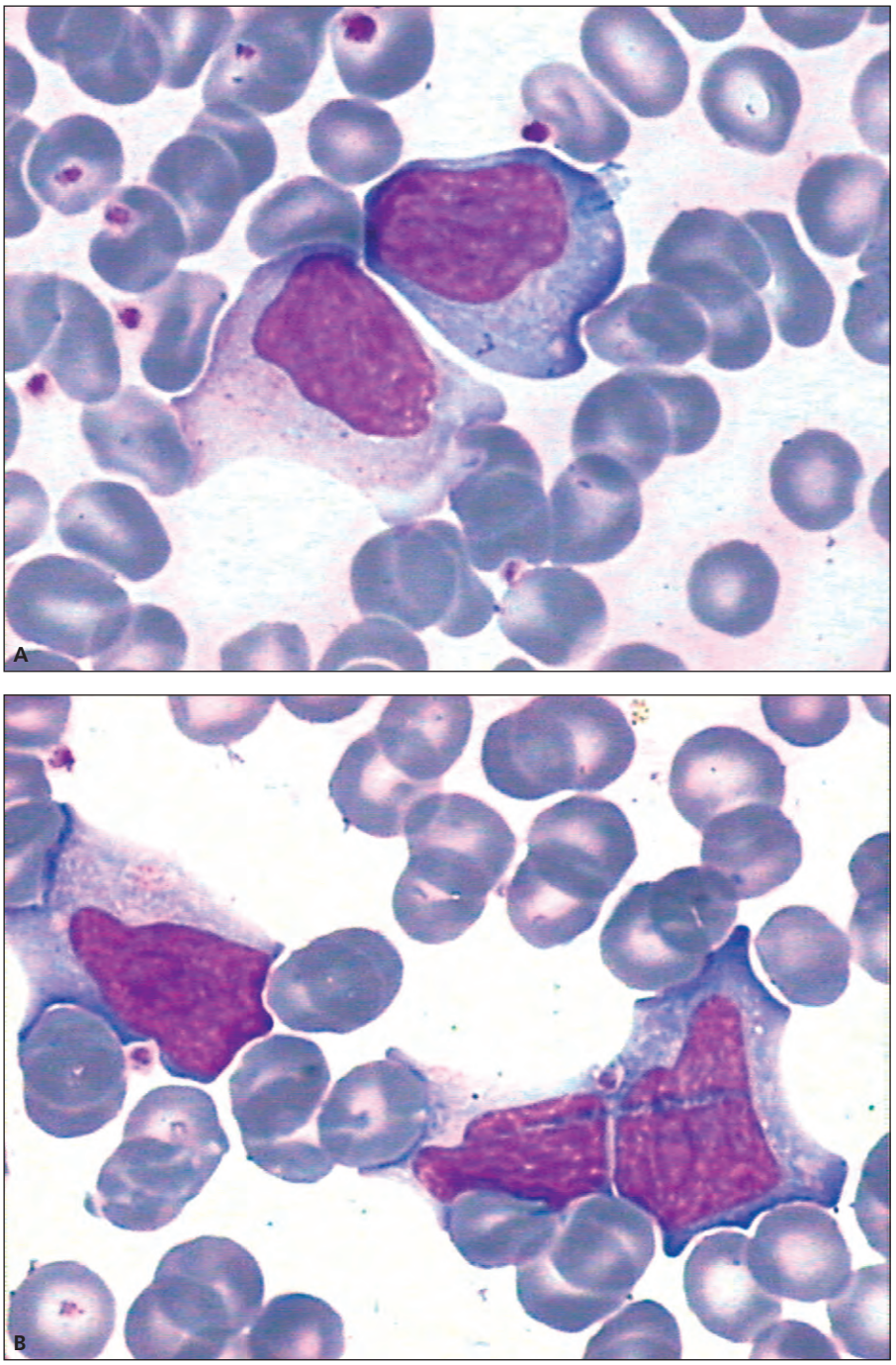



**Fig. 1.44** Particularly marked morphological changes in a case of infectious mononucleosis. (A) The large lymphocyte on the left has pale cytoplasm, which seems to contain very fine granules, no different from those of monocytes; it can, however, be distinguished from a monocyte by the presence of a broad clear projection on the right, and also by the nuclear morphology, which is typical of a large lymphocyte, with condensed chromatin; the cell on the right is an immunoblast with the typical peripheral reinforcement of cytoplasmic basophilia. (B) The nuclei of these immunoblasts appear immature and have large nucleoli. The morphology of the cytoplasm, blue tinged with peripheral enhancement is, however, characteristic and permits a correct cytological identification, which can be confirmed by flow cytometric immunophenotyping.

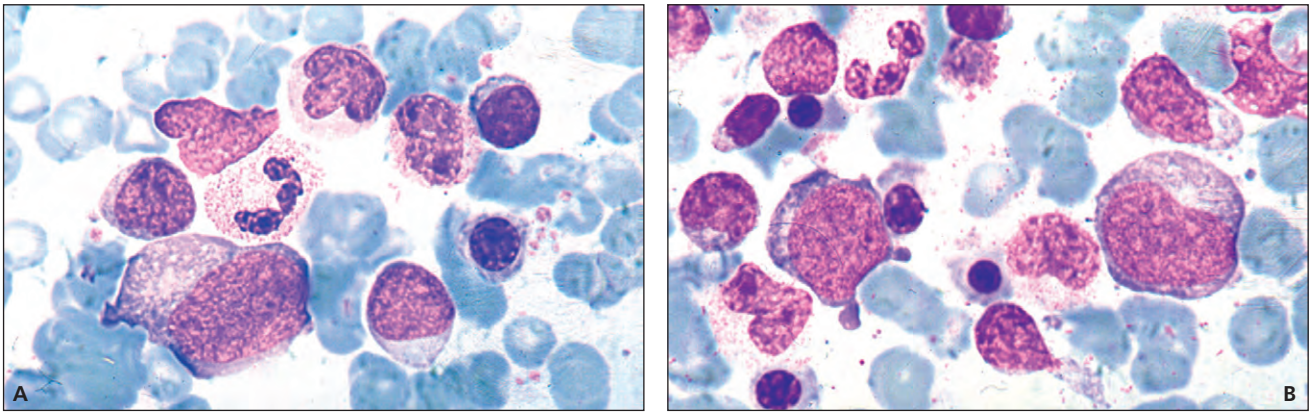



**Fig. 1.45** Infectious mononucleosis, bone marrow aspirate. This examination, performed because of associated thrombocytopenia, shows in the context of a cellular marrow in which all three haemopoietic lineages are well represented, a lymphoid population of around 15%: the typical morphological alterations of mononucleosis can, particularly in a bone marrow aspirate, arouse the erroneous suspicion of lymphoma. (A) The larger cell is a typical immunoblast. There are also two lymphocytes with immature nuclei, similar to small blast cells and, top right, a plasmacytoid lymphocyte. Lower down is a polychromatic erythroblast. (B) In addition to some erythroblasts, two large immunoblasts with hyperbasophilic cytoplasm and several small lymphoid cells that appear almost blastic.

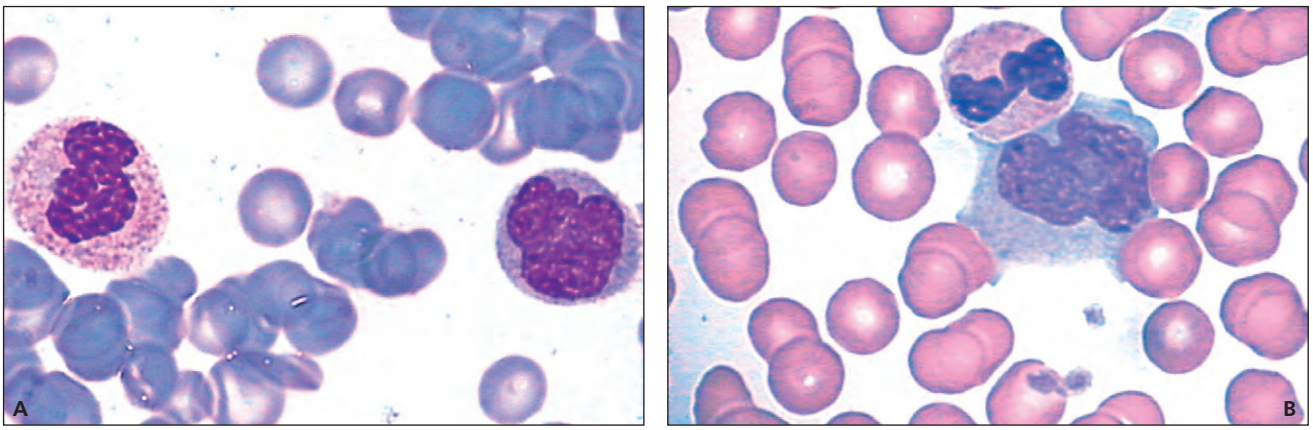



**Fig. 1.46** Morphology of monocytes in the peripheral blood. (A) On the left, a neutrophil granulocyte. On the right, a monocyte: the cytoplasm is basophilic and relatively scanty, differing somewhat from what is usually seen in the peripheral blood (this rather compact cytoplasm is more common in monocytes seen in bone marrow aspirates), but it has the typical very fine sandy appearance that distinguishes monocyte cytoplasm; the nuclear chromatin is not homogeneous and the nuclear outline is irregular with many incisions. (B) At the top, a neutrophil band form. At the bottom, a monocyte with plentiful weakly basophilic cytoplasm, with a sandy appearance created by the presence of numerous very fine, diffuse azurophilic granules; there are no vacuoles; the nucleus has paler chromatin than that of the neutrophil and a typical indented outline.

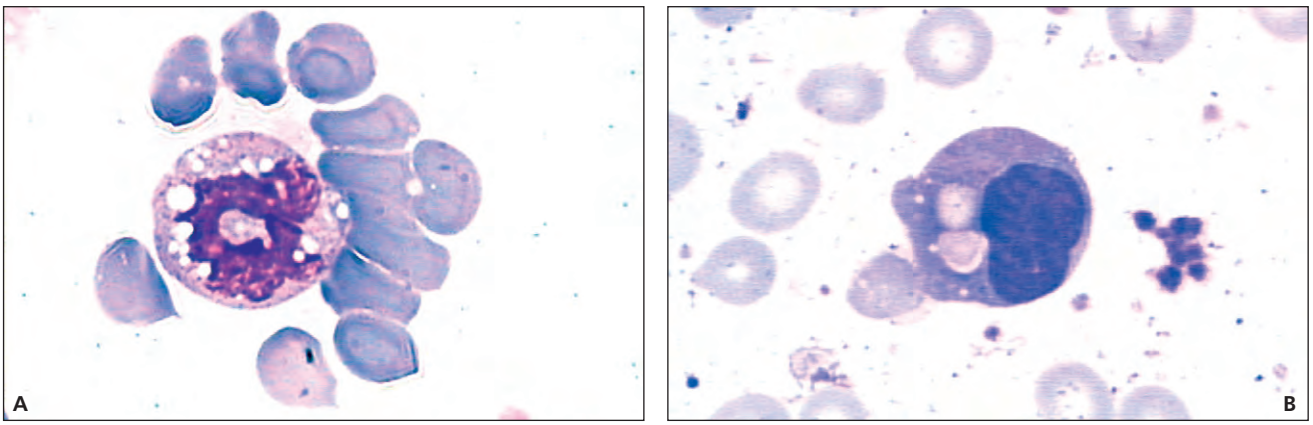



**Fig. 1.47** Other features of monocyte morphology in peripheral blood films. (A) A monocyte with a horse-shoe shaped nucleus, the two extremities of which are superimposed on each other; the plentiful cytoplasm appears dusty and is perforated by numerous vacuoles of variable size, which also distort the nuclear outline. (B) Small circulating monocyte with a compact nucleus; the cytoplasm is rich in fine azurophilic granules and contains two ingested erythroblasts; another red cell is adherent to the lower left pole of the monocyte. Erythrophagocytosis is observed rarely in the peripheral blood in conditions of immunological activation and in autoimmune haemolytic anaemia.

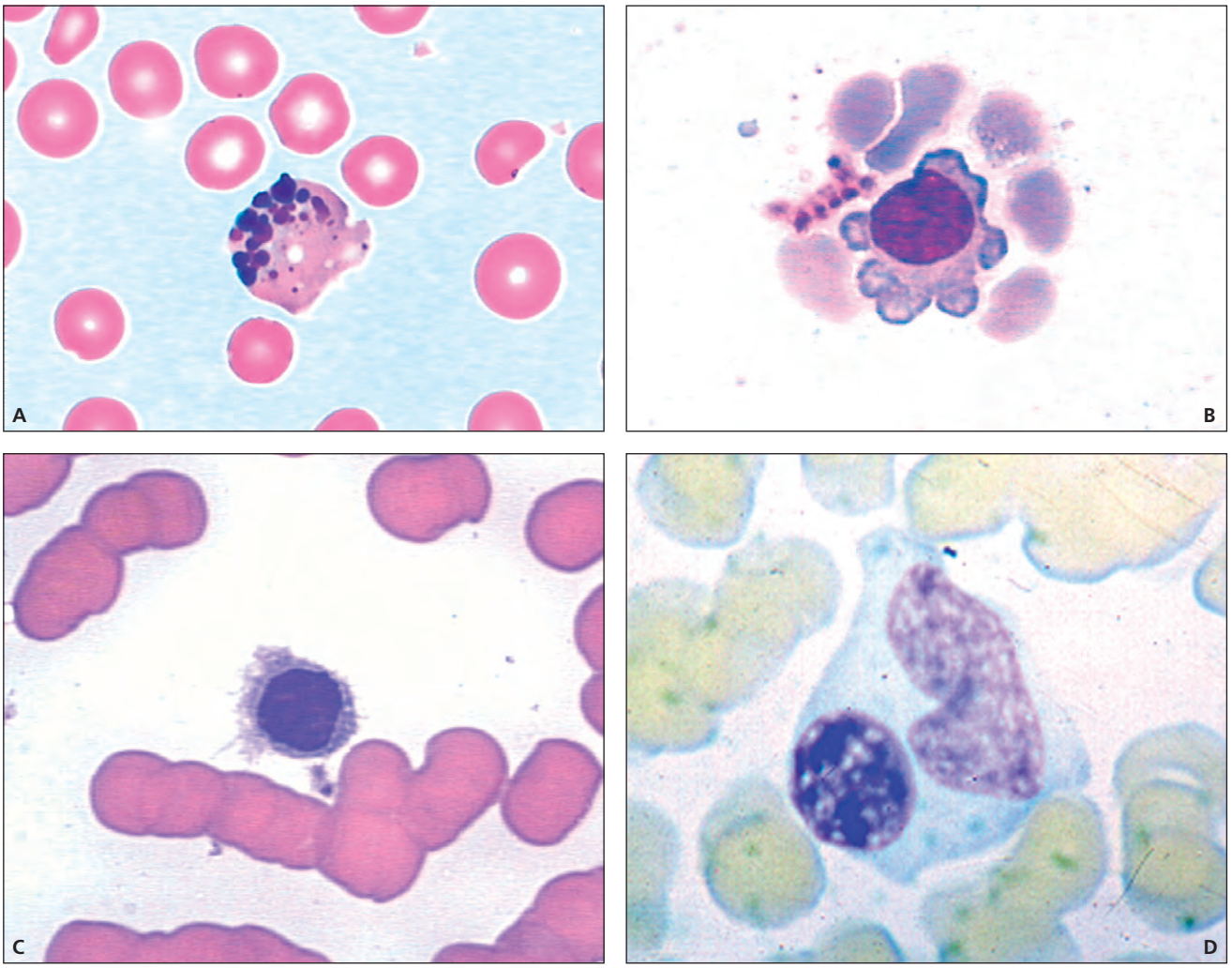



**Fig. 1.48** Non-specific cytological abnormalities in peripheral blood films. (A) An apoptotic cell, with the nucleus fragmented into rounded masses of pyknotic chromatin and the cytoplasm transformed into a homogeneous hyaline mass (a frequent observation if the blood film has been made after a prolonged period of storage of the blood specimen). (B) A cell that is certainly lymphoid, with basophilic cytoplasm forming multiple rounded blebs. This change is caused by cellular stress and resembles clasmacytosis. (C) A lymphocyte with a plasma membrane that is forming numerous large villous projections, in the film of a patient with dysproteinaemia. This is an artefact that should not be confused with villosity of hairy cell leukaemia or splenic lymphoma with villous lymphocytes. One notes also the rouleaux formation. (D) A bizarre cell with plentiful basophilic cytoplasm and two nuclei, one typical of the lymphoid series, the other resembling that of a monocyte. Given that hybrid lymphocyte-monocytes do not exist, this may represent phagocytosis of a nucleus with condensed chromatin by a monocyte (a phenomenon which recalls spontaneous lupus erythematosus [LE] cell formation; see Chapter 11).

**Table 1.8** Morphological characteristics associated with erythroid differentiation (four cellular divisions)

- Reduction in cell size
- Reduction in nuclear size and the nucleocytoplasmic ratio
- Final expulsion of the nucleus
- Characteristic increasing density of the chromatin, finally becoming pyknotic
- Modification of the cytoplasmic colour from basophilic (due to ribosomes) to acidophilic (due to haemoglobin)
- Clear passage from one stage to the next: in contrast to the granulocytic series, the individual stages can be easily distinguished from each other
- Erythroblastic islands: various generations of erythroblasts surrounding a macrophage

endoplasmic reticulum, demonstrable by supravital staining. A proportion of the red cells present in bone marrow are reticulocytes: their liberation into the circulation occurs two or three days after their formation.

In bone marrow erythroblasts of healthy subjects with adequate iron stores, it is possible to observe minor erythroid atypia. This lacks any diagnostic significance, being similar to that seen in the myelodysplastic syndromes (binuclearity, cytoplasmic bridges, defects in haemoglobinisation, basophilic stippling and irregularity of the nuclear outline).[49–51] These anomalies are, however, present in only a small proportion of erythroblasts, less than 10%.

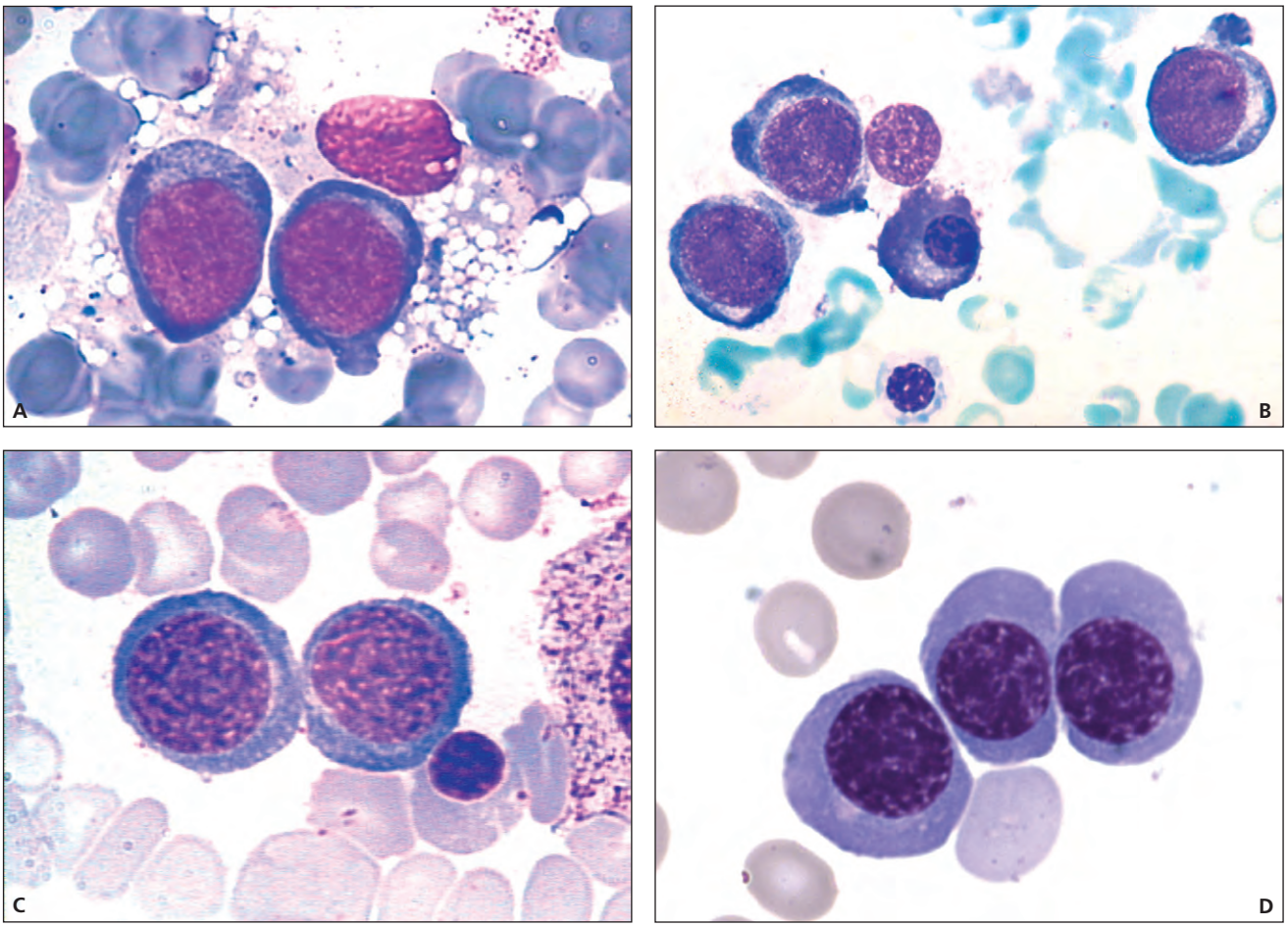



**Fig. 1.49** Erythroid precursors in a bone marrow aspirate. (A) Two proerythroblasts surrounded by the plentiful vacuolated cytoplasm of a macrophage, the spongy nucleus of which can be seen immediately above the proerythroblast on the right. These precursors, which are the earliest recognisable erythroid cells, have numerous distinctive cytological features: their large size, the strong cytoplasmic basophilia, the pale arcoplasm, the distinct regular outline of the nucleus, which does not touch the cytoplasmic membrane and the homogeneous chromatin composed of small clumps. In these cells the nucleoli are incomplete and barely visible. On the right, a cytoplasmic protrusion or bleb, a feature quite often seen in these cells. (B) Three proerythroblasts with more condensed chromatin and hypertrophic arcoplasm; in the cell top right, with a large bleb, the nucleus is displaced from the centre and almost touches a considerable part of the internal surface of the cytoplasmic membrane, something that occurs quite rarely. In the centre, a plasma cell with cytoplasm that is almost lilac and has a ragged outline; lower down, a polychromatic erythroblast. (C) A pair of basophilic erythroblasts, characterised, in comparison with proerythroblasts, by smaller size, chromatin condensed into more definite clumps, absence of nucleoli and much less visible arcoplasm. The nucleus is always round, as is usual in all erythroid precursors in the bone marrow; this feature is seen also in the orthochromatic erythroblast lower right, which has chromatin condensed into a single mass with very few pale spaces; in this cell the eccentricity of the small nucleus suggests its imminent expulsion. (D) Three early polychromatic erythroblasts with nuclei that are still similar to those of the preceding phase of maturation (but with chromatin condensed and compacted into larger masses than are seen is the basophilic erythroblasts in [C]); the cytoplasm has lost the intense basophilia of the more immature stages and is starting to have a grey-lilac or leaden tinge. These cells show slight asynchrony with cytoplasmic maturation apparently more advanced than that of the nucleus: a feature that is not rare among patients without any haematological disorder.

**The granulocytic series** (Table 1.9; Figs 1.55 through to 1.62)

The first morphologically recognisable cells of the granulocytic series are the myeloblasts. They are irregularly rounded or oval with a very high N:C ratio. The nucleus is large, oval or trapezoidal, not regular as it is in the erythroid series; it occupies most of the cell and is usually slightly eccentric. The chromatin is pale, delicately reticular, granular, diffuse and homogeneous, without large areas of increased density. The nuclei contain one or more nucleoli, often not demarcated by a line of denser chromatin and sometimes with indistinct outlines that blend with the rest of the chromatin. The nuclear membrane is delicate and indistinct. The cytoplasm, not abundant but well represented, is uniformly and moderately basophilic. The cytoplasm of the myeloblast may not contain granules (and in this case the cells are not distinguishable from blast cells of other lineages)

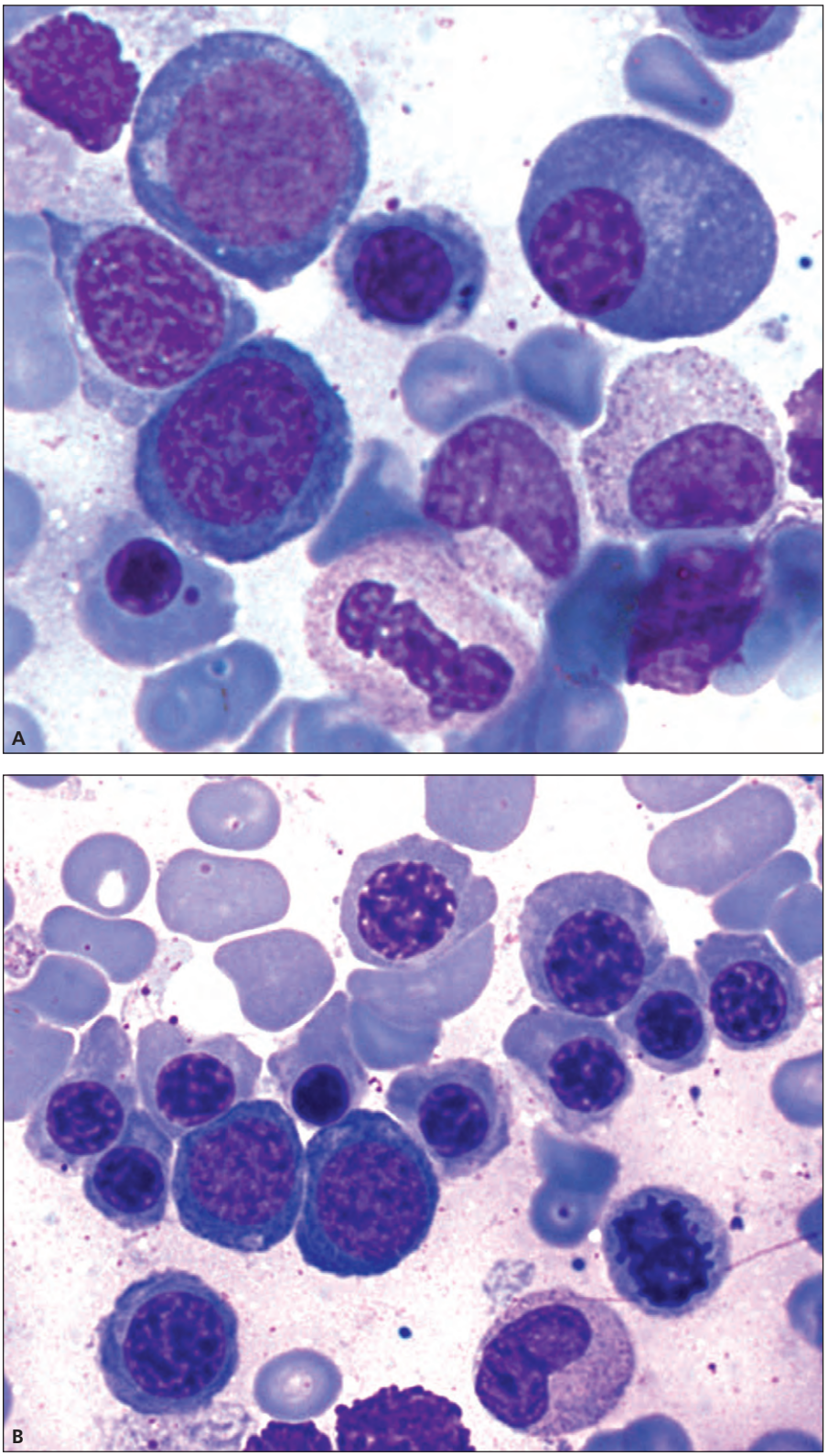



**Fig. 1.50** Normoblastic erythropoiesis: morphology of erythroblasts in bone marrow aspirates. (A) Top left: a proerythroblast, a polychromatic erythroblast and a plasma cell, the latter recognisable from its eccentric nucleus and plentiful basophilic cytoplasm with a large pale Golgi zone. Lower down, on the left: an orthochromatic erythroblast with an almost pyknotic eccentric nucleus and cytoplasm that is almost identical to that of a mature red cell; immediately above, a basophilic erythroblast (one notes that the basophilic staining is darker and the nucleus more compact than in a proerythroblast), a band neutrophil, a metamyelocyte and a neutrophil myelocyte. (B) Erythroid precursors at all stages of maturation, with the exception of proerythroblasts. The progressive condensation of the chromatin is clearly seen, as is the reduction in nuclear size and the changing colours of the cytoplasm. The large erythroblast at bottom left can be considered a transitional form between a basophilic and a polychromatic erythroblast. Among the more mature forms, ignoring the staining of the cytoplasm, which can be difficult to appreciate, the cells that show complete or almost complete nuclear pyknosis without any significant pale intranuclear spaces can be considered orthochromatic erythroblasts. Bottom right, a mitotic figure in an erythroid cell and a neutrophil metamyelocyte.

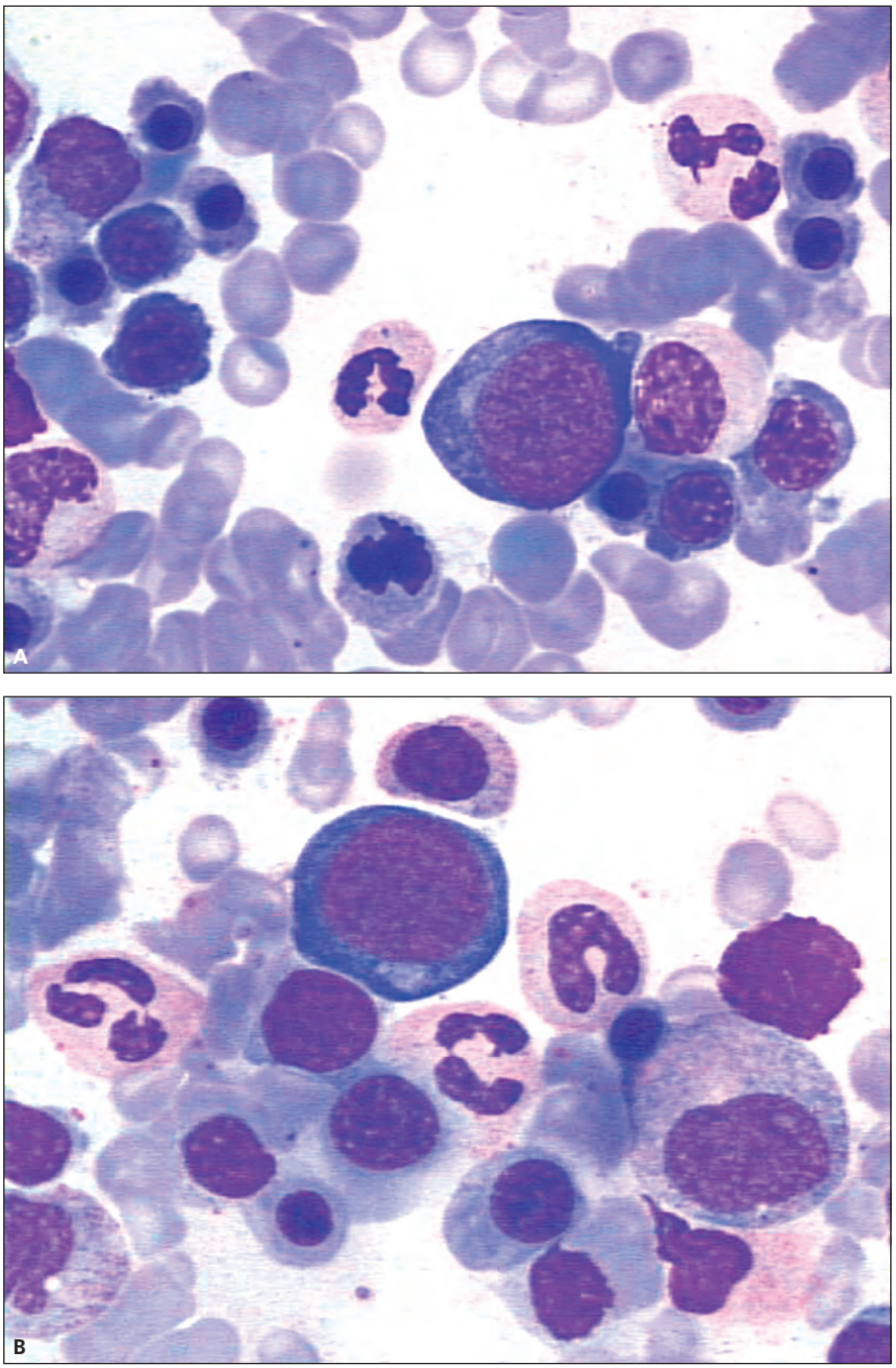



**Fig. 1.51** Normoblastic erythropoiesis: morphology of erythroblasts in bone marrow aspirates. (A) A large proerythroblast, four polychromatic erythroblasts, six orthochromatic erythroblasts and, in the centre at the bottom, a mitotic figure. With regard to the polychromatic erythroblasts, the two on the left appear more basophilic than those on the right, particularly because the cells in that area are less spread out. (B) At the top, a large, quite typical proerythroblast, and below it two polychromatic and two orthochromatic erythroblasts. The cell immediately below the erythroblast may also be a polychromatic erythroblast, but it is not easily recognised; the cell immediately above is a small myelocyte. The other cells of granulocyte lineage are, from left to right, two segmented neutrophils, a band neutrophil and a large promyelocyte with an eccentric nucleus, reticular chromatin, a nucleolus, a Golgi zone and fine azurophilic granules disposed in an orderly manner in cytoplasm that retains its weak basophilia.

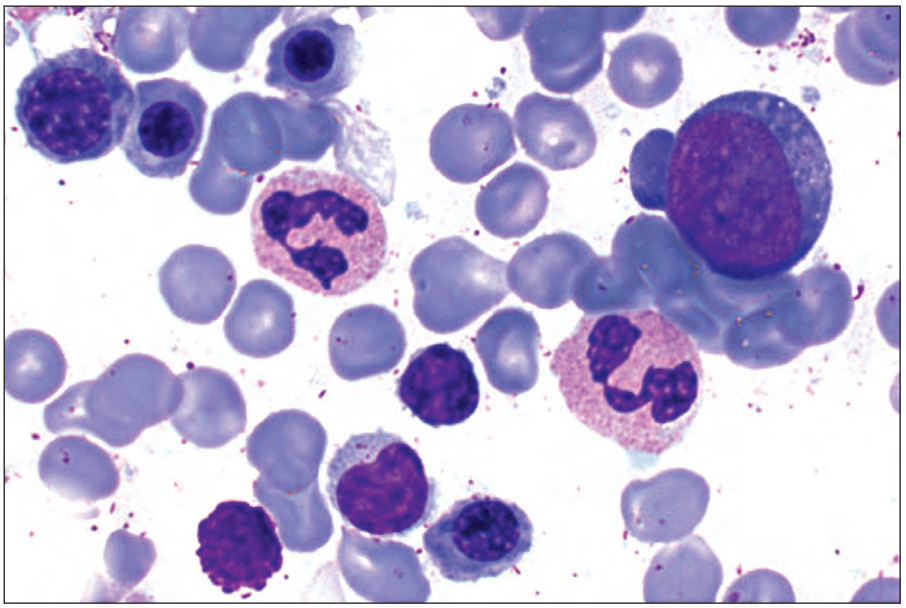


**Fig. 1.52** A proerythroblast with a large, globular cytoplasmic projection; opposite, an early polychromatic and two orthochromatic erythroblasts. Below the two segmented neutrophils (one notes that the one on the right has a veil-like extension of agranular cytoplasm, similar to a uropod) are two lymphocytes, one with negligible cytoplasm and one with the characteristics of a large granular lymphocyte, and another polychromatic erythroblast.

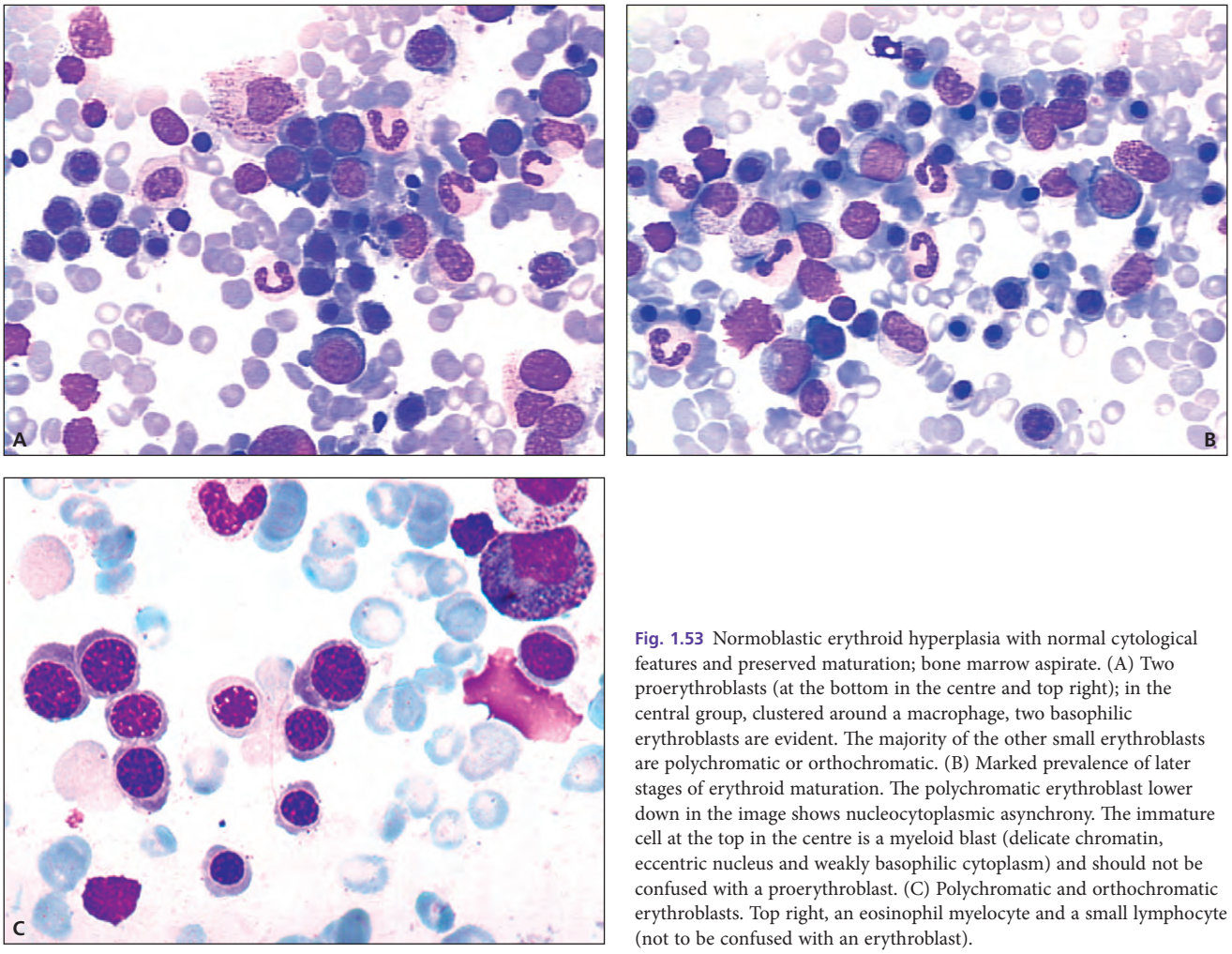



**Fig. 1.53** Normoblastic erythroid hyperplasia with normal cytological features and preserved maturation; bone marrow aspirate. (A) Two proerythroblasts (at the bottom in the centre and top right); in the central group, clustered around a macrophage, two basophilic erythroblasts are evident. The majority of the other small erythroblasts are polychromatic or orthochromatic. (B) Marked prevalence of later stages of erythroid maturation. The polychromatic erythroblast lower down in the image shows nucleocytoplasmic asynchrony. The immature cell at the top in the centre is a myeloid blast (delicate chromatin, eccentric nucleus and weakly basophilic cytoplasm) and should not be confused with a proerythroblast. (C) Polychromatic and orthochromatic erythroblasts. Top right, an eosinophil myelocyte and a small lymphocyte (not to be confused with an erythroblast).

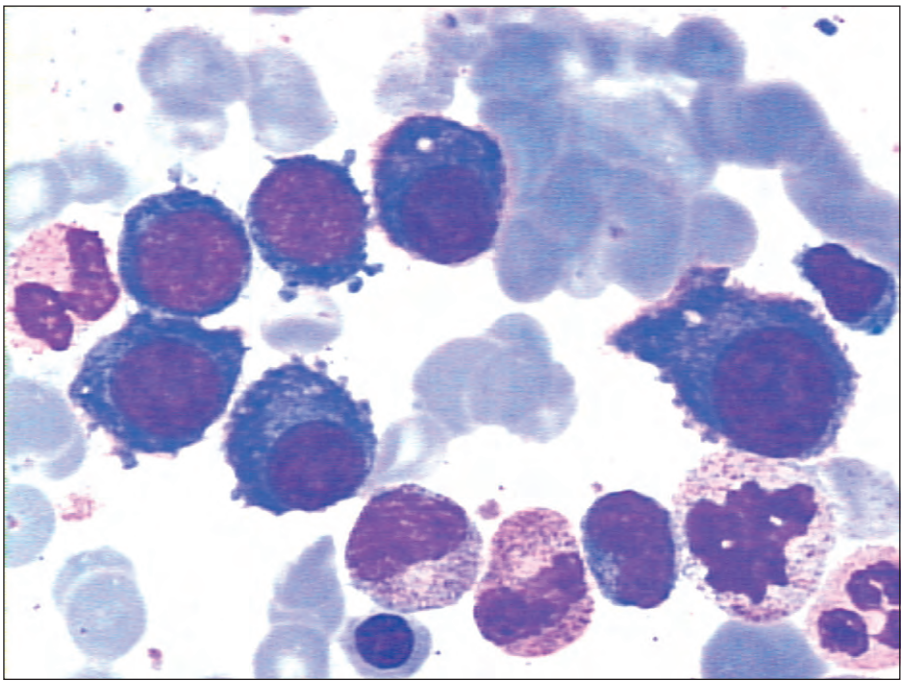


**Fig. 1.54** Cytological criteria for distinguishing different types of cell with intensely basophilic cytoplasm. The basophilic erythroblasts (there are two, top left) have a fairly high N:C ratio with a nucleus that tends to be central and numerous blocks of chromatin occupying the whole nucleus; the Golgi zone is barely visible. The plasma cells (there are four with different levels of maturation) have eccentric nuclei, more plentiful cytoplasm and a lower N:C ratio; the chromatin forms larger and less numerous blocks, which tend to be arranged around the inner surface of the nuclear membrane. This feature is clearly seen in the mature plasma cell a little below and to the left of centre, adjacent to a neutrophil myelocyte; this cell has a large, pale, arch-shaped, paranuclear Golgi zone. These features are similarly shown by the plasma cell higher up in the centre. The two plasma cells half way up and to the left and right respectively show an intermediate level of maturation and are less easily distinguishable from the erythroblasts. At the bottom, partly cut off at the edge of the image, is a small orthochromatic erythroblast.

**Table 1.9** Morphological characteristics associated with granulocytic differentiation (four to five cellular divisions)

- Condensation of nuclear chromatin
- Reduction of the space occupied by the nucleus
- Indentation and segmentation of the nucleus
- Loss of cytoplasmic basophilia
- Hypertrophic Golgi zone in the intermediate stages (promyelocyte, myelocyte)
- Appearance of azurophilic granules
- Modification of staining characteristics of the granules
- Appearance of specific granules
- Similar size in all stages (12–20μm), sometimes slightly larger at the promyelocyte stage
- Gradual passage from one stage to another, continuous without sudden changes, there being cells of varying stages of maturity within the same stage

or there may be a small number of peroxidase-positive azurophilic granules, within basophilic cytoplasm in which no clear area corresponding to the Golgi apparatus has yet developed: this final criterion is the only one that differentiates the blast cell from the promyelocyte.

Promyelocytes are slightly larger than myeloblasts, and represent the next intermediate stage of differentiation. They have a lower N:C ratio because of the increased cytoplasmic volume. The nucleus is oval and often shows a definite concavity toward the centre of the cell; the nucleus is eccentric as if displaced by a large pale Golgi zone, which at this stage of maturation reaches its maximum development. The chromatin starts to condense in small areas and nucleoli are reduced in size and number, although remaining clearly evident. Cytoplasmic basophilia appears to be partly replaced by weak acidophilia, but basophilia usually persists at the periphery of the cell, which still lacks granules. Azurophilic granules, which are an intense light red, are clearly visible: these often stop at the edge of the Golgi zone, lining up at its periphery without appearing to penetrate it (the image of a rising sun). In the more mature promyelocytes, the cytoplasm is almost completely acidophilic, because of the disappearance of ribosomes and the production of invisible neutrophil-specific granules; a proportion of the primary granules now have their definitive brick-red colour.

The next stage of maturation is that of the neutrophil myelocyte. These are smaller than the promyelocytes, and are round or oval. The nucleus is smaller, eccentric and round, oval or reniform; the chromatin is heterogeneous, condensed into large masses separated by paler areas; the nucleoli have disappeared. Mitotic figures are frequent. There is ample cytoplasm, now

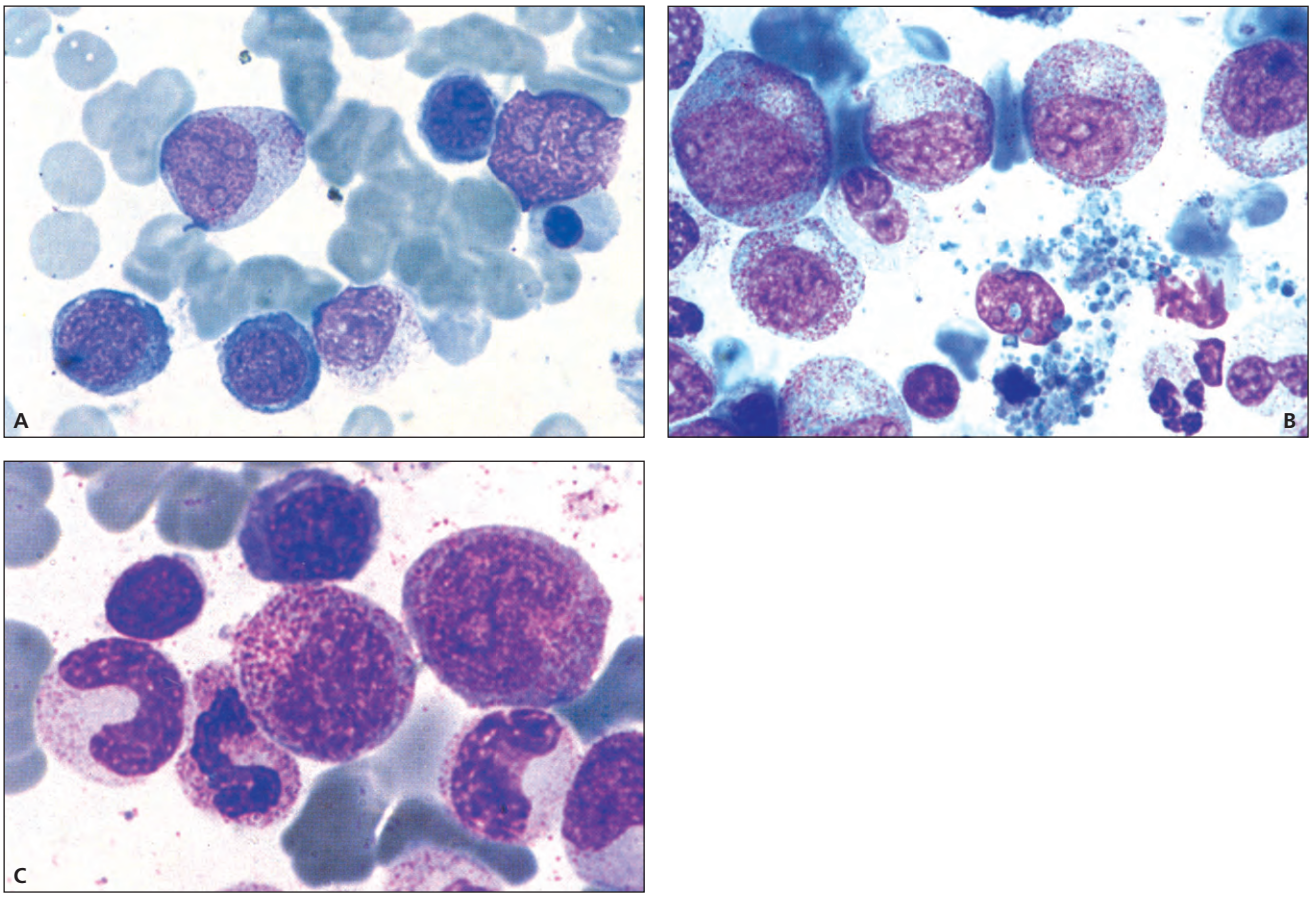



**Fig. 1.55** Granulocytic series; bone marrow aspirates from subjects with normal haemopoiesis. (A) Top left, a myeloblast: the chromatin is reticular with two stamped out nucleoli; the cytoplasm is weakly basophilic with a small pale Golgi zone and fine azurophilic granules. Bottom left, two basophilic erythroblasts; top right, a polychromatic erythroblast, a damaged nucleus and an orthochromatic erythroblast with an almost pyknotic nucleus. (B) Top left, a large promyelocyte, characterised by an irregularly oval nucleus with early chromatin condensation and two nucleoli; the cytoplasm is basophilic, except in the Golgi zone, around which are crowded numerous newly formed azurophilic granules. Continuing at the top, towards the right, there is a neutrophil myelocyte (the nucleoli have disappeared and there is more chromatin condensation, enlargement of the colourless Golgi zone and the persistence of weak peripheral basophilia), another slightly more mature promyelocyte and another myelocyte; a third neutrophil myelocyte is found on the left, immediately beneath the large promyelocyte. In the bottom right corner, a band neutrophil. Towards the centre of the image, an oval macrophage nucleus surrounded by plentiful pale cytoplasm with indefinite margins, full of large blue-grey granules and containing, at the bottom, the remains of a phagocytosed cell. To the left of this cell, a small lymphocyte. (C) Top right, a promyelocyte, to the left of which there is a neutrophil myelocyte, a neutrophil metamyelocyte with a horse-shoe shaped nucleus and, crushed between these two cells, a band neutrophil; beneath the promyelocyte is another neutrophil metamyelocyte. In the centre at the top, an early polychromatic erythroblast; to its left, a lymphocyte.

almost completely acidophilic, with a small or invisible Golgi zone; sometimes there is a persisting basophilic tinge at the periphery. Myelocytes contain numerous darker red-brown granules similar to those of mature neutrophils. A clear paranuclear area is still present but is much less prominent than in a promyelocyte.

Neutrophil metamyelocytes are distinguished from the myelocytes that precede them by the shape of the nucleus, which is now distinctly curved, almost into a horse-shoe shape; this concavity, which often appears almost concentric with the cellar membrane, corresponds to the Golgi zone, which is, however, usually barely visible. Chromatin is condensed into coarse clumps, but does not yet have the prepyknotic homogeneity of the mature neutrophil. The cytoplasm is acidophilic, pale pink, with the typical granules of mature neutrophils.

Slight dysgranulopoiesis, mainly cytoplasmic hypogranularity, has been described in the bone marrow aspirates of healthy subjects by some authors, but not by others.[49–51]

Eosinophil promyelocytes, rarely observed except in patients with hypereosinophilia, have characteristics not dissimilar to those of neutrophil promyelocytes, from which they are distinguished mainly by the presence, together with azurophilic granules, of a small number of large proeosinophilic and eosinophilic granules, the colour of which ranges from dark violet to the

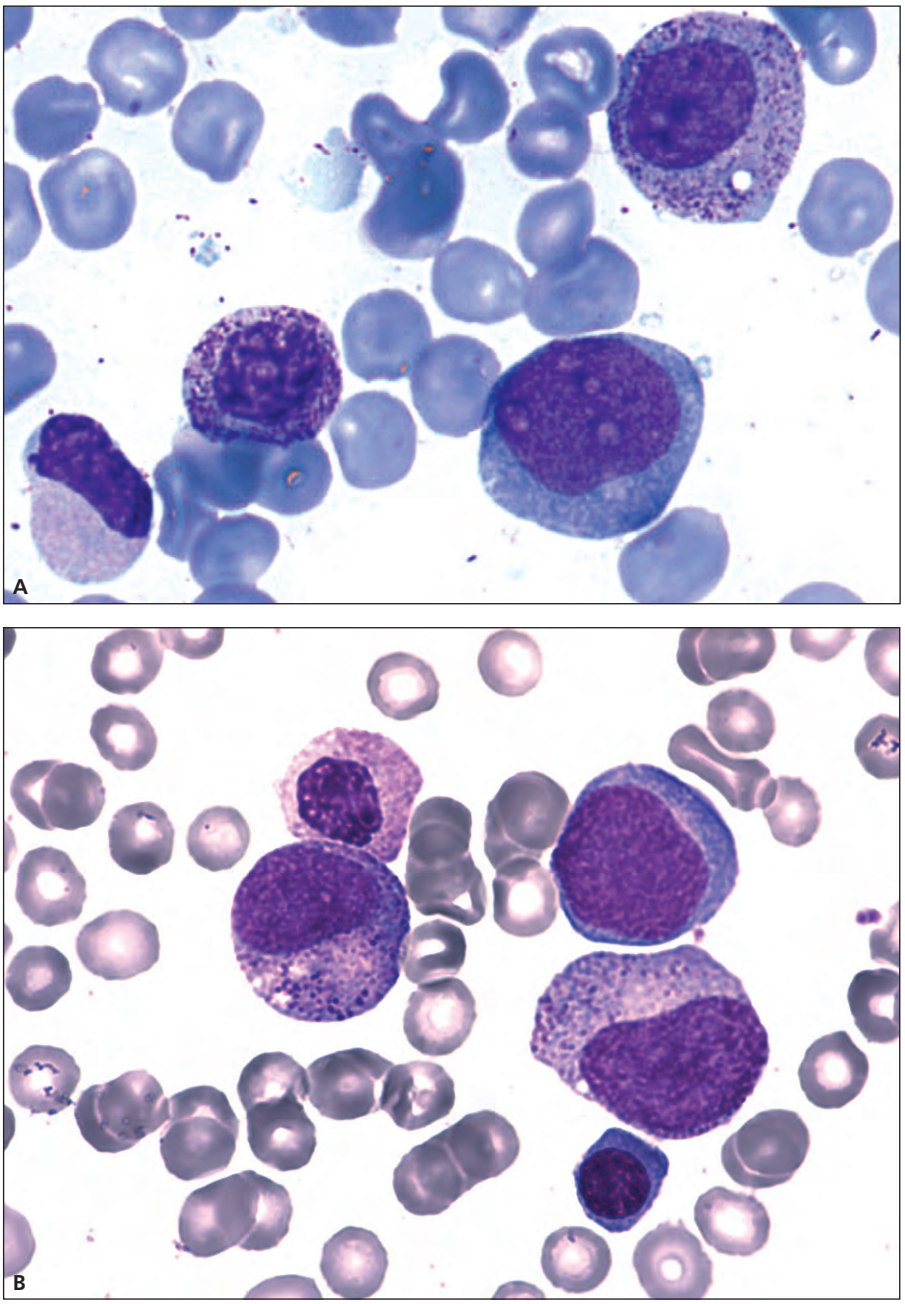



**Fig. 1.56** Neutrophil granulocyte precursors in bone marrow aspirates: the presence of intermediate forms between one stage and another is common, related to the fact that maturation is a continuous process and does not occur in jumps. (A) Bottom right, a myeloblast, with a large nucleus with delicate chromatin, four nucleoli and a polygonal outline with long straight sections; the cytoplasm shows a small Golgi zone and is cerulean with numerous fine pale circles. The cell at top right could be regarded as a late promyelocyte, particularly if the basophilia of the cytoplasmic background is noted or, alternatively, as an early neutrophil myelocyte, if one takes account of the homogeneously distributed dark granules and the fact that a pale paranuclear Golgi zone is not visible. Similarly, the granule-rich cell on the left can be regarded as a late neutrophil myelocyte; some might classify it as a metamyelocyte because of its smallness, but the fact that the nucleus is not curved is against this. In the bottom left corner, a late metamyelocyte, in which the granules have become very fine and are not individually visible. (B) From left to right and clockwise, a late neutrophil promyelocyte; a metamyelocyte; an agranular myeloblast (the intensely basophilic cytoplasm of which resembles that of an erythroid cell which, however, is unlikely because of the angular outline and the eccentricity of the nucleus), another promyelocyte that is more spread out and thus easier to recognise (eccentric nucleus with granular cytoplasm, a Golgi zone resembling a rising sun, azure cytoplasmic background with granules that are sometimes fine and pale and sometimes larger and darker); a polychromatic erythroblast.

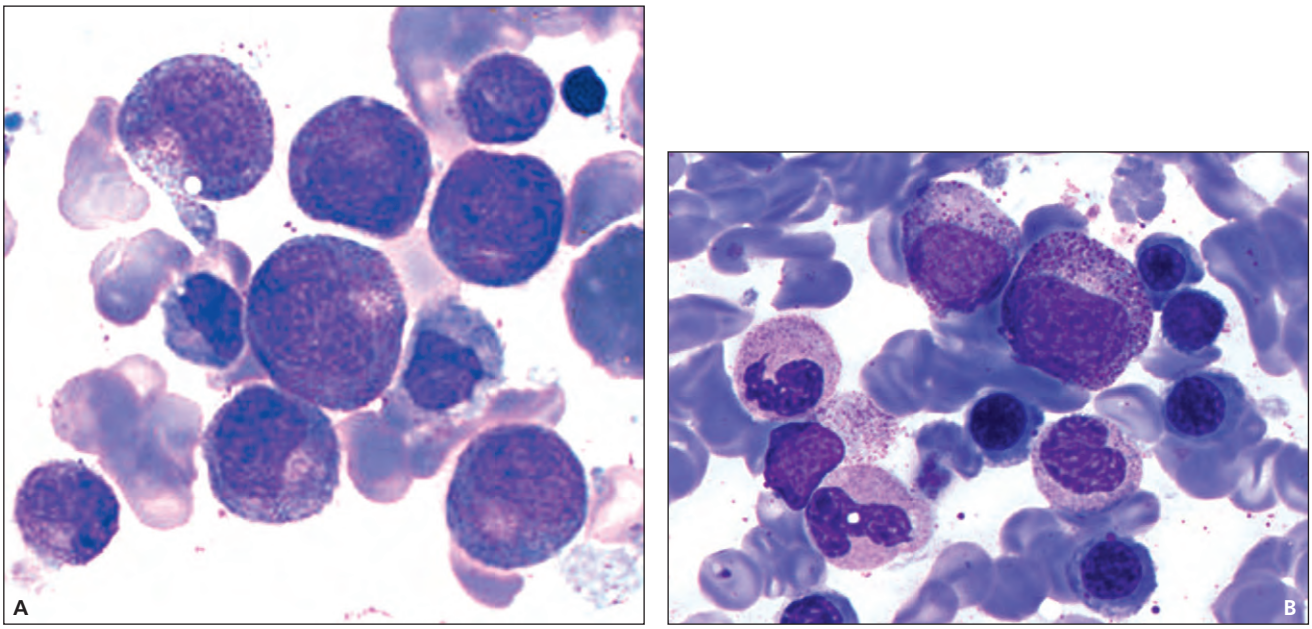



**Fig. 1.57** Features of maturing neutrophil granulocytes in bone marrow aspirates of subjects with normal haemopoiesis. (A) Mainly promyelocytes, rich in granules and with a particularly prominent Golgi zone, which stands out from the background cytoplasmic basophilia. (B) The two granulocyte precursors at the top could be classified as neutrophil myelocytes, particularly because of the condensation of the chromatin. There are also two band neutrophils, a small metamyelocyte, various polychromatic erythroblasts and, on the right, a lymphocyte (distinguishable because of the absence of small pale spaces in the nucleus and because of the colour and the scantiness of the cytoplasm).

characteristic orange. In eosinophil myelocytes and metamyelocytes (Figs 1.63, 1.64 and 1.65) the morphology of the nucleus is identical to that of cells of the corresponding stage of maturation in the neutrophil lineage. The cytoplasm is now packed with eosinophil granules admixed, particularly in some of the myelocytes, with the immature large violaceous proeosinophilic granules.

Promyelocytes with true basophil granules are almost never observed outside the context of acute or chronic leukaemia with basophil differentiation (Fig. 1.65). Basophil myelocytes and metamyelocytes are also rare in normal subjects (Fig. 1.66), but they are often seen in chronic myeloid leukaemia and in acute myeloid leukaemia with basophil differentiation. Because the nucleus may be obscured by the granules, it is not always easy to distinguish them from mature basophils.

### The monocyte/macrophage series

Cells of the monocyte/macrophage lineage are derived from haemopoietic stem cells oriented towards granulocyte/monocyte differentiation. The migration of monocytes into tissues leads to their transformation into macrophages, a process mediated by numerous biologically active substances, mainly as a response to local factors and conditions.

The monocytes that can be seen in a bone marrow aspirate are generally similar to those in the circulation, but they appear smaller: the nucleus has more compact chromatin and the cytoplasm is less voluminous and more basophilic with fewer vacuoles. Monocyte precursors, monoblasts and promonocytes, are described in relation to myelodysplasia and chronic myelomonocytic leukaemia in Chapters 3 and 7.

Macrophages (Figs 1.67 through to 1.74) represent the final stage of monocyte differentiation and are tissue cells. They are present in normal bone marrow and are present in increased numbers when there is increased haemopoiesis, particularly if it is ineffective. Bone marrow macrophages are larger than monocytes (diameter 25–50 μm) and have a much lower N:C ratio. The nucleus is more regular, oval or bean shaped, in a clearly eccentric position; it appears more immature because of the delicate, dispersed, reticular chromatin, formed of a loose network of intertwined filaments; this network is denser beneath the nuclear membrane, which is therefore well outlined; often one or two pale basophilic nucleoli are apparent. There is very plentiful cytoplasm, which may be pale grey or almost colourless. It is non-homogeneous with a pale central area of the Golgi zone, fine azurophilic granules and areas of basophilia and acidophilia. The cell outline is often ill-defined and there may be signs of pinocytotic activity or phagocytosis, in the form of vacuoles of various sizes – more numerous close to the cell membrane – and the residues of phagocytosed cells and granules of haemosiderin. Sometimes macrophages are surrounded by a corona of erythroblasts at various stages of maturation

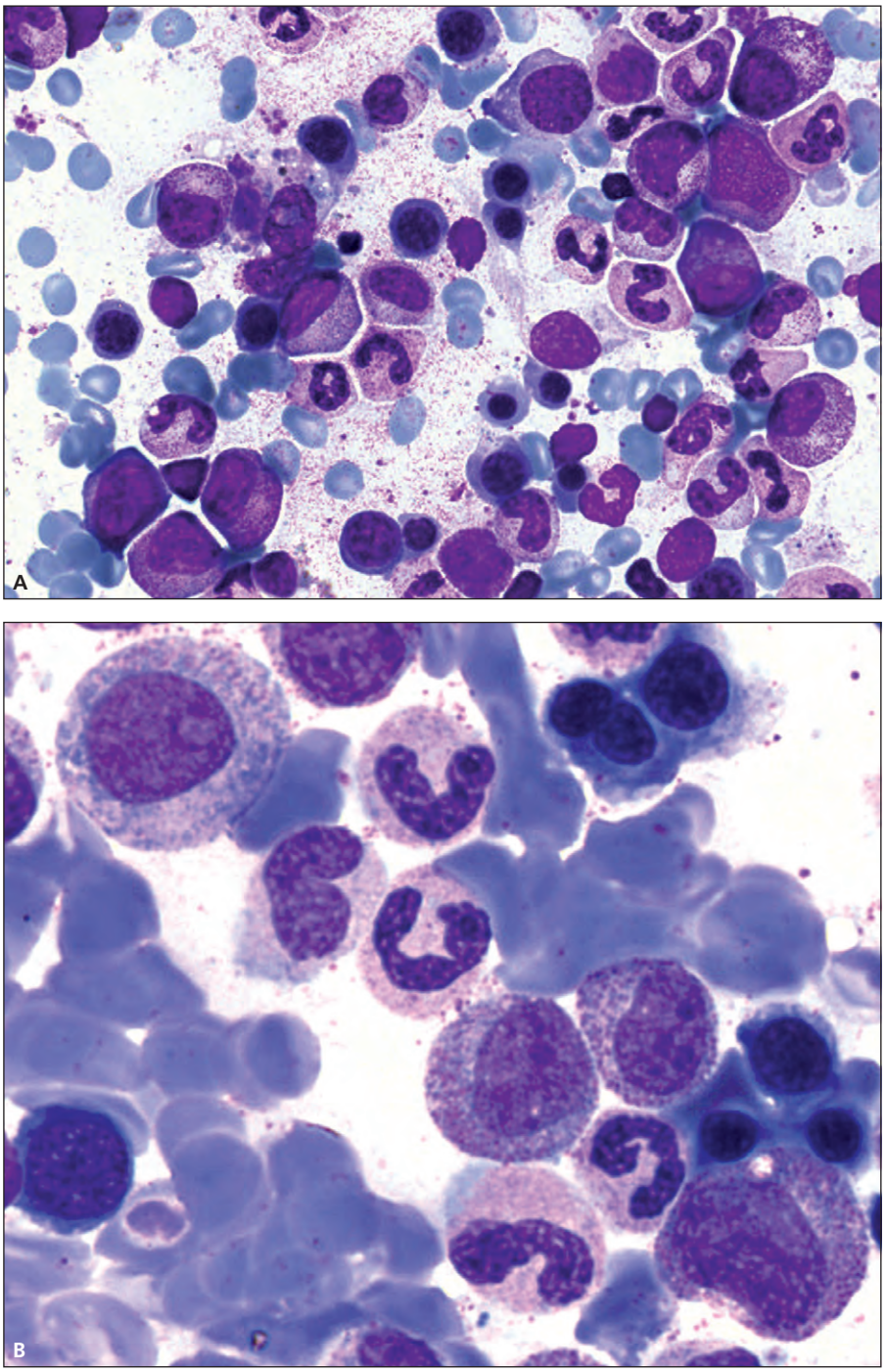



**Fig. 1.58** Features of maturing neutrophil granulocytes in bone marrow aspirates of subjects with normal haemopoiesis. (A) In this cellular field there are granulocyte precursors at all stages of maturation, in addition to numerous basophilic, polychromatic and orthochromatic erythroblasts. At the bottom, on the left, for example, there is a myeloblast and two promyelocytes; other promyelocytes, myelocytes and metamyelocytes are distributed through the rest of the image. The mature neutrophils, quite frequent on the right of the image, are mainly band forms, as is more often observed in the marrow. (B) Top left, a promyelocyte; descending along the diagonal that extends to the opposite bottom right corner, a metamyelocyte, a neutrophil band form, two myelocytes, another two band neutrophils, beside three small erythroblasts and a fairly large neutrophil myelocyte.

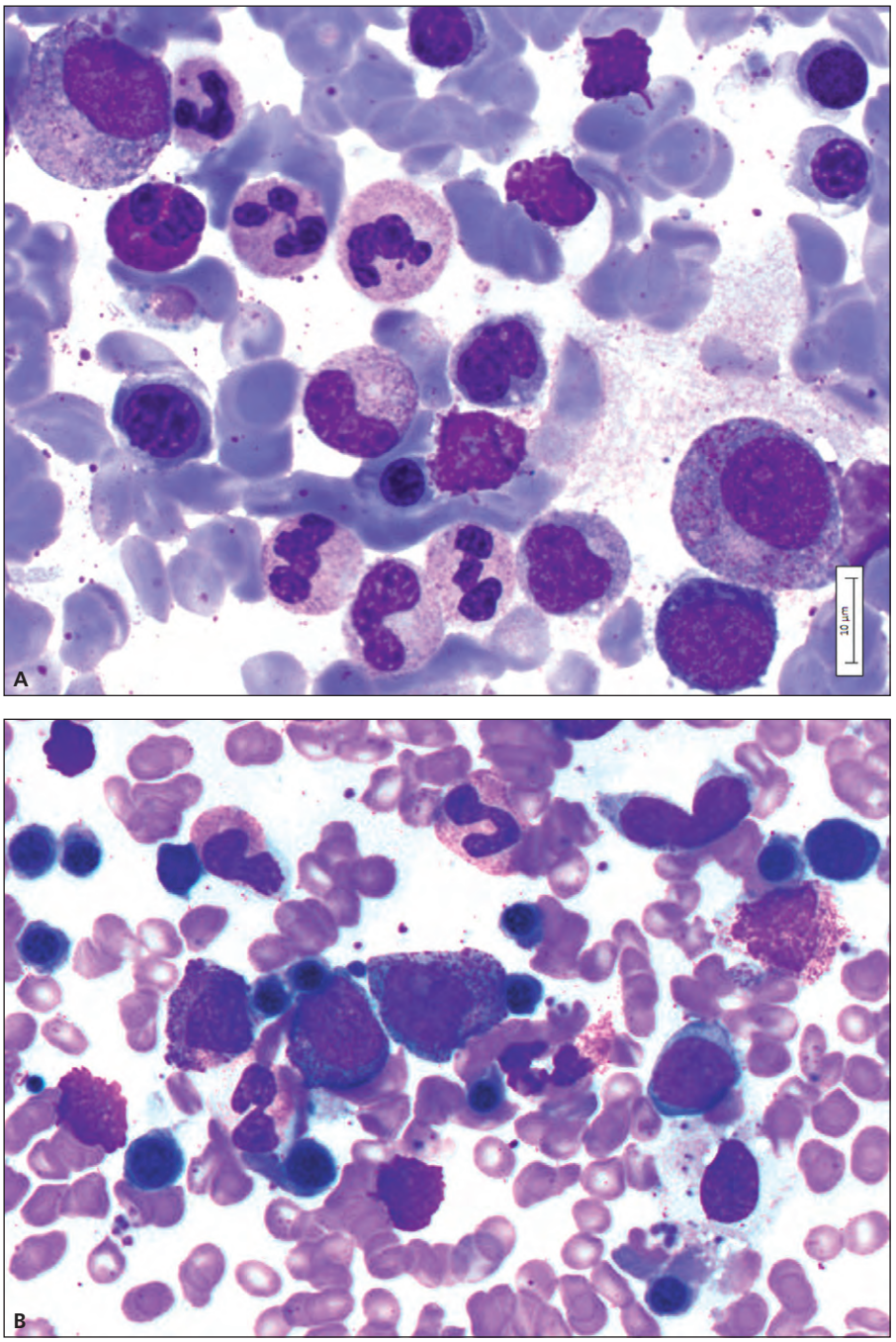



**Fig. 1.59** Features of maturing neutrophil granulocytes in bone marrow aspirates of subjects with normal haemopoiesis. (A) Top left, a promyelocyte, a mature eosinophil and two segmented neutrophils in which a drumstick appendage is clearly seen. In the centre of the image, a rather compact monocyte and, on the left, a metamyelocyte with a bean-shaped nucleus. Bottom right, another morphologically very typical promyelocyte and a small proerythroblast, recognisable even though the nucleus is eccentric because of the roundness of the nucleus, the loose chromatin network and, naturally, the intense cytoplasmic basophilia. The diameter of cells in this image can be evaluated by comparison with the 10-µm indicator in the bottom right corner. (B) On the left, various polychromatic and orthochromatic erythroblasts, two slightly dysmorphic promyelocytes (disorderly but numerous granules), an agranular myeloblast and a macrophage with indistinct margins. This picture, which could suggest a shift to the left of the maturation curve, is from a patient with Gram-negative sepsis and moderate neutropenia.

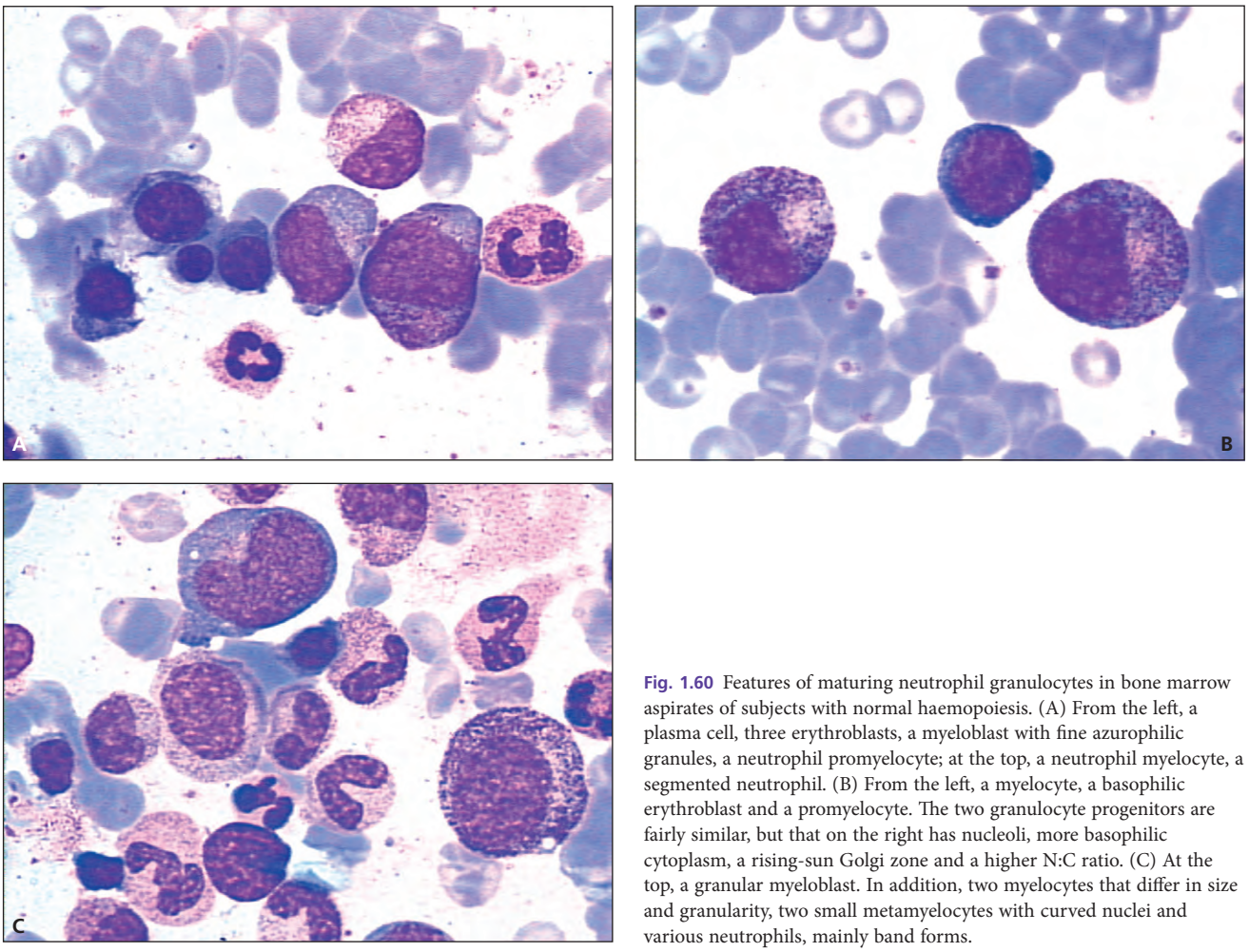



**Fig. 1.60** Features of maturing neutrophil granulocytes in bone marrow aspirates of subjects with normal haemopoiesis. (A) From the left, a plasma cell, three erythroblasts, a myeloblast with fine azurophilic granules, a neutrophil promyelocyte; at the top, a neutrophil myelocyte, a segmented neutrophil. (B) From the left, a myelocyte, a basophilic erythroblast and a promyelocyte. The two granulocyte progenitors are fairly similar, but that on the right has nucleoli, more basophilic cytoplasm, a rising-sun Golgi zone and a higher N:C ratio. (C) At the top, a granular myeloblast. In addition, two myelocytes that differ in size and granularity, two small metamyelocytes with curved nuclei and various neutrophils, mainly band forms.

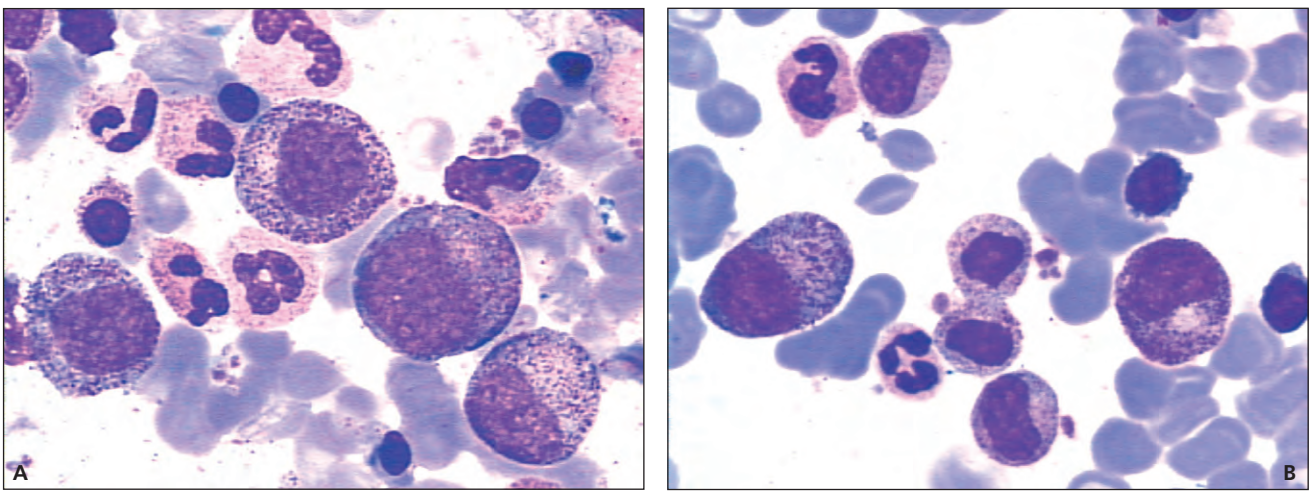



**Fig. 1.61** Features of maturing neutrophil granulocytes in bone marrow aspirates of subjects with normal haemopoiesis. (A) The less mature cells are a promyelocyte (the larger cell with very numerous fine azurophilic granules on a blue background) and three myelocytes. On the left, a small granulocyte with a compact spherical nucleus, similar to that of an erythroblast: it is not rare to see cells such as this in normal bone marrows, as a result of the physiological process of apoptosis; this should not be confused with a pseudo-Pelger cell, the hallmark of dysplasia. (B) On the left, a promyelocyte; in the centre, a mitotic figure in telophase in a cell of neutrophil lineage; lower down, a mature neutrophil and a metamyelocyte with a bean-shaped nucleus; on the right, an early neutrophil myelocyte and two lymphocytes.

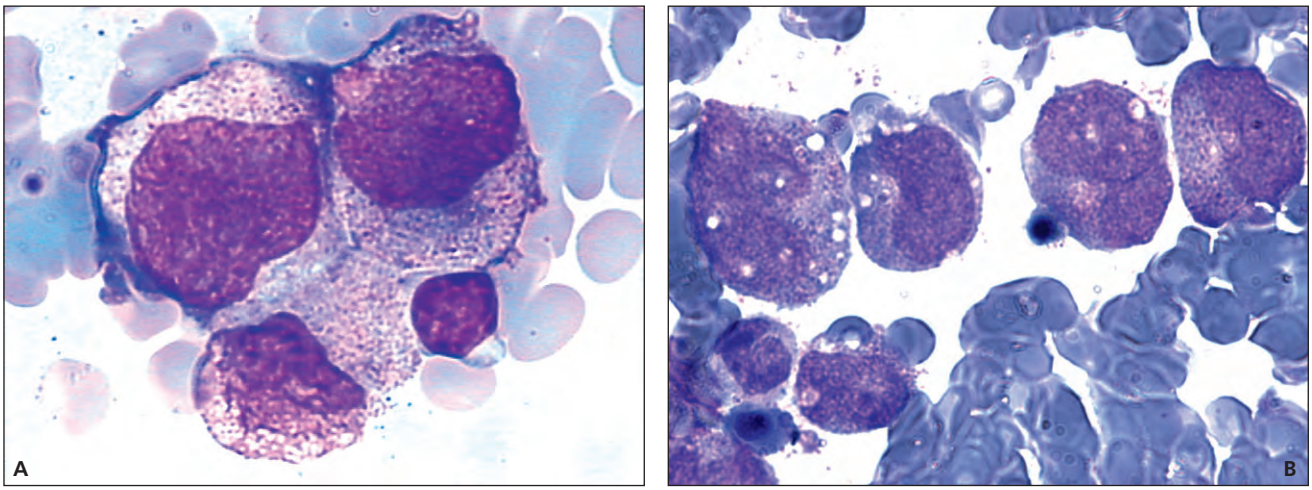



**Fig. 1.62** Gigantism and dysmorphism of granulocyte precursors in two patients being treated with G-CSF following autologous stem cell transplantation for non-Hodgkin lymphoma; bone marrow aspirates. (A) The two large cells at the top, although they are atypical, can be recognised as a myelocyte (no nucleoli, pale cytoplasm) and a promyelocyte (nucleoli apparent, granular basophilic cytoplasm). Bottom right, a lymphocyte. The same case as in Fig. 1.33C. (B) At the top, four large dysplastic promyelocytes with intense cytoplasmic basophilia and irregular nuclear outlines, with lobes and notches. In cases such as this the clinical history, in particular the fact that the patient is receiving a growth factor, is essential for avoiding an erroneous suspicion of a dysplastic or leukaemic condition.

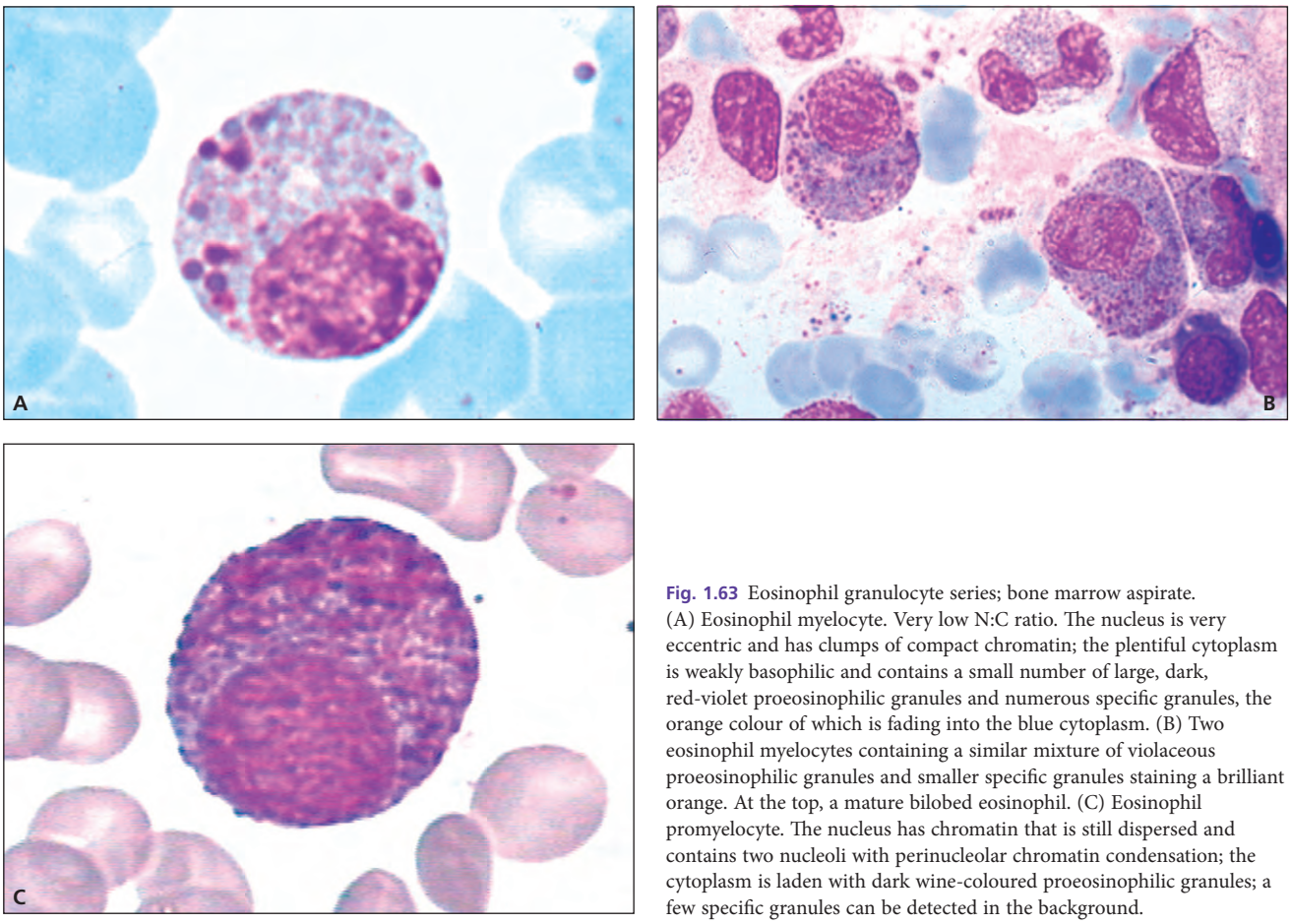
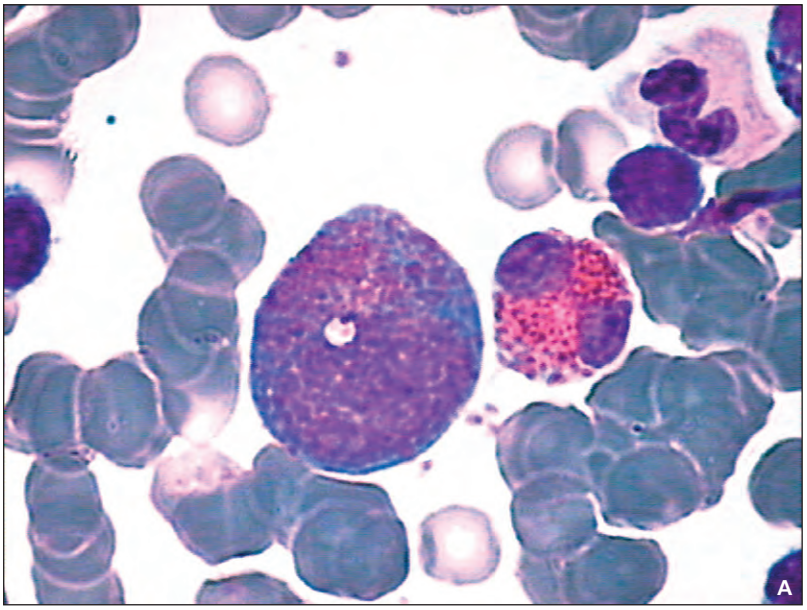
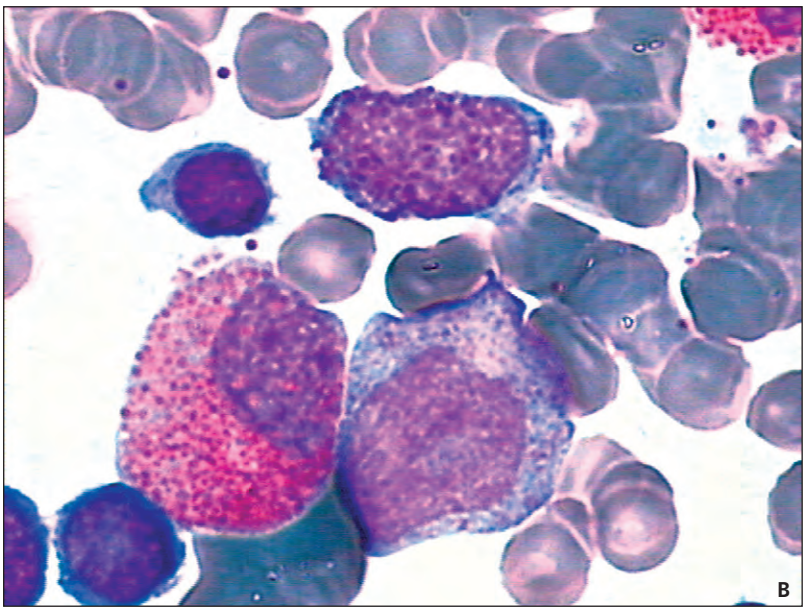
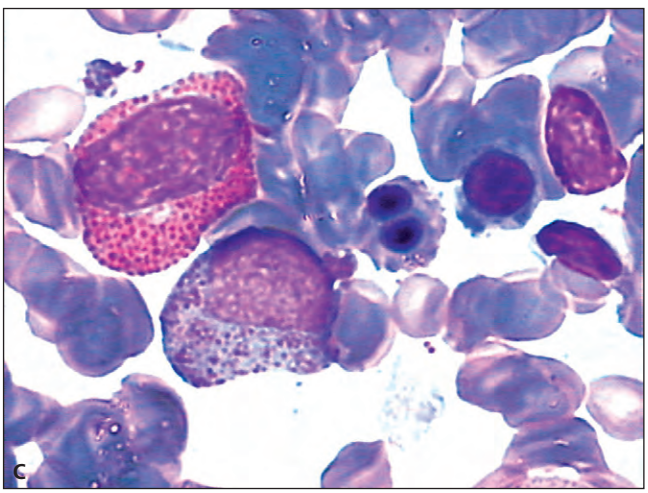



**Fig. 1.63** Eosinophil granulocyte series; bone marrow aspirate. (A) Eosinophil myelocyte. Very low N:C ratio. The nucleus is very eccentric and has clumps of compact chromatin; the plentiful cytoplasm is weakly basophilic and contains a small number of large, dark, red-violet proeosinophilic granules and numerous specific granules, the orange colour of which is fading into the blue cytoplasm. (B) Two eosinophil myelocytes containing a similar mixture of violaceous proeosinophilic granules and smaller specific granules staining a brilliant orange. At the top, a mature bilobed eosinophil. (C) Eosinophil promyelocyte. The nucleus has chromatin that is still dispersed and contains two nucleoli with perinucleolar chromatin condensation; the cytoplasm is laden with dark wine-coloured proeosinophilic granules; a few specific granules can be detected in the background.



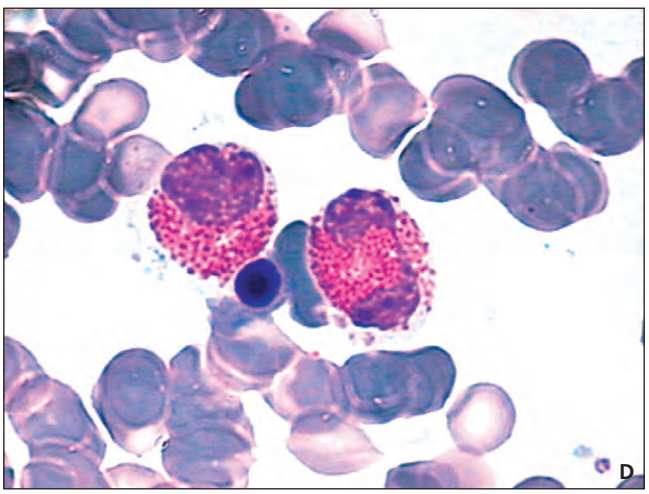
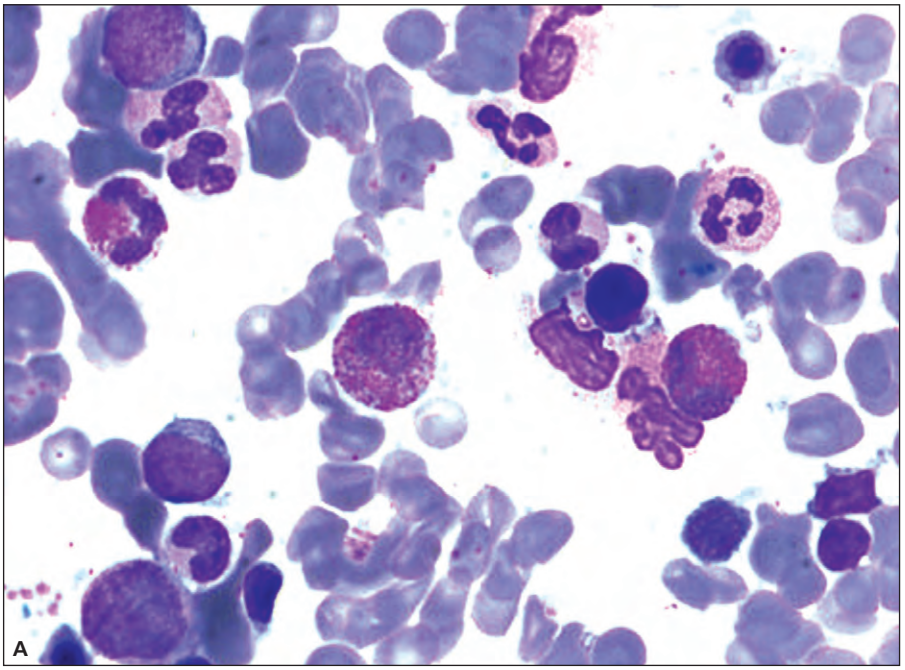




**Fig. 1.64** Eosinophil granulocyte series; bone marrow aspirate. (A) An eosinophil promyelocyte with intensely basophilic cytoplasm and numerous orange specific granules; a few proeosinophilic granules are visible on the right; there is a vacuole in the area of the Golgi zone. On the right, a mature eosinophil with a bilobed spectacle-shaped nucleus. (B) On the left, an eosinophil myelocyte. On the right, a myeloblast with an angulated nucleus, dispersed chromatin and scanty azurophilic granules within the basophilic cytoplasm. (C) From the left: an eosinophil myelocyte with mature cytoplasm; a dysmorphic promyelocyte, belonging to the neutrophil lineage, with large disorderly scattered granules; a small binucleated polychromatic erythroblast; a polychromatic erythroblast. (D) Two mature eosinophils: that on the left is a band form, the other is bilobed.



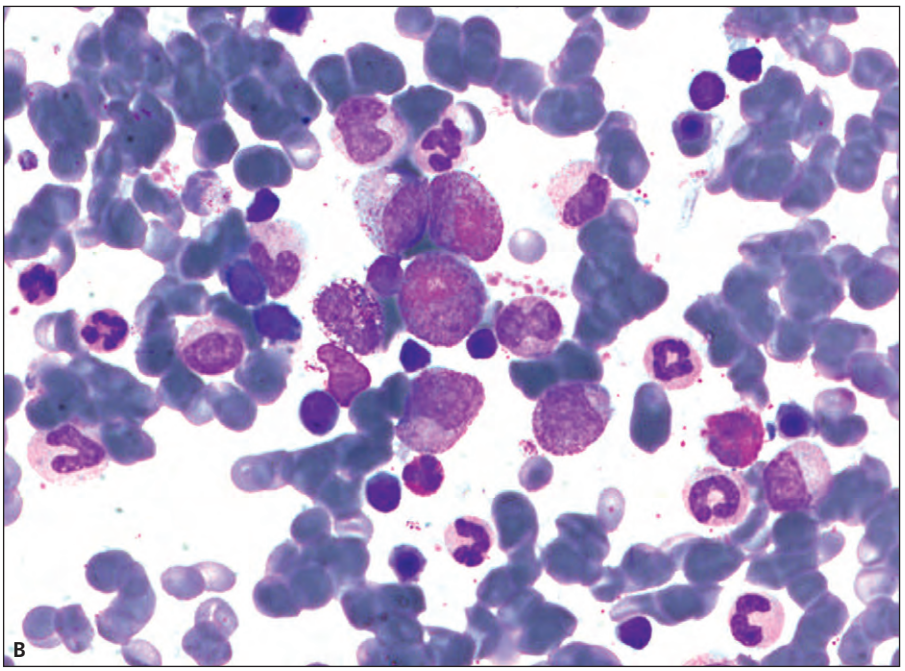
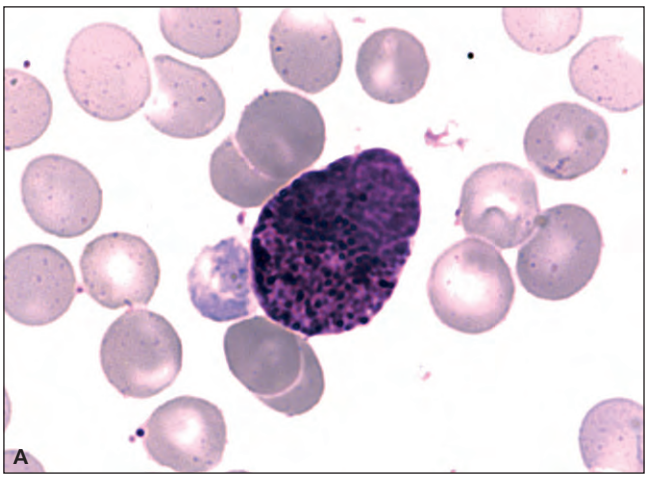




**Fig. 1.65** Bone marrow aspirate from a patient with eosinophilia of undetermined nature. (A) From the left, among other cells, an eosinophil with a band nucleus, an eosinophil myelocyte in the centre and an eosinophil metamyelocyte. (B) The marrow is cellular with a mixture of cell types, with erythroblasts, neutrophil granulocytes and eosinophils in various stages of maturation, a basophil and some lymphocytes.



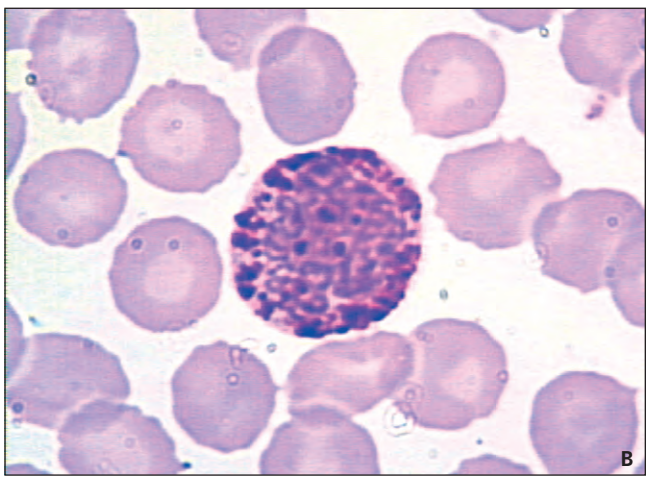




**Fig. 1.66** (A and B) Basophil precursors in a bone marrow aspirate. These cells, recognisable from the presence of a rounded non-lobed nucleus, are rarely seen in normal subjects, while they are seen quite often in myeloproliferative and myelodysplastic conditions.

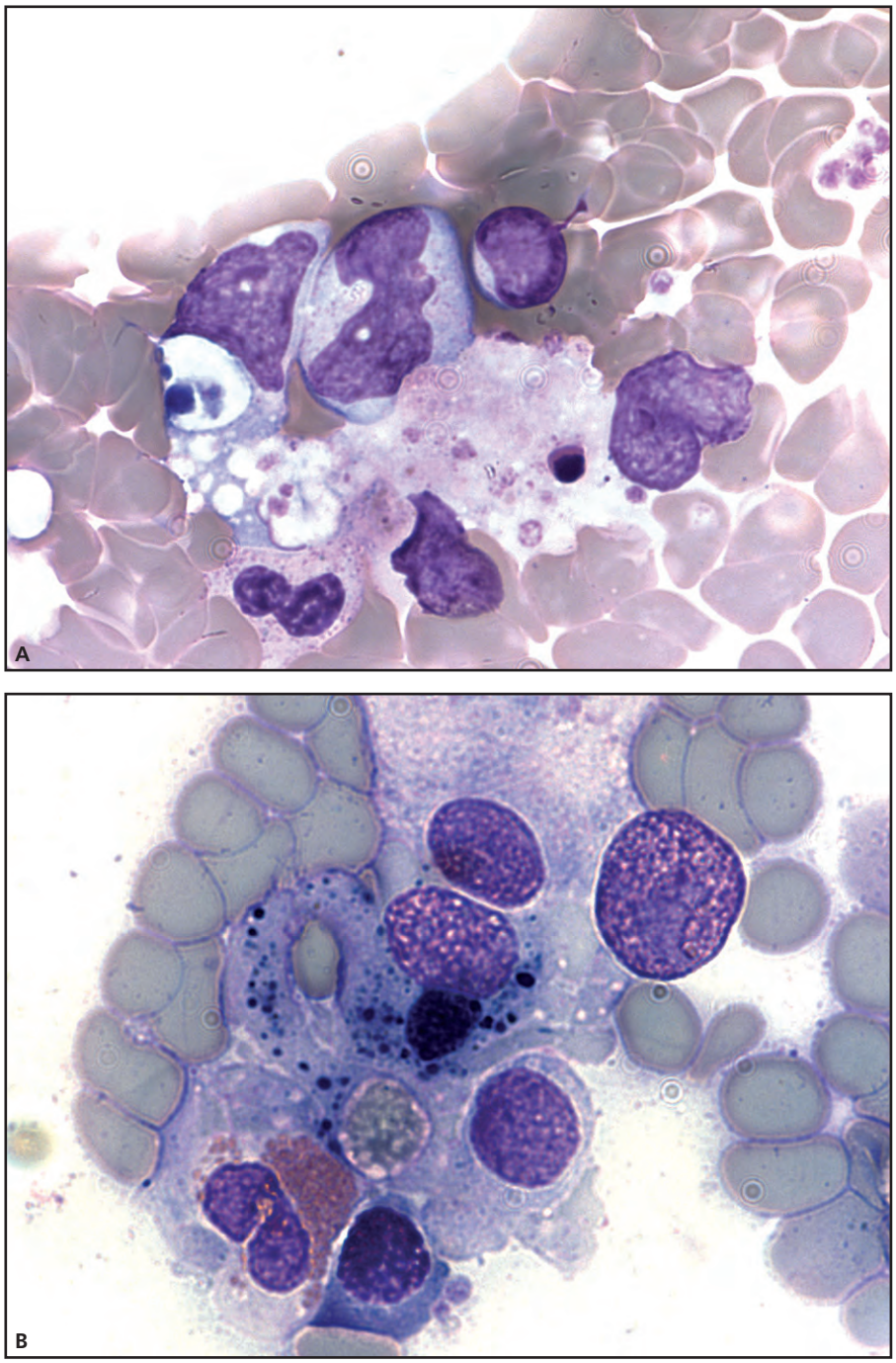



**Fig. 1.67** Monocytes and macrophages in bone marrow aspirates. (A) Transformation from a monocyte to a macrophage. The cell in the centre of this small group is a monocyte, with a contorted nuclear outline and weakly basophilic cytoplasm containing very fine azurophilic granules. To its left, a quite similar cell is altering its morphology, being in the course of transformation into a macrophage: the cytoplasm is still basophilic, but in the lower part there is a large vacuole with ingested necrobiotic cellular material; other vacuoles are present lower down, some of which contain platelets. On the right, adjacent to a neutrophil band form, plentiful granular pink cytoplasm containing other phagocytosed fragments, which belongs to one of the adjacent damaged nuclei: its appearance is quite typical of a mature macrophage. (B) The large spongy nucleus on the right, with an enormous irregular cyclamen-coloured nucleolus, belongs to a macrophage, the cytoplasm of which is merging at the top with that of another macrophage; its nucleus, to the left, is similar but smaller and more ovoid. Immediately below, in the centre of the image, there is another macrophage with a characteristic spongy nucleus and well-preserved cytoplasm, from which one can perceive a tentacle-like prolongation which is extending downwards and to the left, enclosing a red cell; within the macrophage cytoplasm there are numerous dark granules and a large blackish inclusion, which is likely to correspond to an ingested nucleus. Another red cell is seen within a large vacuole that is expanding the distal extension of the tentacle, which is in contact – or possibly fused – with the cytoplasm of another small macrophage. Lower down, an eosinophil and a deformed basophilic erythroblast.

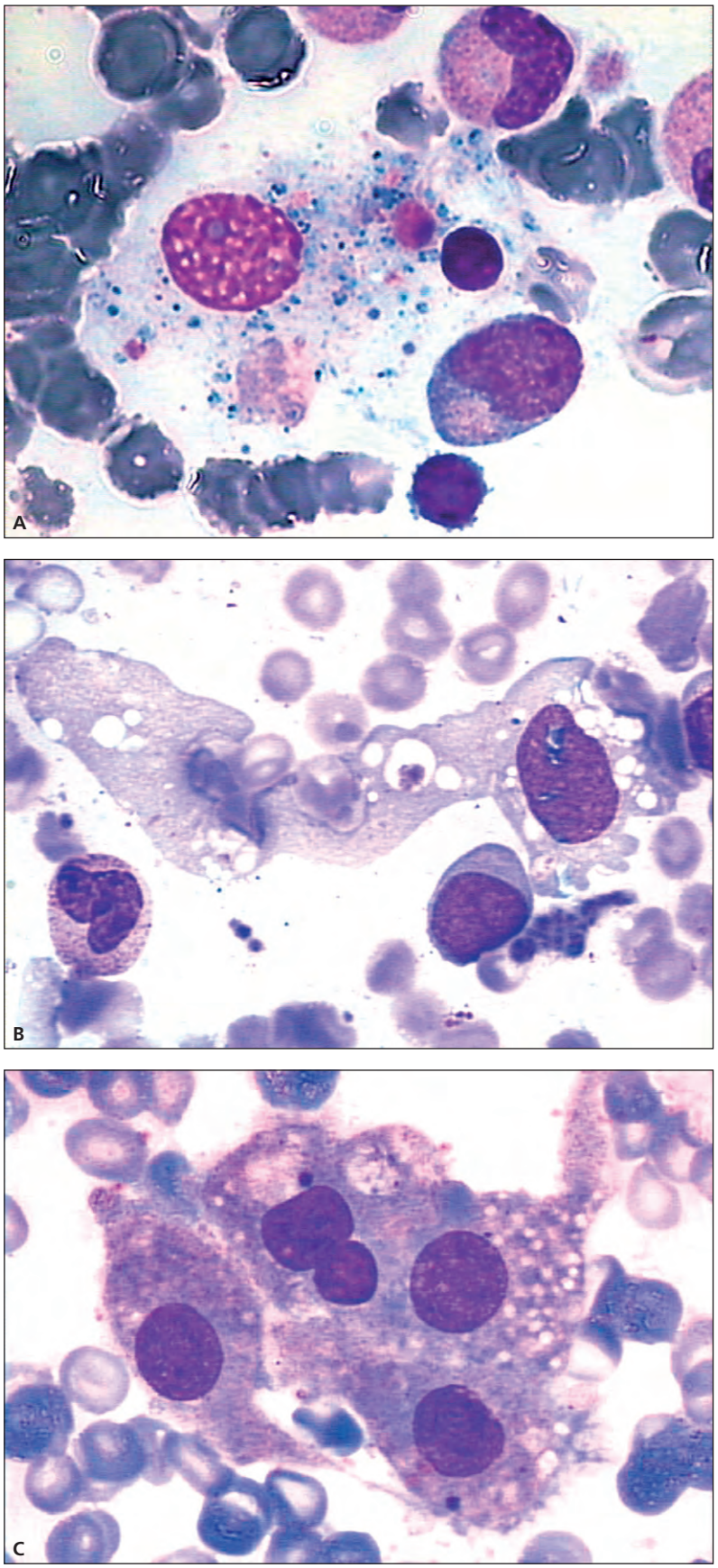



**Fig. 1.68** Various morphological features of macrophages in bone marrow aspirates. (A) This is a fairly common appearance: there is an oval nucleus with a cyclamen-coloured stamped out nucleolus, eccentrically placed in cytoplasm with ill-defined ragged margins, recognisable particularly because of the pale blue colour of the cytoplasm which differs from the background of the preparation, and the blue granular material that it contains; bottom right, phagocytosed necrobiotic material. At the top, a metamyelocyte; at the bottom, an early promyelocyte and a lymphocyte. (B) Bone marrow macrophage with a nucleus that has reticular chromatin made spongy by the presence of numerous pale circles. The cytoplasm is very plentiful, blue-grey, dusty appearing because of the presence of very numerous small granules, and heavily vacuolated with one of the vacuoles containing a platelet. The cell margins are very distinct, an uncommon observation in these cells; the upper surface is invaginated, almost enclosing two erythroblasts. Lower down, a metamyelocyte to the left and an agranular blast cell to the right. (C) A small group of macrophages which appear almost as if fused to form a syncytium. The cytoplasm has a pink tinge and is rich in small granules and vacuoles, with a phagocytosed erythrocyte at the top and a number of cytoplasmic fringes. The colour of the macrophage cytoplasm and the morphological characteristics of the granular material they contain are very variable, depending on their functional state and the degree of activation.

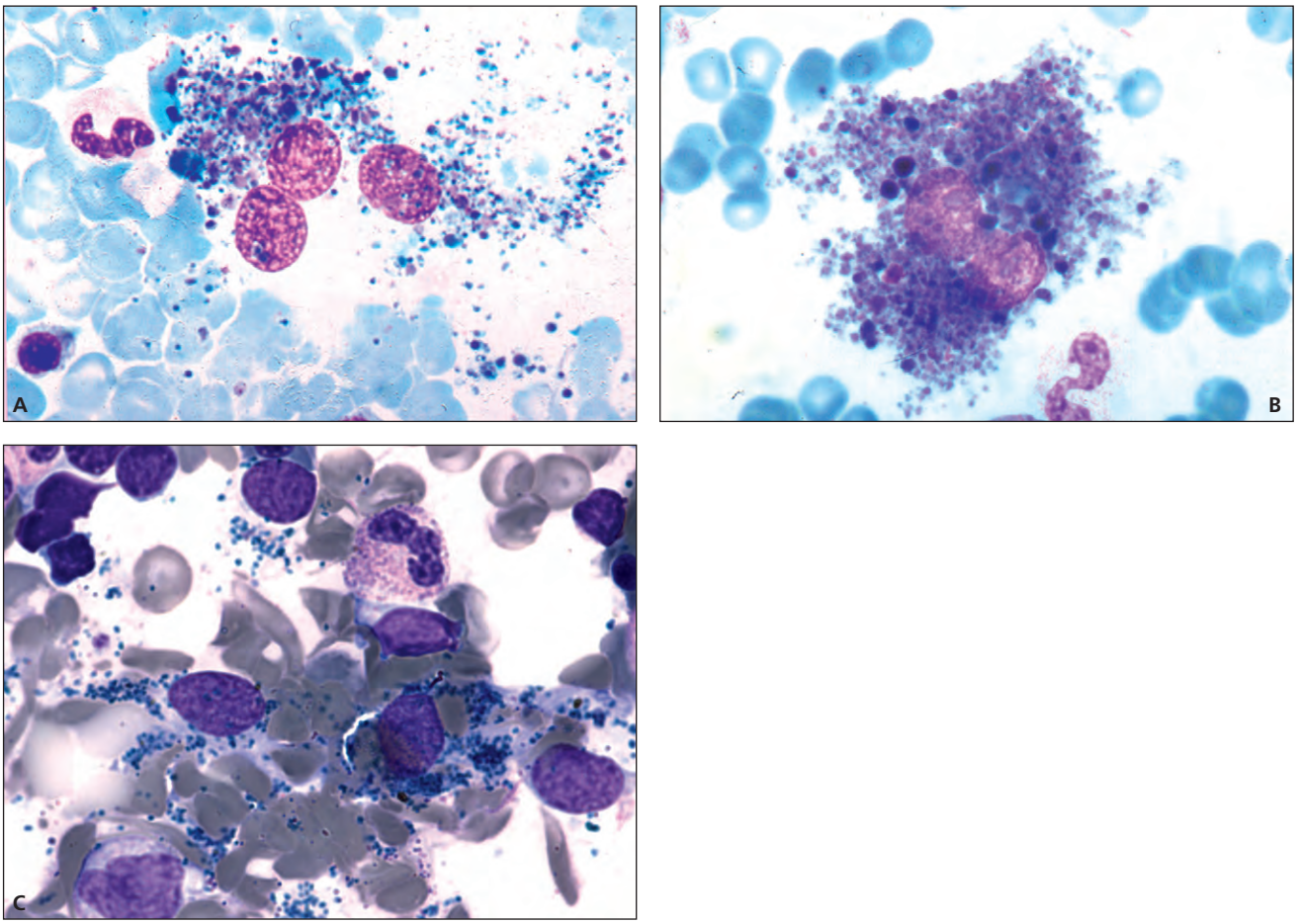



**Fig. 1.69** Bone marrow macrophages with prominent dark granules and showing erythrophagocytosis. (A) The frayed cytoplasm of these macrophages does not have a clear margin so that it is not clear whether there is a trinucleate cell or two or three distinct cells. The nuclear morphology is characteristic and there are numerous dark granules of various sizes admixed with cellular debris. (B) Large binucleate macrophage laden with dark granules and large blackish droplets, at least in part exogenous (the remains of phagocytosed cells). (C) The cytoplasm of the two macrophages, in which nuclei are recognisable in a central position, is indistinct and full of dark blue granules; it appears to be composed of thin elongated projections that are surrounding the majority of the adjacent erythrocytes, as single cells or, more often, in small groups.

(erythroblastic islands; see Chapter 2), to which they donate ferritin molecules by the process of rhopheocytosis (from the Greek term for 'suck out' or 'draw out'); they also phagocytose pyknotic nuclei after these have been expelled from erythroblasts. When macrophages are increased in number and contain abundant cellular debris, amorphous nuclei, platelets and erythrocytes, one refers to haemophagocytosis.

Osteoclasts are specialized macrophages that have the role of resorbing bone and maintaining the integrity of the intramedullary cavity that is necessary for the establishment of various types of haemopoietic niche. Mice that are genetically deprived of osteoclasts suffer from osteopetrosis and have complete obliteration of the marrow cavity. Osteoclasts can also function as negative regulators of the haemopoietic system.[52] They are visible in about 20% of bone marrow aspirates from healthy subjects,[50] being seen more often in children, in hyperthyroidism, when the marrow shows reactive changes, in plasma cell and other haemopoietic disorders and when there are bone marrow metastases. They appear as giant multinucleate cells (the diameter may exceed 100 μm) (Figs 1.75, 1.76 and 1.77); the cytoplasmic staining characteristics can vary from weakly basophilic to intensely acidophilic, with many reddish granules that vary in size and shape; often they contain coarse dark deposits of basophilic detritus. They have numerous separate nuclei, usually more than four, which are oval with spongy chromatin, a clearly defined nuclear membrane and one or two nucleoli. Typical, and useful for making a distinction from megakaryocytes, is the tendency of the nuclei to be central and overlapping, appearing almost transparent.

Langhans giant cells (Fig. 1.78) are formed from monocytes and macrophages by a process of cell fusion. There are multiple nuclei generally arranged at the periphery of a large Golgi zone.

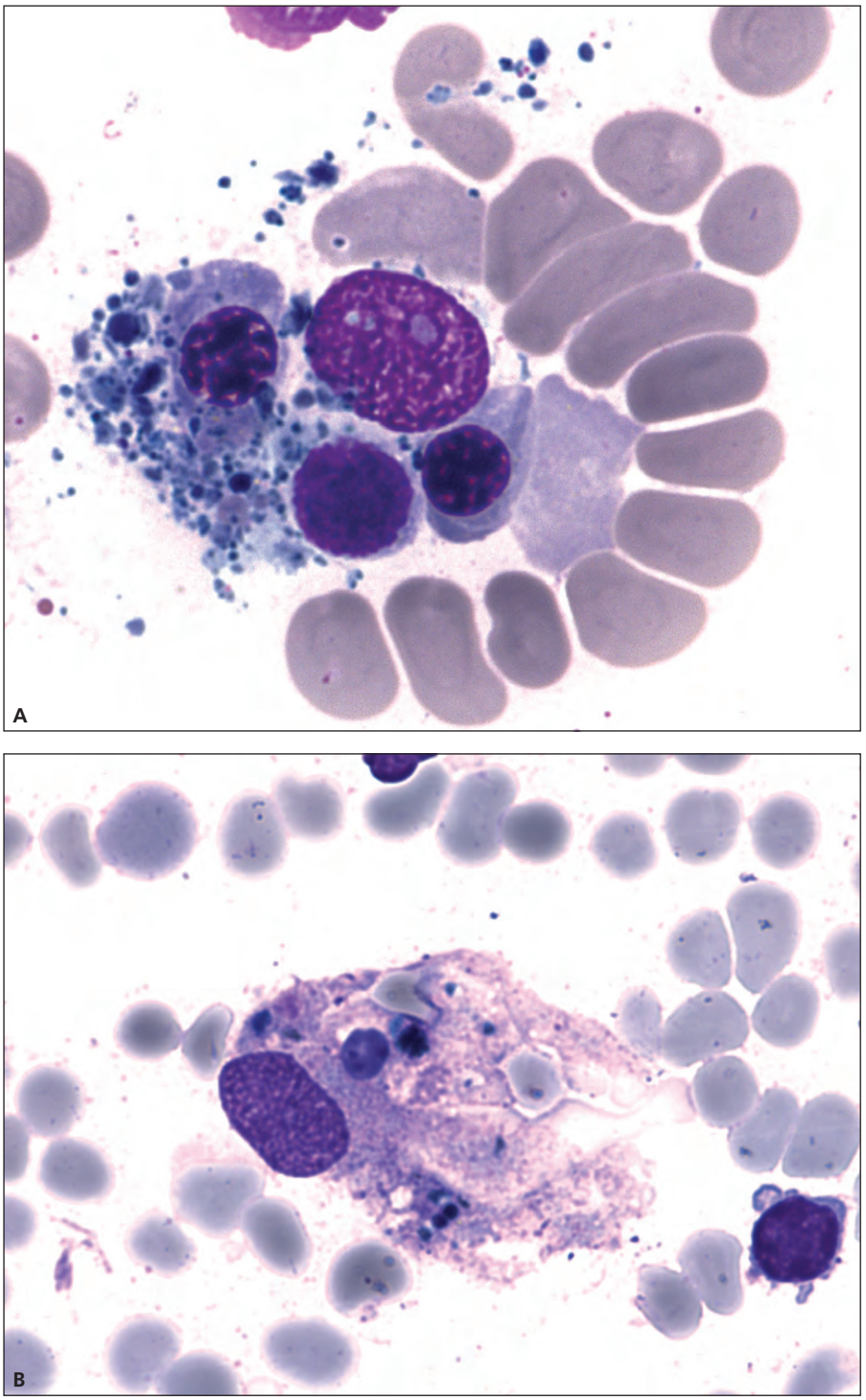



**Fig. 1.70** Bone marrow macrophages. (A) The nucleus of this macrophage is typically spongy, oval and eccentric. The cytoplasm, rich in dark granules, is expanding at the top into a long tentacle with indefinite margins, containing two well-preserved polychromatic erythroblasts and a pyknotic mass corresponding to the nucleus of a cell that is no longer recognisable. (B) The polar position of the nucleus and the plentiful ragged cytoplasm extending into a tail that contains red cells and nuclear debris makes this cell resemble a comet.

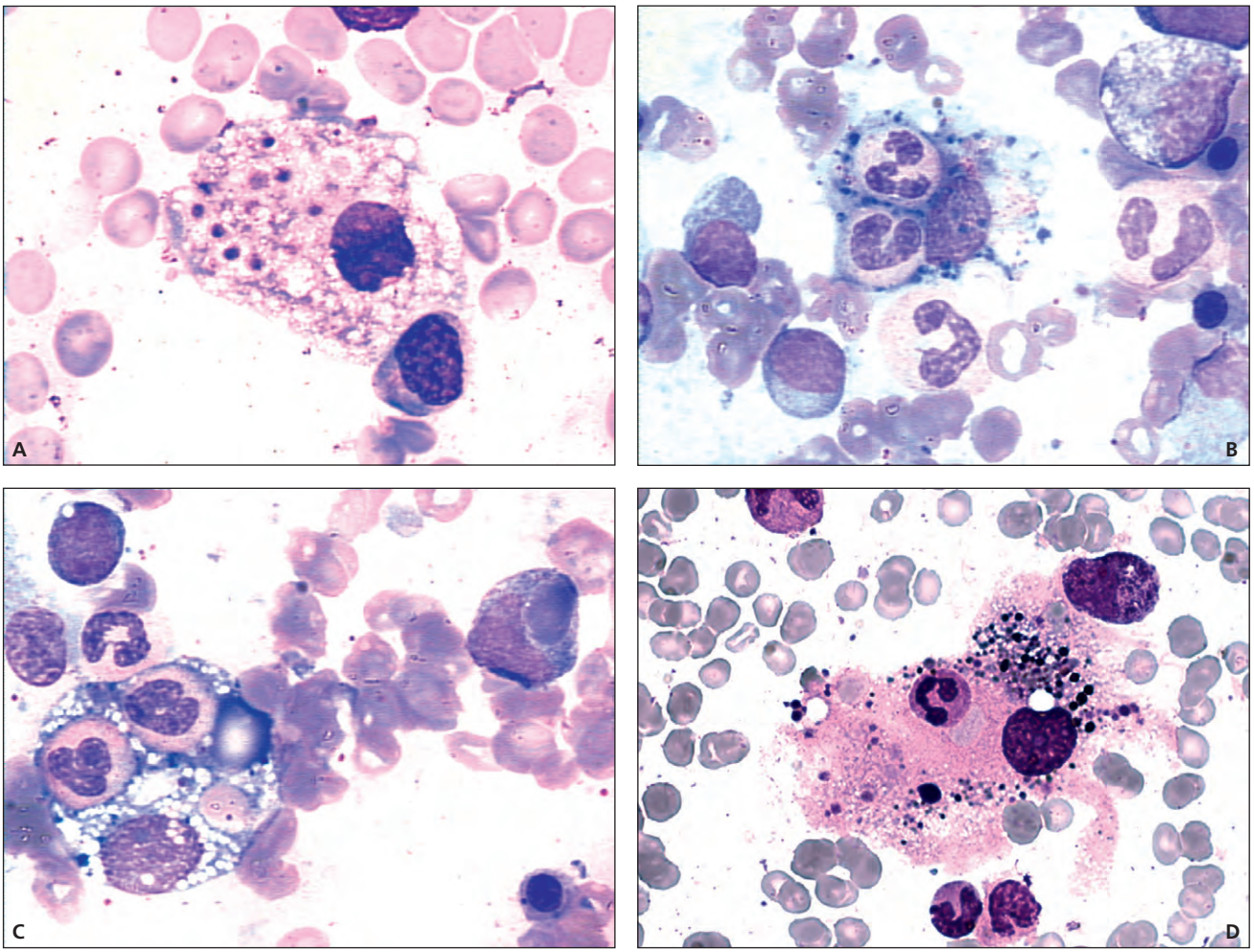



**Fig. 1.71** Bone marrow macrophages showing haemophagocytosis. (A) The plentiful cytoplasm of the cell has a reticular appearance because of the presence of numerous almost confluent vacuoles, many of which contain phagocytosed platelets that have not yet been broken down. (B) This small macrophage has phagocytosed two mature neutrophils, one segmented and the other a band form. From a patient with systemic leishmaniasis and with amastigotes of *Leishmania donovani* in other bone marrow macrophages. (C) From the same patient as (B). On the left, a macrophage with plentiful vacuolated cytoplasm containing two neutrophils, an erythrocyte and the remains of other cells; the spongy eccentric nucleus is at the lower pole. A state of extreme immune activation is confirmed by the presence, top right, of an LE cell that has formed spontaneously: a mononuclear cell – probably a monocyte – has an amorphous inclusion in its cytoplasm, representing an apoptotic nucleus that has been phagocytosed. (D) This macrophage contains, together with debris, an erythrocyte and a well-preserved neutrophil.

They are observed in tuberculosis, leishmaniasis, brucellosis and other chronic infections.

**The megakaryocyte/platelet series** (Table 1.10; Figs 1.79 through to 1.85)

In the bone marrow cavity, megakaryocytes are localised adjacent to adventitial reticular cells, liberating platelets directly into the sinusoidal lumen by means of apertures in sinusoidal walls. The first stage of maturation in this lineage can be recognised, albeit with difficulty; megakaryoblasts are rare in the normal bone marrow. These cells, directly derived from the CFU-MK progenitors, have a clearly blastic appearance, with a large immature nucleus, a fairly dense chromatin network and visible nucleoli; the N:C ratio is high and there is scanty pale cytoplasm often with typical protrusions (blebs). Megakaryoblasts, which are very rare in healthy subjects, are easier to detect when there is accelerated platelet production and in acute megakaryocytic leukaemia; they can be recognised with certainty with immuno cytochemistry using monoclonal antibodies specific for platelet membrane glycoproteins (CD41, CD42 or CD61) or by ultrastructural examination for platelet peroxidase, which is typically positive in the perinuclear area and in the endoplasmic reticulum.

Promegakaryocytes are somewhat more differentiated precursors. These cells are not yet very large (diameter 15–30 μm), with a nucleus that is deeply indented or moderately lobulated

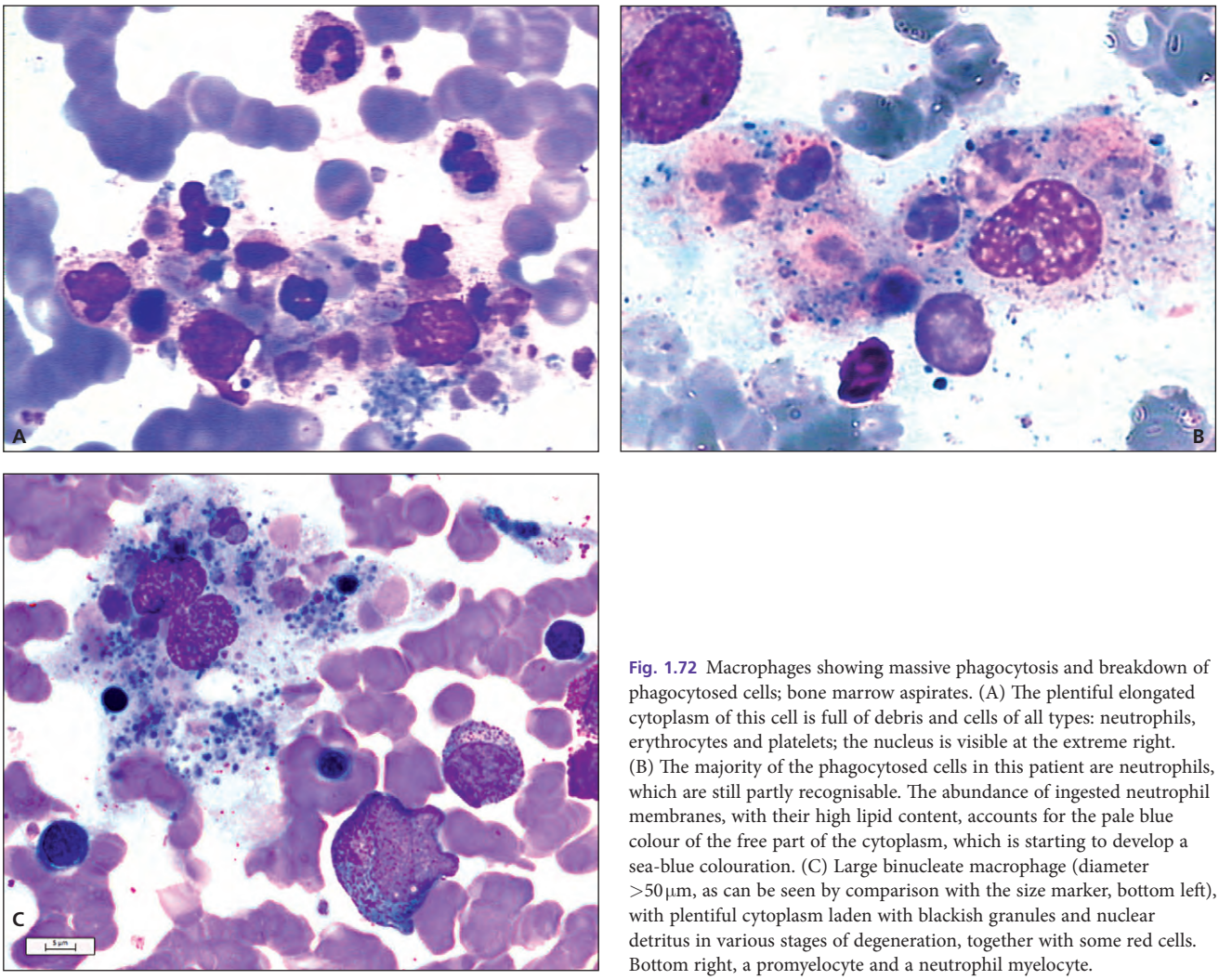



**Fig. 1.72** Macrophages showing massive phagocytosis and breakdown of phagocytosed cells; bone marrow aspirates. (A) The plentiful elongated cytoplasm of this cell is full of debris and cells of all types: neutrophils, erythrocytes and platelets; the nucleus is visible at the extreme right. (B) The majority of the phagocytosed cells in this patient are neutrophils, which are still partly recognisable. The abundance of ingested neutrophil membranes, with their high lipid content, accounts for the pale blue colour of the free part of the cytoplasm, which is starting to develop a sea-blue colouration. (C) Large binucleate macrophage (diameter >50 µm, as can be seen by comparison with the size marker, bottom left), with plentiful cytoplasm laden with blackish granules and nuclear detritus in various stages of degeneration, together with some red cells. Bottom right, a promyelocyte and a neutrophil myelocyte.

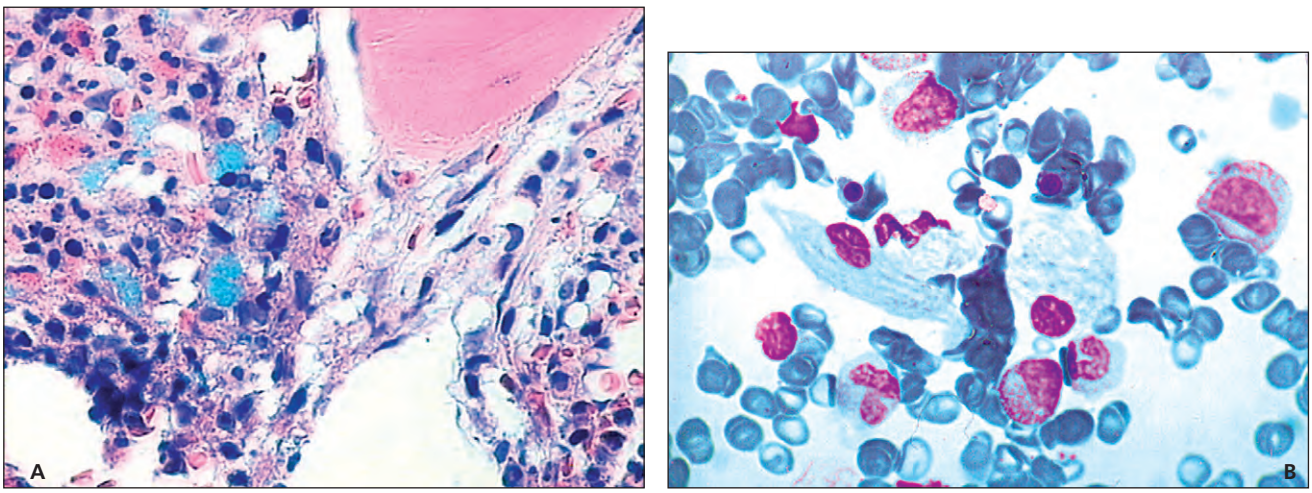



**Fig. 1.73** Sea-blue histiocytes, pseudo-Gaucher cells and Gaucher cells; bone marrow aspirates. (A) Various sea-blue histiocytes, resembling Gaucher cells, in a histological section from a patient with a myeloproliferative neoplasm. (B) Gaucher cells, with characteristic pale blue cytoplasm, which has an onion-skin appearance as a result of the tendency of the lipid, which has accumulated as the consequence of inherited cerebrosidase deficiency, to form thin concentric strata; from a patient with Gaucher disease.

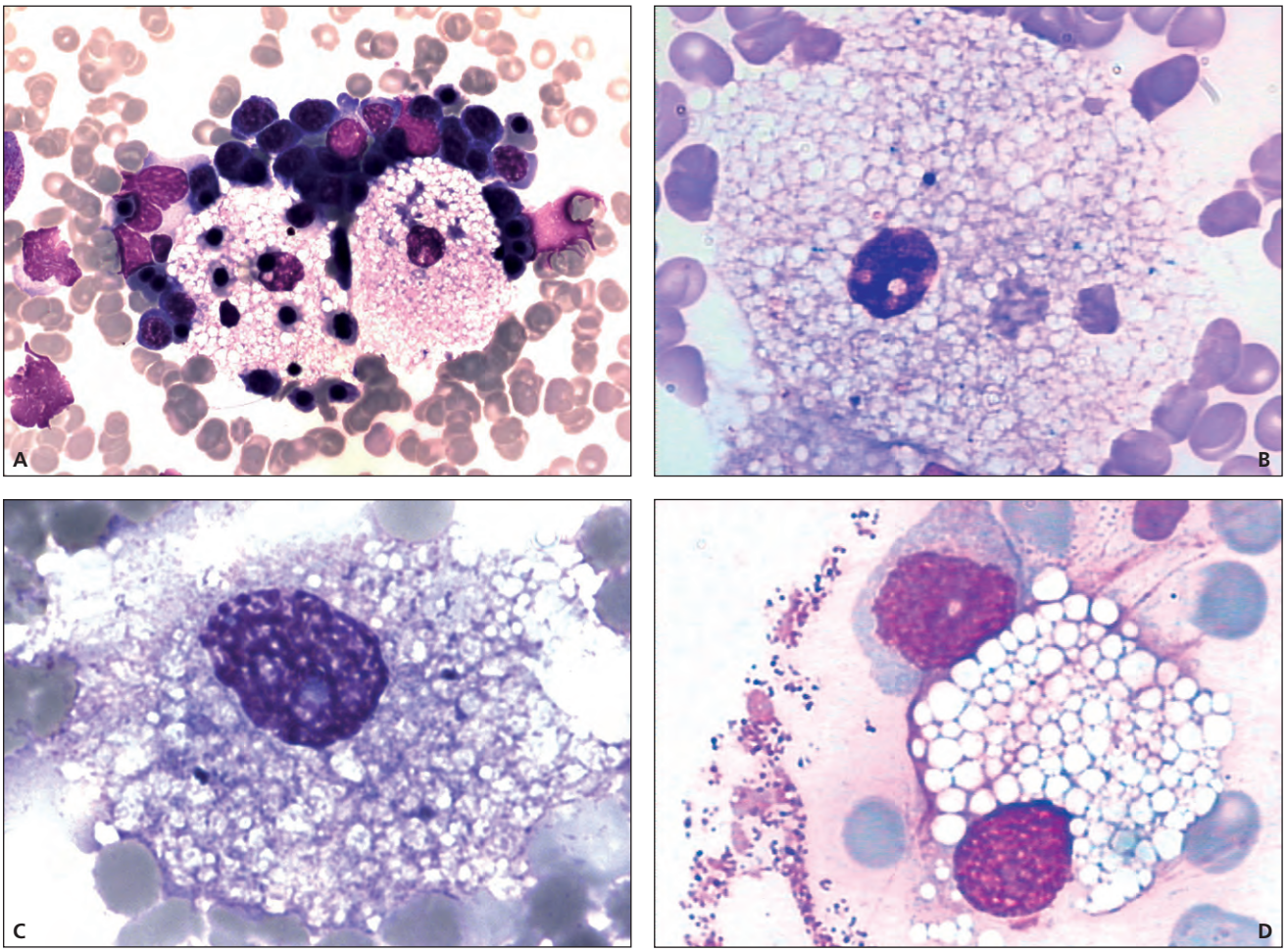



**Fig. 1.74** Foamy macrophages; bone marrow aspirates. Cells with this morphology do not generally show evidence of active phagocytosis. (A) The cytoplasm of these two macrophages has a reticular structure, due to the presence of vacuoles separated by thin septa that completely occupy the cytoplasm. The nuclei, as expected, are oval and spongy, but occupy a central position. The two cells are in contact with a nest of tightly packed erythroblasts, some of which seem to have penetrated the macrophage on the left. (B) Another macrophage with expanded foamy cytoplasm. Also in this case, the nucleus is not eccentric. (C) Macrophage with reticular cytoplasm. The nucleus contains a cyclamen-coloured nucleolus. (D) In another macrophage, the nucleus is eccentric and the cytoplasm is expanded by very numerous, optically empty, globular vacuoles.

and cytoplasm that is intensely basophilic, indicating that the cell is rich in ribosomes; one can see the first granules appearing, particularly surrounding the pale unstained Golgi zone.

Megakaryocytes are usually regarded as having two phases of maturation. The intermediate megakaryocyte (also called a granular megakaryocyte) is large (diameter 25–100 μm). Its cytoplasm, in which granules are steadily increasing in number, shows the first platelet zones outlined by the membranes of the demarcation system, the latter being identifiable by electron microscopy. There is a marginal zone, continuous and clearly defined, composed of hyaline material without any granules, beneath the cytoplasmic membrane: the N:C ratio is now lower and the eccentric nucleus occupies only a small part of the entire cell. The nucleus is now divided into large unequal lobes, joined by broad bands or thin bridges of chromatin. Mature megakaryocytes are even larger, with a diameter of 30–100 μm. The marginal zone is no longer visible and the cytoplasm contains many demarcated fields that represent the future platelets; these show concentrations of granules separated by fine hyaline areas of cytoplasm; platelet formation may be apparent as masses and strips of platelets that detach themselves from the cytoplasmic periphery. In a small percentage of megakaryocytes in healthy subjects one can observe minor dysplastic features, such as the presence of large megakaryocytes with non-lobulated nuclei or with two separate nuclei.[51]

The apparently bare nuclei of megakaryocytes, whether or not associated with cytoplasmic remnants, represent the last signs that platelets have been liberated into the lumens of the

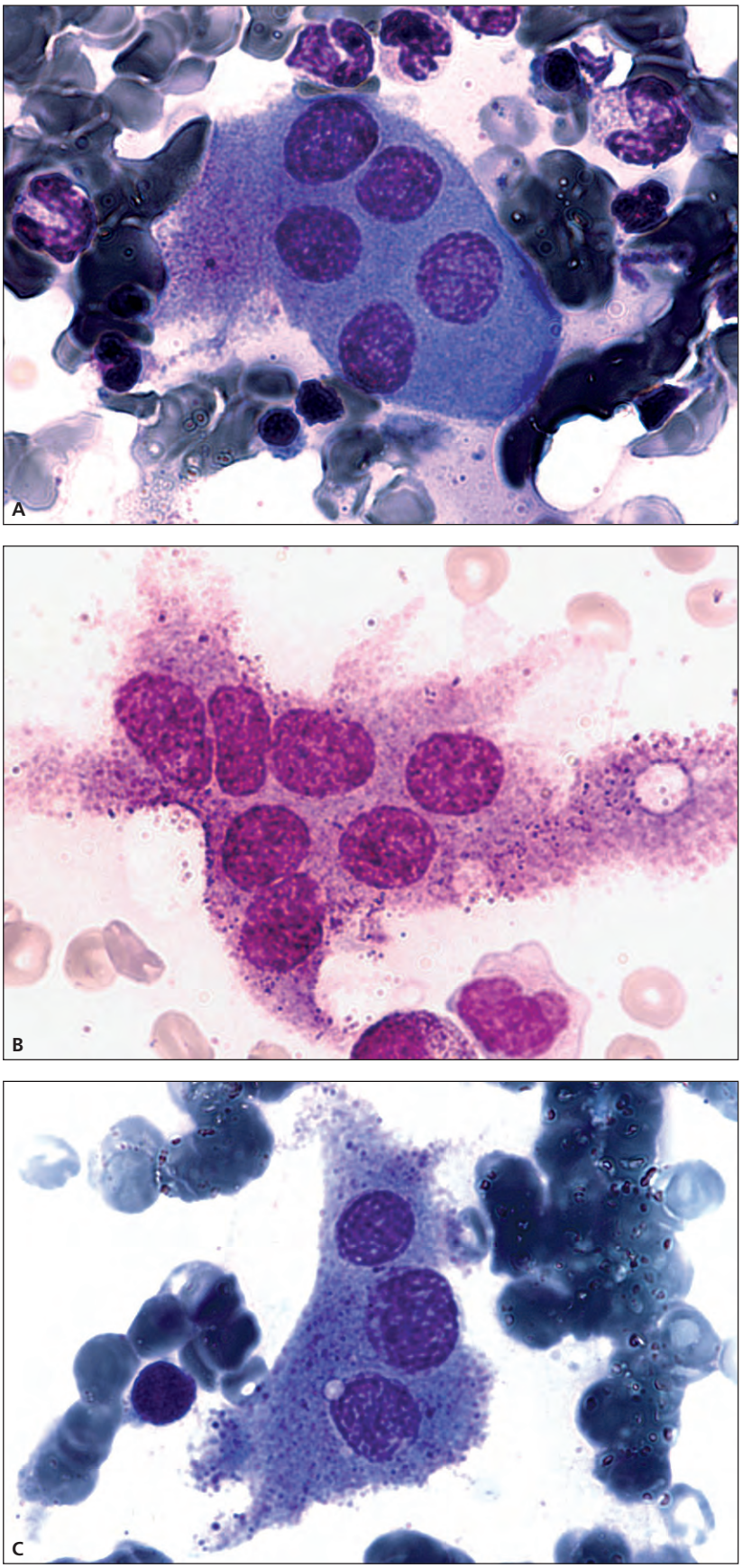



**Fig. 1.75** Osteoclasts in bone marrow aspirates. (A) The morphology of osteoclast nuclei is similar to that of macrophage nuclei. The cytoplasm is plentiful, varying from pink to violet to blue; it always contains fairly large granules, but only rarely cellular debris. The margins in general are better defined that the indistinct margins of many macrophages. (B) In this medium-sized osteoclast there are numerous spongy nuclei that are tending to deform each other where they are in contact, but without being superimposed. The cytoplasm is rich in granules and is extending into pointed projections. (C) Medium-sized osteoclast with three nuclei and basophilic cytoplasm with a clearly outlined margin.

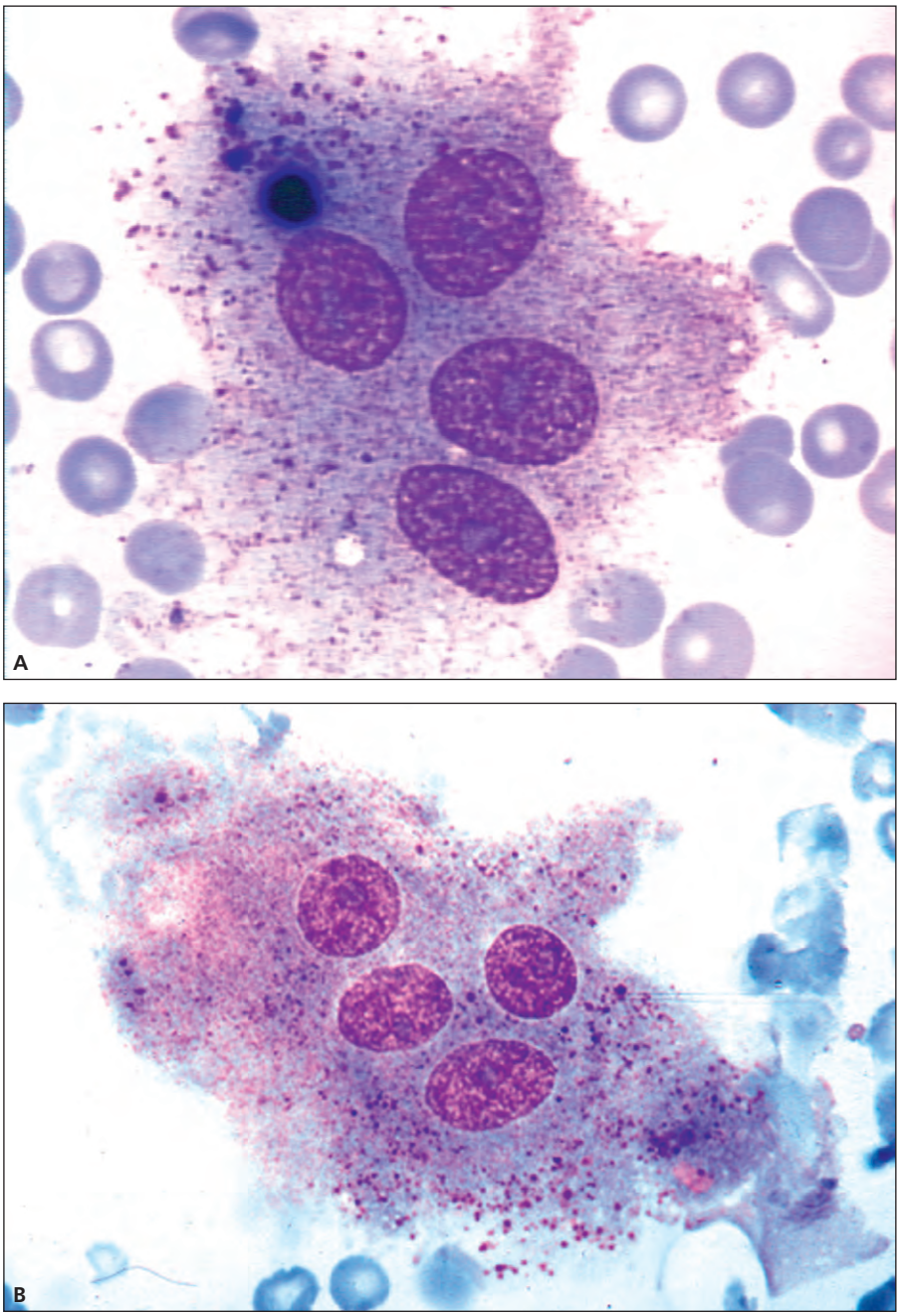



**Fig. 1.76** Osteoclasts in bone marrow aspirates. (A) In this osteoclast the nuclei, with a typical chromatin structure, are hypertrophic and have a large stamped out nucleolus; the granule-rich cytoplasm with a background colour tending to pink, particularly at the periphery, contains the digested nucleus of a phagocytosed cell. (B) A cell with spongy nuclei and numerous granules, very similar to the preceding cell; it does, however, appear larger if a comparison is made with the surrounding red cells and it lacks the detritus of phagocytosed cells.

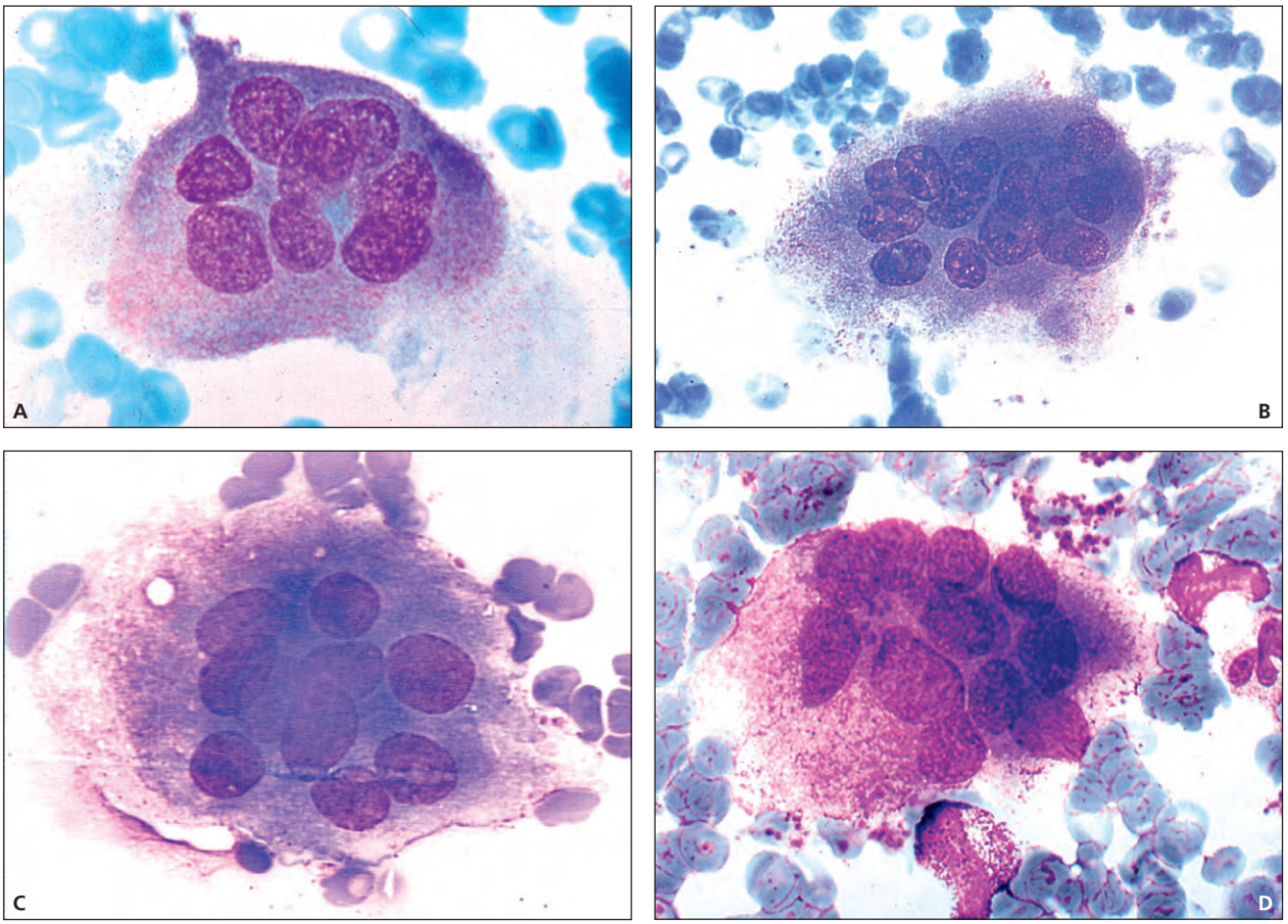



**Fig. 1.77** Osteoclasts in bone marrow aspirates. (A) A basophilic osteoclast with fine dispersed granules and eight or nine nuclei that are tending to cluster in the centre of the cell and, typically, appear transparent when they are superimposed on each other, so that the outline of the underlying nucleus can be discerned. (B) Large multinucleated osteoclast. There are numerous nuclei, crowding together in the centre, superimposed on each other and seemingly transparent; the cytoplasm is weakly basophilic and contains numerous, fairly large, azurophilic granules. The periphery of the cell is irregular and ragged: the platelets that are adhering to it should not lead to its erroneous identification as a megakaryocyte. (C and D) Other large osteoclasts. One again notes the tendency of the nuclei to cluster in the centre and to appear transparent when superimposed.

sinusoids. Bare megakaryocyte nuclei can also sometimes be recognised in the peripheral blood in conditions of accelerated or abnormal thrombopoiesis.

## Other cells of haemopoietic origin

***Plasma cells*** (Figs 1.86 and 1.87; see also Fig 10.5 and subsequent figures)

The frequency of plasma cells in a normal bone marrow aspirate is around 1% (0.5–3%). They are round or, more often, oval or pear shaped (length from 9 to 20 μm). The N:C ratio is very low: the cytoplasm is in fact abundant, usually an intense blue; this strong basophilia indicates the large number of ribosomes, both free and attached to the endoplasmic reticulum. There is always a pale paranuclear zone that corresponds to a hypertrophic Golgi apparatus; often one can also observe numerous small clear areas that correspond to the negative images of mitochondria. The nucleus is small, eccentric, apparently pushed by the hypertrophic cytoplasmic structures to one pole of the cell, usually the narrower pole if the cell is pyriform. The nucleus is most often oval with its long axis perpendicular to the principal axis of the cell. The chromatin is condensed into coarse blocks, vaguely polygonal, separated by thin pale spaces, which tend to have a radial arrangement, like the plates of a tortoise's shell: the classical 'cart-wheel' appearance is more apparent in sections of paraffin-embedded tissues than in bone marrow films. It is not rare to find binucleate forms. Sometimes the cytoplasm has a violaceous-red tinge, particularly at the edge of the cell (flaming plasma cell: this appearance is the result of the superimposition of blue, reflecting the presence of ribosomes, on red attributable to the presence of glycoprotein, which is abundant in the endoplasmic reticulum).

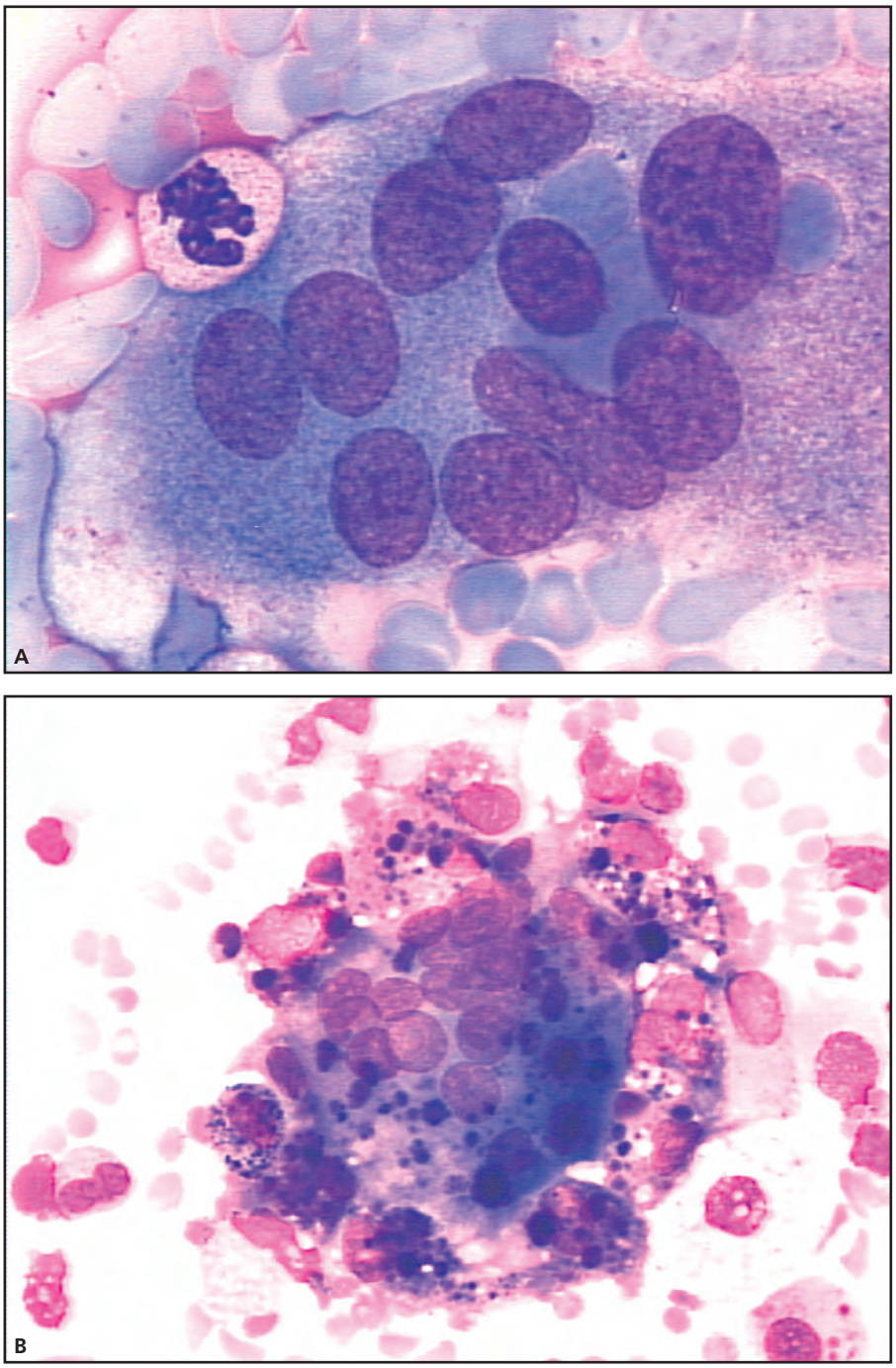



**Fig. 1.78** Foreign body giant cells; bone marrow aspirate. (A) This giant cell contains ten oval nuclei with a spongy chromatin structure and no tendency to central clustering. The cytoplasm is basophilic with very fine lilac granules. Cells of this type are closely related to osteoclasts, with which they share a monocyte–macrophage origin, being formed by cell fusion; however, they are observed mainly in patients with granulomatous disorders such as sarcoidosis or tuberculosis. (B) Granuloma in the bone marrow aspirate of an intensive care ward patient with recurrent sepsis. The foreign body giant cell in the centre is surrounded by a dense ring of macrophages laden with blackish granules and the detritus of phagocytosed cells.

**Table 1.10** Morphological characteristics associated with megakaryocytic differentiation

- Increase in size
- Increase in size and ploidy of nucleus as a result of endomitosis
- Segmentation of the nucleus into unequal lobes
- Increased volume of cytoplasm
- Appearance of cytoplasmic granules
- Increasing density of cytoplasmic granules in a small area that corresponds to future platelets
- Separation of newly formed platelets
- The size of a megakaryocytes and the number of platelets formed correlate with the ploidy, but not with the number of nuclear lobes

***Mast cells*** (Figs 1.88, 1.89, 2.136, 2.137 and 5.74)
The average frequency of mast cells in a normal bone marrow aspirate is very low. They must, however, be included in the myelogram and expressed as a percentage. The name mast cell, 'mast' in German indicating 'full of fat', was coined by Ehrlich, who maintained that the characteristic granules were the metabolic products resulting from inflammation. Mast cells are of very variable size, usually being slightly larger than circulating basophils (diameter 5–20 μm). The shape is also heterogeneous: round, polygonal, fusiform or very elongated. The nucleus, often not visible because it is covered by the granules, is fairly small,

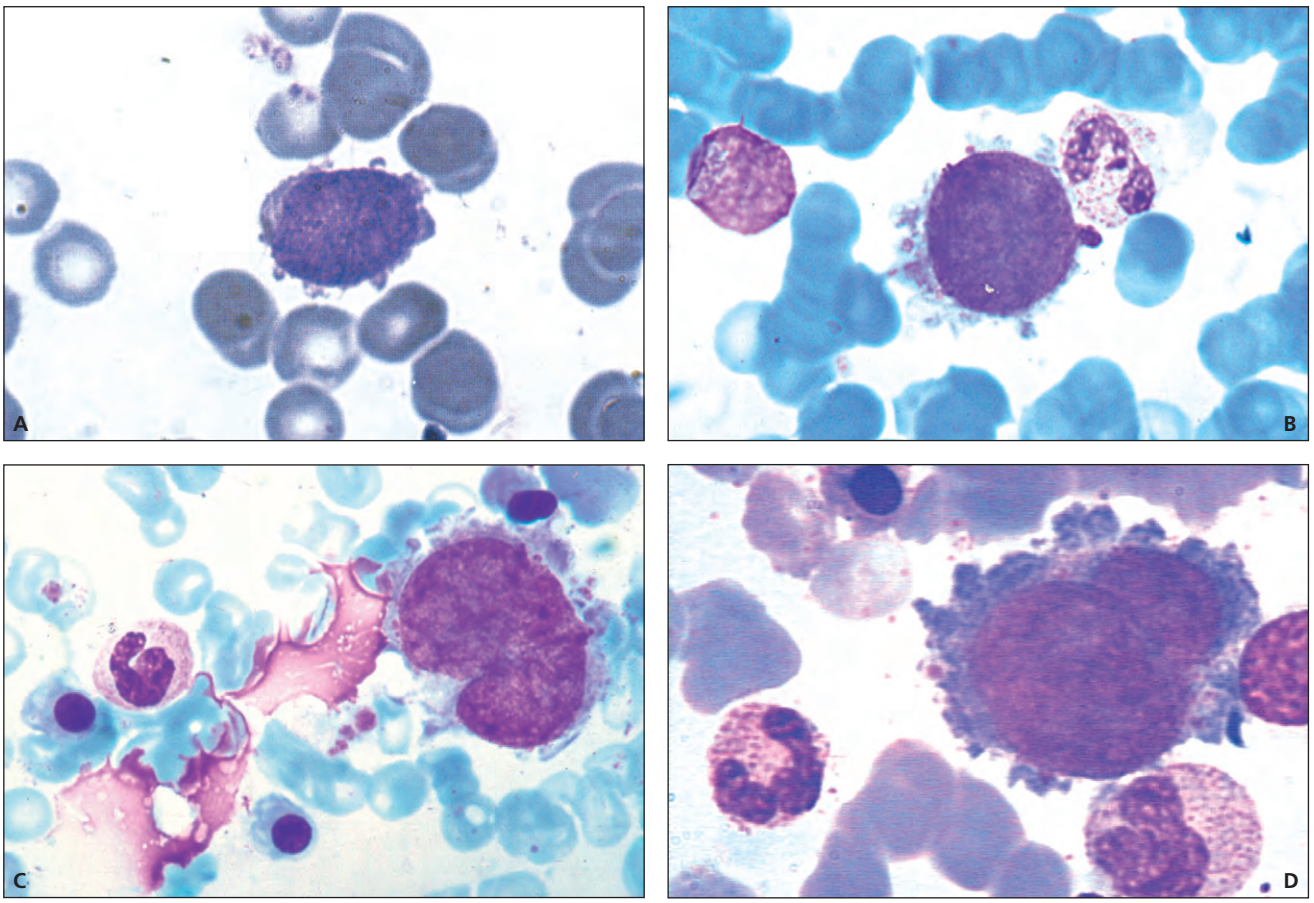



**Fig. 1.79** Immature precursors of megakaryocyte lineage; bone marrow aspirate. (A) Megakaryoblast. Large nucleus with a chromatin network that appears thick for an immature cell; the scanty, weakly basophilic cytoplasm is extending into rounded vesicular projections (blebs). Immature precursors of megakaryocyte lineage are rarely observed other than in clonal disorders involving this lineage. They can be difficult to recognise, appearing in most cases like undifferentiated blast cells without any morphological signs of differentiation. (B) This megakaryoblast has clear signs of being an early precursor of megakaryocyte lineage: the N:C ratio is very high, the nucleus is hyperdiploid with compact chromatin, without clear spaces, irregularly condensed (resembling a slice of salami); the very scanty cytoplasm is mainly visible as peripheral extensions that seem in some instances to already have the morphology of a mature platelet. Because of these signs of differentiation, some observers designate these cells promegakaryocytes. (C) Promegakaryocyte. A large cell with a diameter equivalent to five or six neutrophils. The nucleus is hyperdiploid, as the result of endomitosis, and is polylobated with two large lobes that have chromatin showing typical heterogeneous condensation. The cytoplasm forms a thin basophilic rim around the nucleus, but is also forming numerous projections that in part have the appearance of newly formed platelets. (D) Promegakaryocyte with a nucleus that is already divided into two unequal lobes; the chromatin is dense and heterogeneous, but without clear spaces or nucleoli. The cytoplasm is still intensely blue, with an accumulation of azurophilic granules; the periphery of the cell shows extensive bleb formation, emphasising that there is differentiation towards a platelet-producing cell.

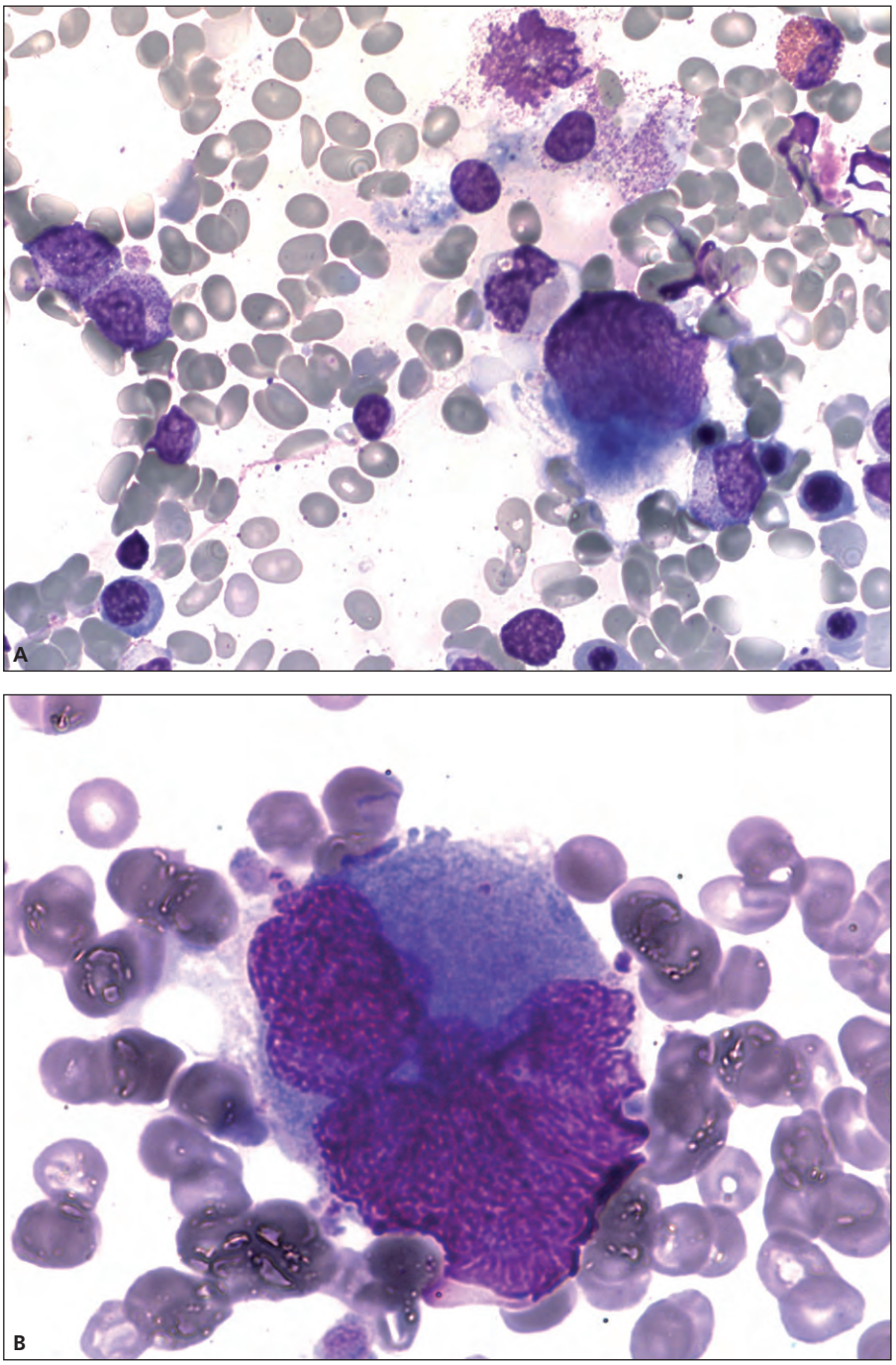



**Fig. 1.80** Immature precursors of megakaryocyte lineage; bone marrow aspirate. (A) The large cell on the right is a promegakaryocyte: the nuclear chromatin is condensed into worm-like structures and the cytoplasm, extending outwards on one side, is still intensely basophilic and not very abundant. On the left, two neutrophil myelocytes; at the top in the centre, two macrophages beneath a damaged nucleus; top right, an eosinophil; bottom right, erythroblasts at various stages of maturation. (B) Promegakaryocyte with similar characteristics to the preceding cell: increased N:C ratio; chromatin condensed into elongated worm-like structures; hyperdiploid nucleus already segmented into large unequal lobes; the cytoplasm is intensely basophilic and shows minimal signs of platelet production.

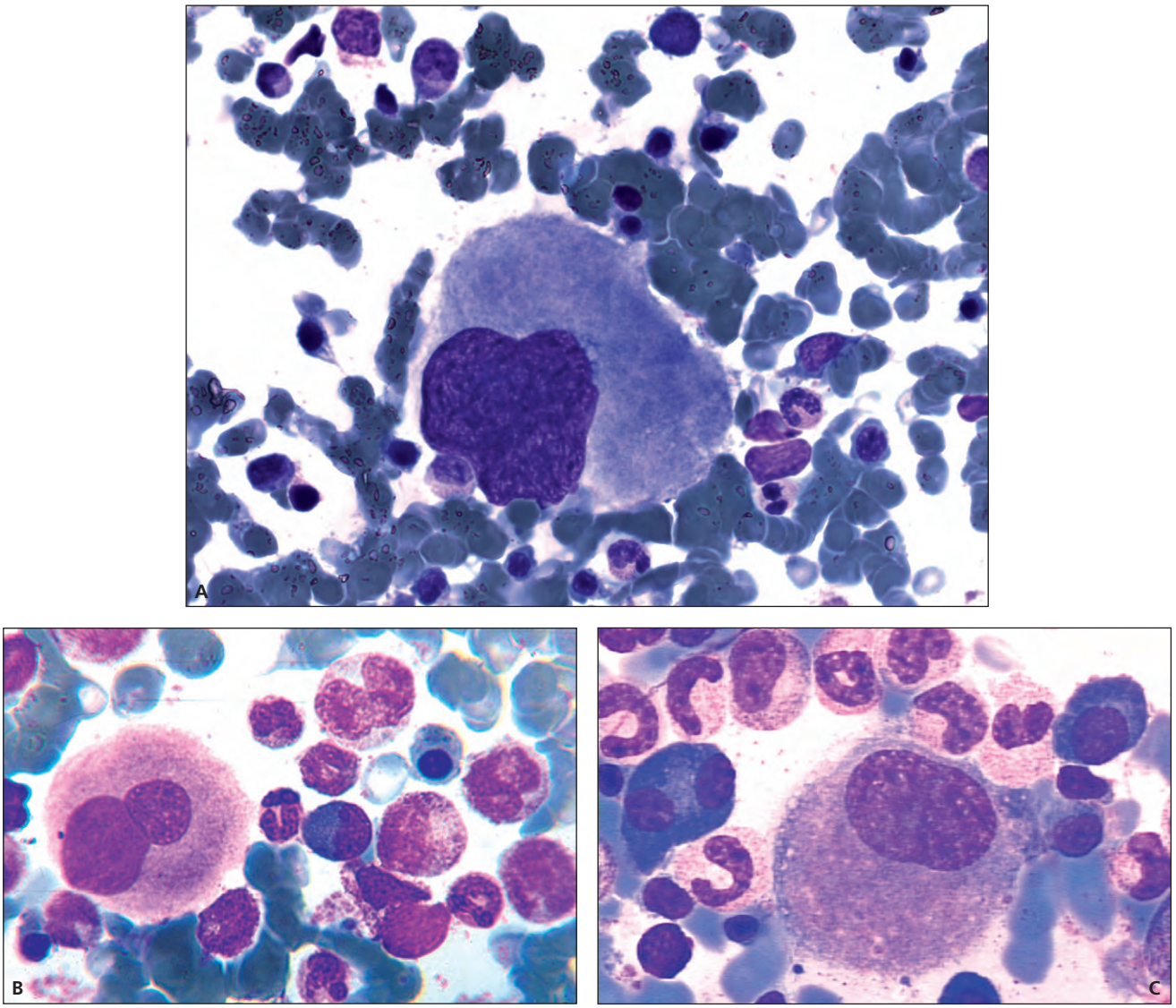



**Fig. 1.81** Intermediate megakaryocyte; bone marrow aspirate. (A) This cell can be classified as an intermediate megakaryocyte because of its large size (which is easily appreciated by comparison with the surrounding cells) and the N:C ratio, which is lower because of the presence of extensive cytoplasm. The cytoplasm is, however, intensely basophilic and the granules that are characteristic of the intermediate phase are not yet very evident. There are some zones of more intense basophilia separated by septa that can just be distinguished because they are paler: these areas correspond to zones of platelet demarcation. The nucleus is eccentric, very large, hyperdiploid and indented. (B) Intermediate megakaryocyte, not particularly large, with a low N:C ratio, a hyperdiploid nucleus with two lobes that are quite different in size and cytoplasm that is rich in granules, which are clustering to form the chromomere of the future platelets. The cell margin, however, does not show any projections. (C) An earlier intermediate megakaryocyte, small with a hyperdiploid but non-segmented nucleus; the cytoplasm is rich in granules, which in some areas are accumulating into platelet fields; on the left, a basophilic marginal zone that is rich in small vacuoles. From a patient with sepsis. This cell is morphologically similar to the megakaryocytes with non-lobated nuclei of a myelodysplastic syndrome with isolated del(5q). At the top are a metamyelocyte and a band neutrophil; at the sides are two plasma cells, one of which is binucleated.

oval or round, not lobated, usually centrally sited, with pale chromatin or, more frequently, very dense pyknotic chromatin. The cytoplasm is packed with metachromatic granules, which are purplish-red or deep violet. Mast cells are increased in numerous infective and inflammatory conditions, in certain dermatoses, in urticaria pigmentosa, in systemic mastocytosis and in Waldenström macroglobulinaemia and other lymphoproliferative disorders.

### *Lymphocytes*

Lymphocytes are present in the bone marrow in variable numbers, constituting up to 15–20% of cells. Their morphology,

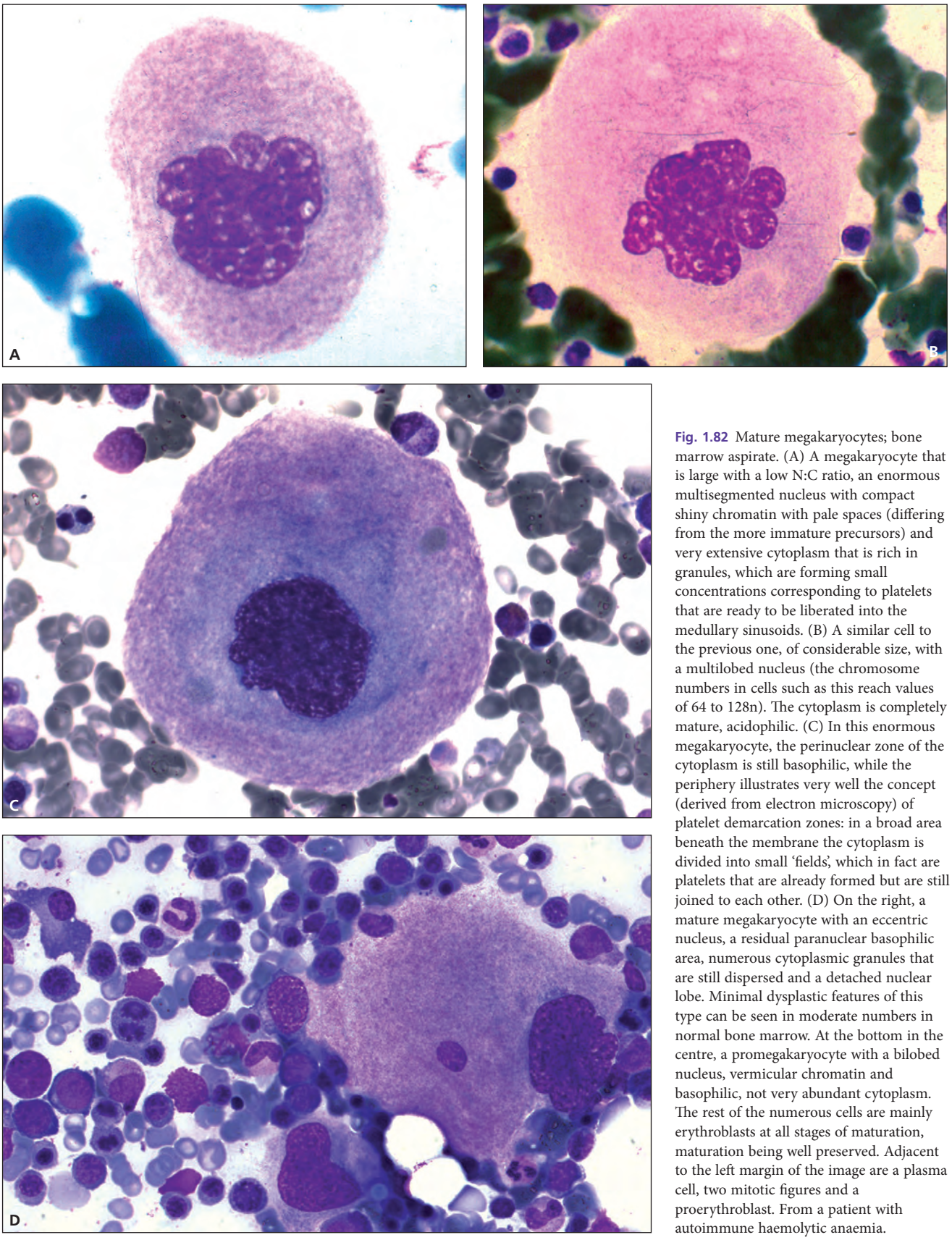



**Fig. 1.82** Mature megakaryocytes; bone marrow aspirate. (A) A megakaryocyte that is large with a low N:C ratio, an enormous multisegmented nucleus with compact shiny chromatin with pale spaces (differing from the more immature precursors) and very extensive cytoplasm that is rich in granules, which are forming small concentrations corresponding to platelets that are ready to be liberated into the medullary sinusoids. (B) A similar cell to the previous one, of considerable size, with a multilobed nucleus (the chromosome numbers in cells such as this reach values of 64 to 128n). The cytoplasm is completely mature, acidophilic. (C) In this enormous megakaryocyte, the perinuclear zone of the cytoplasm is still basophilic, while the periphery illustrates very well the concept (derived from electron microscopy) of platelet demarcation zones: in a broad area beneath the membrane the cytoplasm is divided into small 'fields', which in fact are platelets that are already formed but are still joined to each other. (D) On the right, a mature megakaryocyte with an eccentric nucleus, a residual paranuclear basophilic area, numerous cytoplasmic granules that are still dispersed and a detached nuclear lobe. Minimal dysplastic features of this type can be seen in moderate numbers in normal bone marrow. At the bottom in the centre, a promegakaryocyte with a bilobed nucleus, vermicular chromatin and basophilic, not very abundant cytoplasm. The rest of the numerous cells are mainly erythroblasts at all stages of maturation, maturation being well preserved. Adjacent to the left margin of the image are a plasma cell, two mitotic figures and a proerythroblast. From a patient with autoimmune haemolytic anaemia.

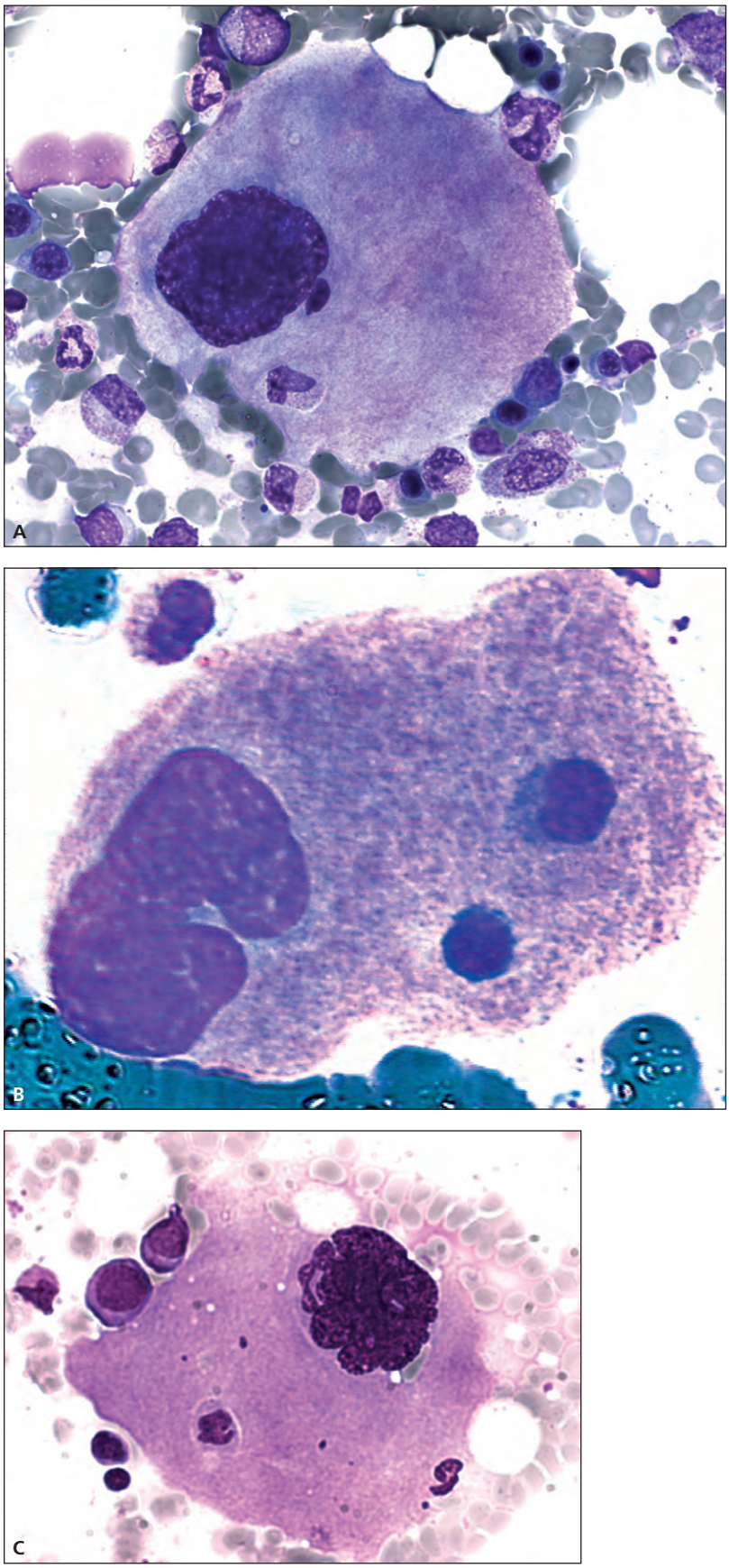
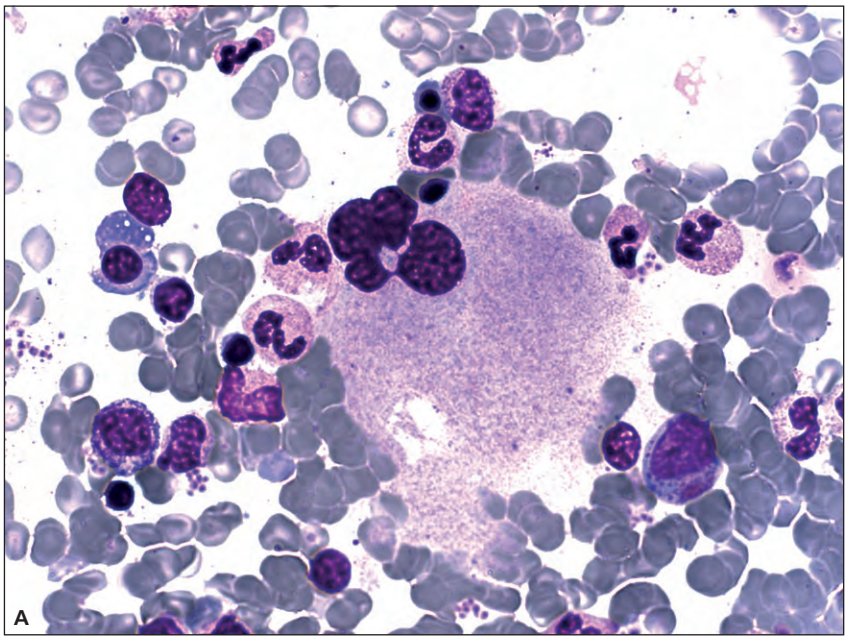
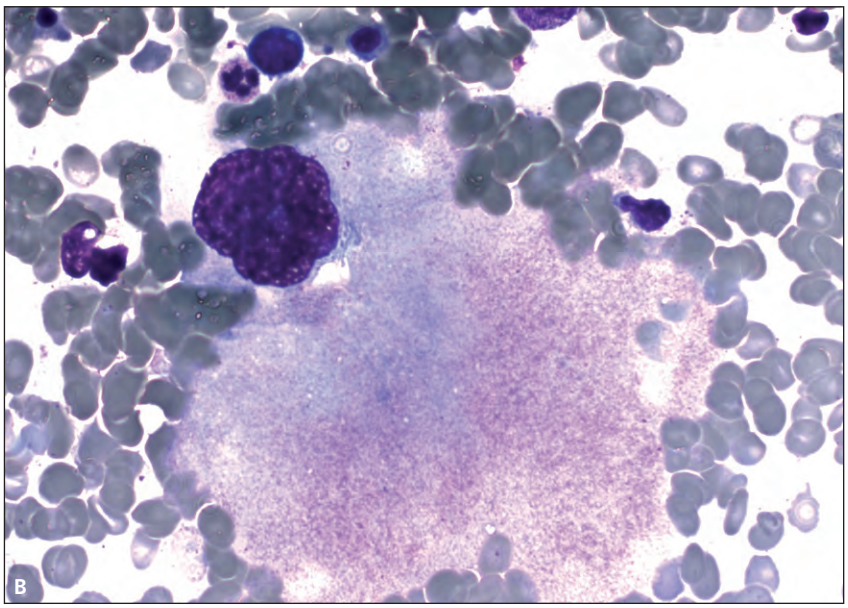



**Fig. 1.83** Emperipolesis (from the Greek *em-*, inside, *-peri-*, around, and *-polemai*, wandering) is not a rare phenomenon and does not have any specific pathological significance; it consists of the active entry into the surface-connected canalicular system of the ample megakaryocyte cytoplasm of intact cells of various types; bone marrow aspirate. (A) The not completely granular cytoplasm of this very large megakaryocyte has been traversed at the bottom by a neutrophil myelocyte. (B) Mature megakaryocyte with an eccentric nucleus; the cytoplasm shows obvious platelet demarcation zones, each containing clusters of granules, and contains two morphologically unaltered cells, a monocyte and a lymphocyte. (C) Neutrophils are the cells which, possibly by virtue of their vigorous motility, most often show the phenomenon of emperipolesis.



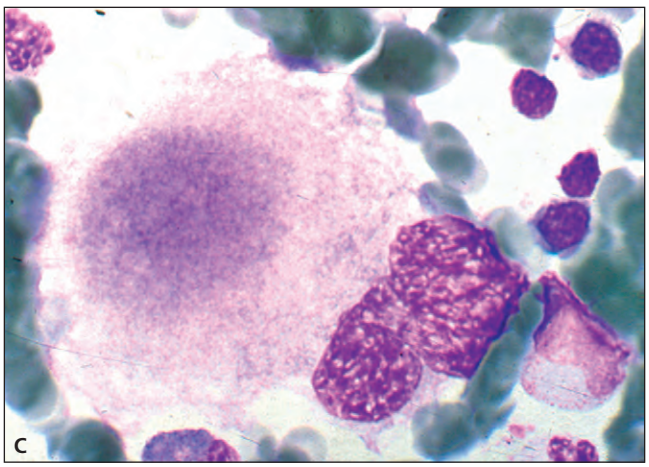




**Fig. 1.84** Megakaryocytes in the act of liberating their completely mature cytoplasm; bone marrow aspirates. (A) The nucleus is very eccentric, the chromatin is very condensed and the nucleus is partly extruded from the cytoplasm which is breaking apart to liberate mature platelets. This phenomenon usually occurs within sinusoids, where the megakaryocytes tend to localise: this topographical feature is rarely observable in cytological preparations of bone marrow aspirates. (B) This dark compact nucleus, which is now almost totally detached from the cytoplasm, still retains a small basophilic perinuclear area. The accumulation of violet granules in the right-hand side of the cytoplasm permits individual platelet fields to be visualised. (C) Also in this case, the detachment of the nucleus is almost complete, even if the cytoplasm seems to be forming a single granular sphere.

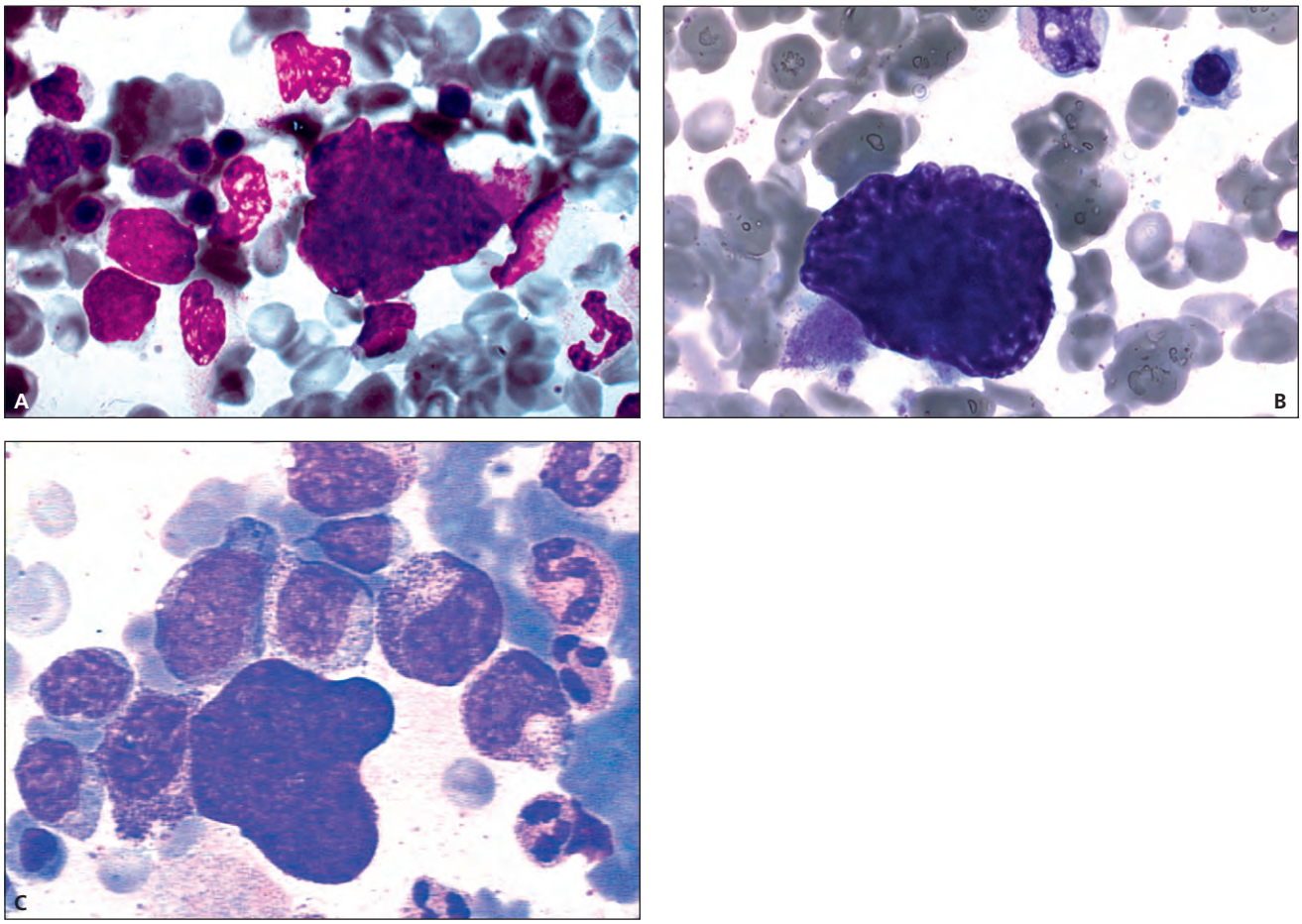



**Fig. 1.85** Bare megakaryocyte nuclei, the final vestiges of the cell after platelet formation; bone marrow aspirate. (A) The multilobed mass of chromatin is now detached from the cytoplasm. (B) This hyperdiploid, already almost pyknotic nucleus still retains a small tail of mature cytoplasm. (C) Bare nuclei of megakaryocytes are seen more often in the bone marrow when there is accelerated platelet production, as in thrombocytopenic purpura of immune or microangiopathic origin. They can also be observed in peripheral blood films, particularly when there is myelofibrosis and extramedullary haemopoiesis.

excluding that seen in lymphoproliferative disorders, can be heterogeneous and not dissimilar to that observed in the peripheral blood. Large granular lymphocytes are relatively more numerous. On histological examination, the presence of lymphoid follicles is not rare and occasionally these are also seen in a bone marrow aspirate, particularly in the elderly and in women beyond the fourth decade of life, in association with reactive bone marrow plasmacytosis and in conditions of chronic immunological stimulation.[53–55]

***Stromal cells***

Stromal cells, such as osteoblasts and the endothelial cells of sinusoids, have a very important regulatory role in relation to haemopoietic stem cells. Stroma is a collective term, derived from the Greek term for 'mattress', indicating 'support'. The stromal cells that can be encountered, which constitute an integral part of haemopoietic tissue, are heterogeneous and are found in marrow aspirates in variable numbers; they are seen particularly in a variety of pathological conditions (Fig. 1.90). In contrast to macrophages and plasma cells, which represent respectively the mature tissue phase of monocyte and B-lymphocyte lineages and are thus derived from haemopoietic stem cells, stromal cells are of mesenchymal origin (osteoblasts) or endothelial origin. These cells are embedded in an intercellular matrix composed of macromolecules, fibrous proteins, glyoproteins, glycosaminoglycans and heparan sulphate, providing effective sites for cytokine binding.

*Osteoblasts*

Osteoblasts are a fundamental constituent of the haemopoietic niche and are found in contact with the endosteum.[11] They interact closely with a small population of adjacent haemopoietic stem cells, regulating their activity and their differentiation, and producing growth factors to maintain them. In bone marrow films they are medium sized (diameter up to 30 μm), oval in shape and often observed in small groups, particularly

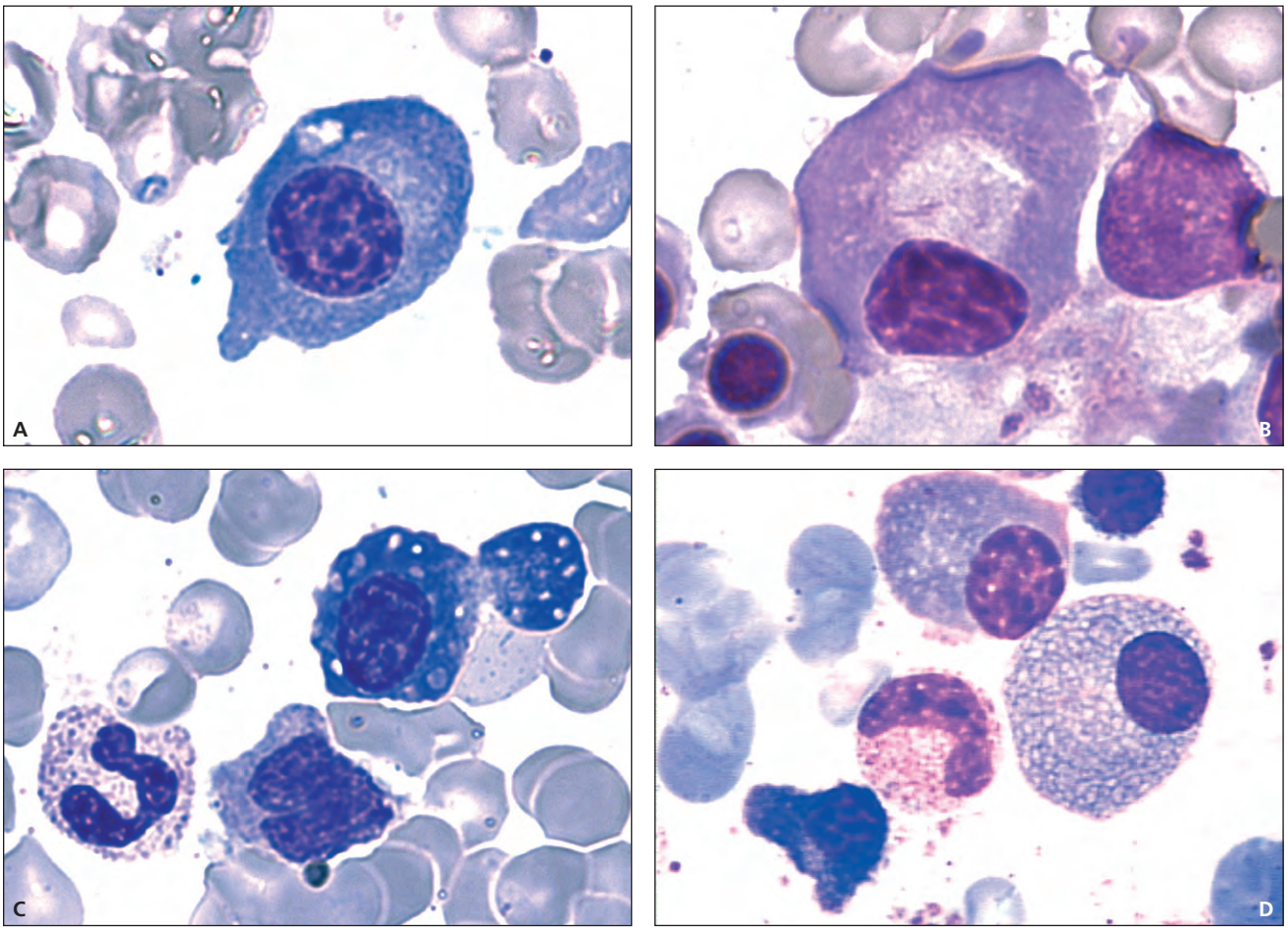



**Fig. 1.86** Plasma cells in bone marrow aspirates. (A) Mature plasma cell. The nucleus, less eccentric than usual, is ovoid with the long axis perpendicular to that of the cell as a whole. The chromatin is condensed into large, not very numerous blocks. The cytoplasm is plentiful and intensely basophilic with a Golgi zone or arcoplasm that is very evident. (B) Even in the absence of haematological disease, bone marrow plasma cells can show dysmorphic features resulting particularly from activation (increased production of immunoglobulin) and 'storage cell' formation (failure to secrete the immunoglobulin): in this cell the cytoplasm is plentiful and stains lilac because of the accumulation of glycoprotein, and the Golgi zone, where the glycoprotein is assembled, is hypertrophic. (C) From the left: a band neutrophil, a monocyte and a plasma cell showing degeneration (vacuolisation and clasmatosis). (D) Three plasma cells (and a neutrophil metamyelocyte) in the bone marrow aspirate of a patient with rheumatoid arthritis. The cell on the right shows reticular transformation of the cytoplasm, which indicates an accumulation of immunoglobulin molecules.

in the tail of the film (Figs 1.91, 1.92 and 1.93). There is plentiful cytoplasm, which is moderately blue and rarely contains a few red granules. The presence of a pale Golgi zone is typical; in contrast to what is observed in plasma cells, the Golgi zone is slightly separated from the nucleus. There is a single nucleus, small in relation to the amount of cytoplasm, definitely eccentric, and in general oriented parallel to the major axis of the cell; the chromatin is regular and less dense than that of a plasma cell; usually there are at least two nucleoli.

*Endothelial cells of sinusoids*

The vascular area at the centre of the medullary cavity consists of a fine network of fenestrated sinusoids. The walls of the sinusoids, rich in openings, are composed of an internal layer of endothelial cells, a basal membrane and an external adventia formed of reticular cells designated because of their location, adventitial cells. The endothelial cells are flattened and elongated (Figs 1.94 and 1.95), with a tendency to taper towards the two extremities. The nucleus is spongy. In bone marrow films they generally appear to be joined to each other at their tapering ends, forming shorter or longer bands of cells, often maintaining a close relationship with other stromal cells.

*Adventitial reticular cells*

These have an oval spongy nucleus and cytoplasm that forms elongated processes, which in life create a network in the interstices of which the haemopoietic cells are located. The enlargement and transformation of reticular cells into adipocytes is the principal phenomenon that determines the change, reversible in appropriate conditions, of active red haemopoietic marrow into fatty yellow marrow. In bone marrow aspirates they are sometimes seen as isolated cells or as small strands of

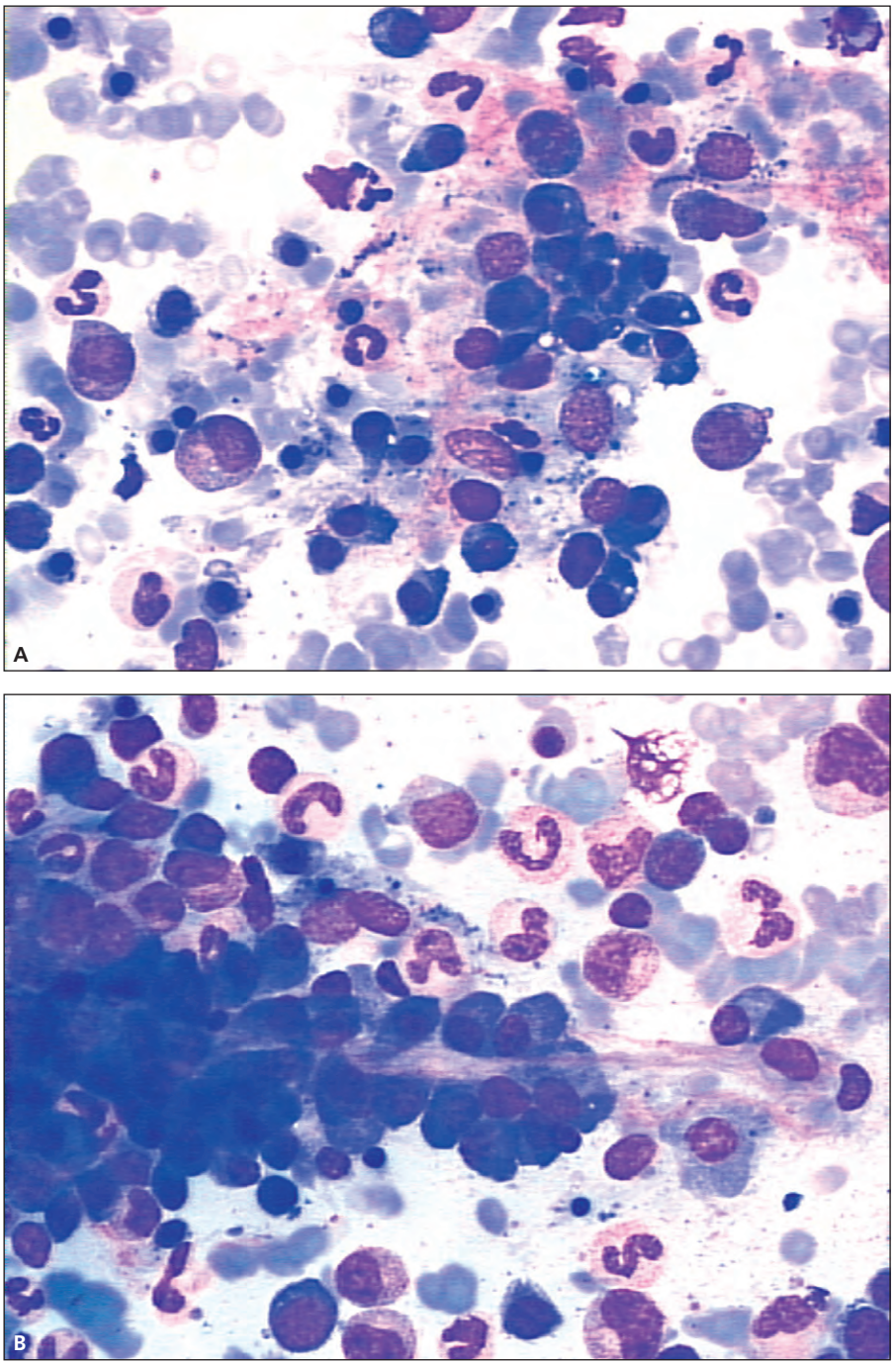



**Fig. 1.87** Plasma cell clusters. Plasma cells tend to accumulate in specific sites, often around blood vessels: the inclusion of too many of these areas when performing a differential count of bone marrow cells can lead to an overestimation of plasma cell numbers. (A) Numerous plasma cells admixed with macrophages and haemopoietic precursors, in a small fragment with abundant background hyaline material. (B) A large cluster of plasma cells surrounding a sinusoid, which is recognisable from the parallel filamentous structures in the centre that terminate in the nucleus of an endothelial cell. Immediately above there are macrophages.

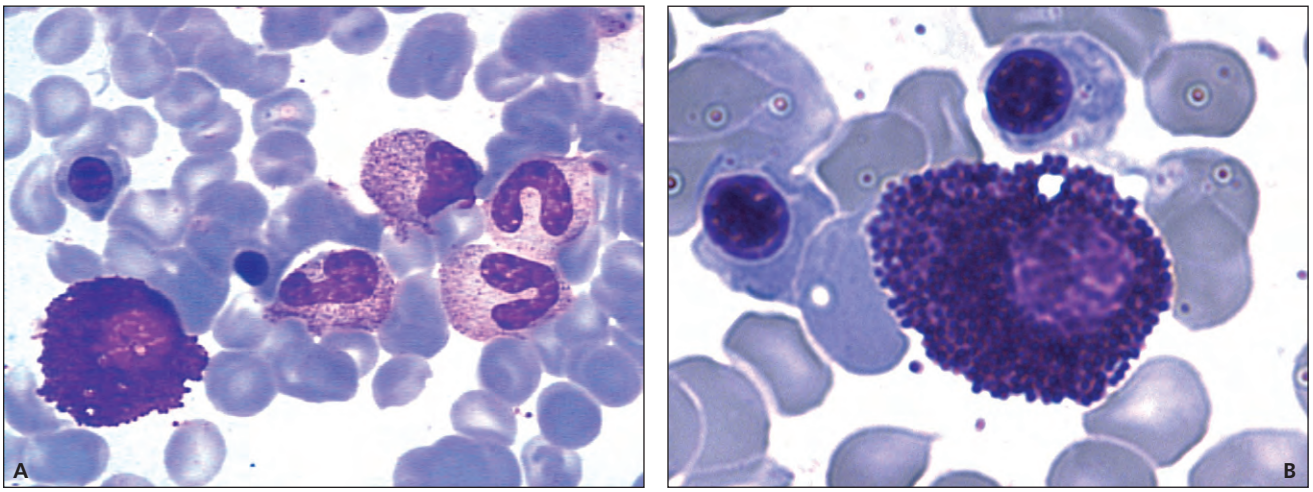



**Fig. 1.88** Mast cells in bone marrow aspirates (see also Chapter 5). (A) A mast cell, bottom left, together with a polychromatic and an orthochromatic erythroblast, a metamyelocyte and three neutrophil band forms. The mast cell is trapezoidal; the cytoplasm is packed with dark violet-red granules, smaller and more tightly packed than those of basophils. (B) Differing from the granules of basophils, mast cell granules tend not to be superimposed on the nucleus, which thus appears rounded, non-lobed and surrounded by granules. The shape of the mast cell in this case, which is very similar to a *menhir* (a standing stone) with its base to the right and its point to the left, is fairly characteristic.

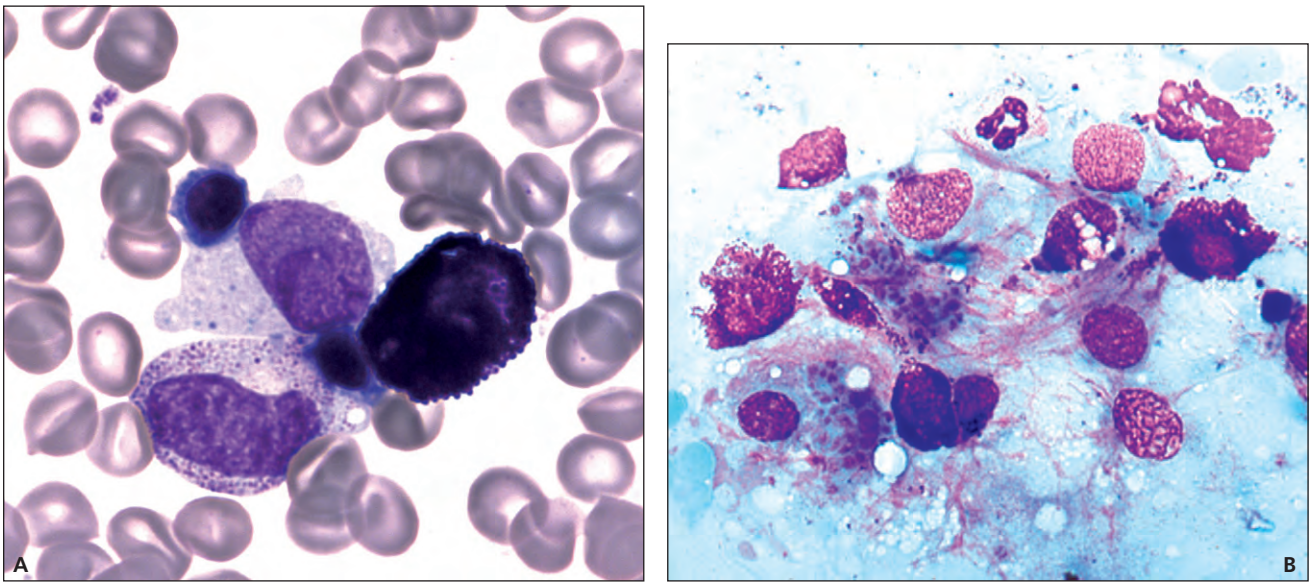



**Fig. 1.89** Mast cells in bone marrow aspirates. (A) A neutrophil metamyelocyte, two erythroblasts, a young macrophage and a mast cell. (B) A small mass of hyaline material in a bone marrow aspirate, within which can be seen a number of polygonal mast cells and a fourth smaller, more fusiform mast cell (adjacent to the larger cell on the left), several macrophages, a plasma cell and masses of platelets. Features such as these can indicate an inflammatory myelitis.

two or three cells, particularly along the edges or in the tail of the film (Figs 1.96, 1.97 and 1.98). They are large (diameter 20–30 μm) and sometimes polygonal or rhomboidal; often the cytoplasm, greyish with a basophilic tinge and delicate azurophilic granules, appears tattered, extending as thin, elongated processes; sometimes the cytoplasm is indistinct and barely visible and sometimes it is detached from the nucleus as a result of the damage inflicted during aspiration. The nucleus, which thus often appears bare, is oval with very delicate chromatin, composed of a regular network of fine filaments, without any condensation. In general, there are two or three prominent pale blue nucleoli, outlined by a circle of denser chromatin.

*Adipose cells*

These are large cells with a hyperchromatic nucleus flattened at the periphery of a very large amount of optically empty cytoplasm (Figs 1.99 and 1.100).

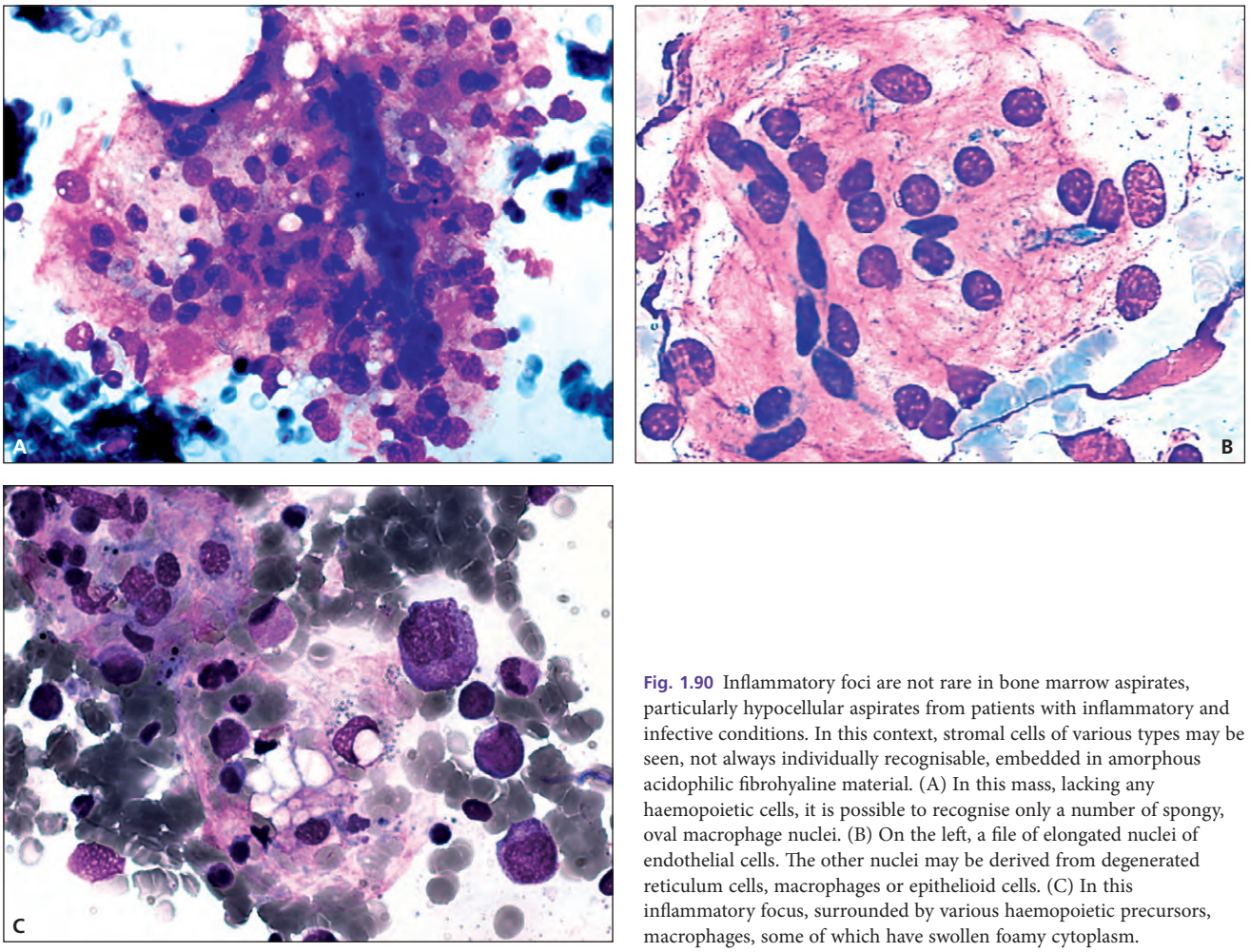



**Fig. 1.90** Inflammatory foci are not rare in bone marrow aspirates, particularly hypocellular aspirates from patients with inflammatory and infective conditions. In this context, stromal cells of various types may be seen, not always individually recognisable, embedded in amorphous acidophilic fibrohyaline material. (A) In this mass, lacking any haemopoietic cells, it is possible to recognise only a number of spongy, oval macrophage nuclei. (B) On the left, a file of elongated nuclei of endothelial cells. The other nuclei may be derived from degenerated reticulum cells, macrophages or epithelioid cells. (C) In this inflammatory focus, surrounded by various haemopoietic precursors, macrophages, some of which have swollen foamy cytoplasm.

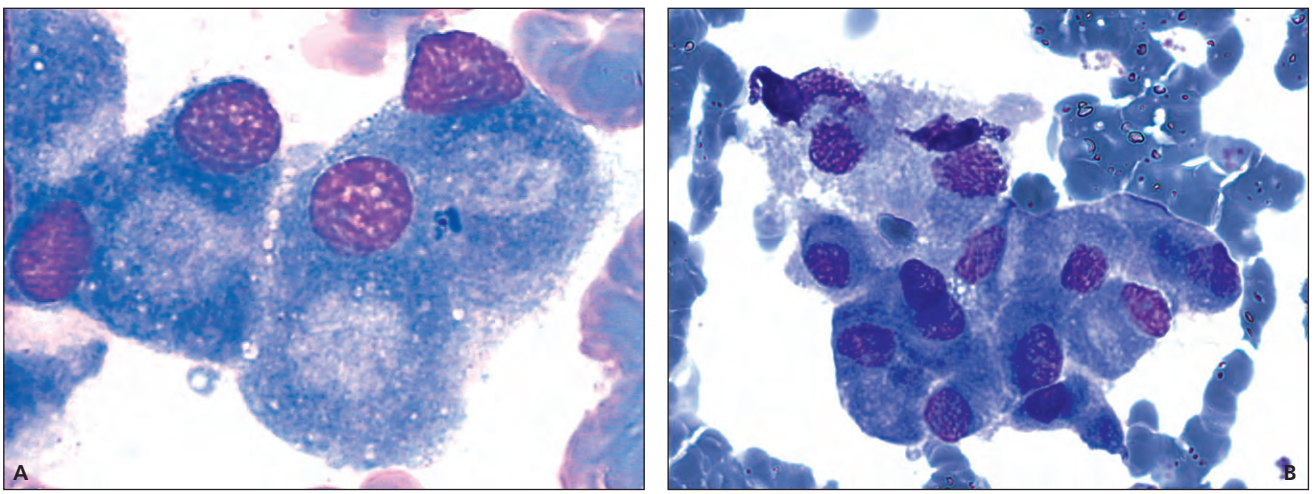



**Fig. 1.91** Osteoblasts in bone marrow aspirates. (A) The very typical morphological details that permit recognition are: (1) the strongly eccentric position of the nucleus, which has a spongy structure, often with a single nucleolus, not dissimilar to that of a macrophage with its oval shape and its main axis most often parallel to the main axis of the cell (a feature that distinguishes it from a plasma cell); (2) the morphology of the cytoplasm, blue in colour and usually an elongated egg shape or an irregular rectangle; (3) the shape and position of the Golgi zone, which is usually an irregular square right in the centre of the cell, thus being clearly separate from the nucleus which is located at one pole. (B) Often osteoblasts are found in cohesive groups.

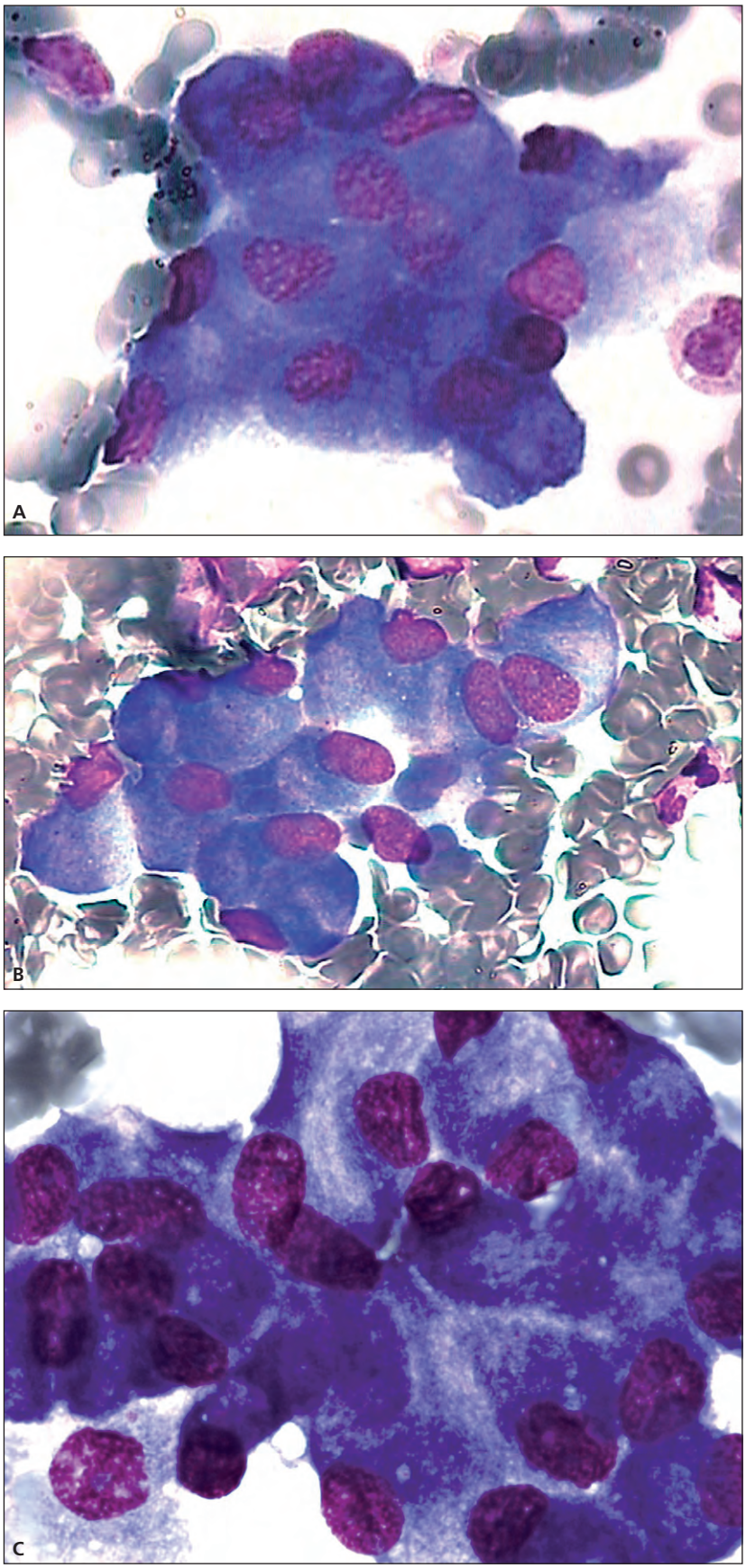



**Fig. 1.92** Small groups of osteoblasts. An arrangement in more or less compact groups is very frequent in bone marrow aspirates and reflects the localisation of these cells in sheets and chains along the endothelial surface of bony spicules. (A) A very compact group with cytoplasm that is more intensely basophilic than normal. The morphology, which can misleadingly suggest that of plasma cells, can be seen to be typical of osteoblasts with regard to the oval spongy nucleus, as is clearly seen in the cell at bottom left, which shows a rectangular Golgi zone clearly distant from the polar nucleus. (B) Osteoblasts in this group are more spread out and thus their morphological characteristics are more easily recognisable. Some nuclei have a single stamped out nucleolus. (C) Sometimes, as in this instance, the compactness of a mass of osteoblasts can suggest an erroneous interpretation as metastatic cells from a non-haemopoietic tumour.

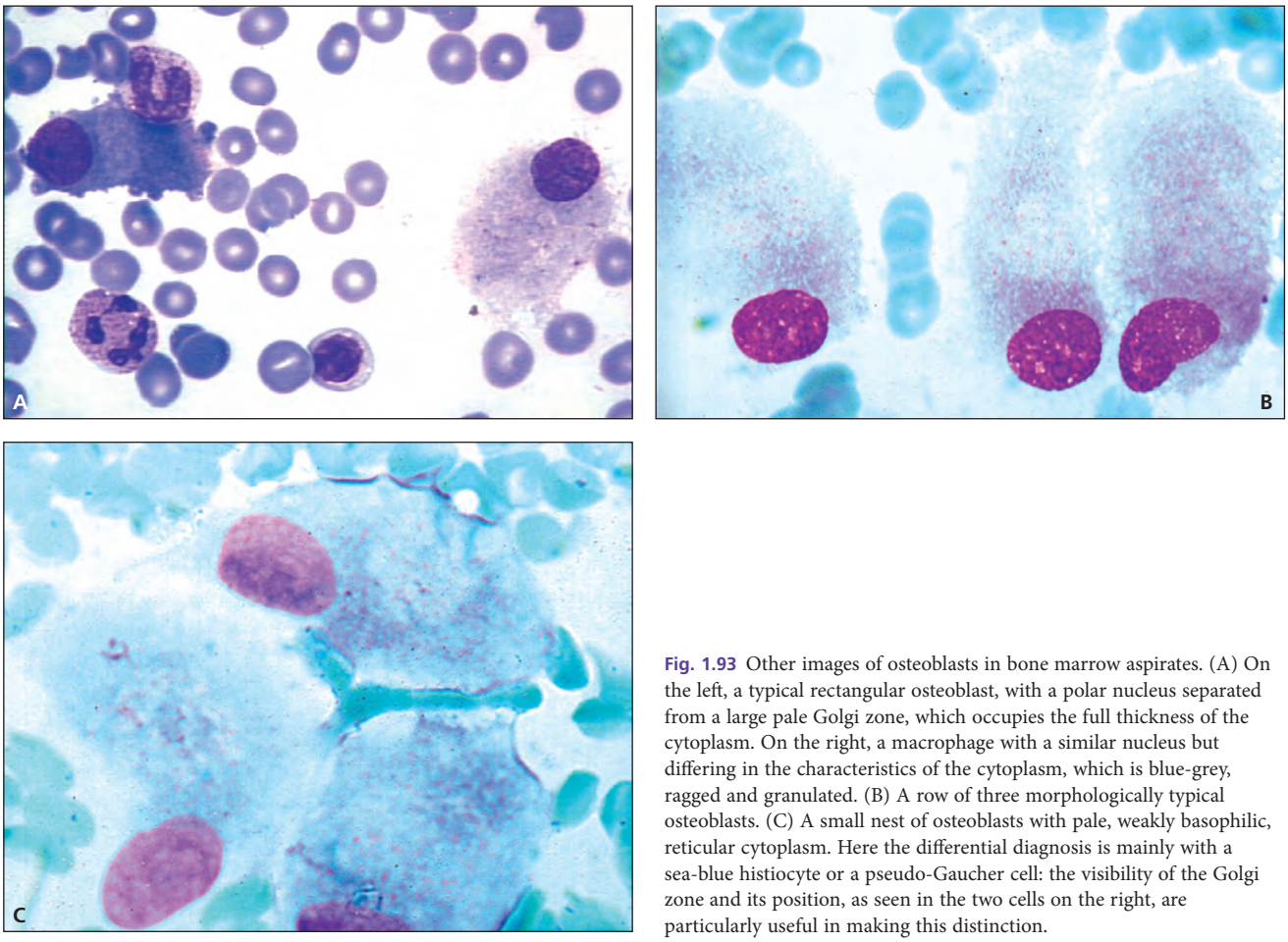



**Fig. 1.93** Other images of osteoblasts in bone marrow aspirates. (A) On the left, a typical rectangular osteoblast, with a polar nucleus separated from a large pale Golgi zone, which occupies the full thickness of the cytoplasm. On the right, a macrophage with a similar nucleus but differing in the characteristics of the cytoplasm, which is blue-grey, ragged and granulated. (B) A row of three morphologically typical osteoblasts. (C) A small nest of osteoblasts with pale, weakly basophilic, reticular cytoplasm. Here the differential diagnosis is mainly with a sea-blue histiocyte or a pseudo-Gaucher cell: the visibility of the Golgi zone and its position, as seen in the two cells on the right, are particularly useful in making this distinction.

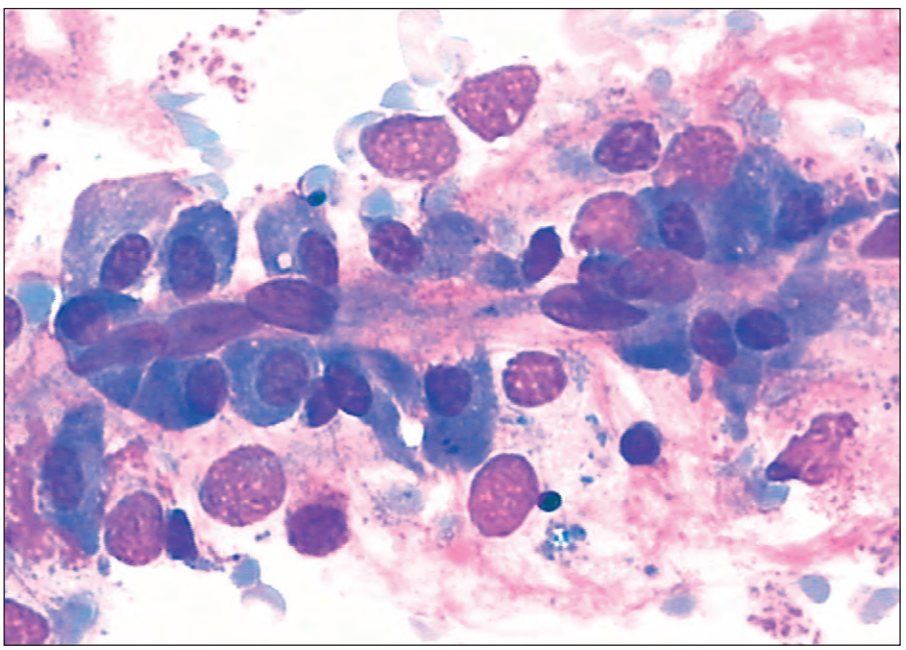
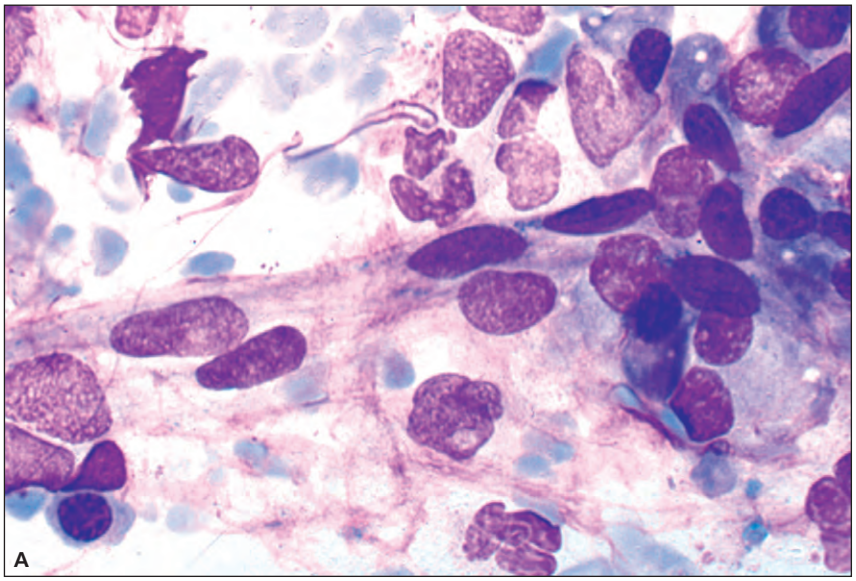
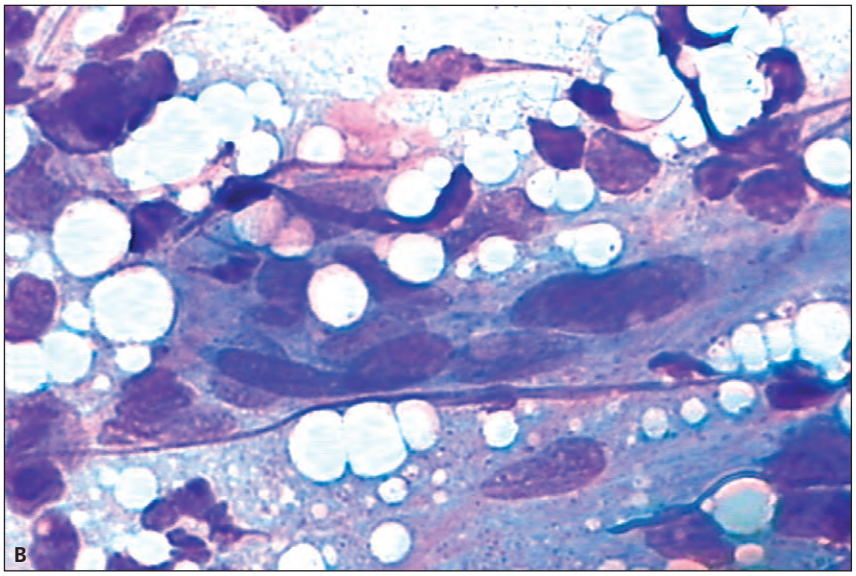


**Fig. 1.94** The fusiform nuclei of these endothelial cells of a sinusoid are arranged in Indian file across the centre of the image. Around them are crowded, in an orderly manner, numerous plasma cells. Further away are the spongy nuclei of macrophages or reticulum cells.



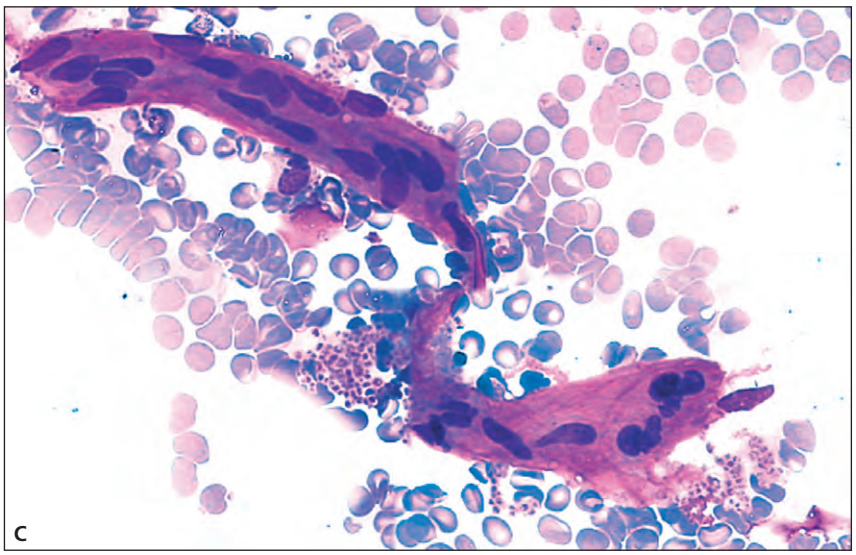




**Fig. 1.95** (A–C) Endothelial cells of sinusoids in bone marrow aspirates. These stromal cells are fusiform and are usually only recognisable when they have been pulled out during bone marrow aspiration and are lined up in the long apparently syncytial files that form the wall of the sinusoids.

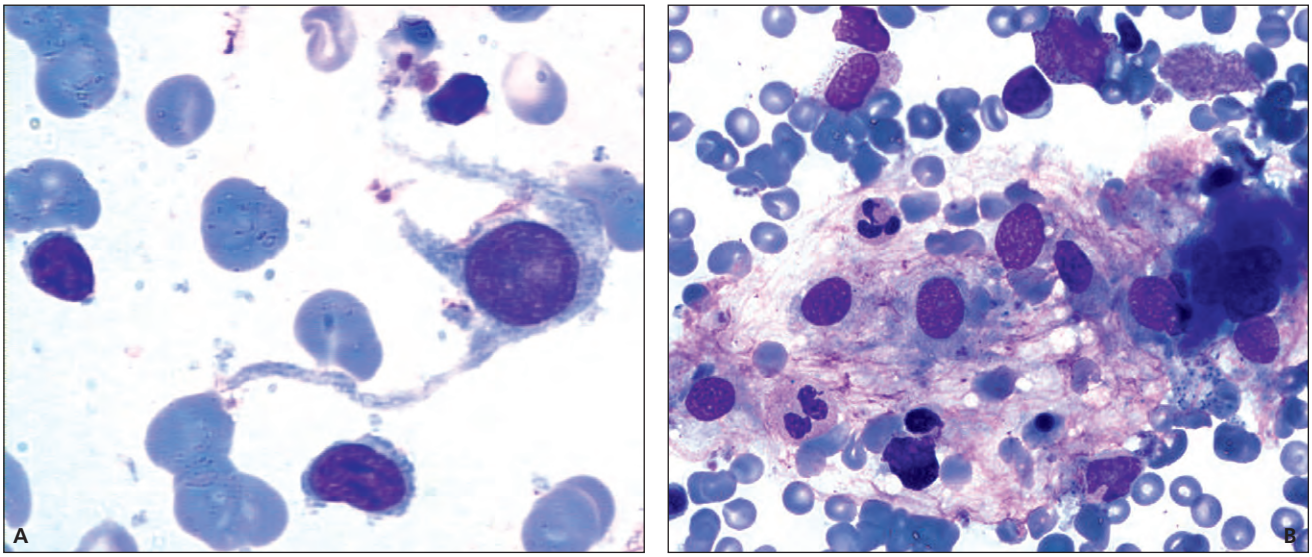



**Fig. 1.96** Adventitial reticulum cells in bone marrow aspirates. (A) The features of this cell are typical: spongy oval nucleus, relatively scanty cytoplasm forming long, thin, tentacle-like projections, which in fact are the result of the process of aspiration. (B) In this fibrohyaline structure the nuclei of the reticulum cells appear almost bare with pale greyish cytoplasm. On the right, a young basophilic megakaryocyte.

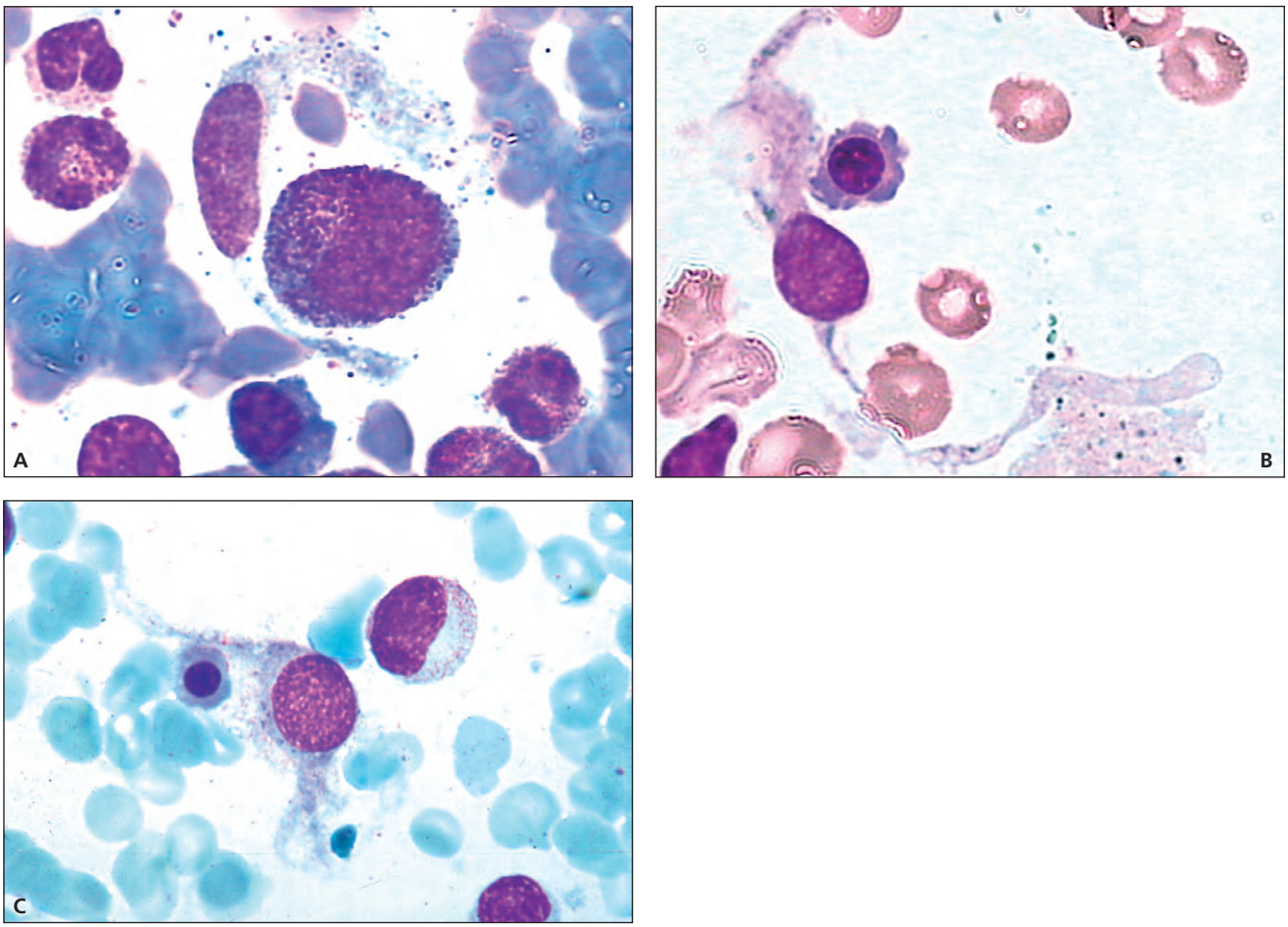



**Fig. 1.97** Adventitial reticulum cells in the bone marrow. (A) A reticulum cell with a fusiform nucleus appears to surround a neutrophil promyelocyte by means of its two frayed cytoplasmic tails. (B) One notes in particular the delicacy of these long strands of cytoplasm; adjacent to the reticulum cell, a polychromatic erythroblast. (C) The spongy nucleus and the frayed cytoplasm are characteristic of cells of this lineage.

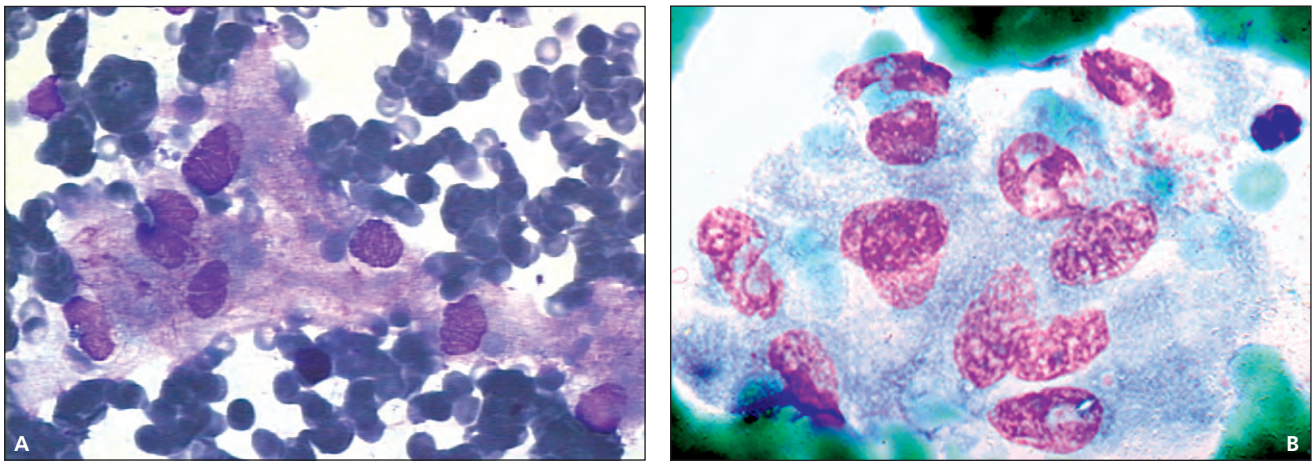



**Fig. 1.98** Small groups of reticulum cells in bone marrow aspirates. Images of this type, because of the compactness of the cluster, the lack of signs of differentiation and the monomorphism, can easily lead to the suspicion of bone marrow metastases, which can be excluded by careful evaluation of the cellular morphology and the cytological context. (A) A syncytium of reticulum cells. (B) A three-dimensional mass of reticulum cells, partly fused to each other. One notes the transparency of the nuclei when they are superimposed, a feature which would not be at all common in metastatic cells.

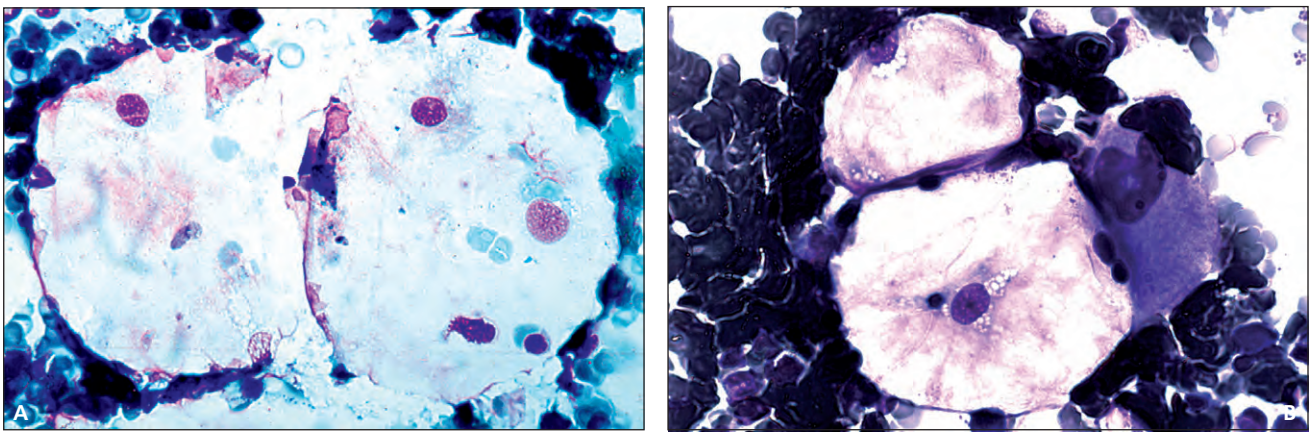



**Fig. 1.99** Adipocytes in bone marrow aspirates. (A) A pair of adipocytes: same shape, same size, eccentric nuclei curiously located at the same level. (B) Two well-preserved adipose cells with typical features. The cytoplasm is swollen and almost empty; the nucleus appears spongy: in the cell at the top, the nucleus appears to be squashed at one pole, while in the lower cell it is in a central position. On the right, a megakaryocyte with a lobed, folded nucleus and dark cytoplasm.

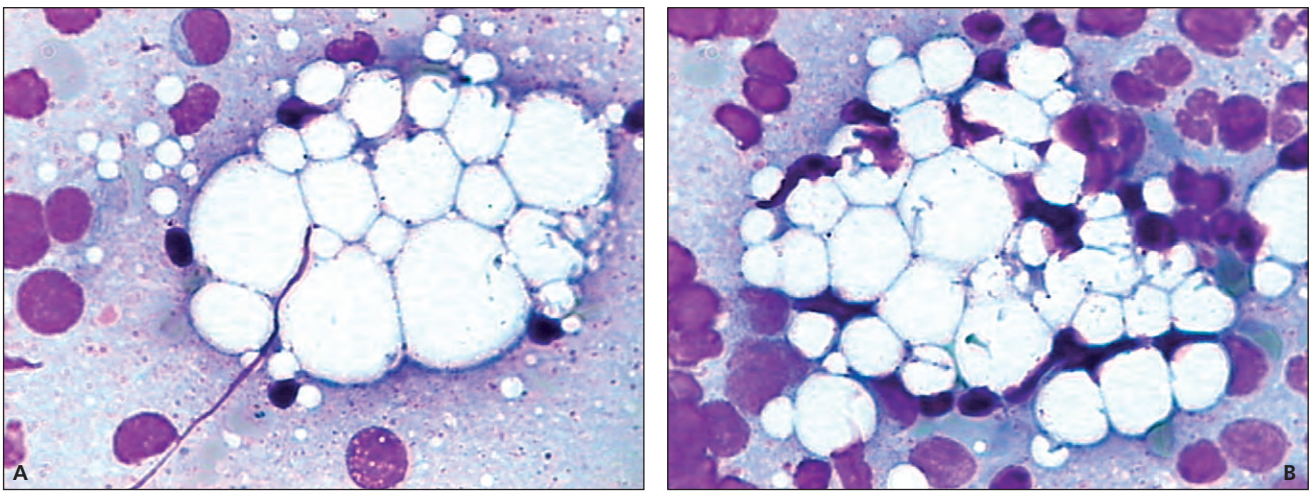
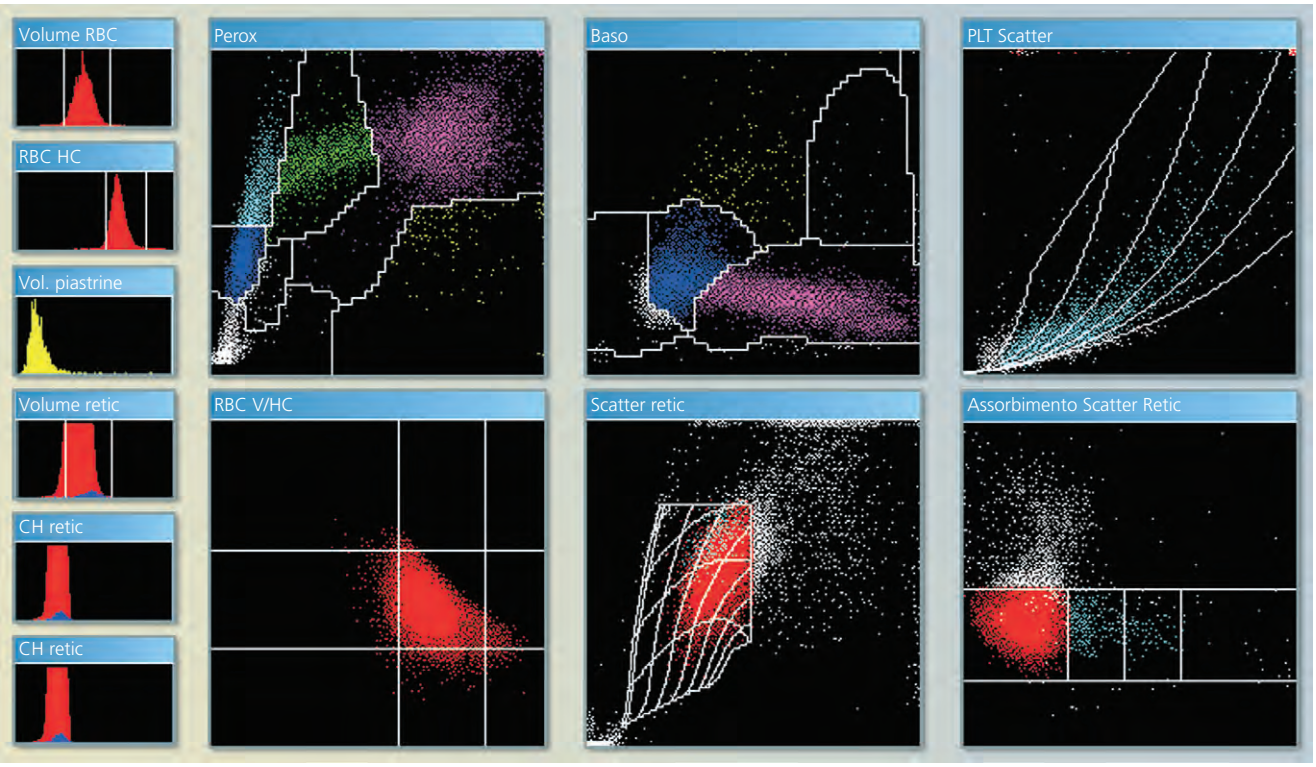



**Fig. 1.100** (A and B) Formation of adipocytes. The accumulation of lipid within the cytoplasm of reticulum cells leads to the formation of deposits of fat, which after the dissolution caused by fixation in alcohol appear optically empty.

## REPORT 1.1 Infectious mononucleosis in a 19-year-old girl




| Parameter | Value | Units | Flags |
|---|---|---|---|
| HGB | 121 | g/l | |
| RBC | 4.28 | $\times 10^{12}$/l | |
| HCT | 0.37 | l/l | |
| MCV | 86.4 | fl | |
| MCH | 28.3 | pg | |
| MCHC | 328 | g/l | |
| CHCM | 324 | g/l | |
| RDW | 13.4 | % | |
| HDW | 2.46 | g/dl | |
| HYPO | 2.0 | % | |
| HYPER | 0.5 | % | |
| MICRO | 1.0 | % | |
| MACRO | 0.2 | % | |
| RBC fragments | 0.02 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 0.79 | % | |
| RET | 33.6 | $\times 10^{9}$/l | |
| CHr | 28.3 | pg | |
| IRF (M + H) | 6.9 | % | |
| PLT | 278 | $\times 10^{9}$/l | |
| MPV | 8.4 | fl | |
| PDW | 48.7 | % | |
| Large platelets | 0.0 | $\times 10^{9}$/l | |
| MPC | 21.7 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flags |
|---|---|---|---|
| WBC Baso | 11.61 | | |
| WBC Perox | 12.01 | | |
| NEUT | 3.89 | 33.5 | |
| LYMPH | 5.27 | 45.4 | |
| MONO | 1.13 | 9.7 | |
| EOS | 0.14 | 1.2 | |
| BASO | 0.16 | 1.4 | |
| LUC | 1.03 | 8.9 | ATYP ++ |
| NRBC | 0.00 | 0.0 | |
| LI | 2.06 | | |
| MPXI | 6.1 | | |
| MN | | 53.4 | |
| PMN | | 44.8 | |
| BLASTS | | 2.3 | Blasts + |
| Perox HPX | | 1.4 | |
| Perox SAT | | 0.7 | |

Moderate lymphocytosis with an increase of lymphocytes and LUC, which in the Perox cytogram form a short, stubby but pointed cluster. In the Baso cytogram it is interesting to note the increased density of signals at the bottom at the extreme right of the PMN tail, similar to that produced by erythroblasts with pyknotic nuclei. The erythrocyte, reticulocyte and platelet parameters do not show any anomalies.

**Morphology.** The white cell differential count is characterised by 30% of reactive lymphocytes with the morphological characteristics of infectious mononucleosis in florid stage. The ATYP flag is the result of increased LUC (>4.0%), and this is the reason that a BLAST flag is also present, although there are only 2.3% of events to the left of the threshold in the Baso cytogram. The signals to the right of the PMN tail correspond to apoptotic lymphocytes, which are observed in the blood film, with nuclei having disintegrating chromatin.

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CHAPTER 2

# Erythropoiesis and Anaemia

Is anaemia a symptom or a disease? The response to this classic question, which is neither obsolete nor purely academic, is not straightforward: the answer can emerge only case by case, following a path that is sometimes simple and sometimes intricate, creating a diagnostic picture and classification. Establishing the diagnosis of an anaemia indicates:

1. Identifying in the individual patient the nature of the specific condition that is altering his erythropoietic balance;
2. Recognising whether the anaemia is secondary, that is, a direct effect of a non-haematological disorder or, on the contrary, is a primary disorder with specific aetiological factors that are responsible for the deviation from normal erythropoiesis.

The importance of the two-fold approach (primary or secondary?) is found in its therapeutic relevance, indicating a specific therapeutic strategy directed either at the cause of a primary haematological disorder or, in the case of a secondary anaemia, at the underlying illness. In the latter instance, supportive care, in particular blood transfusion, can be of critical importance to avoid the effects of poor tissue oxygenation while awaiting the favourable impact of treatment directed at the underlying disorder.

The erythroid system[1,2] is a complex, dynamic system that has the primary role of transporting oxygen to all the tissues of the body: this system, despite its structural fluidity and the absence of anatomical limitations, must be considered an organic and functional unit, which includes:

- Bone marrow erythropoiesis – the haemopoietic stem cells and erythroid progenitors and precursors;
- Circulating erythrocytes;
- The cellular, humoral and biochemical controls of erythropoiesis;
- The essential raw materials, particularly iron, vitamin $B_{12}$ and folic acid.

Each of these fundamental components is divided in turn into various elements, which need to be co-ordinated to maintain the harmonious efficiency of the whole. All anaemias are the result of defects, more or less specific, primary or secondary, located at specific points in erythroid development, which determine the block in erythropoiesis and the resultant changes, which are often compensatory but sometimes lead to further damage. The diagnostic effort is directed at identifying the stage of the process at which the defect has occurred.

Erythropoiesis can be defined as the process, occurring in the bone marrow, by which reticulocytes are produced: these then enter the circulation, rapidly completing their maturation into fully functional erythrocytes. The haemopoietic matrix is composed of a dynamic mass of cells in various stages of maturation: pluripotent stem cells, erythroid progenitors and morphologically recognisable erythroblasts, the latter often organised into erythroblastic islands with a central macrophage, the visible correlate of the erythropoietic niche[3] (Figs 2.1, 2.2 and 2.3). The denucleation of the erythroblast is the process that leads to formation of a reticulocyte, the final bone marrow component of the erythroid series. The rate of formation is two million reticulocytes per second. After three days, the bone marrow reticulocytes emerge into the peripheral blood, with the daily release of 250 billion cells, which in stable conditions balance the number of erythrocytes removed from the circulation at the end of their life. This medullary matrix of erythroid precursors, although enormous, is ten times smaller than the mass of erythroid cells that circulate in the blood stream, which in the adult is equal to 2 litres (24–32 ml/kg in men and 21–27 ml/kg in women). The red cells, the mature functional crux of the system, are continuously renewed, with an average survival of individual cells of 100–120 days and a rate of renewal in basal conditions of around 1% of cells per day. In normal circumstances, the process is remarkably stable, but in order to maintain the constant final product, the output of erythrocytes can increase, with suitable stimulation, to ten or even 20 times the basal rate.

Homeostasis of the erythroid system is of fundamental importance, because of the marked dynamicity of the process, which renders it labile and susceptible to interference at all levels. Tissue oxygenation is at the same time the primary aim of erythropoiesis and the essential signal that achieves homeostasis, both in basal conditions and when there is a response to physiological or pathological processes. The sensors of oxygen pressure are found in the renal parenchyma. Local ischaemia induced by ligation of the single renal artery induces a general-

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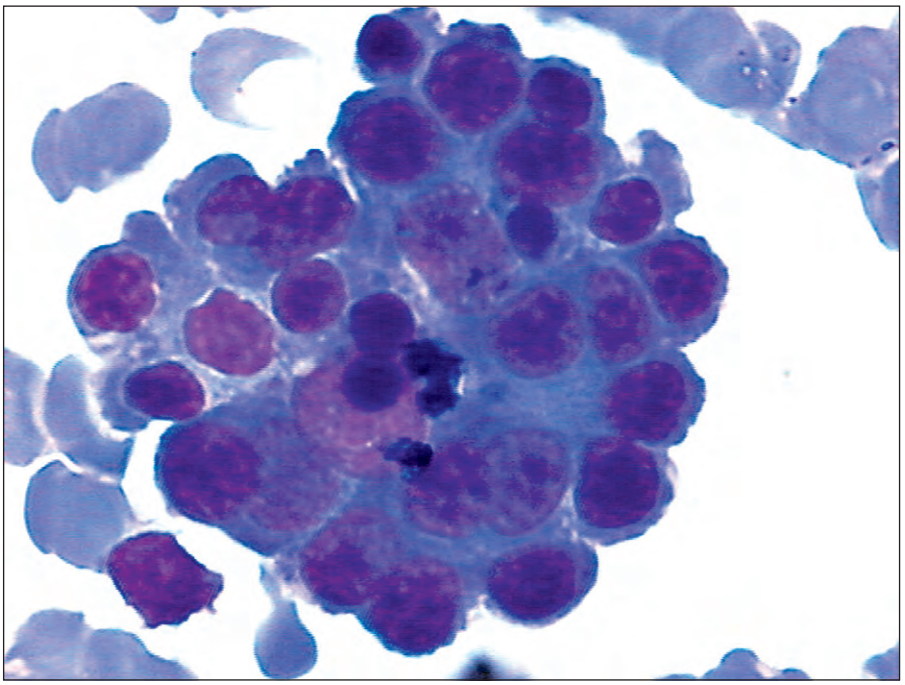


**Fig. 2.1** Erythroid island with more mature cells to the periphery. Bone marrow aspirate. This island is composed mainly of cells in the early phases of erythroid maturation. The lightly staining nucleus in the centre, covered by other small pyknotic nuclei, belongs to a macrophage. It is surrounded by proerythroblasts, basophilic erythroblasts and, towards the periphery, polychromatic erythroblasts.

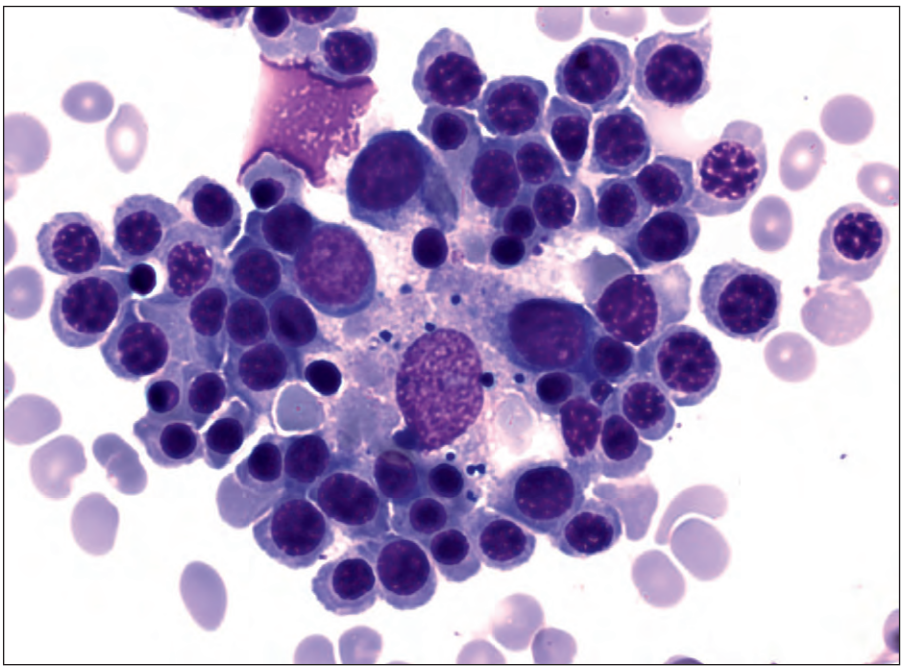


**Fig. 2.2** Bone marrow aspirate. A large erythroid island with cells in an advanced stage of maturation, these being mainly polychromatic and orthochromatic erythroblasts. There are some basophilic erythroblasts in the centre, in contact with a macrophage, the latter showing signs of phagocytic activity and having in its cytoplasm a pyknotic nucleus and the cytoplasm of erythroid cells.

ised erythroid response. The cells that play this role are similar to highly specialised fibroblasts, localised in small groups outside the basal membrane of the renal tubule, in the interstitial spaces of the inner cortex and the outer medulla. The concentration of haemoglobin in the circulating blood is the principal factor that determines the tissue pressure of oxygen: if this varies, as in anaemia, the oxygen available to the kidney is altered, bringing homeostatic forces into play. This is explained by a major feedback loop activated by intracellular hypoxia and based on the production and liberation into the circulation, by these sensing cells, of a peptide of 166 amino acids, 30% glycosylated, which is the hormone and stimulating factor for

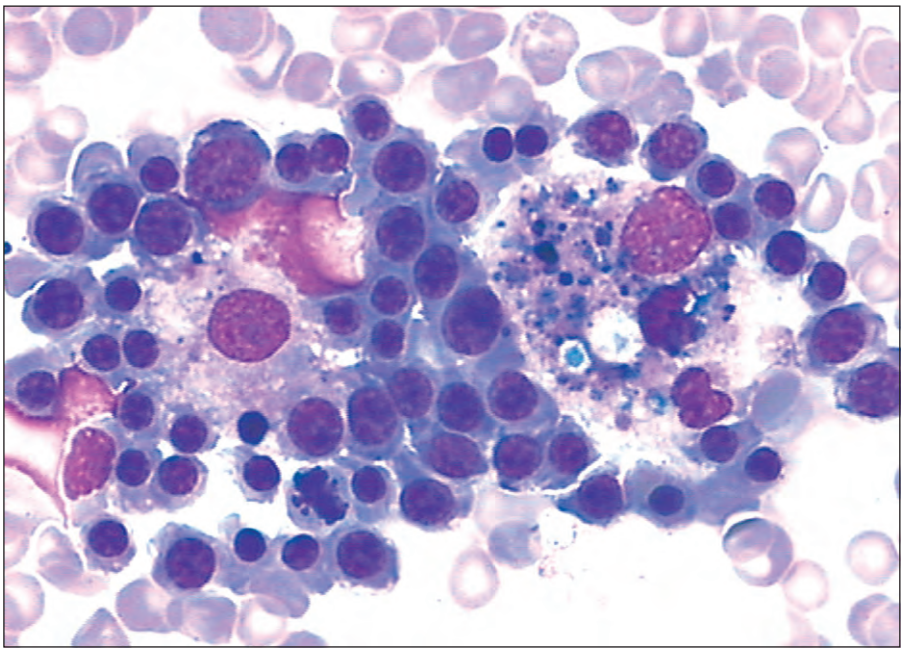


**Fig. 2.3** Bone marrow aspirate. Erythroid islands are usually composed of heterogeneous groups of erythroblasts arranged around a central macrophage. There are two macrophages, recognisable from their plentiful cytoplasm with poorly defined cell margins and, particularly, from the spongy chromatin of the nucleus with a prominent bluish nucleolus, and from the well-defined nuclear membrane. The macrophage on the left, in which the cytoplasm appears pale and heterogeneous, but contains only one red cell and a few granules, is clearly surrounded by erythroid cells: these are mainly polychromatic and, to a lesser extent, orthochromatic erythroblasts; a basophilic erythroblast stands out at the top and a polychromatic erythroblast arrested in metaphase is seen below the macrophage. The picture suggests that this macrophage is a 'nurse cell', which furnishes iron and other molecules to the erythroblasts, by means of the process known as rhopheocytosis. The macrophage on the right, on the other hand, appears to function more as a 'road-sweeper'; there are fewer surrounding erythroblasts, polychromatic and orthochromatic, but its cytoplasm contains numerous inclusions, including phagocytosed nuclear material, which has certainly been derived from the final phase of erythropoiesis, the loss of the nucleus. The global picture suggests moderate dyserythropoiesis.

erythropoiesis, designated erythropoietin (EPO). In man, only 7% of EPO is synthesised by hepatocytes.

In stable conditions, the production of EPO is reduced to the minimum, so that the erythrocyte mass and the haemoglobin concentration (Hb) remain stable in the face of daily losses through red cell senescence. A lack of oxygen increases the number of oxygen-sensing cells in the kidney, but in particular leads to numerous modifications within these cells that finally result in increased transcription of the *EPO* gene, i.e. synthesis of the relevant mRNA, and thus the synthesis and secretion of the newly synthesised EPO. The molecular mechanisms through which oxygen tension acts are known[4]: the protein HIF-1 (hypoxia-inducible factor 1), and in particular its subunit HIF-1α, is constitutively produced by these cells, but survives for only a brief time, because of its immediate binding to the Von Hippel-Lindau protein (VHL), which leads to its degradation through the ubiquitin-proteosomal complex.[5] For this to happen, the hydroxylation of the proline of HIF-1α is essential and this requires an enzyme that has oxygen as a cofactor. If there is insufficient oxygen, HIF-1α is not degraded and can thus bind to HIF-1β, forming a heterodimer, which in turn binds to the enhancer of the *EPO* gene on chromosome 7, activating it.

In this way the production of EPO is directly regulated by the availability of oxygen. In this very efficient system, it is not the blood concentration of EPO that is the regulating factor, but the end result of the process it activates, i.e. the tissue availability of oxygen, which controls the release of EPO by the sensing cells. There are various elements in the feedback loop, in addition to variation in the Hb, that can influence the tissue pressure of oxygen and thus the control of the system: reduced arterial oxygen saturation because of cardiac or pulmonary disease, or living for some time at high altitude, for example, can induce erythrocytosis; the same happens when globin gene mutations, leading to a high oxygen affinity haemoglobin, prevent the release of an adequate amount of oxygen to tissues. Initial effective compensatory mechanisms for anaemia or hypoxia are represented by an increased heart rate and respiratory rate, and an increase in erythrocyte 2,3-diphosphoglycerate (2,3-DPG) that favours the release of oxygen to tissues.

The increased concentration of EPO induced by hypoxia acts on the bone marrow erythroid progenitors, leading to an increase in the number of erythroblasts in the process of differentiation and maturation and thus increasing the mass of circulating erythrocytes, so that the tissue effects of hypoxia are amelio-

rated.[6,7] The interaction between the EPO molecule and its cellular receptor (EPO-R) is another crucial point in the control of erythropoiesis. The first progenitors committed to erythropoiesis, known as BFU-E (erythroid elements that form 'bursts', explosive colonies on a tissue culture plate), still have only a low sensitivity to EPO, since the density of receptors on their membrane is low. The later progeny, both the CFU-E (forming smaller colonies) and the proerythroblasts (the first morphologically recognisable erythroid precursors) are, however, rich in EPO receptors: CFU-E and proerythroblasts are thus the principal cellular targets of EPO. In the pathway of maturation from this stage onwards, EPO-R progressively diminish, until they disappear at the stage of orthochromatic erythroblasts. Each EPO molecule binds to two EPO-R on the cell membrane, inducing a conformational change that brings their intracytoplasmic domains into close proximity to each other (homodimerisation), thus activating the phosphorylating function of the JAK2 tyrosine kinase with which they associate. The phosphorylation of these and other substrates, such as STAT1 and STAT5 proteins, activates their transcription factor function for numerous genes implicated in the erythroid response to EPO.

In the erythroid series, proliferation and differentiation-maturation occur in a co-ordinated and harmonious fashion.[8] Investigations of *in vitro* cultures have shown that the first type of erythroid progenitor, the BFU-E, in 6–8 days gives rise to its progeny, the CFU-E, and it is these in turn that, after another 5–7 days of proliferation and differentiation, give rise to proerythroblasts. EPO acts particularly on these cells to inhibit apoptosis and thus promote survival: in other words, in the absence of EPO, erythroid progenitors undergo massive programmed cell death, which EPO is able to prevent, thus permitting the process of proliferation and differentiation to continue. Less well established, on the other hand, is whether EPO also has an instructive effect, directing differentiation of pluripotent stem cells towards erythropoiesis. In any event, in the presence of EPO, the CFU-E and their progeny show generalised metabolic activation with increased synthesis of RNA, of globin chains and of proteins of the membrane cytoskeleton.

The proerythroblasts proliferate with a series of three, four or five cellular divisions, which occur simultaneously with two fundamental cytoplasmic processes that distinguish erythroid differentiation: nuclear degeneration and the cytoplasmic synthesis of haemoglobin.[9] From one proerythroblast originate a number of orthochromatic erythroblasts, varying from eight, with three mitoses, to 32, if there are five mitoses (Fig. 2.4). The orthochromatic erythroblast has completely condensed

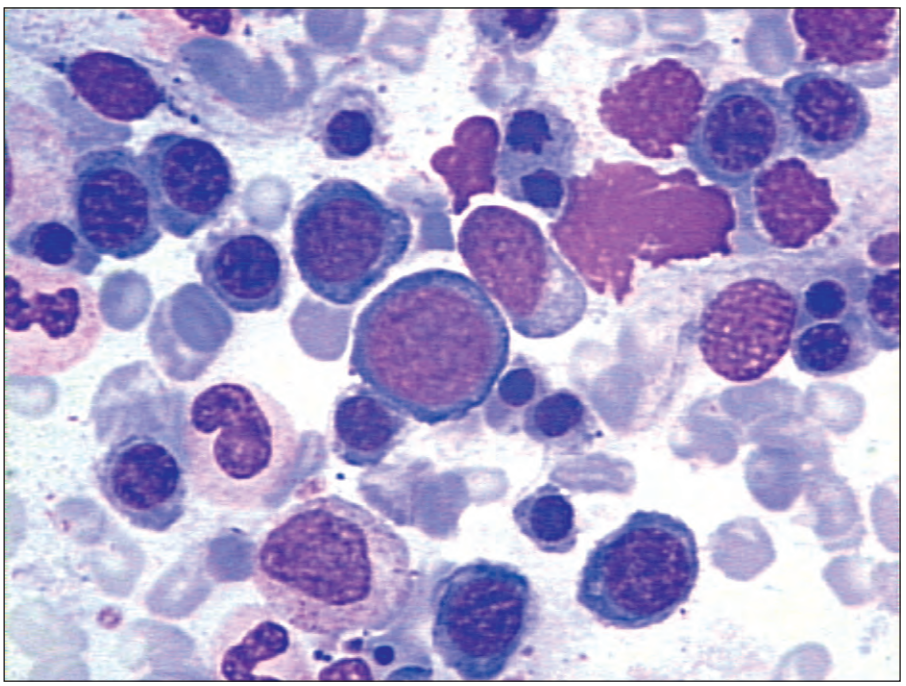


**Fig. 2.4** Normoblastic erythropoiesis. Bone marrow aspirate. The erythroid precursors in this field show cells at all stages of maturation: a proerythroblast, with plentiful blue cytoplasm and a large round nucleus that is distant from the cell membrane, with homogeneous chromatin apart from small areas of condensation and some nucleoli. Above it to the left, a much smaller basophilic erythroblast with denser chromatin. Still further to the left, three polychromatic erythroblasts, two early and one mature, the nuclei of which have a thick nuclear membrane and clumps of chromatin separated by pale spaces. On the right and below the central proerythroblast, small orthochromatic erythroblasts and on the right and above, a blast cell that does not show any signs of differentiation. Given that the bone marrow appears normal, it could be a multipotent myeloid progenitor. Above this cell, an erythroblast with almost orthochromatic cytoplasm, in telophase. In the top left corner, a cell with an ovoid nucleus and elongated cytoplasm that represents an adventitial reticulum cell.

chromatin, pyknotic and inactive: it is no longer capable of synthesising DNA or RNA, and thus cannot divide any further. The expulsion of the nucleus, which is an active process similar to cytodieresis, is promoted by the action of actin. It signals the birth of the reticulocyte, in which the synthesis of haemoglobin continues at a slower rate for another three days of intramedullary life. At the moment of release into the circulation, the reticulocytes have accumulated a quantity of haemoglobin more or less equivalent to that of the mature erythrocyte.[10]

The morphological characteristics of erythroid differentiation have been described in Chapter 1.

## Definition of anaemia

The numerous possible definitions of anaemia have a unifying element: the reduced capacity of the blood to supply oxygen to tissues. For example, the last edition of *Wintrobe's Clinical Hematology* defines anaemia by a functional approach, as the presence of "an erythrocyte mass that is insufficient to transport and release the necessary quantity of oxygen to the tissues".[11]

Similarly useful on the conceptual and physiological plain, but of little use in daily laboratory practice because of the technical difficulties in its measurement, is the definition of anaemia as "reduction of the total content of haemoglobin in the body".

Adopting, on the other hand, a more appropriate operational and clinicopathological approach, anaemia can be defined as "the condition in which the concentration of haemoglobin, measured by automatic methods and expressed as grams per unit volume (g/dl or g/l), is reduced below the reference limit specific for the age and gender of the individual". The World Health Organization (WHO) has defined anaemia in adults (above 15 years of age) as "a value of haemoglobin below 13 g/dl in men and below 12 g/dl in women".[12] These values correspond to the lower limit of a reference range that represents the central 95% of values in a healthy adult population, expressed as the mean ±2 standard deviations, and equal to 15 ± 2 g/dl in men and 13.5 ± 1.5 g/dl in women (i.e. 150 ± 20 g/l in men and 135 ± 15 g/l in women).[13] A recent authoritative re-evaluation, however, suggests increasing significantly the value for Hb below which a diagnosis of anaemia is made: 137 g/l for adult white males between 20 and 50 years; 132 g/l for adult white males above the age of 60 years; 122 g/l for all white women above the age of 20 years. In black subjects (African-Americans) these limits must be reduced by 6–8 g/l.[14] These limits were established from population surveys and reflect intra-individual variation. It is predictable that in the future the quantitative diagnosis of anaemia could be based on an evaluation that took into account intra-individual variation, using the concept of the delta check (variation observed between one measurement and another in the same subject)[15] and using longitudinal profiles analysed according to the rules of Bayesian statistics, thus extending into the diagnostic field techniques until now only applied in the biological passports of athletes.[16,17]

Among the quantitative measurements supplied by haematological analysers, the determination of Hb in a venous blood sample taken into EDTA is the most efficacious method for establishing both the presence and the severity of any anaemia. All instruments do this by measurement of optical density or absorbance of light by an aliquot of the blood sample haemolysed by the addition of specific reagents. These reagents lead to the transformation of haemoglobin into stable derivatives, which give reproducible results by spectrophotometry or colorimetry, measurements in general being made at 534–540 nm. With the most recent completely automated instruments, the analytical error in the measurement of Hb is extremely small, around 1%, a great deal less than the degree of error that is acceptable for diagnostic purposes.[18] The results of the estimation can be expressed, as suggested by the International Council for Standardization in Haematology (ICSH), in grams per litre (g/l) but are also sometimes expressed in grams per decilitre (g/dl). In fact, converting one unit to the other merely requires a simple multiplication or division by a factor of ten: for example, an Hb value of 10 g/dl corresponds to 100 g/l. The adoption of molar concentration (μmol/l) does not have any particular advantage, and with the exception of a small number of countries, it has not come into general use in practice or in the scientific literature.

As for other quantitative haematological values, the haematocrit (Hct) and the red cell count, inter-individual variability in Hb in the context of reference values – in the absence of disease – is much greater than intra-individual variation. The Hb, Hct and red cell count of each individual are determined, in large part, by inherited genetic factors, as has been demonstrated in studies in twins.[19] Recently, this has been explored further by automated scanning of the entire genome, seeking loci for quantitative traits, which has shown a link between red cell variables and a locus at 6q23.[20]

The Hct provides a quantitative estimate of the capacity of the blood to transport oxygen to tissues; it is equivalent to the Hb, but it is less reliable for both pre-analytical and analytical reasons. Historically, the Hct indicates the proportion of a column of whole blood occupied by red cells after a blood sample has been centrifuged in a graduated glass tube. This method, designated packed cell volume (PCV), was originally standardised by Wintrobe in 1929. The techniques of centrifuging anticoagulated blood in a graduated glass tube or a capillary tube (microhaematocrit) have today been largely abandoned, both because of the inherent relative inaccuracy and imprecision attributable to the variability of the amount of plasma that remains between the packed red cells (the 'trapped plasma'), and because automated methods actually measure the volume of erythrocytes directly and calculate the Hct only indirectly as the product of the red cell count (RBC) and the mean cell volume (MCV). Some instruments, such as those of Sysmex, carry out

a more 'direct' measurement of Hct as the sum of the electronic impulses measured by the transducer; from this value, divided by the RBC (or rather the number of impulses), the MCV can be calculated: there do not seem to be any implications, in terms of quality, of this different approach.

The Hct values obtained in this manner appear to be an underestimation in comparison with a microhaematocrit corrected for trapped plasma, to the extent of interfering with the correct monitoring of patients with polycythaemia vera.[21] From the diagnostic point of view, however, the Hct does not add useful information for determining the presence or severity of anaemia, and, being calculated indirectly from direct measurements (MCV and RBC), is more sensitive to artefacts than is the Hb.[22] Among these artefacts, the most common is the swelling of red cells as the result of storage of samples for too long a time or at an inappropriate temperature; this can increase the MCV. The widespread idea that the Hct is a better measure of the 'red cell mass', and therefore is more useful in the diagnosis and monitoring of polycythaemia, is false; even though it has no scientific basis, this idea is nevertheless difficult to get rid of. In effect the Hct, like the Hb, is a measure of the concentration in the plasma (in this case, not of molecules but of the cells that contain them): as such the Hct and Hb respond in the same way to alterations in the plasma volume associated with haemodilution – increased plasma volume (as occurs in endurance athletes or during pregnancy), or with haemoconcentration – reduced plasma volume (e.g. immediately after very intense exercise or in so-called 'stress polycythaemia'). The use of the Hct as a way of screening for anaemia can produce an increased number of false negatives.[23] In the anaemia of malaria, for example, the Hct obtained by centrifugation underestimates the prevalence and the severity of anaemia, particularly in children.[24,25] This is important for assessment of anaemia in under-developed countries with a high incidence of illnesses causing anaemia; in these countries, the use of the microcentrifuge can be convenient and permits the practice of estimating the Hb by dividing the microhaematocrit by three.

Before the introduction of automated methods, the RBC was determined using a counting chamber and a microscope, after dilution using a graduated pipette: its principal use was for calculating the haemoglobin index or colour index, using the formula: [haemoglobin (percentage of normal)/(2 x RBC) (first two figures of the RBC)]. For example, in a normal male, the colour index was equal to $[100/(2 \times 50)] = 1$. The colour index, still cited on American websites as useful in the classification of anaemia, is a relic of the past. The RBC, however, cannot be used to evaluate anaemia, since it does not reflect the capacity for oxygen transport in microcytic anaemias (in which it tends to overestimate this capacity) or in the macrocytic anaemias (in which it tends to underestimation). For example, a patient with beta thalassaemia minor and an MCV of around 65 fl can have an increased RBC but an Hb that is actually below normal. The only value of the RBC is in quality control of automated instruments and in the differential diagnosis of microcytic anaemia, when a value above $5 \times 10^9$/l has a high degree of specificity for the diagnosis of thalassaemia trait.[26,27]

The measurement of Hb, while representing the best indicator of the capacity for oxygen transport, can nevertheless be inadequate for estimating tissue hypoxia in certain conditions. With ongoing massive haemorrhage, for example, the loss of as much as a litre of whole blood, does not immediately modify the Hb: the measured Hb accurately reflects the loss of red cell mass only after there has been sufficient time for extracellular tissue fluid to enter the blood stream (sometimes accelerated by the therapeutic administration of intravenous fluid), restoring the plasma volume and the total blood volume. The reduction of Hb attributable to acute blood loss may not be fully manifest, in the absence of treatment, for as long as 48–72 hours. Furthermore, alteration of Hb can reflect alterations in plasma volume rather than in the circulating red cell mass. A false impression of 'anaemia' from an Hb measurement can result from hypervolaemia during pregnancy or in patients with massive congestive splenomegaly ('pseudo-anaemia'). A minor increase in plasma volume, rarely clinically significant, can occur in supine patients in comparison with ambulatory patients. This can similarly occur in athletes during the first weeks of training and as a result of intense prolonged exercise, as in bicycle stage races. Conversely, the Hb can give a false impression of the total red cell mass, with an underestimate of the degree of tissue hypoxia, or creation of a spurious polycythaemia, when there is hypovolaemia due to severe dehydration, as in severe protracted diarrhoea in children, in severe burns when fluid is lost from cutaneous lesions or immediately after very intense physical effort. In the case of haemolysis, whether occurring within the blood stream in life or, alternatively, within the specimen tube in the laboratory, the Hb, although measured accurately and precisely, represents the sum of the haemoglobin in the red cells and in the plasma. In the colorimeter of the instrument, in fact, the red cells are completely lysed by the addition of the relevant reagents and the photometric measurement cannot make any distinction between plasma haemoglobin and erythrocyte haemoglobin. This situation can cause paradoxical changes in the red cell indices, their nature depending on the specific instrument. In instruments that measure erythrocyte haemoglobin concentration directly by an optical method, such as the ADVIA system (see Report 2.1), there is a discrepancy between the calculated mean cell haemoglobin concentration (MCHC), which is falsely high, and that measured directly, the cellular haemoglobin concentration mean (CHCM), which is not subject to interference from the free plasma haemoglobin.[28]

## First key diagnostic steps: erythrocyte dimensions

The diagnosis of anaemia is an organised process, sometimes simple, on other occasions more complex. It is a good idea to proceed step by step. The first step is to establish that there is

actually an anaemia, noting the reduction in Hb and assessing its severity. Measurement of Hb is precise and accurate, and given that there is adequate quality control in place, there is excellent standardisation within and between laboratories. However, the diagnosis of anaemia does not stop here: it is always necessary to proceed to classification, by means of a process resembling an inverted pyramid. The reduction of Hb with the resultant tissue hypoxia can be related to a fairly narrow range of essentially haematological or secondary disorders: with the use of standard instruments producing Hb and red cell indices and, if necessary, proceeding to more sophisticated tests, a systematic classification identifying the underlying cause becomes possible. The classification orients the investigator towards aetiology, pathogenesis and finally, when it is possible, indicates a specific efficacious therapy.

The now historical morphological classification of anaemia was tied to the above-mentioned colour index (of minor importance in hypochromic anaemias and of major importance in hyperchromic anaemias). The observation of red cells in the peripheral blood film permitted, in the pre-automation era, an evaluation of the diameter and intensity of staining of red cells; the idea of 'chromia' was considered together with an assessment of size (microcytic or macrocytic). Starting with Wintrobe's observations, based on standardisation of the method for measurement of the Hct, this method of classifying anaemias became more efficacious when it was possible to calculate red cell indices.

The three red cell indices, in the pre-automation era, were based respectively on dividing the PCV by the RBC to give the MCV, dividing the Hb by the RBC to give the mean corpuscular haemoglobin or MCH, and dividing the Hb by the PCV to give the MCHC. The study of red cell indices in large series of patients confirmed the existence of a group of hypochromic microcytic anaemias, already recognised morphologically for some time on the basis of the appearance of the erythrocytes in stained peripheral blood films: the simultaneous reduction of MCV, MCH and MCHC suggested iron deficiency anaemia or one of the numerous varieties of thalassaemia. The intuition of Wintrobe, which today appears elementary, represented a fundamental step in the evolution of modern diagnostic haematology. However, with the superior accuracy achieved today, the first key to the classification of anaemia, using the automated measurements available, is a single measurement: the mean corpuscular, or mean cell, volume (MCV; expressed in femtolitres).

Thirty years later, when the Coulter impedance method was applied to measuring the volume of red cells, it became evident that measurements made with the new technology showed marked discrepancies in comparison with measurements on the same blood samples using manual methods: in particular, with the automated instrument measurements, there was a systematic underestimation of the MCV and the Hct, which was more marked for microcytic specimens. The electronic measurement of erythrocyte volume, in fact, was more accurate than its calculation from the Hct obtained by centrifugation as a result of the phenomenon of trapped plasma. This caused an increase in manual measurements of 1.5–3% in normal blood samples; with less deformable erythrocytes, such as those from patients with iron deficiency anaemia, the proportion of trapped plasma rose to 5–6%.[29] The overestimation of the manual Hct led to an overestimation of the MCV, which made this measurement less sensitive for the detection of early changes in erythropoiesis: Wintrobe's original 1934 publication had already noted that 30% of subjects with iron deficiency anaemia and 45% of those with pernicious anaemia had a normal MCV. Another classical study had emphasised that a normal MCV was not rare among patients with mild or moderate iron deficiency anaemia.[30] The MCV measured directly by the impedance method, freed of the artefact of plasma trapping and independent of the RBC, was rapidly found to be more sensitive than the manual technique, e.g. as an early indicator of altered erythropoiesis in iron deficiency.

The precisely measured MCV is now the key element, in the presence of a reduced Hb, that permits the classification of anaemia in physiopathological terms. The minimal value of 80 fl and the maximum of 100 fl are derived from the scientific literature and are habitually used because of their ease of application. Some authors give slightly different reference intervals (e.g. $92 \pm 9$ fl, equal to a range of 83–101 fl).[13] There are no gender differences in the red cell indices. The precise automated measurements now available have permitted original observations: thus, for example, carriers of haemochromatosis have a tendency, within the range of normal, to slightly higher values for MCV and MCH in comparison with the population lacking these mutations.[31]

In the course of bone marrow erythroid maturation, the signal that leads to the condensation of chromatin into heterochromatin, no longer active once genes are no longer being transcribed, derives from the quantity of newly synthesised haemoglobin that accumulated in the cytoplasm. In fact, it is the haemoglobin that regulates the maturation of the nucleus and finally its expulsion. It penetrates through the pores of the nuclear membrane and, having reached a concentration of around 200 g/l within the nucleus, interacts with nuclear histones, favouring inactivation of the chromosomes and condensation of the chromatin.[32] This effect of haemoglobin on erythroblast nuclear chromatin explains the differences in cell size observed in various types of anaemia and explains the diagnostic value of the MCV. In particular, when the efficiency and velocity of haemoglobin synthesis are reduced or compromised because of a lack of iron or genetic defects in the synthesis of globin chains, the critical concentration of haemoglobin for the disactivation of nuclear function is delayed: the erythroblasts can thus divide one further time, generating the microcytes that are characteristic of iron deficiency and thalassaemia. It has recently been suggested that reticulocytes and erythrocytes, after their release from the bone marrow, undergo a further progressive reduction in their volume and haemoglobin content

and concentration, until a threshold of MCHC is reached that leads to their removal from the circulation.[33] This biological process would, in microcytic anaemias, undergo a compensatory slowing down, which it is possible to quantitate using statistical models that permit an early diagnosis of latent or compensated iron deficiency and its distinction from thalassaemia trait.[34] Conversely, in situations in which erythropoiesis is accelerated by an increased erythropoietin drive, the speeding up of haemoglobin synthesis leads to earlier nuclear degeneration and one or two nuclear divisions are missed: this explains the macrocytosis, often mild, that is seen in anaemia with a strong regenerative drive, in which reticulocytes in the circulation are numerous and immature. On the other hand, in macrocytosis due to megaloblastic erythropoiesis, one finds slowing down of nuclear function and cell division, without any compromise in haemoglobin production.

Values for MCV and MCH (like those for the RBC) show marked inter-individual variability in healthy subjects, but are relatively stable in a single individual. Recent studies have shown that these values, in the absence of any pathological process, are genetically determined in an analogous manner to the quantitative measurements, Hb and RBC, but independently of them: the genetic control of MCV, in particular, can be linked to a specific region of chromosome 11, close to the beta globin gene cluster,[35] and to a region of chromosome 6, adjacent to genes involved in erythroid expansion.[36]

The MCV is the first major contribution from automated instrumentation to determining the cause of anaemia. From this measurement alone, when the Hb is reduced, an effective differential diagnosis can be derived. The calculated MCH correlates closely with the MCV,[37,38] and does not have any independent diagnostic value. The value of the MCHC, calculated from the relationship between the Hb and Hct (and thus derived from three direct measurements), tends to remain constant over a wide range of values for MCV, reducing only in cases of severe protracted iron deficiency and increasing mainly in hereditary spherocytosis and in other rare related inherited haemolytic anaemias. In practice, the MCHC has become an excellent indicator of the stability of instrument calibration, being affected by pre-analytical and analytical anomalies that influence one or more of the three directly measured values from which it is calculated (MCV, RBC, Hb).

The primary classification of anaemia is thus now volumetric. In this way a straightforward evaluation of the MCV, already included in the full blood count (FBC) together with the Hb, permits the recognition among the multiple possible causes of anaemia that a case belongs to one of three groups: normocytic anaemia (MCV 80–100 fl), microcytic anaemia (MCV $<$80 fl) or macrocytic anaemia (MCV $>$100 fl). Within each of these groups, one has a much narrower range of diagnostic possibilities, and thus investigations can be targeted and specific for individual conditions.

A reduced MCV indicates, in addition to reduced size, a reduction in the haemoglobinisation of erythrocytes: the reduction in the total quantity of haemoglobin in the circulation and the total erythrocyte mass is proportionally greater that the reduction in the number of red cells. It is the production of haemoglobin that is defective, while the proliferative mechanisms of the erythroblasts are preserved. Microcytosis is therefore the mark of a defect in the synthesis of normal haemoglobin molecules; the abnormality may be either quantitative or qualitative (due to a lack of iron, thalassaemia or a haemoglobinopathy such as haemoglobin E with a reduced rate of beta chain synthesis). In subjects with mild or moderate anaemia, the reduction in MCV is generally more marked in those with thalassaemia trait than in those with iron deficiency; on the other hand, when iron deficiency becomes severe, one can see MCV values below 55–60 fl. The RBC – a poor quantitative index of anaemia – underestimates the severity of tissue hypoxia when there is microcytosis and can be normal or even increased in thalassaemia trait.

When the red cell population is analysed by automated instruments, the term macrocytosis is reserved for those samples in which the MCV is above the upper limit of normal, usually taken as 98–100 fl. An MCV of 100–130 fl is normal in neonates and it can be even higher in premature neonates; in the first year of life there is a progressive reduction to 70–85 fl.

An increased MCV indicates a defect in cellular proliferation because of impaired synthesis of nucleic acids, as is seen in megaloblastosis: the reduced availability of vitamin $B_{12}$, folic acid or both in this condition slows down and deflects the process of DNA replication and cellular proliferation. Synthesis of haemoglobin is unimpaired, and the concentration that determines chromatin condensation and nuclear extrusion is reached when fewer mitoses than normal have occurred; many erythroid precursors do not reach a mature functional state and die in the marrow; others give rise to gigantic red cells, fragments and dysmorphic forms, often with nuclear remnants.

Macrocytosis with an analogous mechanism is observed after the intake of certain drugs. In fact, the most common cause of macrocytosis in hospital inpatients and outpatients is the intake of anticancer drugs (antifolates, purine and pyrimidine antagonists and alkylating agents) or antiretrovirals (which alter the synthesis of nucleic acids): macrocytosis is frequent and marked, particularly with the use of hydroxycarbamide (previously hydroxyurea) and zidovudine. In addition, anticonvulsant medications, antidepressants, oestrogens and sulphonamides can cause macrocytosis.

Macrocytosis, which is usually only moderate, is also caused by erythropoietic regeneration with a marked reticulocytosis, as in autoimmune haemolytic anaemia.

A mild isolated macrocytosis, with an MCV between 100 and 110 fl and a normal Hb, is relatively common, particularly at an advanced age. This sometimes remains unexplained even after extensive investigation, but cannot be ignored, since it may indicate early pernicious anaemia, occurring in advance of neurological signs.[39] The sensitivity of the MCV to vitamin $B_{12}$

deficiency, in the absence of anaemia or with only mild anaemia, is inferior to the sensitivity of morphological examination of the blood film and biochemical investigations.[40] In elderly patients with macrocytosis, abnormalities of haemopoietic stem cells have been described that suggest a relationship to the myelodysplastic syndromes.[41] A macrocytic anaemia associated with thrombocytosis is typical of the myelodysplastic syndrome associated with deletion of 5q.

Alcohol abuse is one of the most common causes of macrocytosis in apparently healthy adults (even in the elderly): the recognition of an MCV above normal is considered a useful sign of alcoholism.[42] Acetaldehyde, the primary metabolite of ethanol, is the main agent responsible for macrocytosis due to alcohol, because of its ability to form stable bonds with cellular proteins.[43] In 25–30% of cases of anaemia associated with alcohol excess, one can find ring sideroblasts in the bone marrow, the evidence of an alteration of mitochondrial metabolism and haem synthesis.[44] Very thin macrocytes, designated leptocytes, can also be the result of alteration in the lipid content of the erythrocyte membrane, as in liver disease, in which there can also be acanthocytes. Hypothyroidism also can cause an increased MCV. Macrocytosis is likewise observed in certain rare constitutional disorders, as in the erythroid hypoplasia of Blackfan-Diamond syndrome, congenital dyserythropoietic anaemia (types I and III) and Down syndrome.[45,46]

Finally, macrocytosis represents one of the most common laboratory artefacts attributable, among other things, to prolonged storage of the specimen without refrigeration. Extreme and bizarre changes in MCV (and even more so in other red cell indices) can be attributable to cold agglutinins and, rarely, can occur in other types of autoimmune haemolytic anaemia.

When the MCV is increased, the reduction in the RBC is generally more marked in relation to the reduction in the total mass of haemoglobin and thus the RBC overestimates the severity of the anaemia.

A normal MCV characterises normocytic anaemias, which include a large number of conditions that do not have a pathogenetic mechanism in common. The majority of anaemias secondary to systemic illness, acute or chronic, are normocytic, as long as the mechanism is not iron deficiency. Anaemia of chronic disease is normocytic with a tendency towards reduction of the MCV, which can sometimes fall to microcytic levels. Other normocytic anaemias have a tendency to a high MCV, sometimes reaching macrocytic levels (liver disease, hypothyroidism, post-haemorrhagic anaemia and acute haemolysis).

Determining the pathogenesis of a normocytic anaemia has two key steps:

- A reticulocyte count or at least the recognition of reticulocytosis;
- Assessment of the morphological features of a peripheral blood film.

Sometimes it is necessary to have recourse – in addition to other supplementary investigations – to a bone marrow aspirate, which may be supplemented by a trephine biopsy.

## Next key diagnostic steps: the erythropoietic response

In cases in which the immediate diagnostic picture, based on the MCV, is insufficient for a diagnosis and, particularly, for further evaluating the large intermediate category of normocytic anaemias, the second key step is the pathogenetic characterisation of anaemia according to the number of reticulocytes in the circulation. The reticulocyte count, whether expressed as a percentage or in absolute numbers, is the principal indicator of recent production of new erythrocytes. It thus provides a current reflection of bone marrow erythropoietic activity and, if the Hb is reduced, shows whether there is an effective response to the anaemia.

Reticulocytes are cells in transition between the nucleated erythroblasts, which normally reside in the bone marrow, and the mature red cells. The phase of maturation of reticulocytes, as has been established by studies of kinetics or radiolabelled iron, lasts on average, in stable conditions, for four days, three of which are generally passed in the bone marrow.[47] Thus, in stable conditions the reticulocyte spends just 24 hours in the circulation. Reticulocytes are defined by the presence of cytoplasmic material, composed mainly of ribosomal RNA, which is precipitated and stained by supravital stains such as new methylene blue or brilliant cresyl blue, appearing as granules and filaments[48] (Fig. 2.5). In addition to ribosomal RNA, these precipitates can contain various other organelles, including mitochondria, saccules of the endoplasmic reticulum, centrioles, ferritin molecules, pinocytotic vesicles and autophagic vacuoles. These degenerating structures are the morphological expression of the elimination of cellular components that are no longer necessary for erythrocyte maturation. In contrast to mature red cells, reticulocytes have an irregular, sometimes lobulated, outline, reflecting their capacity for contractile movement.[49] They are about 20% larger than mature red cells,[10,50] but diminish in size in the circulation as the result of the loss of 10–14% of the membrane, equivalent to what is lost during the four months of life that remain to the red cell as it ages in the circulation.[51] Reticulocytes are not visualised directly on blood films stained with panoptic stains, but their presence can be suspected when polychromasia is observed. In this instance, one is often dealing with young reticulocytes, laden with granulofilamentous material, released from the marrow as the result of intense erythropoietin stimulation (stress reticulocytes) (Fig. 2.6).

Nowadays, reticulocyte counts are performed mainly by cytometry, with cytofluorometers or red cell counters that employ supravital stains or fluorochromes. Technological evolution has led to a much wider and more effective use of a measurement, which in its original microscopic version was imprecise and approximate (but valuable from the point of view of understanding pathophysiology). The reticulocyte count is now reproducible even at very low levels. The sensitivity and precision of these electronic measurements thus permit diagnostic

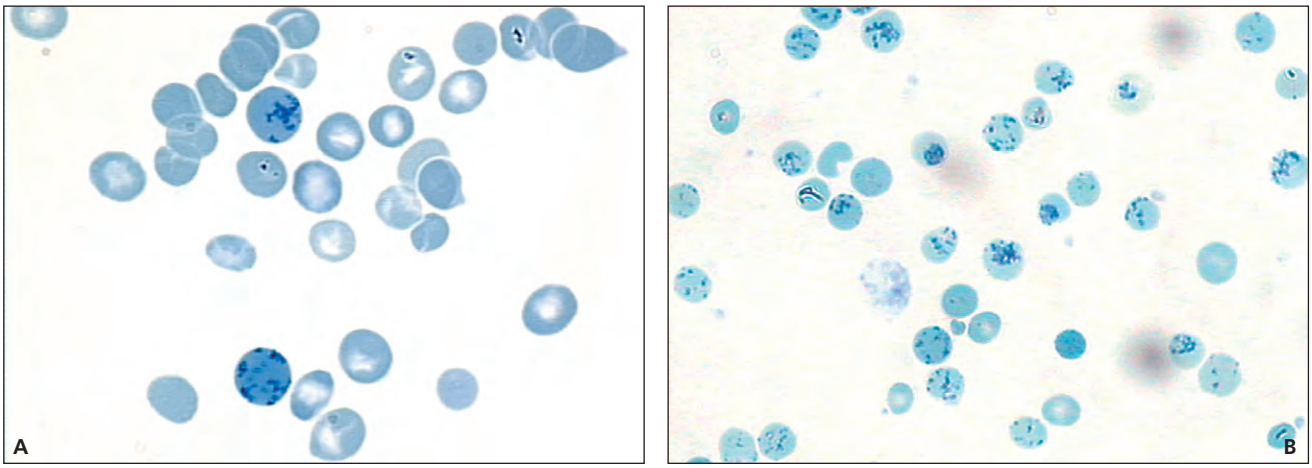



**Fig. 2.5** Peripheral blood, supravital stain with new methylene blue, dried films. (A) Two reticulocytes, recognisable from the relatively abundant granulofilamentous material, which in the upper cell is tending to be denser centrally, while in the lower cell it is distributed more uniformly and is adhering in part to the inside of the cell membrane. (B) Marked reticulocytosis in a patient with autoimmune haemolytic anaemia. The majority of the red cells are reticulocytes at various stages of maturation. This vigorous erythropoietic response shows the extraordinary capacity of the bone marrow progenitors to accelerate their maturation and proliferation as a response to the stimulus of hypoxia. In some cells, the granulofilamentous material is condensing into 'pseudo-nuclei', while in others it is very abundant but more dispersed: it is these cells that are classified as immature reticulocyte fraction (IRF) by automated instruments.

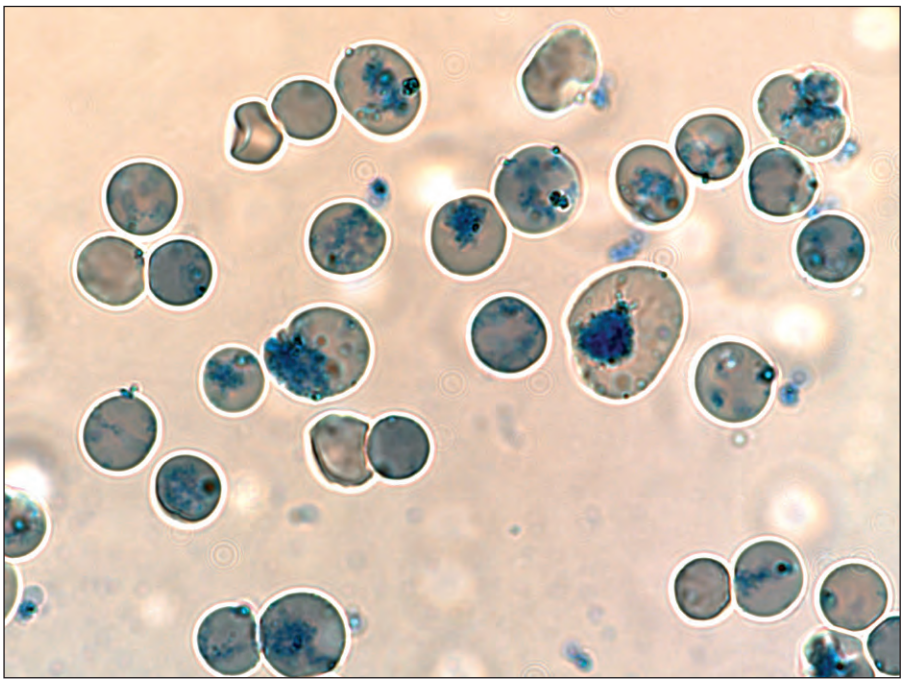


**Fig. 2.6** Massive reticulocytosis. Supravital stain of the peripheral blood using new methylene blue, observed without fixation; from a patient in an acute haemolytic crisis of favism. The dark blue precipitate is visible in almost all the red cells in this field, but varying in quantity and distribution. In some cells the granulofilamentous material is so abundant that it is condensing into the form of a 'pseudo-nucleus'.

evaluation even in cases with reticulocytopenia or when deviations of the count from normal are relatively small.[52]

Assessment of reticulocytes is fundamental for a physiopathological approach: they indicate the integrity both of the erythropoietin response and of the capacity of the erythroid marrow to respond effectively to erythropoietin. With a maximal hypoxic stimulus, the erythropoietic marrow is capable of increasing the production of reticulocytes, and thus of mature red cells, to more than ten times basal production.[2] In the face of a reduced Hb, a reticulocyte response that is less than

expected indicates a quantitative defect in progenitors (reduced production, hyporegenerative anaemia), or a qualitative defect in which the progenitors, although present in normal or increased numbers, are unable to produce and release into the circulation sufficient numbers of reticulocytes and mature functioning erythrocytes (ineffective erythropoiesis). The reticulocyte count permits the separation of anaemias, particularly normocytic anaemias, into (1) a group without reticulocytosis, caused by, or associated with, reduced production or erythrocytes (because of defects of various types in bone marrow progenitors), and (2) a group with reticulocytosis, caused by, or associated with, increased destruction of red cells (haemolysis) or loss of red cells (haemorrhage), with intact and efficient progenitors, but with these being insufficient to cope with the peripheral loss.

In the past, and still continuing in some Anglo-Saxon countries, various indices were calculated to correct the reticulocyte percentage for the Hct or for the supposed period that the reticulocytes themselves spent in the circulation (reticulocyte production index [RPI]). The theoretical base of this calculation is the fact that the reticulocyte percentage expresses the relationship between the number of reticulocytes and the total number of red cells, reticulocytes included, in a unit volume. If there is an absolute reduction in the number of red cells, as in anaemia, the increase in reticulocytes could be only relative, not actually associated with an increased erythropoietic drive, but merely reflecting the reduction in mature red cells. It is also possible that the reticulocytes could be released prematurely from the bone marrow and thus their time in the circulation would be more prolonged. These corrections are no longer necessary: the absolute reticulocyte count (obtained by multiplying their percentage, divided by 100, by the number of red cells, and reported $\times 10^9$/l in the blood count) provides a quantitative estimate that is correct and sensitive even in anaemic patients. For the differential diagnosis of anaemia, and also for the evaluation of the erythropoietic response to iron or vitamins or of bone marrow recovery following chemotherapy, it is more useful to base clinical judgement on the absolute reticulocyte count.

The classical reference interval of the reticulocyte count is $1 \pm 0.5\%$ (0.5–1.5%) for the percentage and $50 \pm 30 \times 10^9$/l (20–80%) for the absolute count, independent of gender and age (with the exception of cord blood of neonates, which has higher numbers).

As well as being easy to remember, these ranges are diagnostically useful. Since the introduction of flow cytometry, numerous studies have been published, which have produced heterogeneous results, even if in general they are not dissimilar; in part, the differences are dependent on the technology and the choice of stain or fluorochrome used.[53] Reference ranges reported in the latest edition of *Dacie & Lewis Practical Haematology*, for example, are 0.5–2.5% and $50–100 \times 10^9$/l, which appear to be overestimates compared with current automated flow cytometric counts.

Anaemia can be the result of reduced red cell production or reduced survival in the circulation (due either to early destruction or to blood loss). The pathogenesis of anaemia can be seen as a continuum with two different mechanisms at the extremes (at one end aplasia, at the other haemolysis) and between these a series of mixed states in which both processes are involved with one gradually prevailing over the other. Anaemia in which the erythrocyte size is altered, in particular, is often recognised as having mixed pathogenesis despite the single cause; for example, a defect in production, whether due to iron deficiency or a genetic defect, can be associated with reduced red cell survival. In microcytic and macrocytic anaemia, in which the main diagnostic feature is the MCV, the reticulocyte count in general does not give diagnostically useful information, being usually normal or slightly below normal in anaemias due to lack of iron and normal or slightly above normal in the thalassaemias and macrocytic and megaloblastic anaemias. However, in the normocytic anaemias, the reticulocyte count is often valuable both for establishing the pathogenetic mechanism and for diagnosis. The normocytic anaemias with reticulocytosis are, in fact, those due to haemolysis or blood loss. Normocytic anaemias without reticulocytosis are more variable in their aetiology and pathogenesis and can be caused by:

- Damage to the erythropoietic marrow (hypoplasia, erythroblastopenia or aplasia; infiltration by leukaemic cells, myeloma, lymphoma or non-haemopoietic malignant cells);
- An intrinsic defect in erythropoiesis (myelodysplasia, often macrocytic and dimorphic; paroxysmal nocturnal haemoglobinuria), sometimes associated with disturbed granulocytic or megakaryocytic maturation;
- Alteration secondary to another pathological process (anaemia of chronic disease);
- Reduced erythropoiesis due to inadequate synthesis (involvement of the erythropoietin-producing cells in renal insufficiency, chronic disease) or reduced need for oxygen (endocrine disorders such as thyroid or adrenal insufficiency, protein-calorie malnutrition, HIV infection).

A normal or reduced reticulocyte count can also be seen in a not insignificant proportion of patients with autoimmune haemolytic anaemia, in up to 30% of cases in some studies,[54,55] resulting from the effect of autoantibodies on erythroid precursors and reticulocytes. Parvovirus B19 infection, moreover, is responsible for the hypoplastic crisis with reticulocytopenia observed particularly in inherited haemolytic anaemias, as well as in immunosuppressed subjects in general, but also in patients with chronic inflammatory anaemias, thalassaemia or iron deficiency.[56,57]

The reticulocyte count can be altered in an anomalous manner through interference of various types, some influencing all methods, both flow cytometric and microscopic, while others affect one method or the other in a more or less specific manner.[58] Well-recognised causes of interference include the presence of erythrocyte inclusions of various types,[59] such as Howell-Jolly bodies (DNA) and Heinz bodies (denatured hae-

moglobin), which are stained by supravital stains and also fluorochromes: these can be distinguished morphologically on a blood film (Howell-Jolly bodies are spherical and isolated, without associated filaments), the position they occupy in the cytoplasm (peripheral and in contact with the cell membrane in the case of Heinz bodies) or their staining characteristics (intensity of staining or various shades of green/blue), but they may be included in an automated reticulocyte count. Pappenheimer bodies, containing iron, are usually small and single and on the blood film stain deep blue (but they do not stain with fluorochromes): they can be identified with certainty by the Perls reaction. The 'golf-ball' inclusions that form as a result of the denaturation of haemoglobin H stain greenish blue. Extreme thrombocytosis and platelet agglutination can cause a factitious increase in the flow cytometry reticulocyte count. Rarely, this also happens with high leucocyte counts, particularly when the leucocytes are small and fragile, such as those of chronic lymphocytic leukaemia, in which very small nucleated cells can overlap into the reticulocyte zone,[53] and with red cells containing malaria parasites[60]: in these instances, observation of the instrument histograms and cytograms is very useful for revealing the presence of interference.

## Further key diagnostic tests: erythrocyte morphology

Three simple basic measurements – Hb, MCV, reticulocyte count – obtained using a well-standardised automated counter thus represent the foundation of the pre-morphological diagnostic pathway. To these one can add a knowledgeable examination of the morphology of the erythrocytes in a blood film, permitting a further enrichment of the diagnostic process of considerable value in specific conditions. As well as being diagnostically important, this can be fundamental for the recognition of interference, which may have rendered the automated counts invalid. Adding on film examination when a diagnostic suspicion has already established permits a targeted, rational application of further more specialised tests: morphological examination of a bone marrow aspirate, investigation of iron status, investigation of the structure of haemoglobin and the genetics of globin chain synthesis, cytogenetic and molecular genetic analysis, immunophenotyping, histology, immunohistochemistry and ultrastructural examination.

The microscopic examination of red cells in a blood film continues to represent, even in this era of advanced technology, a fundamental step in the diagnosis of many anaemias. It can confirm electronic measurements of cell size and permits an appreciation of heterogeneity (providing evidence of variation in haemoglobinisation of cells) and, above all, permits recognition of alterations in shape that characterise various haematological disorders, often with a high degree of specificity.[61,62] It is not rare for there to be erythrocyte dysmorphism that is not detected by automated instruments, since there is no alteration of the size or haemoglobin concentration of the cells and erythrocyte inclusions are absent. The technical aspects of spreading the film and the expert systematic approach of the observer are fundamental, since the morphological features of red cells are very susceptible to artefacts, often attributable to too slow drying of the film, to the presence of precipitates, to excess water in the staining solutions or to the contamination of slides by dust, micro-organisms or other substances. A preliminary low-power observation is indispensable for identification of the optimal area where red cell morphology is best preserved and for detecting the presence of red cell or platelet agglutinates, increased rouleaux formation or abundant weakly stained amorphous material in the background (see Figs 2.54 and 2.55). Artefacts are common in the tail of the film, which is too thin for optimal morphology; the erythrocytes suffer deformation as a result of the forces applied during spreading and often the area of central pallor disappears (causing false ovalocytosis, spherocytosis and hyperchromia). Artefacts are similarly common in the thick part of the film, where red cells are crowded together and the appreciation of the morphology of individual cells is prevented by superimposition and rouleaux formation.

An assessment of red cell morphology must thus be carried out in the part of the film where red cell distribution is optimal, where the cells are close to each other, sometimes touching, but with only a minor degree of overlap of the edges of cells.

### Normal morphology

The red cells in the blood film lack nuclei and have a rounded outline and a pale central zone that occupies about a third of the cell diameter (Fig. 2.7). Morphological evaluation includes an assessment of red cell size, shape, colour and intensity of staining plus the detection of any inclusions or lack of homogeneity of staining.

The diameter of red cells is difficult to evaluate on a blood film in absolute terms. Usually size is fairly uniform; the normally slight variation in size between cells corresponds to a heterogeneity of diameter, based on experimental micrometric measurements, that ranges between healthy subjects from 6.5 to 8.5 μm, with an average of around 7 μm. Red cells that show these characteristics are normocytic.

Cytoplasmic colour is very variable (pink, orange, greyish) depending on the staining technique – both the type and duration of staining. Staining characteristics are a morphological expression of the binding of haemoglobin molecules to the eosin of a panoptic stain. One of the most important morphological features is the presence, the size and the symmetry of the area of central pallor. The peripheral zone is continuous, homogeneous with regard to intensity of staining and apparent thickness, with two concentric outlines, internal and external. Red cells that show these staining characteristics are called normochromic normocytic. The appearance of erythrocytes in the blood film represents in two dimensions the biconcave disc form that they show in three dimensions (when they are not deformed by forces in flowing blood). The form of the area of

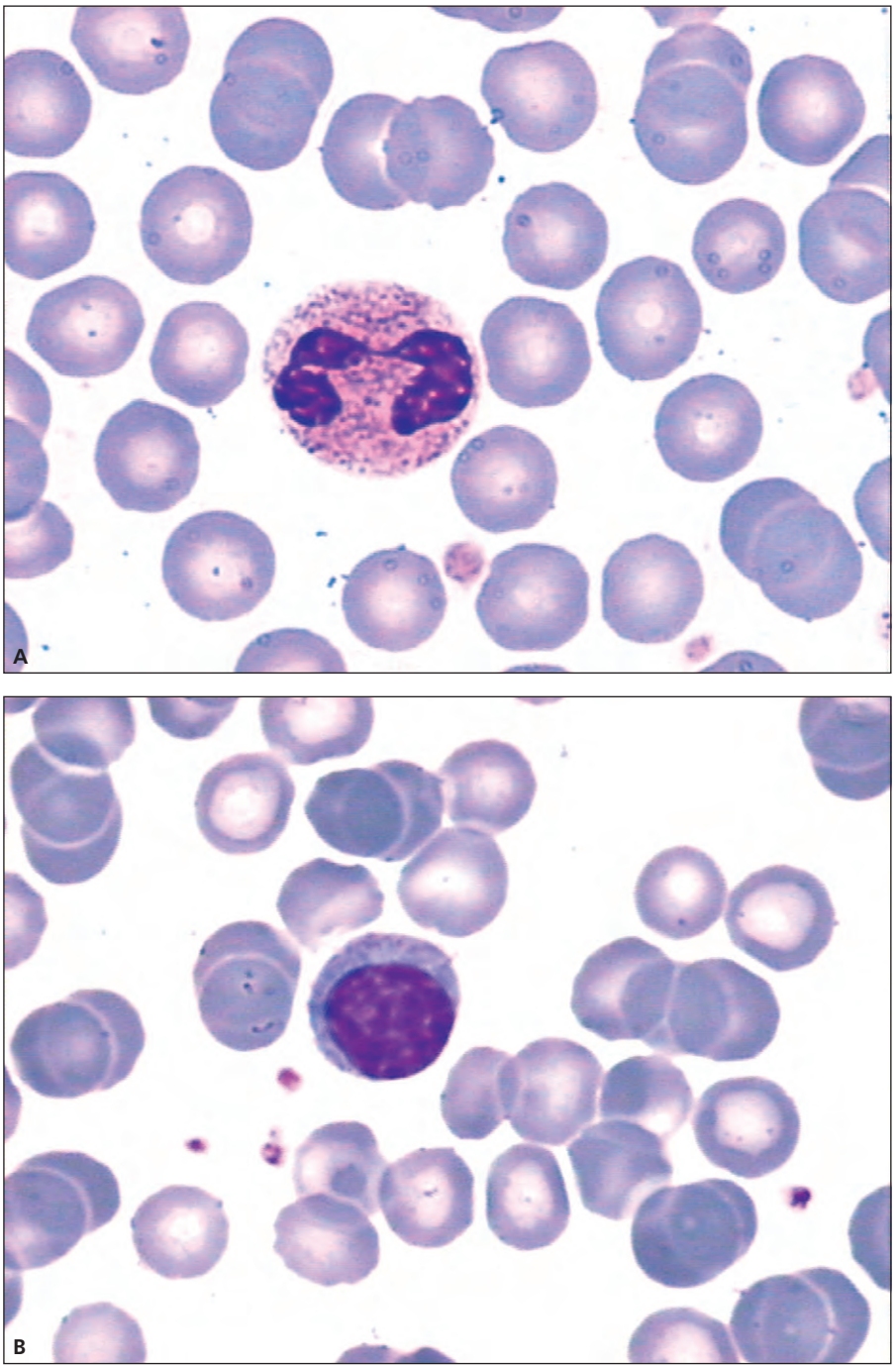



**Fig. 2.7** Normal erythrocyte morphology; peripheral blood film. (A) Around a well-segmented neutrophil granulocyte with very obvious cytoplasmic granules are red cells with a regular rounded outline and relative homogeneity of size and staining characteristics (even though some cells, such as the one on the right, have a slightly lower diameter), no inclusions (with the exception of minute granules superimposed on the cells, which are extraneous in origin, artefactual) and a regular cytoplasmic circumference without any interruptions. Fundamental in the observation of erythrocyte morphology is an evaluation of the presence and nature of the pale central area representing cellular thinning (designated 'the area of central pallor') and the thickness of the peripheral darker rim. The distribution of cells shown in this image is optimal for the assessment of morphology, there being only minimal superimposition. Above the neutrophil, there is an echinocyte with an undulating membrane, without any pathological significance. The platelets appear normal with dispersed granules. (B) The lesser diameter of the oval nucleus of this lymphocyte is a little greater than that of the surrounding red cells, which have an obvious area of central pallor. The cytoplasm of the lymphocyte is basophilic and relatively plentiful. A morphological assessment of cell size must be based on a comparison with cells of known and preferably normal morphology: this lymphocyte shows slight signs of activation, but is nevertheless useful for judging the size of the red cells.

central pallor reflects the fact that in the centre of the cells the thickness reduces in a progressive and regular manner. Often variations in this area (enlargement, reduction or disappearance) do not indicate genuine changes in the haemoglobin concentration, but rather an increase or decrease in cell size, and thus of the thickness of the area of double concavity.

The profile or external border of the red cells is usually regular and continuous, but is sometimes deformed by the effect of spreading or by contact with other cells.

Red cell cytoplasm does not normally contain any particles visible by light microscopy; the presence of inclusions is thus not a physiological feature.

A deviation from this usual normal picture can be the result of production of intrinsically abnormal cells because erythropoiesis is itself abnormal, or can be the result of extrinsic pathological forces to which the erythrocytes are exposed in the circulation. In evaluating the presence and the severity of any apparent abnormality, it is necessary to consider that in the blood films of healthy subjects, in the absence of artefactual changes, it is possible to find isolated poikilocytes, although not exceeding 1–2%; even more rarely, one can find erythrocyte fragments.

Red cell morphological abnormalities will be described in consecutive sections dealing with cell size, colour, shape, the presence of inclusions, the distribution of cells in the blood film and the presence of nucleated red blood cells or artefacts. However, this scheme can be disturbed by the fact that aberrations of erythrocytes are often multiple: alterations of one type, e.g. size, are often associated with alterations of another type, e.g. colour or shape, or the presence of inclusions.

## Variations in size

Microscopic examination for the evaluation of cell size has limited reproducibility, both inter- and intra-observer. The intrinsic subjectivity can be lessened by using an internal calibrator that is normally available in the film, the most constant of these being the size of the nucleus of a small non-activated lymphocyte, which has average dimensions similar to, or slightly greater than, those of a normocyte (Fig. 2.7B). In the absence of interference with electronic measurements of cell size, microscopic examination of red cells in a blood film does not add anything to the accurate and precise MCV measured by an automated instrument.

### Anisocytosis

This term indicates increased variation in erythrocyte size. Within the limits previously described, variation in size can be physiological, being the result of the normal process of erythropoiesis (Fig. 2.8). The histograms of red cell size produced by automated instruments on the blood of healthy subjects show this physiological variation graphically and quantify it in terms of the RDW (red cell distribution width), usually expressed in

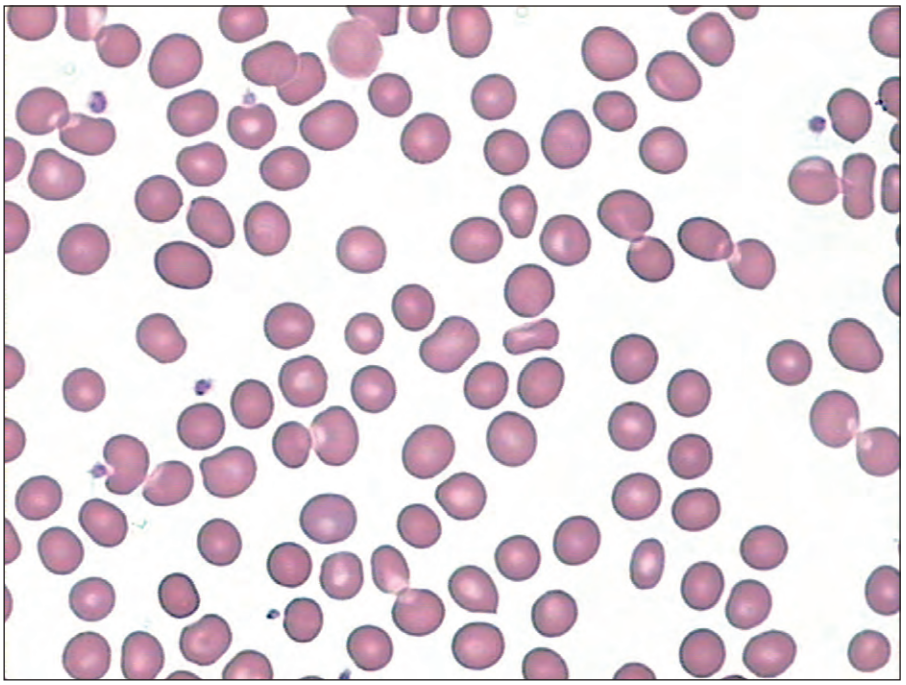


**Fig. 2.8** Physiological variation in cell size; peripheral blood. The diameters of these cells show a certain amount of variation, almost at the limit of normal. The area of central pallor is variable, depending on the characteristics of the film and the part of it that is assessed: here, for example, the red cells are well separated and this is likely to be a region close to the tail of the film. A minimal amount of variation in cell shape, less than 1% poikilocytes, is also physiological: one notes, for example, a small red cell in the centre at the bottom that has the features of a dacrocyte.

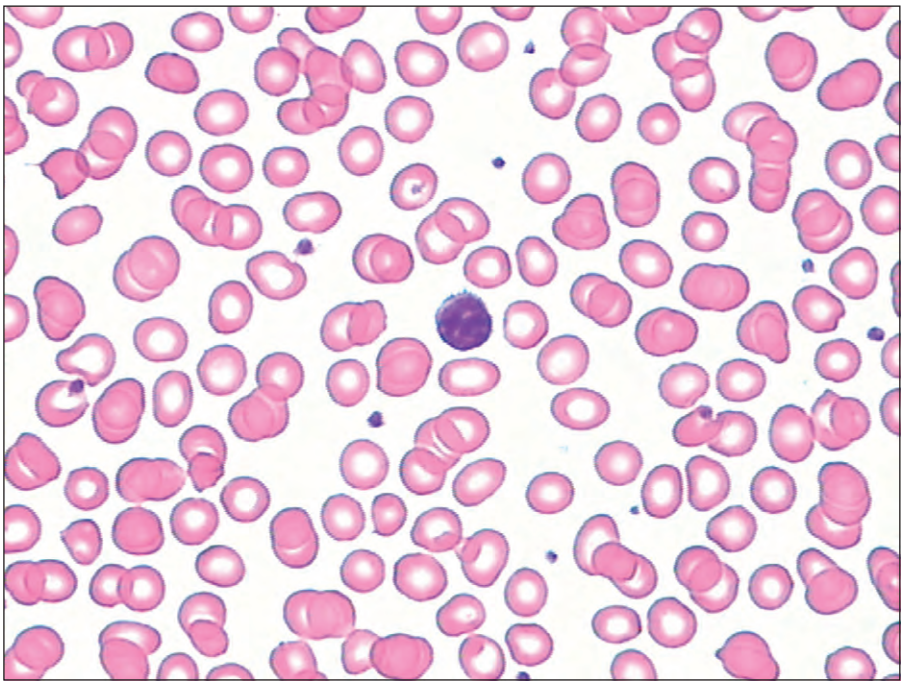


**Fig. 2.9** Microcytosis in the peripheral blood film of a carrier of beta thalassaemia. The diameter of these red cells is a little less than that of the nucleus of the small lymphocyte in the centre, but the increased area of central pallor suggests a population of poorly haemoglobinised microcytes. There is a tendency to form small rouleaux and there are numerous small, hyperchromatic platelets. In these cases, there can be poikilocytosis: top left, a hyperchromic cell that evokes the morphology of a dacrocyte.

percentage terms as a co-efficient of variation (or a standard deviation) with regard to the median MCV. Increased variation in size visible on a blood film is associated with an increasing RDW, with a broadening of the base of the histogram and eventually asymmetry. Anisocytosis and an increased RDW are significant, but not specific, since they can result from various types of disturbance of erythropoiesis. In the case of microcytic anaemias, there is a partial dissociation between microscopic observation, which in thalassaemia trait shows a greater frequency of atypical erythrocytes, target cells and basophilic stippling, and iron deficiency in which there is a more marked change in the RDW, shown by automated instruments but harder to appreciate with the microscope. A basic approach to the differential diagnosis of microcytic anaemia can be based on the variable profile of the MCV and RDW[63]: the more marked anisocytosis of iron deficiency reflects well the gradual exhaustion of iron stores with a steady worsening of haem synthesis, which differs from the defect in globin synthesis in thalassaemia trait, which remains constant rather than being in a state of evolution.

### Microcytosis

When the red cell population is analysed with automated instruments, the term microcytosis is applied to samples with an MCV below the lower limit of the reference range, usually taken as 80–82 fl. At the single cell level on blood films, one can define as microcytes those cells that have a diameter clearly less than that of the nucleus of a small lymphocyte (<6–7 μm) (Fig. 2.9). Generally, microcytes also appear hypochromic on blood film examination, because of thinning of the biconcave part of the cell and thus an increase in the area of central pallor: sometimes the zone of haemoglobinisation is reduced to a very thin peripheral rim of colour (Fig. 2.10). On blood film examination, it is often difficult to appreciate and quantify any reduction in red cell diameter, particularly when the reduction is slight and uniform. Microscopic examination is, however, sometimes useful for identifying a microcytic population that is too small to appreciably alter the MCV (early iron deficiency) or morphological alterations of single cells that will produce no abnormality on automated instruments (poikilocytosis, basophilic stippling or elliptocytosis). In most cases of microcytosis, there is a generalised defect in haemoglobin production. This is characteristic of iron deficiency, thalassaemia, haemoglobin E and congenital sideroblastic anaemia. In anaemia due to lead poisoning, the red cells are often hypochromic and microcytic, in addition to showing the typical basophilic stippling, which is the result of aggregation of polyribosomes that have not been degraded because of the enzymatic defect induced by the lead.

In the anaemia of chronic disease the MCV is usually close to the lower limit of the reference range: in the majority of cases

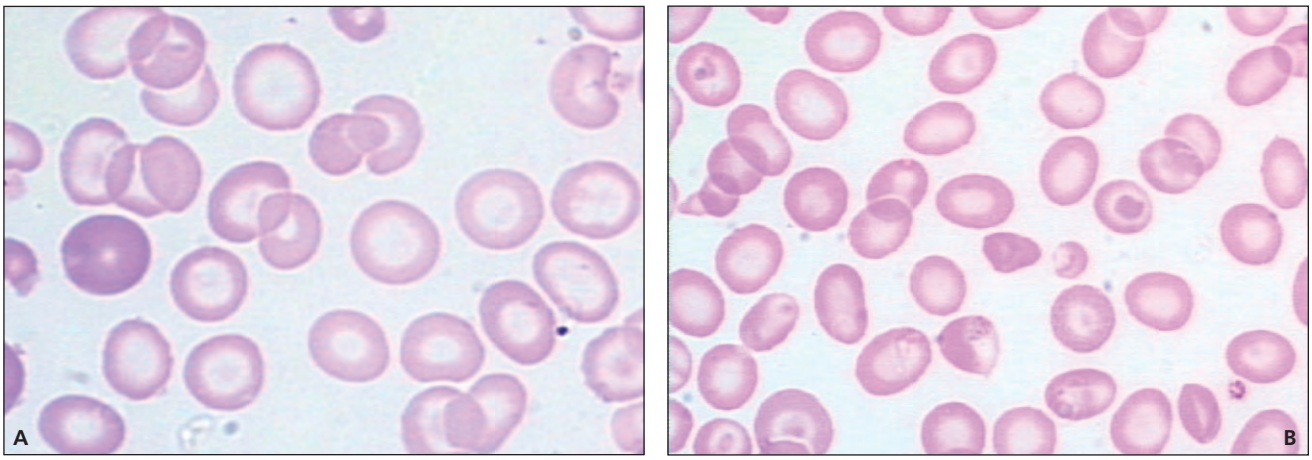



**Fig. 2.10** Iron deficiency anaemia; peripheral blood film. (A) In this patient, at presentation, the area of central pallor in the majority of the erythrocytes occupies four fifths of the cell, as a result of a lack of haemoglobin and reduced cell thickness. The peripheral stained rim is variable but often very thin. There are various ovoid cells, a not uncommon observation in severe iron deficiency anaemia. There is a single large hyperchromic cell on the left. In a field such as this, it is easy to appreciate the lack of haemoglobinisation, while it is more difficult to evaluate the cell size because of the lack of a nucleated cell for comparison. (B) Microcytosis with moderate anisopoikilocytosis in a case of overt iron deficiency. In addition to the increased area of central pallor, there are some microcytes and hyperchromic fragments, target cells of various sizes and a tendency to oval or elliptical forms, with their major axes in the direction of the spreading of the film.

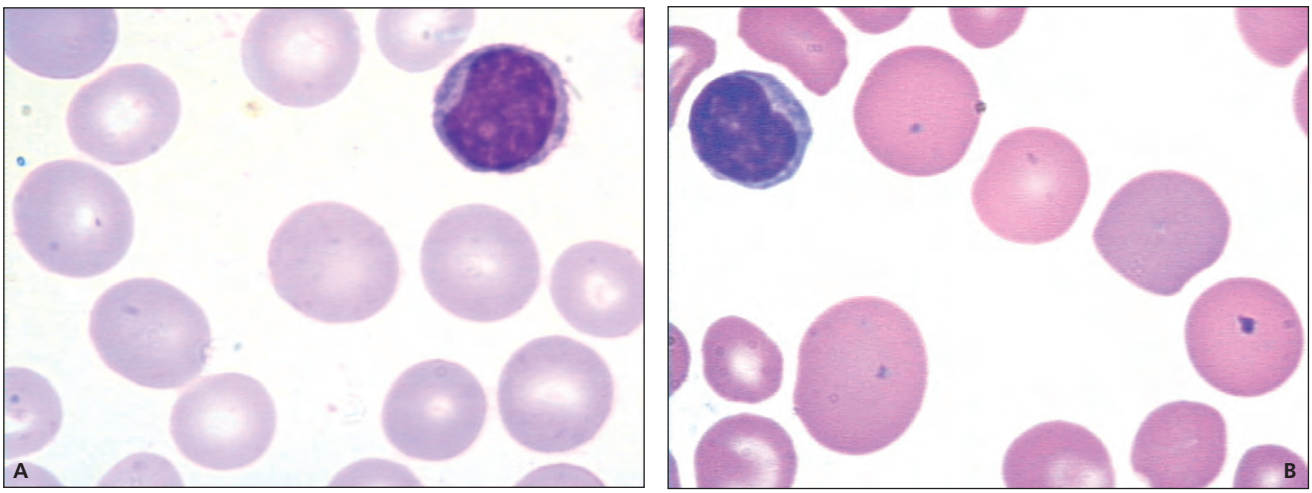



**Fig. 2.11** Examples of macrocytosis in peripheral blood films. (A) Some of the red cells are larger than the nucleus of the lymphocyte. They are tending to oval. The area of central pallor is reduced or totally absent, this hyperchromia being the result of the increased thickness of the cells. (B) Some of these red cells are very large and the area of central pallor is totally absent. In their plentiful cytoplasm, which sometimes has the violet tinge of polychromasia, there are small inclusions that have the appearance of isolated siderosomes (Pappenheimer bodies). The size of the macrocytes contrasts with the size of the normal lymphocyte and a few smaller red cells in the bottom left corner.

one is thus dealing with a normocytic rather than a microcytic anaemia.

### Macrocytosis

At the level of single cells in a blood film one can define macrocytes as red cells that have a diameter clearly greater than that of the nucleus of a small lymphocyte (>8–9 μm). Their morphological identification is easier in the presence of frank anisocytosis, or when there is a significant proportion of normocytic or microcytic red cells. Macrocytes are well haemoglobinised with reduction or disappearance of the area of central pallor attributable to the increased thickness of the cell, to the extent that single cells, observed in a blood film, can appear hyperchromic (Fig. 2.11). This appearance, which is accompanied by an increase in MCH that parallels the increase in MCV, is not an expression of a genuine increase in the intracellular concentration of haemoglobin, but only of the increased volume: the MCHC, in fact, remains within normal limits.

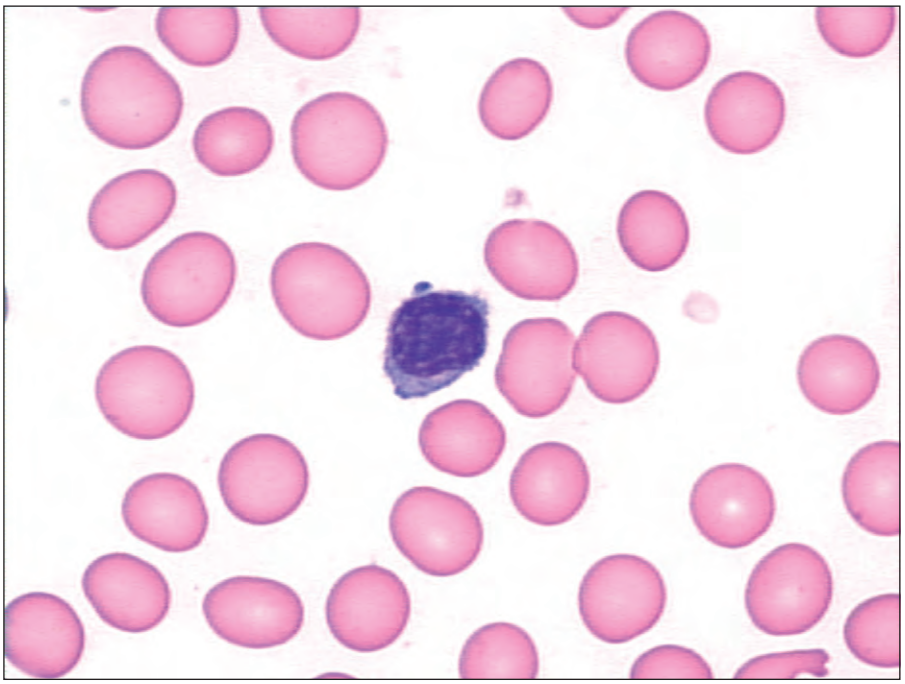


**Fig. 2.12** Macrocytosis in a case of megaloblastic anaemia; peripheral blood. The red cells are hyperchromic ovalocytes, homogeneously stained or with a small remaining area of central pallor. The smaller cells are those that are more strongly stained. The lymphocyte, giving the impression of being very small but actually of normal size, has delicately villous cytoplasm, which in all probability is an artefact, attributed to the dense pink background matrix that indicates dysproteinaemia.

Macrocytosis can be associated with acute stimulation of erythropoiesis, with immaturity of the red cell population as in haemolytic anaemia with massive reticulocytosis: when due to increased erythropoiesis, the increase in MCV is generally moderate, but in cases with the greatest erythropoietic drive one can observe large polychromatic erythrocytes, which correspond to immature reticulocytes.

The most marked increases in MCV are seen when there is megaloblastosis as a result of a grave defect in erythroid maturation resulting from deficiency of vitamin $B_{12}$, folic acid or both; there is pathological slowing, almost an arrest, of the cell cycle. The red cells in these deficiency states can be gigantic and also oval in outline (Fig. 2.12); their bone marrow precursors – characterised by typical granular immature chromatin and nucleocytoplasmic asynchrony – are known as megaloblasts. Observation of the peripheral blood film often permits a distinction to be made between macrocytosis that results from megaloblastosis, and macrocytosis associated with normoblastic erythropoiesis. Megaloblastosis is often associated with striking peripheral blood abnormalities, including anisocytosis, poikilocytosis, red cell fragmentation and the presence of inclusions (Fig. 2.13); in megaloblastic anaemia the RDW is very high, sometimes even early in the course of the deficiency (see Report 2.2). Furthermore, neutropenia and thrombocytopenia are common, as is neutrophil hypersegmentation, one of the most valuable diagnostic features observable in the peripheral blood (Fig. 2.13C).

## Alteration in staining

### Hypochromia

Hypochromia is detected as an increase in the area of central pallor: when it is marked, the individual red cells are pale with a delicate peripheral stained rim and a large area of central pallor. Hypochromia is usually associated with microcytosis and is attributable to the reduced thickness of the central concave area of the cell (see Figs 2.9 and 2.10). Microcytosis is always the result of reduced synthesis of haemoglobin, which characterises iron deficiency, the various forms of thalassaemia and congenital sideroblastic anaemia. The hypochromia of iron deficiency changes over time and is more marked when deficiency is more severe and protracted, while in thalassaemia trait hypochromia is stable with time, as is the microcytosis. Analysis by automated optical methods has shown that the percentage of red cells recognised as hypochromic in iron deficiency anaemia is always higher than the number recognised as microcytic: this phenomenon is not observed in thalassaemia and thus it is useful in the differential diagnosis of microcytic anaemias[27] (see Report 2.3).

#### *Leptocytes*

Marked hypochromia can also be recognised in red cells with a normal or increased diameter: these thin wafer-like cells are known as leptocytes. They are flattened cells with an increased diameter with a small ring of peripheral haemoglobinisation

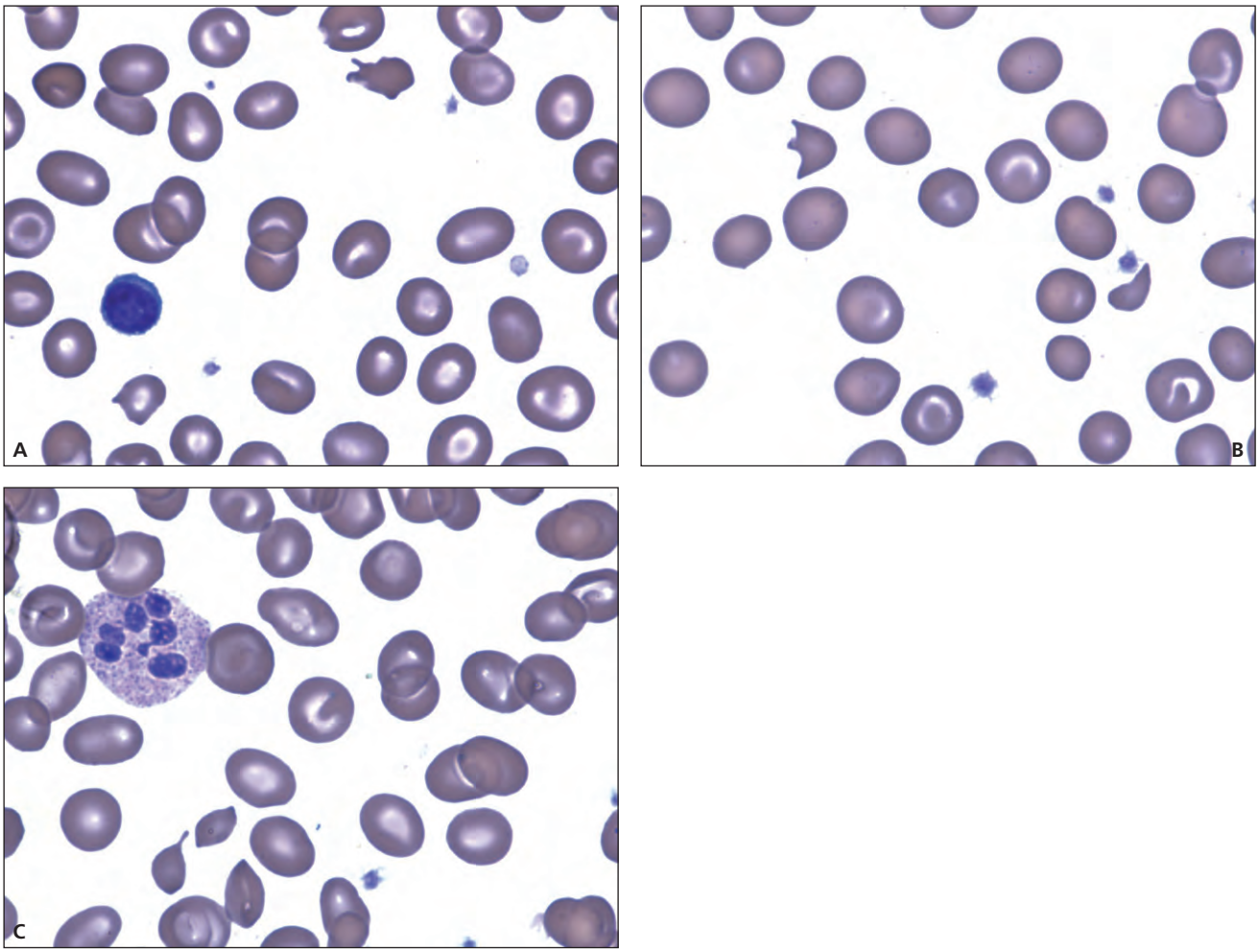



**Fig. 2.13** Macro-ovalocytosis and poikilocytosis in a patient with megaloblastic anaemia with an MCV of 135 fl; peripheral blood. (A) These elliptical macrocytes retain an area of central pallor, but are larger than the small lymphocyte. There are two poikilocytes, which have the appearance of irregular keratocytes, a target cell on the left-hand edge and, higher up, a smaller, almost triangular hyperchromic erythrocyte. (B) In this area of the film many macrocytes appear more definitely hyperchromic. The less large cells resemble spherocytes, while two hyperchromic poikilocytes resemble schistocytes, but would not be classified as such in the absence of a microangiopathic process. There are two large hyperchromic platelets. (C) A giant hypersegmented neutrophil with six lobes, with well-granulated greyish cytoplasm and on the right, a Döhle body. At the bottom, three poikilocytes: cells of this type in megaloblastic anaemia are not formed, as true schistocytes are, by fragmentation as a result of abnormal endothelium, but result from the intrinsic abnormality of bone marrow erythropoiesis.

(Fig. 2.14). They are seen in obstructive jaundice and in thalassaemia, particularly thalassaemia intermedia and major when the synthesis of haemoglobin is gravely reduced and there is marked erythrocyte dysmorphism (Fig. 2.15). Leptocytes sometimes appear folded. Hypochromic macrocytes can also be a sign of megaloblastosis that is associated with an actual or functional iron deficiency leading to reduced synthesis of haem (Fig. 2.16).

### Hyperchromia

The reduction or disappearance of the area of central pallor is a feature of both spherocytes and macrocytes, despite their different aetiologies. Both these cell types are uniformly stained by eosin. In macrocytes the phenomenon is not due to any real increase in the concentration of haemoglobin in the erythrocytes, but rather is an optical effect resulting from increased cell thickness (Figs 2.14, 2.15 and 2.16); the cell is still biconcave and the MCHC remains within normal limits. In spherocytosis, on the contrary, the increase in thickness in the centre of the cell results from the loss of biconcavity with a reduction in the amount of membrane in relation to the cytoplasmic volume: this discrepancy requires its transformation into a sphere (Fig. 2.17). In spherocytosis there is an increase in the MCHC and in the proportion of hyperchromic cells detected by automated analysers. By microscopy the diameter of a spherocyte appears less than normal (slightly so in hereditary spherocytosis, more

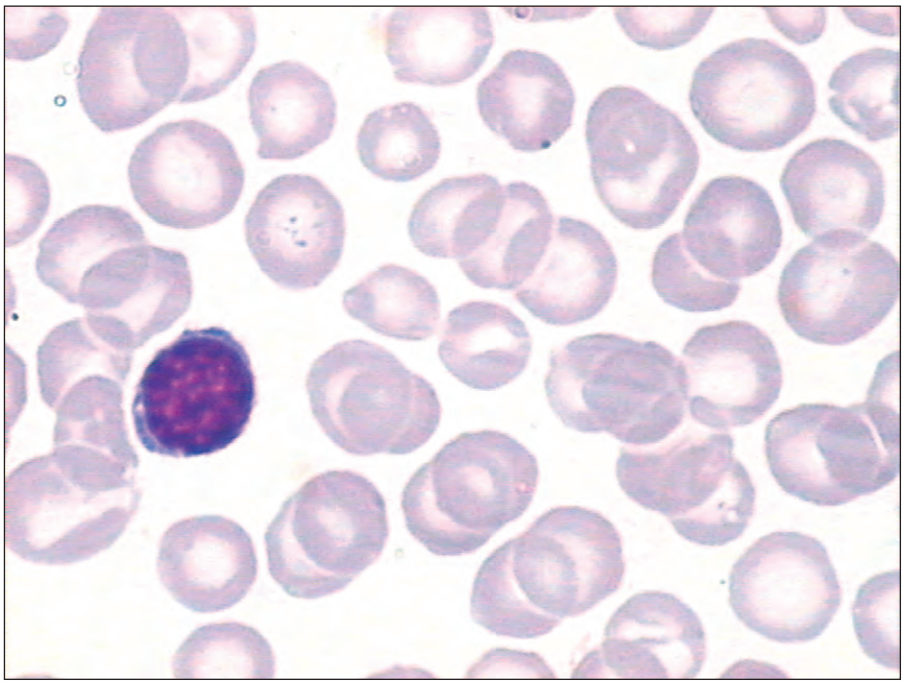


Fig. 2.14 Peripheral blood film. The macrocytes in this elderly patient with hepatic steatosis, differing from those illustrated in previous figures, are hypochromic and can be designated leptocytes. The haemoglobin is confined to a thin peripheral rim. There is mild anisocytosis with a fairly homogeneous red cell population. A single erythrocyte, at the top, is close in appearance to a dacrocyte. Some cells on the left, flattened by contact with a lymphocyte, are tending to fold over on themselves.

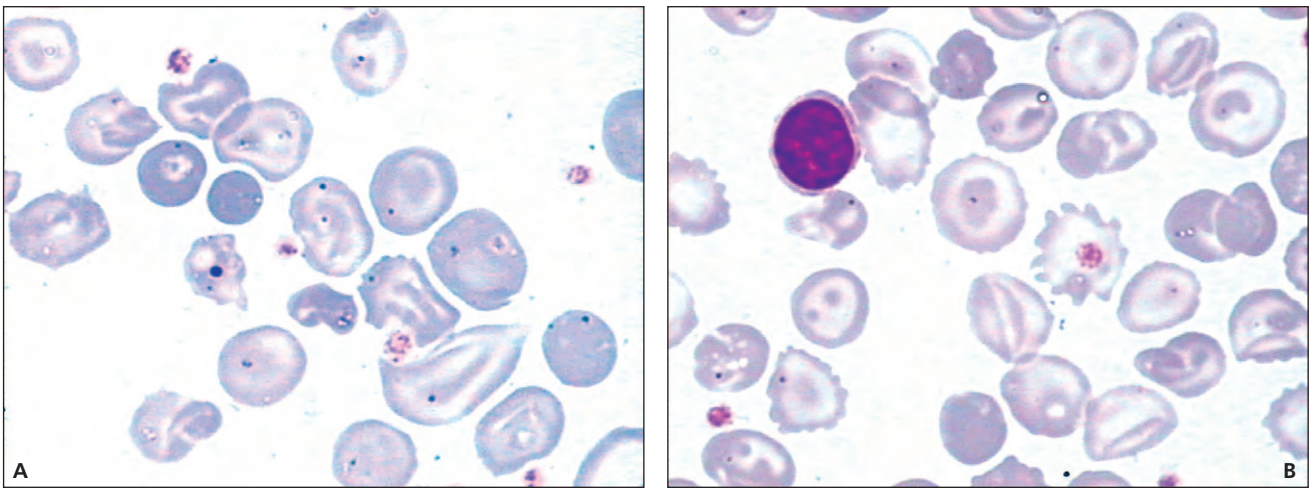



Fig. 2.15 Peripheral blood film from a patient with primary myelofibrosis. (A) The red cell population is characterised by marked anisopoikilocytosis and anisochromasia. There are large hypochromic leptocytes with a tendency to target cell formation, and one microspherocyte. Almost all the cells show cytoplasmic inclusions: some are moderately large, distinct and single, and represent Howell-Jolly bodies (as in the very irregular erythrocyte immediately to the left of centre); others are less distinct, with there being more than one inclusion per cell, and are more similar to siderosomes, as in the better haemoglobinised erythrocyte immediately below the cells that have just been described. (B) There are large folded erythrocytes on the right, bizarre macrocytic dacrocytes, sometimes with two dark central areas or with a reniform dark centre, and some irregularly contracted cells. Many cells have small Howell-Jolly bodies and have the serrated outline of an echinocyte.

marked in the case of microspherocytes produced by fragmentation in thrombotic microangiopathies; in the latter case there is a genuine reduction in cell size as well as an abnormal shape). In spherocytosis the MCV is normal or slightly reduced. Other types of abnormal red cell characterised by hyperchromia are acanthocytes, irregularly contracted cells and many schistocytes.

### Anisochromasia and defects in haemoglobinisation

The term anisochromasia indicates heterogeneity in staining between different red cells in the same blood film (and thus indicates variation in haemoglobinisation). It is particularly frequent in iron deficiency anaemia, in which it is accentuated, finally becoming dimorphic during iron therapy. However, it is observed also in various conditions distinguished by distur-

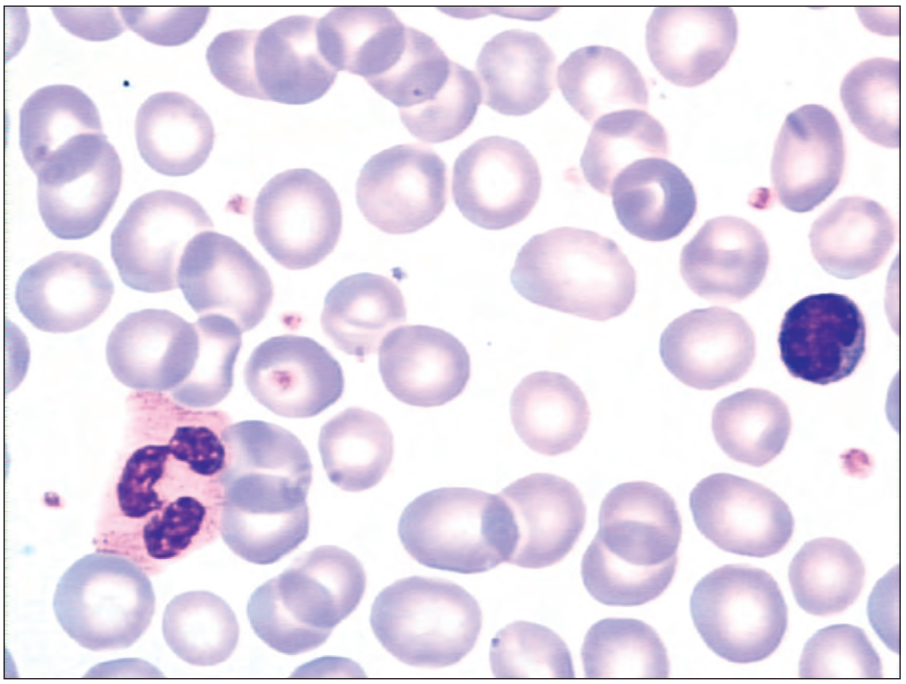


**Fig. 2.16** Peripheral blood film from a patient with megaloblastic anaemia and carcinoma of the stomach. The increased area of central pallor in these macrocytes is attributable to a co-existing iron deficiency. The nucleus of the neutrophil granulocyte on the left has three lobes.

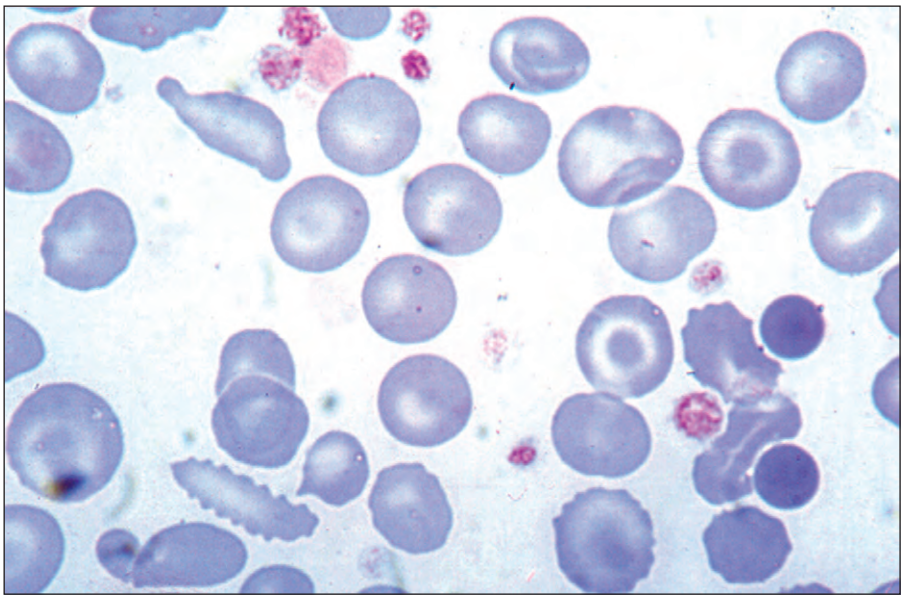


**Fig. 2.17** Anisopoikilocytosis with two small microspherocytes on the right, several target cells, a folded leptocyte, a small dacrocyte and two elliptical echinocytes. The apparent inclusion visible in the large red cell close to the left edge at the bottom is an artefact, probably representing dirt on the lens of the microscope (since it is in a different focal plane). Peripheral blood film.

bance of haemoglobin synthesis, such as the thalassaemias and dyserythropoiesis in general, e.g. in the myelodysplastic syndromes (Figs 2.15 and 2.18). The presence of an irregular asymmetrical pale area of defective haemoglobinisation within an erythrocyte (and similarly within the cytoplasm of the more mature erythroblasts), seen more often in myelodysplastic syndromes and megaloblastosis, is a feature of dyserythropoiesis and is distinct from anisochromasia. A clear distinction must also be made between anisochromasia and the vesiculated cells of the haemolytic crisis resulting from oxidative damage

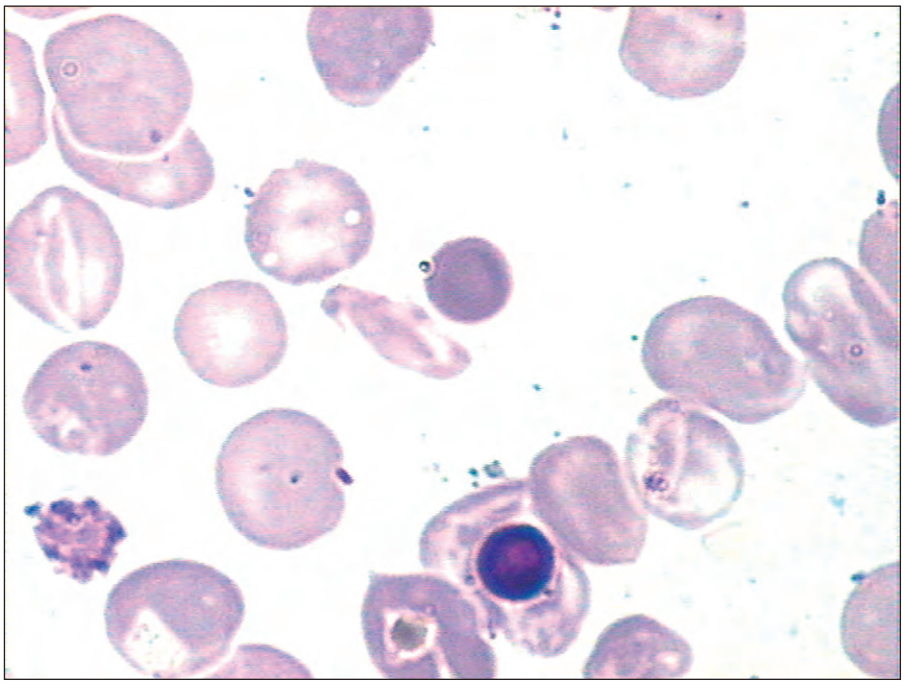


**Fig. 2.18** Anisochromasia. Marked heterogeneity in the content and intracellular distribution of haemoglobin, together with marked variation in red cell size. In the centre is a microspherocyte, adjacent to a cigar-shaped red cell. In the bottom left corner is an irregularly contracted cell. An orthochromatic erythroblast, close to the lower edge, shows an obvious defect in haemoglobinisation. From a case of beta thalassaemia intermedia, peripheral blood film. There are some dark dots superimposed both on red cells and on the white background and thus identifiable as artefacts.

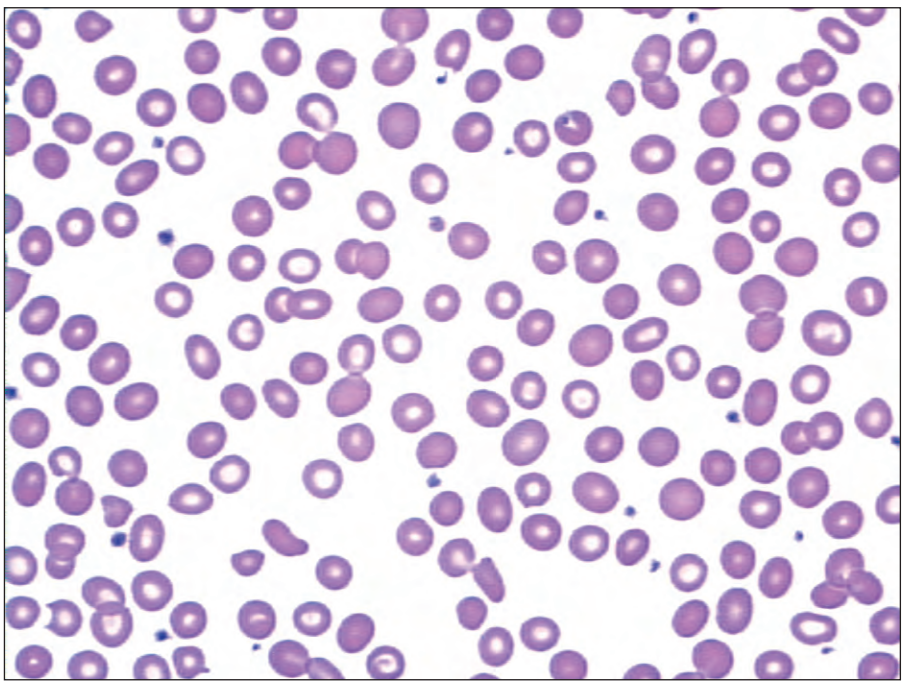


**Fig. 2.19** Dimorphism with anisopoikilocytosis. In this peripheral blood film from a subject with beta thalassaemia trait transfused during surgery there are two morphologically different red cell populations: in addition to red cells of normal size with normal staining chacteristics, although with a tendency to an oval shape, there are larger numbers of cells that are smaller with enlargement of the area of central pallor. Some erythrocytes have the elongated outline of elliptocytes. Towards the bottom, some poikilocytes including a keratocyte in the bottom left corner and two cells with the small tail of a dacrocyte.

occurring in patients with a deficiency of glucose-6-phosphate dehydrogenase (G6PD) in which a pale area is attributable to the precipitation of haemoglobin, leaving part of the red cell membrane virtually empty.

### Dimorphism

The term dimorphism is correctly used when the blood film shows two different populations of red cells that differ in size and staining characteristics, each population however appearing uniform (Fig. 2.19): this definition distinguishes dimorphism from anisopoikilocytosis and from anisochromasia. Typical dimorphism is seen in iron deficiency anaemia after a period of effective therapy: while in the first few days reticulocytes (detected by supravital stains) and polychromatic cells (detected by panoptic staining) appear, after several weeks a significant proportion of new normocytic normochromic red

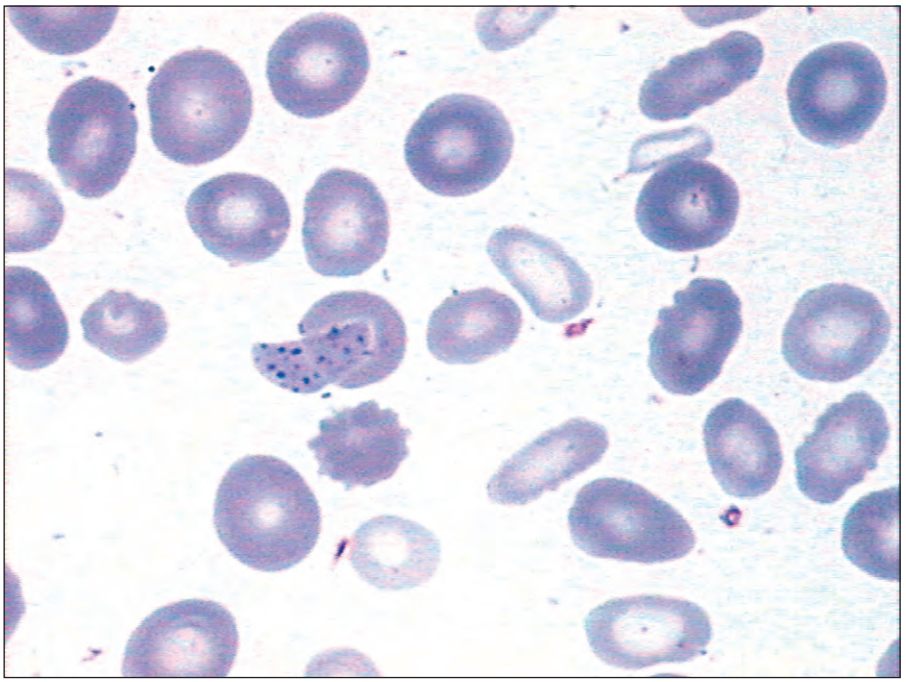


**Fig. 2.20** Dimorphism in iron deficiency anaemia after 3 weeks of therapy; peripheral blood film. The predominant red cell population is comprised of normally shaped cells with a very obvious area of central pallor. The hypochromic microcytes that attest to the previous state of iron deficiency are almost all elliptocytic. One of these, to the left of centre and superimposed on a normal erythrocyte, has very obvious basophilic stippling, with granules that vary in size and shape and are irregularly distributed in the cytoplasm; they correspond to abnormal ribosomes that have been precipitated and fixed. Some erythrocytes are becoming echinocytic.

cells are present in the circulation together with the old sideropenic hypochromic microcytes which are progressing towards the end of their life span (Fig. 2.20). A conceptually similar picture, albeit inverted, is seen during therapy of megaloblastic anaemia; poikilocytes gradually disappear and as the marrow recovers and becomes normoblastic, newly formed normocytes appear alongside residual macrocytes and polychromatic cells. Complex or polymorphic pictures are occasionally observed in megaloblastic anaemia associated with prominent iron deficiency. Cases that are straightforward to interpret are seen when a patient with microcytic anaemia or macrocytic/megaloblastic anaemia is transfused with erythrocytes from a normocytic donor (see Fig. 2.22 and Report 2.3) or when a transfusion into a normocytic recipient is from a donor with thalassaemia trait. Automated size analysis, with an RDW, histograms, cytograms and erythrocyte subpopulations is particularly efficacious in revealing the presence of minor erythrocyte populations that differ in cell size, and permits the increase of a normal population to be followed as it gradually replaces an abnormal population of cells of a different size.

A dimorphic picture, with a normocytic or macrocytic population and a hypochromic microcytic population, is seen in myelodysplastic syndromes, in particular refractory anaemia with ring sideroblasts, and also in congenital sideroblastic anaemia. In certain congenital anaemias characterised by particular erythrocyte morphology, it is possible to observe the co-existence in the film of normal and abnormal red cells (e.g. spherocytes in hereditary spherocytosis or elliptocytes in hereditary elliptocytosis): this particular dimorphism depends on the fact that the reticulocytes and young red cells in these subjects appear normal, the defect being acquired as a result of removal of a portion of the cell by macrophages during passage through the spleen, this occurring proportionately to the severity of the genetic defect.

### Polychromasia

Polychromatic cells have an affinity for more than one of the panoptic stains. They are generally at least a quarter larger than the normochromic cells and their colour tends more towards grey, bluish or violet (Fig. 2.21). Polychromasia can be abolished by treatment with ribonuclease, as also occurs with the granulofilamentous material of reticulocytes: this demonstrates that the staining characteristics are attributable to RNA. RNA has affinity for basic stains, and this staining blends with the acidophilic staining of the haemoglobin molecules by eosin. Polychromatic cells in fact correspond to immature reticulocytes, which are not seen as such with panoptic stains but are revealed by supravital stains. Polychromatic red cells are rich in ribosomes and polyribosomes, and are released prematurely from the bone marrow because of intense stimulation by

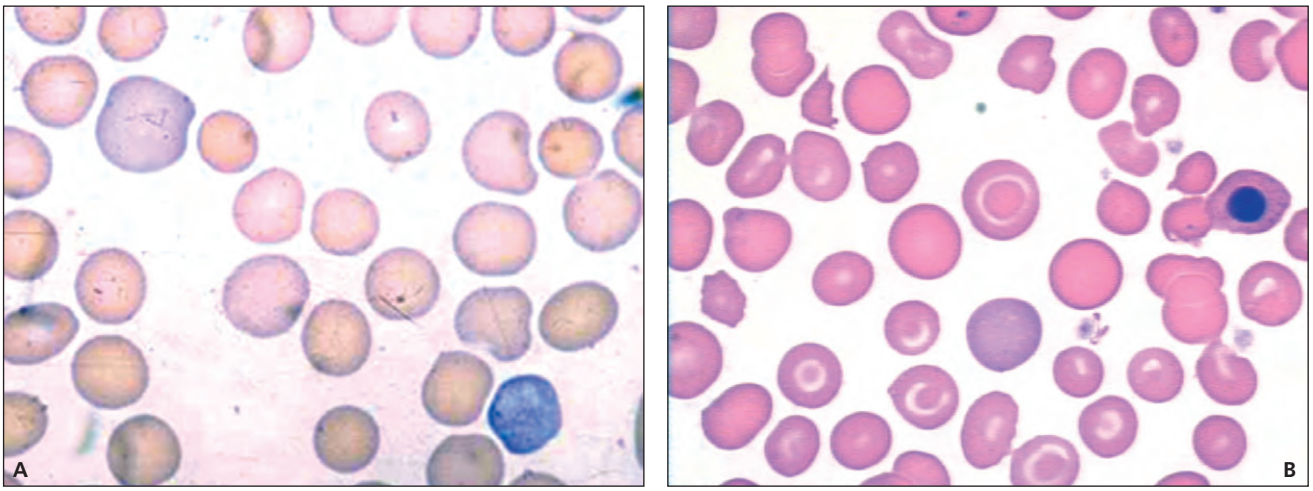



**Fig. 2.21** Polychromasia; peripheral blood film. (A) Two red cells are stained differently from the others. That seen top left is grey-violet and is larger. The one lower down on the right is quite a bright blue. Both are polychromatic cells, corresponding to immature reticulocytes with a high RNA content. (B) Marked anisopoikilocytosis with anisochromasia: a large polychromatic red cell stands out among the target cells, some hyperchromic macrocytes, polymorphic fragments, a small dacrocyte, irregularly contracted cells and an orthochromatic erythroblast with a nucleus that has homogeneously condensed chromatin and fine basophilic stippling.

erythropoietin.[64] In practice, they correspond to the most stainable fraction of the immature reticulocytes (IRF) measured by flow cytometry. By electron microscopy the basophilic material appears to be homogeneously distributed in the polychromatic red cells in blood films fixed and stained by panoptic methods, while it is precipitated and aggregated after supravital staining (i.e. staining of living cells).[49]

Polychromatic red cells are an effective indicator of an erythropoietic response, particularly in haemolytic anaemia and following haemorrhage and during treatment of anaemia due to deficiency of an haematinic agent.[65] They are also seen, but without this indicating regeneration, in association with other erythroid anomalies, in megaloblastic anaemia, in extramedullary haemopoiesis and in some cases of myelodysplasia.

## Variation in the intracellular distribution of haemoglobin

### Target cells

Target cells have also been referred to as codocytes, Mexican hat cells and bull's eye cells. Target cell morphology is seen quite frequently and it appears to be the result of an anomaly of intracellular distribution of haemoglobin, leading to a dark central haemoglobinised area surrounded by a hypochromic ring that is surrounded in turn by a second dark peripheral ring with the same staining characteristics as the central area (Figs 2.21B and 2.22). The principal anomaly of these cells is, in fact, an excess of membrane in relation to the volume of the cell. The target appearance is an artefact that develops when the cell is flattened during the spreading of the blood film: by electron microscopy, target cells have the shape of a cupola or a bell; their shape might even be compared to that of the pasta from Puglia known as orrechiette (little ears)![49] Target cells have reduced osmotic fragility being able to increase their volume more than normal with the ingress of water, thanks to the ample membrane available. Target cells are the principal morphological abnormality in four conditions: thalassaemia, haemoglobin C disease, obstructive jaundice and hyposplenism. They can also be present in sickle cell anaemia, iron deficiency and lead poisoning. They thus do not have a specific diagnostic value.

The membrane redundancy that causes target cell morphology can have two pathogenetic mechanisms.[66] In obstructive jaundice, in intrahepatic cholestasis and in the rare deficiency of lecithin-cholesterol acyl transferase (LCAT), the increase in membrane is absolute and results from an accumulation of phospholipid and cholesterol in the membrane, these deriving from anomalous low density lipoproteins present in the plasma. This type of target cell is thus macrocytic or normocytic. In other conditions, the excess of membrane is relative, resulting from reduced synthesis and therefore reduced content of haemoglobin, and the target cells are microcytic; this is the mechanism of target cell formation in thalassaemias and to a lesser extent in iron deficiency, and also when there is a structural haemoglobin variant (particularly C, but also D or E). In homozygosity for haemoglobin C, in particular, and in compound heterozygous states involving haemoglobin C, thalassaemia and haemoglobin S, target cells are usually numerous; in haemoglobin C homozygotes and in compound heterozygotes for haemoglobin C and haemoglobin S, irregularly contracted cells are often present in association with the target cells (Figs 2.23 and 2.24). Erythrocytes containing haemoglobin C can cause massive pseudoleucocytosis as a result of resistance to lysis in the white cell channel of an automated analyser. An

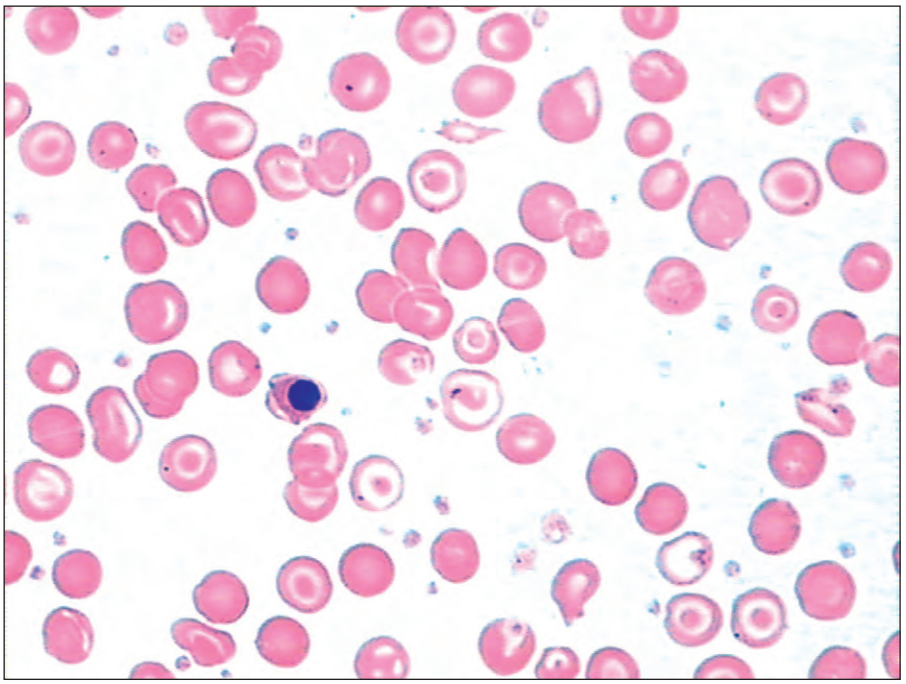


**Fig. 2.22** Dimorphism resulting from transfusion in a severe case of thalassaemia intermedia. The transfused cells appear normocytic and normochromic, while the pathological population is composed principally of hypochromic erythrocytes with target cell morphology, the central condensation of the haemoglobin giving the impression of concentric dark and pale rings. Two larger red cells are polychromatic. A small orthochromatic erythroblast, also showing defective haemoglobinisation, is seen to the left of centre. In the centre, towards the upper edge, there is a small hypochromic poikilocyte, and on its right a macrocyte with a deformed pale area at the periphery. To the left of the poikilocyte is a red cell containing an inclusion resembling a Howell-Jolly body, but difficult to distinguish from superimposed extraneous material: this second possibility is made more likely by the presence of similar refractile granules in other red cells, as in three target cells close to the erythroblast. There are numerous poorly stained platelets.

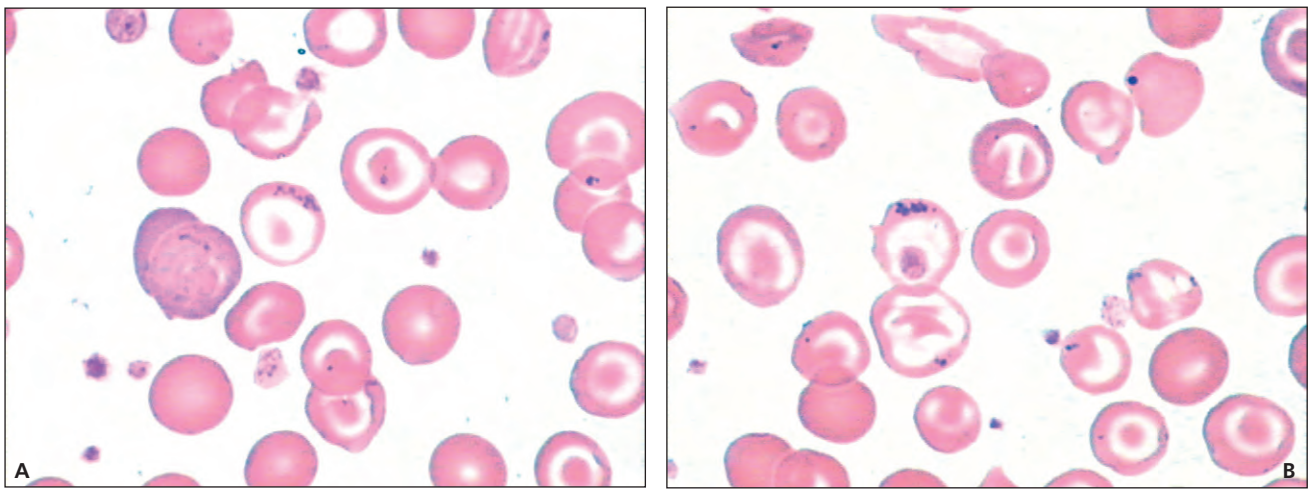



**Fig. 2.23** Polychromasia, target cells and dimorphism; peripheral blood film in a compound heterozygote for beta thalassaemia and haemoglobin C. (A) Also in this patient, the morphologically normal cells are transfused cells. Two large polychromatic red cells are superimposed one on the other. To their right, a hypochromic target cell containing Pappenheimer bodies. The platelets are numerous, heterogeneous and hyperchromatic. (B) The hypochromia of the target cells, typical of this compound heterozygous state, stands out in comparison with the single normochromic cell close to the bottom right corner. In some of the pathological erythrocytes, the area of central pallor has the appearance of an incomplete ring or a semicircular slit; in one of these cells, to the left of centre, there are multiple Pappenheimer bodies; close to the top right corner is a deformed hyperchromic red cell containing a Howell-Jolly body.

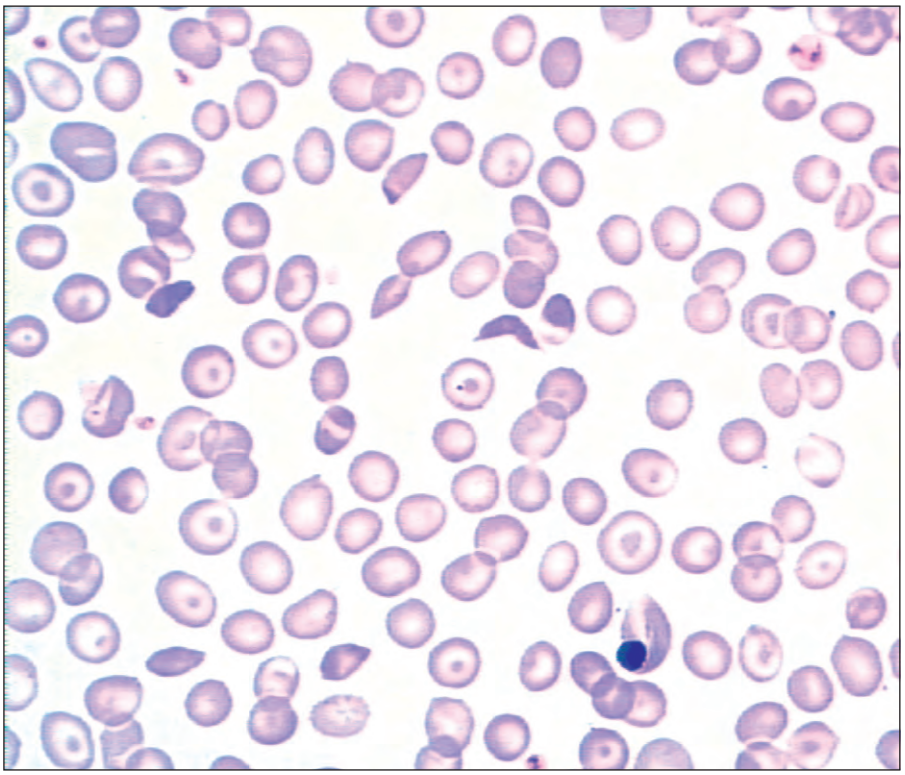


**Fig. 2.24** Target cells and sickle cells in a patient with compound heterozygosity for haemoglobin S and haemoglobin C. The red cell population is hypochromic and microcytic. There are numerous target cells. There is one sickle cell a little above the centre of the image, beside an irregularly contracted cell in which the haemoglobin is concentrated at the upper pole. Another contracted red cell, slightly to the left, has bipolar condensation of the haemoglobin. Other red cells are elongated, boat or oat shaped, while still others, such as the small very dense and irregular cell at about 10 o'clock in the image, are approaching a rectangular shape and resemble SC poikilocytes. A small hyperchromic erythroblast nucleus is visible at the bottom, in contact with a macrocyte that possibly still partly contains it.

analogous artefact has been described in other haemoglobinopathies and recently with target cells observed in patients with malignant neoplasms.[67]

Target cells are readily observed after splenectomy and in other hyposplenic states, as a result of the loss of the spleen's capacity to remove excess erythrocyte membrane.

### Spherocytes

These are dense, rounded red cells of a homogeneous colour (spherical if observed in three dimensions), lacking the area of central pallor; the cellular outline is smooth and regular (Fig. 2.25). This anomaly results from removal of part of the red cell membrane by phagocytosis. This can result from a structural abnormality of the membrane attributable to an inherited defect in the proteins related to the cytoskeleton, or from sensitisation following binding of antibodies and/or complement: this membrane loss leads to transformation from a biconcave disc to a sphere. The diameter of a spherocyte is reduced, while the cell volume, expressed as the MCV, is usually normal or only slightly reduced. Hereditary spherocytosis is a result of an inherited defect affecting a protein of the cytoskeleton or a protein that attaches the lipid bilayer to the cytoskeleton (spectrin, ankyrin or band 3): these defects cause a destabilisation of the membrane with formation of microvesicles and loss of part of the membrane itself. Reticulocytes and young erythrocytes have a normal shape, and the spherocytic morphology develops gradually during the red cell's sojourn in the circulation. This spherocytic transformation makes the cell less deformable, easily trapped in the spleen, leading to further damage to the cell and extravascular haemolysis. The percentage of spherocytes in the blood film varies markedly and can be related to the considerable heterogeneity of clinical expression; the film in general shows dimorphism (normocytes/spherocytes): spherocytes are absent or rare in silent carriers of the defect, are present in only small numbers in each microscopic field in mild cases, represent 20–30% of cells in variants of intermediate severity and are very numerous with marked anisopoikilocytosis and polychromasia in patients with obvious haemolysis, reticulocytosis, hyperbilirubinaemia and early-onset clinical complications. In hereditary spherocytosis the MCHC tends to be increased (>340–350 g/l), correlating in general with the severity of the clinical picture.[68–70] An increase in the percentage of hyperchromic cells

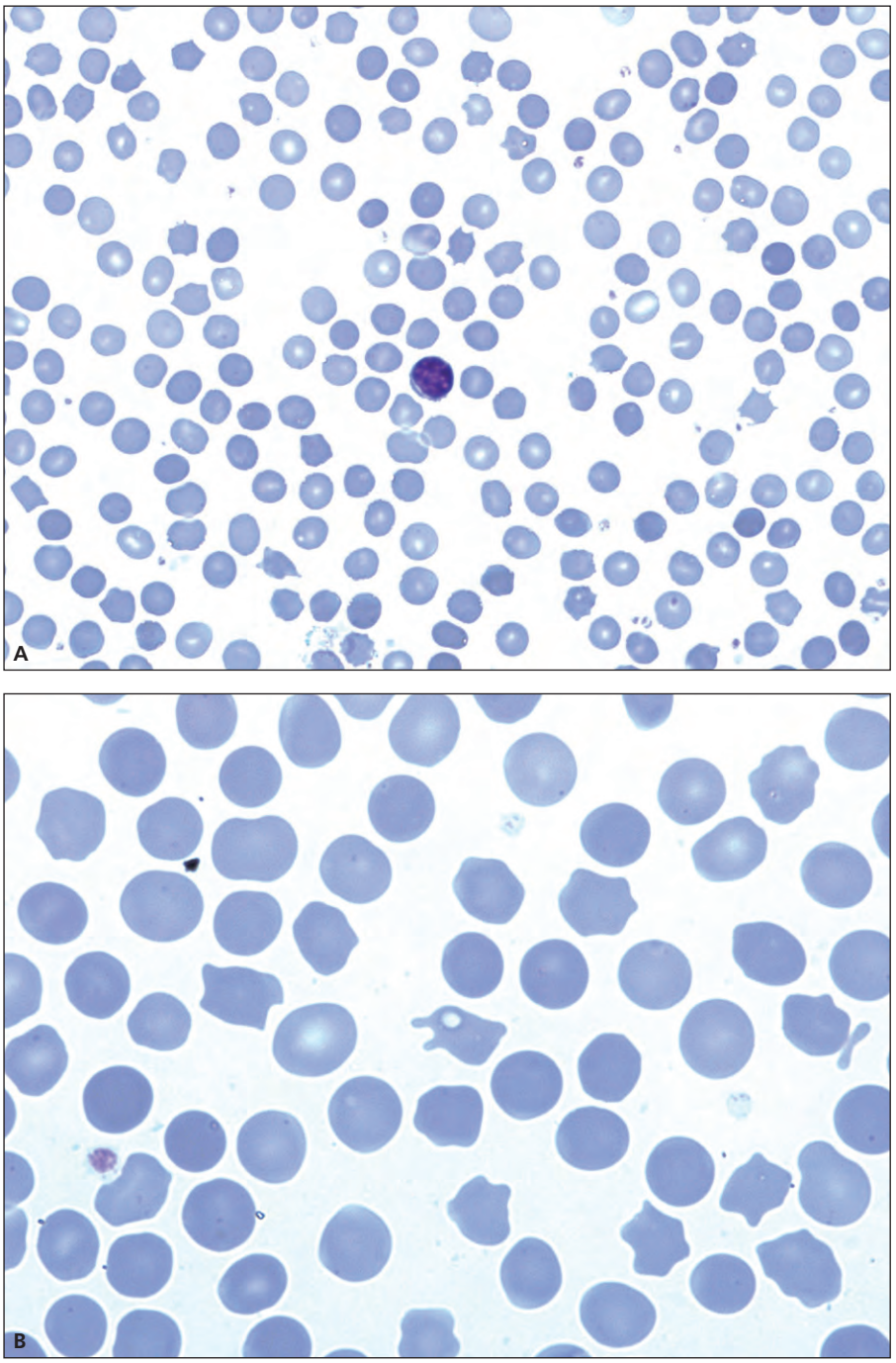



**Fig. 2.25** Hereditary spherocytosis; peripheral blood film. (A) The appearance of the red cell population is dimorphic. A little less than half of the red cells appear normal, the others being typical spherocytes characterised by uniform staining with a lack of central pallor that makes them appear hyperchromic. The size criterion, relating red cells to the lymphocyte in the centre, is useful for differentiating these spherocytes resulting from a congenital abnormality of the red cell membrane from the microspherocytes of microangiopathic haemolysis, which have a smaller diameter (see, for example, Fig. 2.17). Only a few of the spherocytes have an irregular outline with peripheral spicules, which make them similar to acanthocytes. (B) The distribution of normocytes and spherocyte in this film is homogeneous, with a good mixture of the two cell types excluding an artefact. Like the spherocytes with an irregular outline, the vacuolated keratocyte at the centre reflects the membrane damage that leads to spherocyte formation following contact with splenic macrophages. The normal appearing red cells are younger and thus membrane damage is not yet manifest.

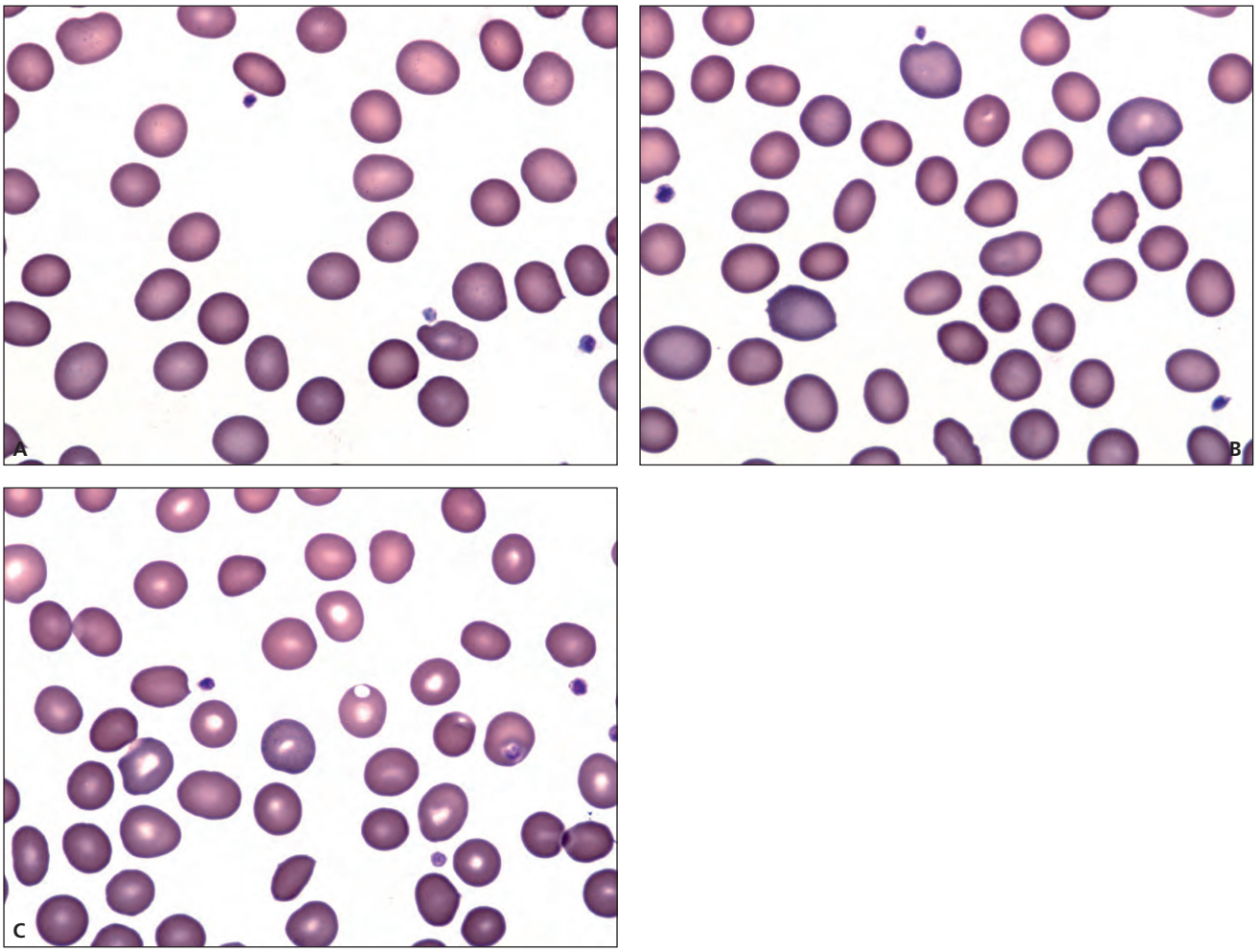



**Fig. 2.26** Elliptocytic spherocytosis; peripheral blood film. (A) Normal red cells, spherocytes and sphero-ovalocytes, some of which still retain slight central pallor, indicating that transformation into spherocytes is not complete. There is one dacrocyte. (B) Three larger polychromatic cells indicate the presence of reticulocytosis, which correlates inversely with the haemoglobin concentration. (C) A red cell with a pale area still very visible with a vacuole just below the cell membrane, anticipating the loss of part of the membrane in the spleen leading to spherocyte formation. Adjacent, in the centre, a slightly polychromatic red cell. The sphero-ovalocytes have the morphology of spherocytes with an oval outline.

measured by automated analysers that use the Mie principle of two-angle light diffraction is an instrumental characteristic of hereditary spherocytosis,[71,72] also correlating with the clinical severity and with the increase of the RDW[73] (see Report 2.4). Spherocytic elliptocytosis is characterised by the presence of spherocytes and sphero-ovalocytes (Fig. 2.26).

The recognition of spherocytes in a blood film does not lead to a specific diagnosis since spherocytes can occur both in hereditary spherocytosis and also in warm autoimmune haemolytic anaemia (more rarely in cold antibody-mediated haemolytic anaemia or in patients with haemolysis associated with the presence of both warm and cold antibodies) (Fig. 2.27), and also in alloimmune haemolysis, as in acute or delayed transfusion reactions; spherocytes can be seen also in severe haemolysis related to enzymatic defects (Fig. 2.28) and in clostridial sepsis. Other lesions than can lead to spherocytes in the circulation include severe burns, hypophosphataemia, intravenous infusion of water, fresh water drowning, snake bite, bartonellosis and the Rh null phenotype. Spherocytes are also more numerous after splenectomy and in hyposplenism. Finally, the absence of the area of central pallor is one of the most common artefacts if the thin tail of the film is examined (pseudospherocytosis).

Even though spherocytes have a reduced diameter and a normal-low normal MCV, the term microspherocyte must be reserved for red cells that show the typical features of spherocytes together with a more marked reduction of the cell diameter, sometimes with irregularity of the outline, with some features suggesting irregularly contracted cells (see Fig. 2.37). Microspherocytes, also called spheroschistocytes,[49] are the result of red cell fragmentation and can represent remodelled

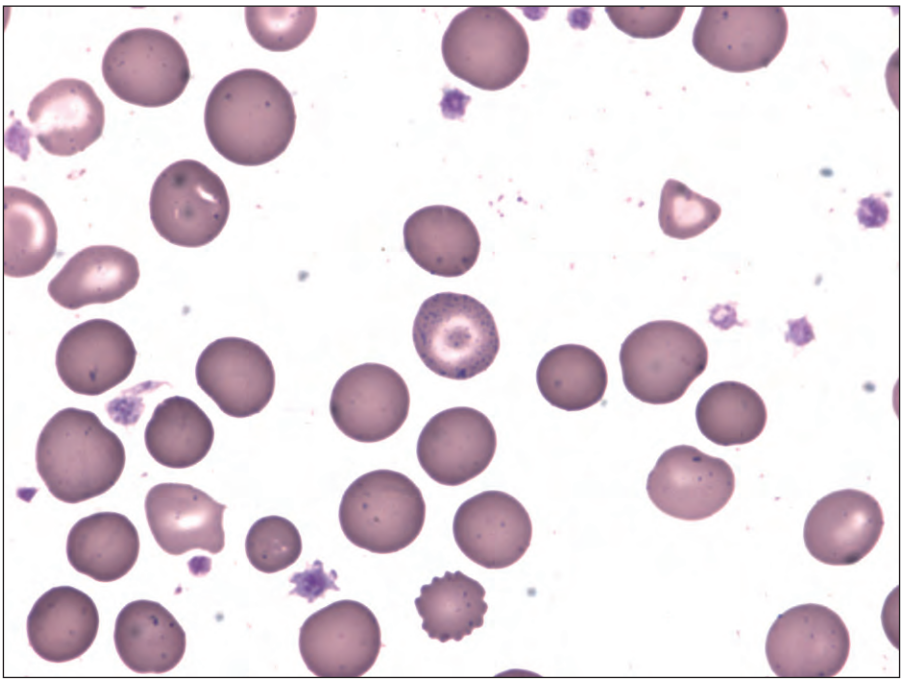


**Fig. 2.27** Peripheral blood film from a case of warm autoimmune haemolytic anaemia. The prevailing abnormality is the presence of hyperchromic spherocytes and microspherocytes. In the centre, a target cell with basophilic stippling; lower down, an echinocyte; on the right, a triangular fragment. Platelets are numerous and show a clear separation of granulomere and hyalomere.

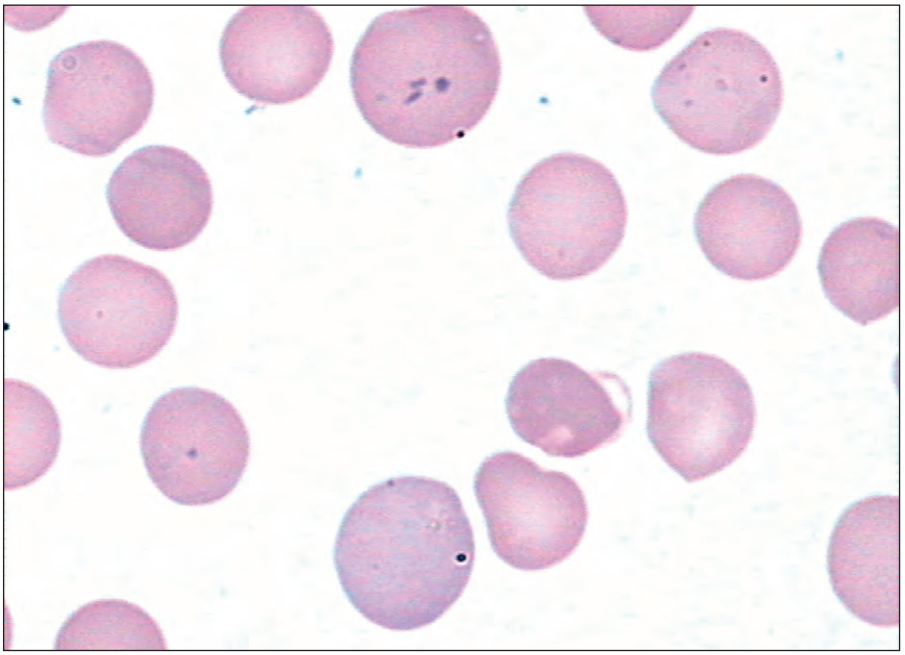


**Fig. 2.28** Peripheral blood film of a patient with glucose-6-phosphate dehydrogenase (G6PD) deficiency and a haemolytic crisis. The red cells are spherocytic, variable in size and lacking an area of central pallor. Some have a pointed extremity. There is also a vesiculated cell, typical of this condition and, at the top, a macrocyte with three not clearly definable inclusions. In the centre, at the bottom, a polychromatic macrocyte in which the apparent inclusion is refractile and can thus be recognised as a superimposed particle of dust.

schistocytes (see Fig. 2.39). They are seen particularly in microangiopathic haemolytic anaemia and mechanical haemolytic anaemia, being accompanied by other features of red cell fragmentation. They are also one of the red cell lesions of extensive burns[61] and occur in severe anaemia with dysmorphic and ineffective erythropoiesis, as in megaloblastic anaemia and thalassaemia major (Fig. 2.29).

### Irregularly contracted cells

Irregularly contracted cells are small hyperchromic erythrocytes, lacking an area of central pallor, which are distinguished from spherocytes by their irregular shape (Fig. 2.29B). They thus show both a staining anomaly and an abnormal cell outline. The distinction from spherocytes, not always easy at the single cell level, is important: irregularly contracted cells do not

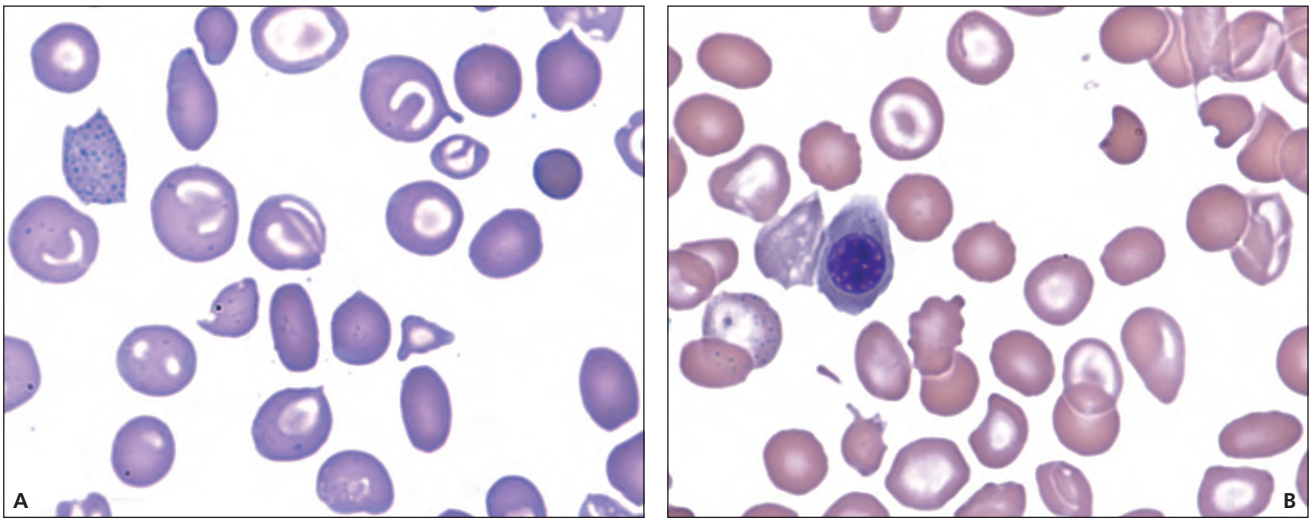



**Fig. 2.29** Peripheral blood film from a patient with thalassaemia intermedia, with marked anisopoikilocytosis and anisochromasia. (A) In the upper right corner, a spherocyte, a dacrospherocyte and a microspherocytes. The area of central pallor of the other red cells often has a bizarre and elongated shape. There is a strange keratocyte with four pointed extremities and basophilic stippling, a triangular fragment and other small hyperchromic red cells, sometimes with small tails. (B) Various irregularly contracted cells, some of which still retain a faint area of central pallor. Top right, a helmet cell and a bite cell. To the left of centre, a polychromatic erythroblast, with the cytoplasm still being greyish and the nucleus having chromatin condensed into large blocks, but still not totally compact. Another two red cells, more to the left, have a polychromatic tinge and one of them shows basophilic stippling.

result from abnormalities of the cell membrane or from sensitisation by antibodies, but are characteristic of oxidant-induced haemolysis, and are seen particularly in haemolytic crises in G6PD deficiency (Fig. 2.30) and in haemolysis attributable to an unstable haemoglobin, such as haemoglobin Hammersmith or Köln.[61] This type of lesion results from precipitation of haemoglobin in masses that deform the cell outline, corresponding to Heinz bodies (demonstrable with supravital stains). Irregularly contracted cells have the same significance as vesiculated cells, with which they often co-exist. They are also a feature of the presence of haemoglobin C (together with target cells and haemoglobin C crystals) and, less conspicuously, occur in heterozygotes and homozygotes for haemoglobin E and in beta thalassaemia major (see Fig. 2.18) or, occasionally, beta thalassaemia minor.[62]

Irregularly contracted cells have been described, together with spherocytes and anisopoikilocytosis with an increase in the RDW, in Zieve syndrome, characterised by an acute haemolytic anaemia in the context of alcohol abuse, fatty liver, hypercholesterolaemia and dyslipidaemia. This has been attributed to acquired modifications of the membrane that make the red cell more susceptible to the action of natural haemolysins.[74,75]

The term 'pyknocyte' has been applied to a type of contracted cell that is seen in small numbers in normal neonates and breast-feeding babies and, in more significant numbers, in babies with a rare form of transient haemolysis known as 'infantile pyknocytosis'.[76]

### Vesiculated cells

These have also been called eccentrocytes, blister cells and hemi-ghosts. They are oval or elliptical erythrocytes, which have an ample clear zone at one of the two poles. They are a variant of irregularly contracted cell, in which the haemoglobin appears very dense, concentrated and retracted towards one pole of the cell, which is thus eccentrically hyperchromic, leaving empty a smaller part of the cytoplasm that corresponds to the other pole (Figs 2.28 and 2.30). They are seen in haemolysis resulting from G6PD deficiency or oxidant exposure and are the precursors of 'bite cells'. They are sometimes seen in sickle cell anaemia. In acute haemolysis in G6PD deficiency one can also sometimes detect ghost cells that have completely lost their haemoglobin.

### Stomatocytes

These are erythrocytes that, on the dried, fixed and stained blood film, have a central pale area that, rather than being rounded as normal, appears elongated into the form of a fissure or an open mouth (a stoma) (Fig. 2.31). This appearance, which reflects hyperhydration of the red cell, corresponds in three dimensions to a cup with a single concavity rather than the normal biconcave disc. Stomatocytes are present in small numbers even in the blood films of healthy subjects, but are seen in larger numbers in a variety of conditions, such as hepatic and biliary abnormalities, acute alcoholic intoxication, chronic obstructive pulmonary disease, and during the use of certain drugs including vincristine and hydroxycarbamide. They can

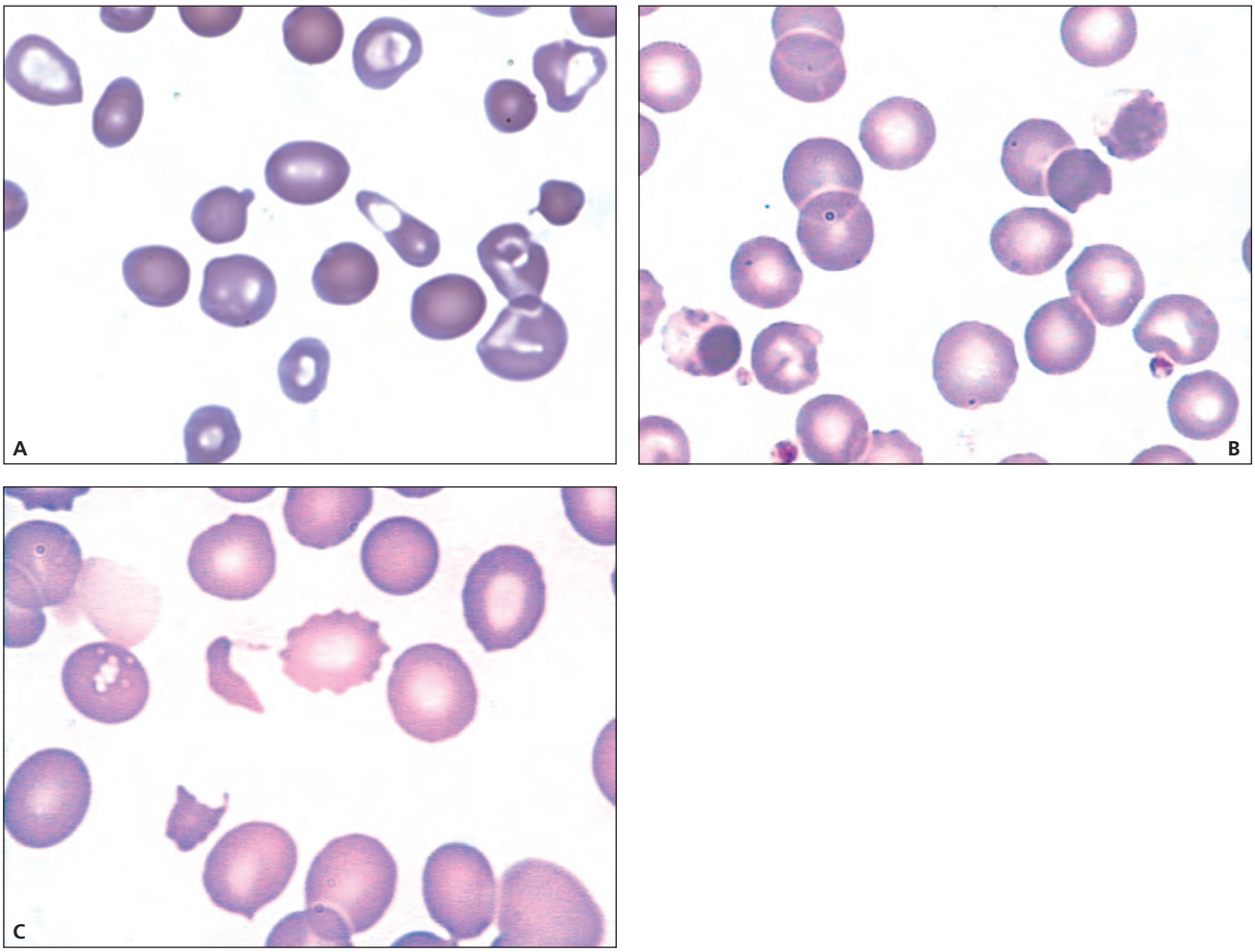



**Fig. 2.30** Peripheral blood films from three cases of haemolytic crisis in G6PD-deficient subjects. (A) There are various contracted cells of variable size and an elongated vesiculated red cell (a blister cell). (B) In addition to the normocytic red cells, there are irregularly contracted cells with a vesicular appearance resulting from polar condensation of the haemoglobin. Top right, the loss of part of the cytoplasm has led to formation of a bite cell. (C) There are fragmented erythrocytes, an echinocyte, a hyperchromic red cell at the top and, on the left, the stroma of an empty erythrocyte (a ghost cell). Beneath the latter, an erythrocyte with numerous small vacuoles.

represent an intermediate form in the transformation of a discocyte to a spherocyte. In the majority of cases, however, stomatocytes are an artefact produced in the blood film when it dries slowly in a humid environment.

More rarely, stomatocytosis is the result of an inherited membrane defect that leads to abnormal cation permeability, with a disturbance in the intra-erythrocytic cation distribution and a resultant increase in intracellular water.[66,76,77,78] Hereditary stomatocytosis includes a heterogeneous group of conditions, from mild to severe, in which the membrane shows increased permeability to sodium, with a resultant increase in intracellular sodium and cytoplasmic hyperhydration, an increased MCV with a normal or reduced MCH and an increased osmotic fragility. A different form of abnormal permeability to cations is seen in the dehydrated form of hereditary stomatocytosis, known as hereditary xerocytosis, in which the defect is loss of potassium with cytoplasmic dehydration and mild or moderate haemolysis.[79,80] In these cases, automated instruments show an increased MCHC, an increase of hyperchromic cells and sometimes a mild reticulocytosis. In the film, one sees stomatocytes, target cells, irregularly contracted cells with cytoplasmic spicules or clumped hyperchromic cytoplasm and polychromasia. Osmotic fragility is reduced and deformability, measured with an ektacytometer, is typically altered.

Stomatocytes have been described in hereditary haemolytic anaemia due to a lack of Rh antigens (Rh null phenotype) and, recently, in association with macrothrombocytopenia, in patients with phytosterolaemia, an inherited abnormality that leads to selective absorption of lipids,[81] in the dyslipidaemia known as Tangier disease and in vibration disease, when

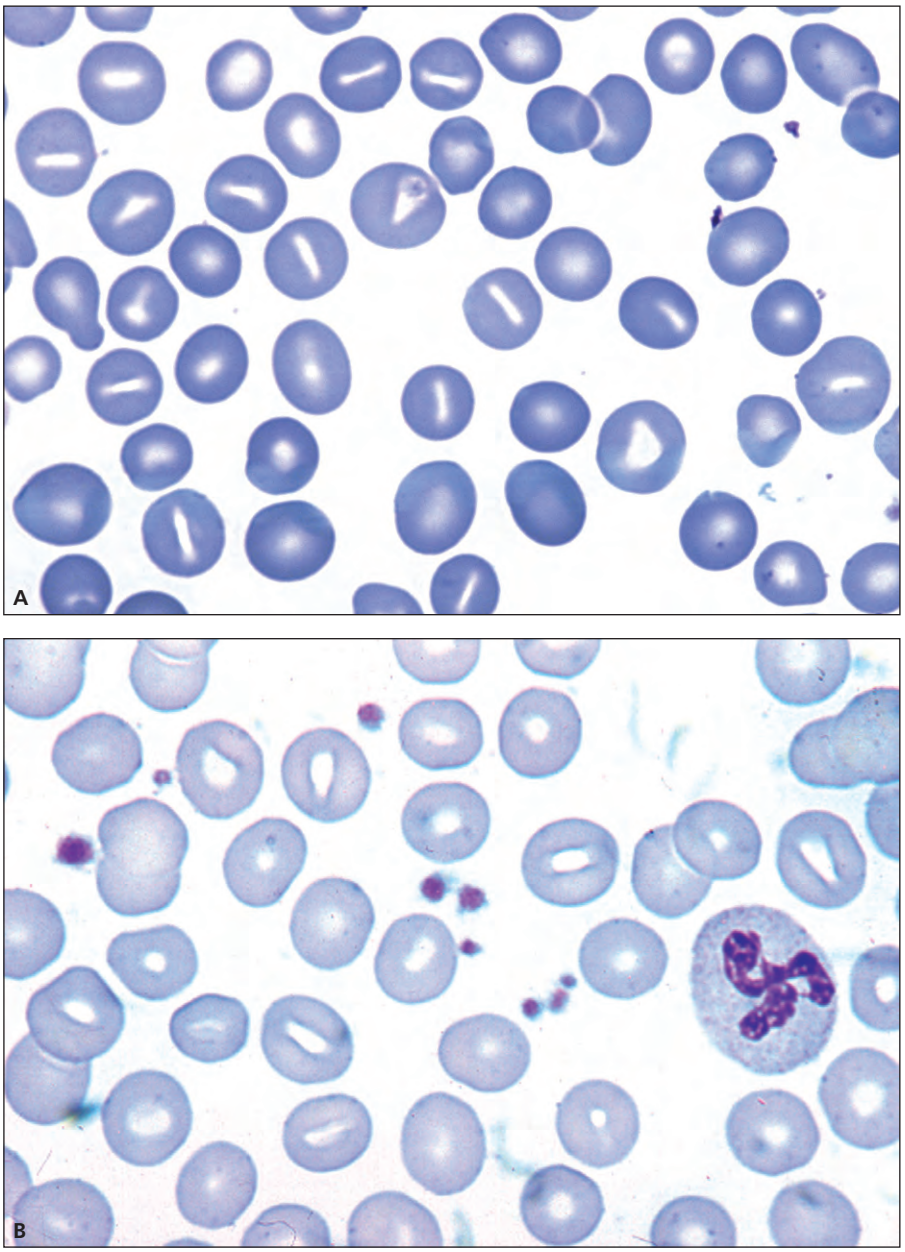



**Fig. 2.31** Hereditary stomatocytosis. (A) The majority of the erythrocytes have a pale central slit. Often stomatocytes are slightly oval with a greater diameter than morphologically normal red cells. (B) A similar appearance to that seen in hereditary stomatocytosis can be seen as an artefact resulting from alteration of the state of hydration of the red cells at the time of spreading and drying of the film.

morphological alteration is favoured by keeping the specimen at a low temperature.[82] Stomatocytes with a double stoma or a Y- or V-shaped stoma are seen, together with macro-ovalocytes, in South-East Asian ovalocytosis.[61,62]

## Variation in shape and cell outline

### Poikilocytosis

This term indicates a generalised atypia of the red cells in the blood film, with multiple different abnormalities being present. It is a generic term, which is best used conservatively to indicate samples that are clearly abnormal (like, for example, those in Figs 2.17 and 2.29). Even in films of normal subjects, one can observe a few elliptocytes or ovalocytes, rare target cells or fragments and very rare spherocytes or irregularly contracted cells. In the evaluation of poikilocytosis, one must first identify any predominant anomaly (e.g. acanthocytes or fragments) and must also ascertain that the anomalies present are not artefactual. It is opportune in the report for the term 'poikilocytosis'

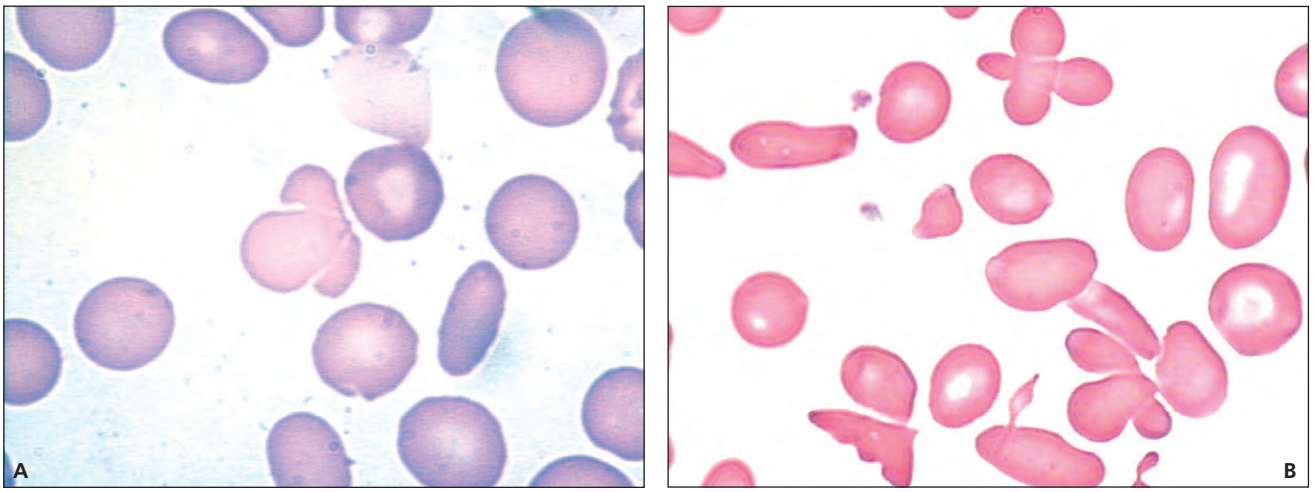



**Fig. 2.32** Anisopoikilocytosis in a peripheral blood film; it sometimes happens that red cells assume interesting shapes. (A) The central image, composed of three red cells, one of which is constricted at the centre, resembles a cherub with its head immediately beneath a ghost cell; from a haemolytic crisis in G6PD deficiency. (B) Extreme anisopoikilocytosis with elliptocytosis, bizarre red cells and triangular fragments; at the top, two elliptocytes form a cross, which has the shape of a dove. At the bottom, a fan-shaped red cell and on the right an image similar to that in (A). The patient from whom this film was obtained had a myeloproliferative neoplasm, specifically, primary myelofibrosis.

to be accompanied by an indication of the specific aberrations that are prevalent.

Evaluation of poikilocytosis can only be done microscopically. Automated instruments do not have the capacity to reveal these morphological deformities, even if there may be a clue, when the changes are marked, in the form of an increased RDW or an abnormal red cell subpopulation. Moderate poikilocytosis can be seen in the common microcytic anaemias, in myelodysplasia, in haemolytic crises of various types and in many other subtypes of anaemia. The most striking poikilocytosis, in which there is marked variation in cell size, either macrocytes and/or microcytes, together with a range of cell shapes which can be bizarre and very individual (anisopoikilocytosis) (Fig. 2.32), is seen in acquired conditions, such as megaloblastic anaemia and primary myelofibrosis, and in hereditary conditions, such as thalassaemia major (homozygotes and compound heterozygotes), hereditary pyropoikilocytosis and congenital dyserythropoietic anaemia.

### Echinocytes

Echinocytes, also referred to as crenated cells, sea-urchin cells and burr cells, are normocytic normochromic erythrocytes, with numerous fine pointed surface projections (between 10 and 40), regularly distributed at a uniform distance from each other. In contrast to acanthocytes, the area of central pallor is retained (Fig. 2.33). The outline reminds one of a bottle cap viewed from above. This is an acquired abnormality of the red cell membrane, attributed to a greater expansion of the external lipid layer in comparison with the inner layer. Often echinocytosis is a technical artefact, which can occur on drying, particularly when the blood specimen has been conserved in EDTA for a prolonged time; it can be induced by many 'echinogenic' factors, among which are contact with glass and physiological solutions, alkalinity or hypertonicity of staining solutions, anionic detergents and lysolecithin (whereas lowering the pH, cationic detergents and phenothiazines favour stomatocyte formation). Particularly when the change is artefactual, the spicules are not apparent in the centre of the cell, but only around the edges (two-dimensional distribution). Echinocytosis is also apparent when films are made one or two days after the blood specimen was obtained and when patients have received transfusions; this is the result of the depletion of ATP that occurs in erythrocytes during storage. Echinocytes can also be seen in inherited non-spherocytic haemolytic anaemia due to pyruvate kinase (PK) deficiency, particularly after splenectomy: also in this case, the change can be attributed to ATP depletion, which favours the loss of potassium and water from the erythrocyte. Echinocytes can form, in association with rare acanthocytes, in pyrimidine 5′-nucleotidase deficiency.

The blood films of patients with uraemia can show echinocytes (Fig. 2.34), which fall in number and disappear following effective dialysis therapy. They have also been described in athletes after long distance runs, in hypophosphataemia and in disseminated carcinoma.

### Acanthocytes

Acanthocytes, also known as acanthus-leaf cells, spiny cells and spur cells, are irregularly spheroidal, dense, hyperchromic and poorly deformable. Like spherocytes, they lack an area of central pallor, but unlike the spherocytes they are characterised by the

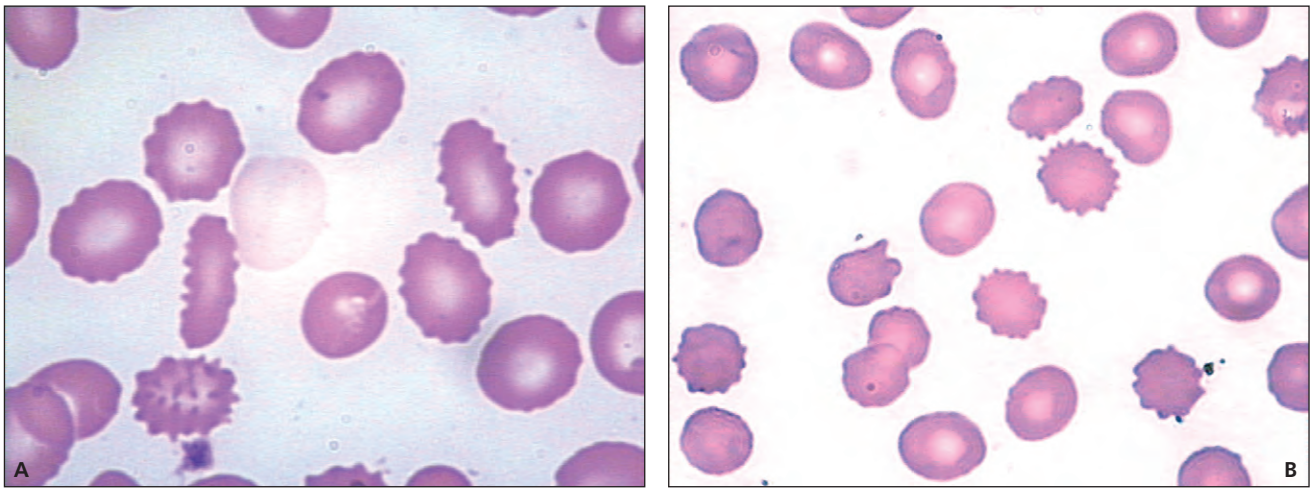



**Fig. 2.33** Echinocytes. (A) Peripheral blood of a patient with renal insufficiency on haemodialysis. Echinocytic transformation is present in almost all the erythrocytes and in some cells is visible in three dimensions. Echinocytes retain an area of central pallor, thus differing from acanthocytes. In the centre of the image, a ghost cell, empty of haemoglobin. (B) Echinocytosis as the result of an artefact. The echinocytes, admixed with a more numerous population of normal cells, have two-dimensional spicules only around their outlines and do not have an area of central pallor. This picture is common in blood films that are too thin and dried slowly.

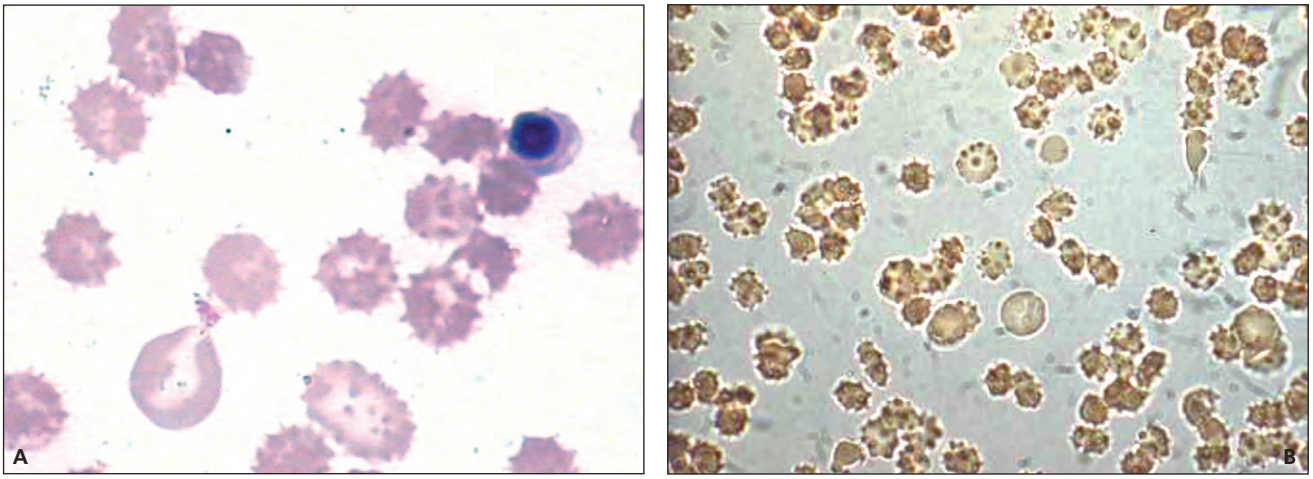



**Fig. 2.34** Generalised echinocytic transformation of a patient with very severe anaemia associated with multiorgan failure. (A) These echinocytes are in effect irregularly contracted cells, hyperchromic and microcytic, with numerous spicules visible on all surfaces of the cell. The area of central pallor is often reduced and sometimes is not visible: the number and the shape of the spicules, however, are such as to exclude acanthocytosis. Normal red cells are absent, but there is a small erythroblast without spicules in the top right corner. (B) In an unfixed preparation, all the erythrocytes, with the exception of one macrocyte, have the appearance of echinocytes, even in the absence of drying out and fixation.

presence of not more than 5–10 finger-like projections or blunt-ended spicules; these are heterogeneous with regard to shape, length and thickness (Fig. 2.35). The distribution of projections over the cell surface appears random rather than uniform, including when seen in three dimensions. Acanthocytosis is an important erythrocyte feature in abetalipoproteinaemia, in which it is related to an inversion of the ratio between sphingomyelin, increased by absorption from the plasma, and glycerophospholipid: normal red cells become acanthocytic when they are transfused into subjects with abetalipoproteinaemia. Acanthocytes are also typical of the syndrome knows as neuroacanthocytosis[83] or choreoacanthocytosis, and of other neurodegenerative conditions, among which is the erythroid phenotype known as the McLeod syndrome (an X-linked disorder caused by a lack of the precursor Kx of the Kell red cell antigen system). In hepatic cirrhosis, particularly that due to alcohol, acanthocytes are associated with an increase in cholesterol and of the cholesterol/phospholipid ratio in the erythrocyte

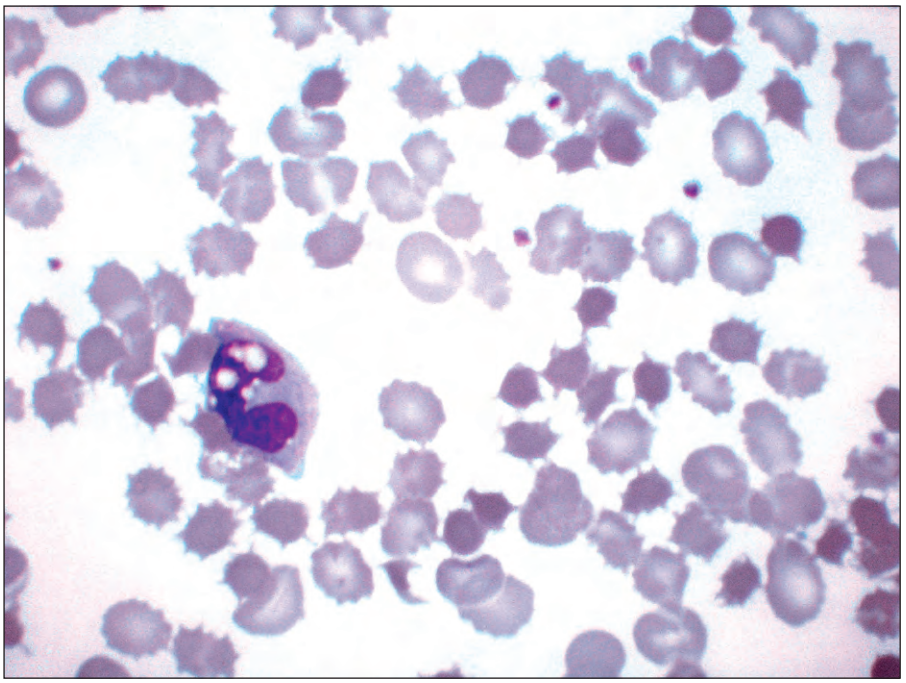


**Fig. 2.35** Peripheral blood film of a patient with abetalipoproteinaemia. There are numerous acanthocytes, characterised by their smallness, very variable morphology, absence of central pallor and presence of a small number of irregular pointed spicules irregularly distributed over the cytoplasmic membrane. The very reduced volume and the hyperchromia of these cells is seen clearly in comparison with a normal red cell a little above the centre (image courtesy of B.J. Bain).

membrane: this lipid profile causes an increase in viscosity and the loss of fragments of red cell membrane in the spleen, with reduced deformability and survival of the spiculated cells thus formed (spur cell haemolytic anaemia of severe liver disease). The formation of target cells, which is more common in liver disease is, on the other hand, linked to an increase in lipid content without any alteration in the cholesterol/phospholipid ratio.

Furthermore, acanthocytes are observable after splenectomy and in hyposplenism in general, in states of malnutrition such as anorexia nervosa, in subjects receiving parenteral nutrition with solution that has a high lipid content, in hereditary spherocytosis with a beta spectrin deficiency, in uraemia, in vitamin E deficiency and in hypothyroidism. In hereditary xerocytosis, the dehydrated form of hereditary stomatocytosis, it is possible to see both echinocytes and acanthocytes.

According to an unconfirmed hypothesis, the molecular basis of acanthocytosis could relate to a change in the conformation of the transmembrane protein, band 3, which determines the alteration of the spectrin molecule from a completely extended form (in echinocytosis) to a contracted refolded form (associated with acanthocytosis).[84]

### Sickle cells

Irreversibly sickled cells are thin bipolar erythrocytes with points at both ends, which recall the shape of a sickle or the moon at the beginning or end of its cycle. The long sides are not parallel but curvilinear and converge towards the two points. The morphological distortion is caused by the polymerisation of molecules of deoxygenated haemoglobin S, rendered insoluble by the replacement of glutamic acid in the sixth position of the beta chain of haemoglobin by valine. Sickle cells are observed in blood films only in homozygotes for haemoglobin S and in compound heterozygotes, such as S/C (Figs 2.24, 2.36 and 2.37), S/beta thalassaemia and S/D-Punjab. A sickling test induces a massive transformation of erythrocytes into sickle cells by subjecting unfixed blood to anoxic conditions (Fig. 2.37D).

### Cigar-shaped cells

Erythrocytes with the shape of a cigar or a boat (boat-shaped cells) or an oat grain (oat-shaped cells, rhomboidal cells) are often seen in sickle cell anaemia together with typical sickle cells (Fig. 2.36). A variant is a Napoleon hat cell, differing in that one of the two edges tends to be straight and the ends are more pointed. They have been described as typical of carriers of haemoglobin S-Oman, identified in Oman and thus given the name of the country.[85] Envelope-shaped cells are another variant of a sickle: these appear large and thin and folded on themselves (Fig. 2.36A).

### Bite cells

These are red cells the outline of which is interrupted as if by a bite, which is smaller than half the circumference (Figs 2.29B,

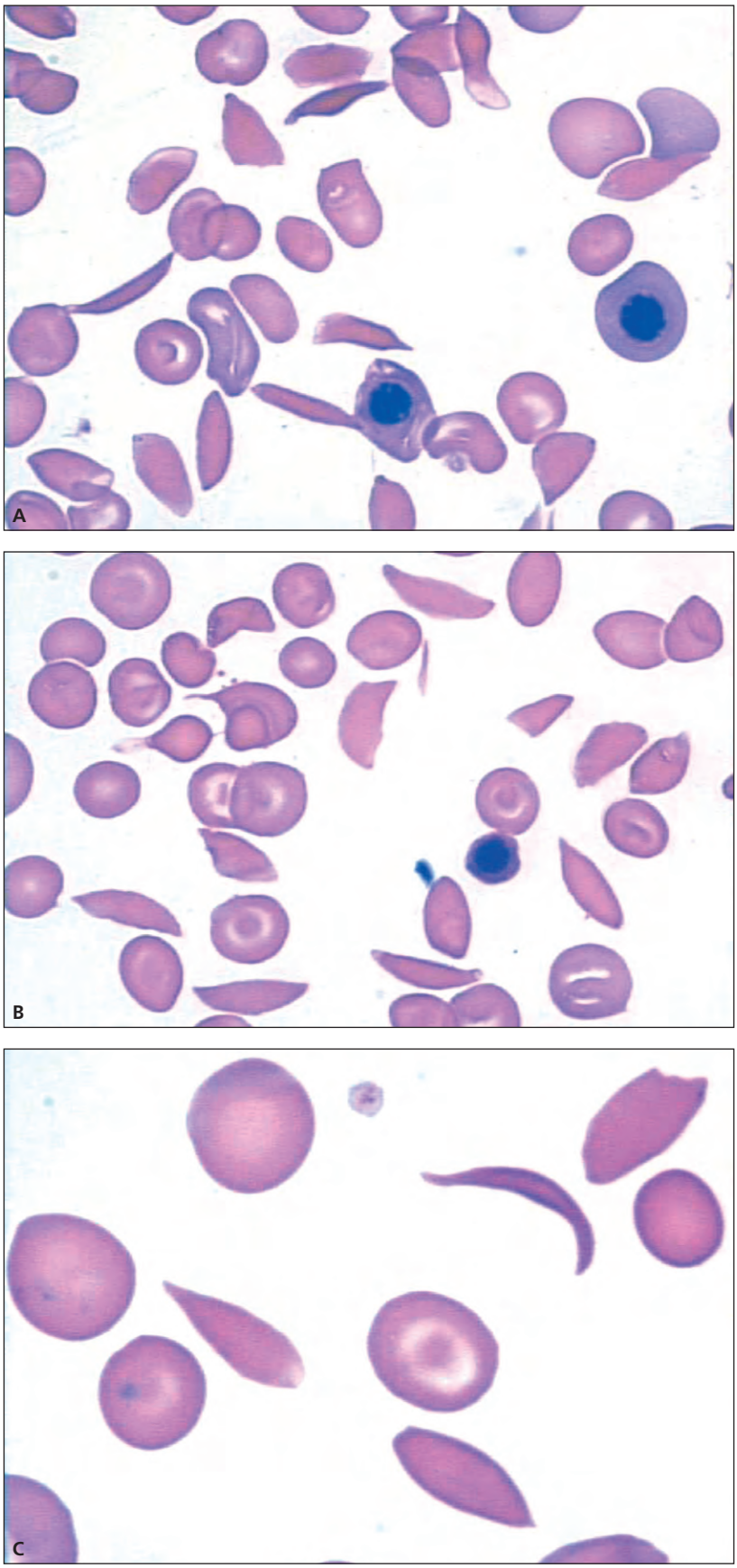



**Fig. 2.36** Morphology of sickle cell disease, target cells and anisopoikilocytosis in a woman with compound heterozygosity for haemoglobin S and haemoglobin C; peripheral blood film. (A) There are a number of sickle cells and also cigar- or boat-shaped cells; some cells, such as that between the two boat-shaped cells in the bottom left corner, have the shape of an oat grain, with an almost rhomboidal outline. The two erythroblasts, one orthochromatic and the other weakly polychromatic, have nuclei with compact violaceous chromatin. (B) In addition to the two semilunar-shaped sickle cells and a cigar-shaped cell, there are various target cells and other hyperdense, contracted cells, two of which appear joined by a filament. Others appear amputated, similar to helmet cells. Bottom right, a macrocyte with two slit-like areas of central pallor. (C) Top right, a classical sickle cell, an amputated boat-shaped cell and a microspherocyte. Nearby there are two rhomboidal red cells and a target cell.

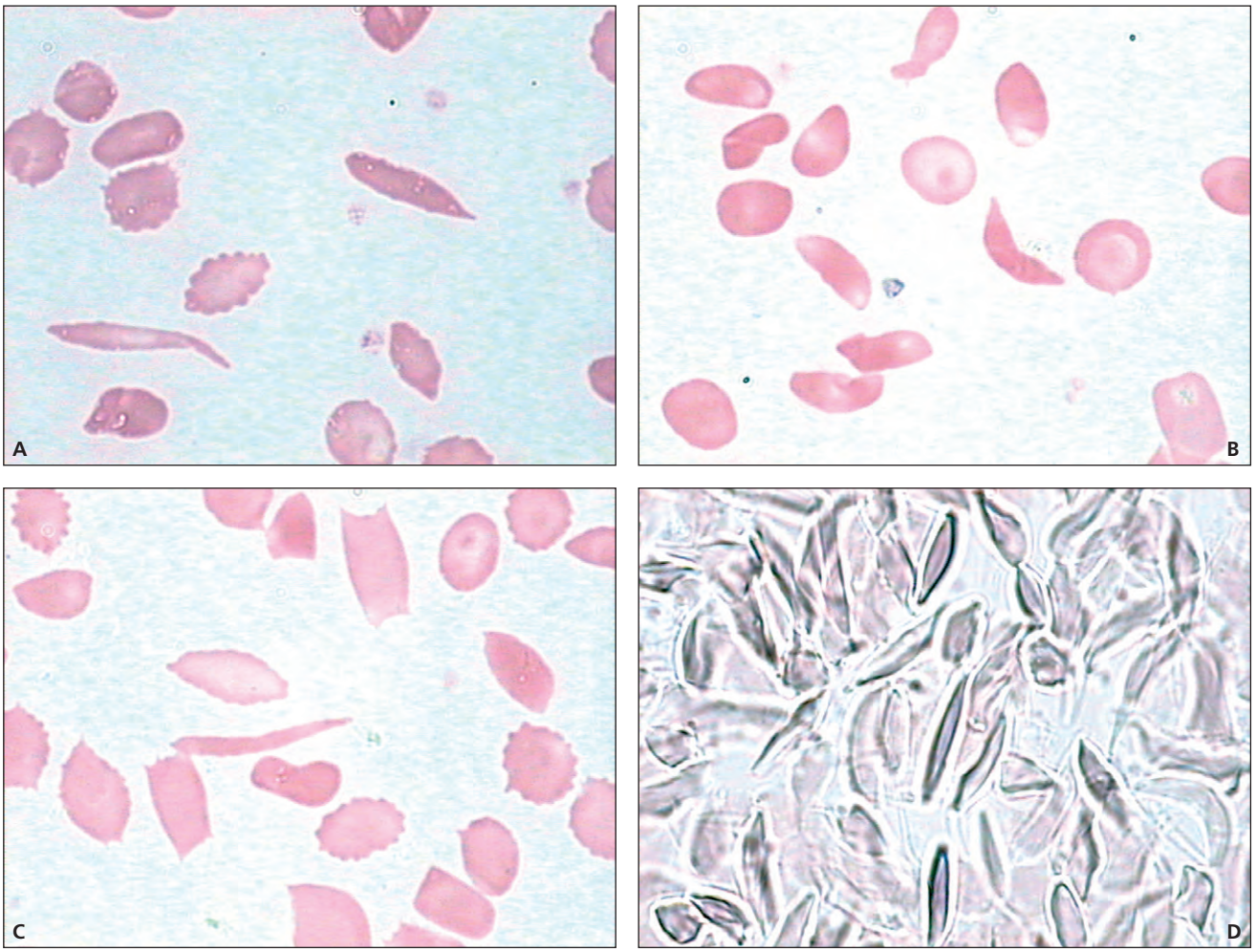



**Fig. 2.37** From a patient with homozygosity for haemoglobin S (sickle cell anaemia) during a sickle crisis. (A–C) There are cells with the shape of a sickle, a pencil, a boat and an oat grain or folded envelope plus target cells and echinocytes. (D) Sickle cell test, unfixed blood observed by phase contrast microscopy. All the red cells have been transformed into sickle cells or cigar-shaped cells.

2.30B and 2.38). The intact part of the membrane retains its normal curvilinear form. This lesion is caused by the removal (pitting) of masses of denatured precipitated haemoglobin (corresponding to Heinz bodies that are visible by supravital staining) by a phagocytic cell of the spleen. These cells are seen, often together with vesiculated cells and irregularly contracted cells, in haemolytic crises in G6PD deficiency, when there is damage from oxidising chemicals and in the presence of unstable haemoglobins, characterised by mutations that alter the interaction between haem and globin, causing denaturation.

### Schistocytes

These are damaged or fragmented erythrocytes, caused mainly by mechanical damage or because of turbulent flow. The prefix derives from the Greek *schistos* (cleaved, split) or from the corresponding verb *schizo*. These terms are often used as a general synonym for any sort of erythrocyte residue or detritus, whether haemoglobinised or not, formed as a result of rupture of the membrane. With a more restricted usage, as indicated by the ICSH guideline[86] for the diagnosis of microangiopathic haemolytic anaemia, excluding fragments that are the result of dyserythropoiesis, autoimmune haemolysis or artefacts, schistocytes are considered to be:

- Very small fragments of red cells which have an outline that is triangular, trapezoidal or moon shaped, but angulated with two or three pointed extremities, with cytoplasm that is often but not necessarily hyperchromic (Fig. 2.39);
- Larger erythrocytes that have the recognised form of a helmet cell or a keratocyte; the small fragments described above actually represent the portions that have been traumatically amputated from these damaged erythrocytes (Fig. 2.40);
- Microspherocytes (or spheroschistocytes) (Fig. 2.40): these are a morphological variant of a schistocyte formed by mechanical damage and are particularly common after

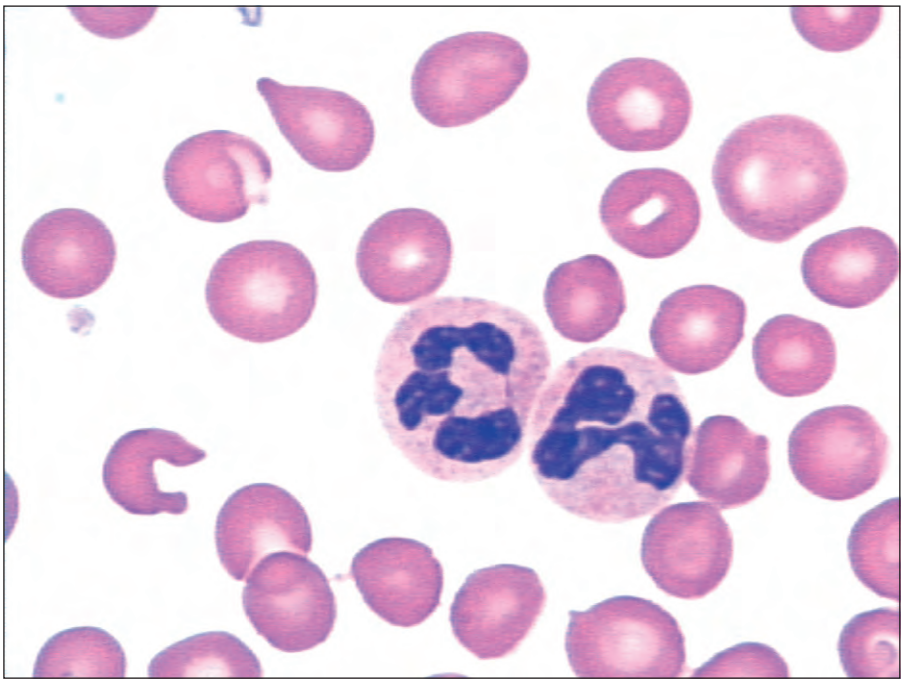


**Fig. 2.38** On the left, a bite cell, produced when the spleen removed haemoglobin that had precipitated during oxidative haemolysis. This cell can be distinguished from a keratocyte, a type of schistocyte characteristic of microangiopathic haemolytic anaemia. A little higher, a red cell with a fractured area of hypochromia, which predicts a similar occurrence. To the right of this cell, a typical dacrocyte and, in the top right corner, a macrocyte with very fine basophilic stippling.

splenectomy; the fragments assume a very small spherical form without any central pallor either *in vivo*, as a feature of ageing, or, as an artefact, in the thinnest part of the blood film[87] where the general appearance may erroneously suggest hereditary spherocytosis or autoimmune haemolytic anaemia; they are also seen when there is very abnormal ineffective erythropoiesis (see Fig. 2.17).

Schistocytes are formed following loss of part of the membrane and the cell content as the result of physical trauma or mechanical rupture of erythrocytes – followed by self-repair – caused by a collision with a fibrin strand augmented by abnormal, forceful blood flow related to damaged endothelium.[88] They occur in thrombotic microangiopathies, such as thrombotic thrombocytopenic purpura (TTP), the haemolytic-uraemic syndromes and, during pregnancy, in the HELPP syndrome; in disseminated intravascular coagulation; in valvular prostheses in the heart and similar foreign structures in the great vessels, particularly when malfunctioning (with a lower frequency today than in the past thanks to better surgical techniques and prosthetic materials) and in numerous other pathological states that alter the structure or the internal surface of the heart or the great vessels, producing vortices and regurgitant flow; in malignant hypertension, in renal diseases such as glomerulonephritis or renal cortical necrosis, in systemic lupus erythematosus; and in large cavernous haemangiomas.[89]

The schistocyte count has recently become clinically important, because of the importance of early diagnosis of TTP, which can now be treated by plasma exchange, a procedure that is effective particularly if initiated before the clinical syndrome is fully developed. For the diagnosis of thrombotic microangiopathy the percentage of schistocytes, determined microscopically on at least 1000 red cells, must be greater than 1%.[86] In the particular context of TTP in patients who have had a haemopoietic stem cell transplant, the prevalence of which is on average not much below 10%, the diagnosis is based on the recognition of more than 4% schistocytes, together with thrombocytopenia, increased lactate dehydrogenase (LDH) and a fall of Hb and haptoglobin concentration.[90]

### Helmet cells

These are a specific morphological variant of a schistocyte, only slightly smaller than an intact erythrocyte. They are characterised by a straight part of the cell membrane with two angulated extremities (Fig. 2.40). They are formed by amputation of a semilunar fragment of membrane and cytoplasm within intravascular networks that are composed mainly of filaments and other deposits of fibrin, or by the frictional forces produced by intravascular lesions or prosthesis that cause vortices, regurgitant flow and turbulence. They must be included in the schistocyte count.

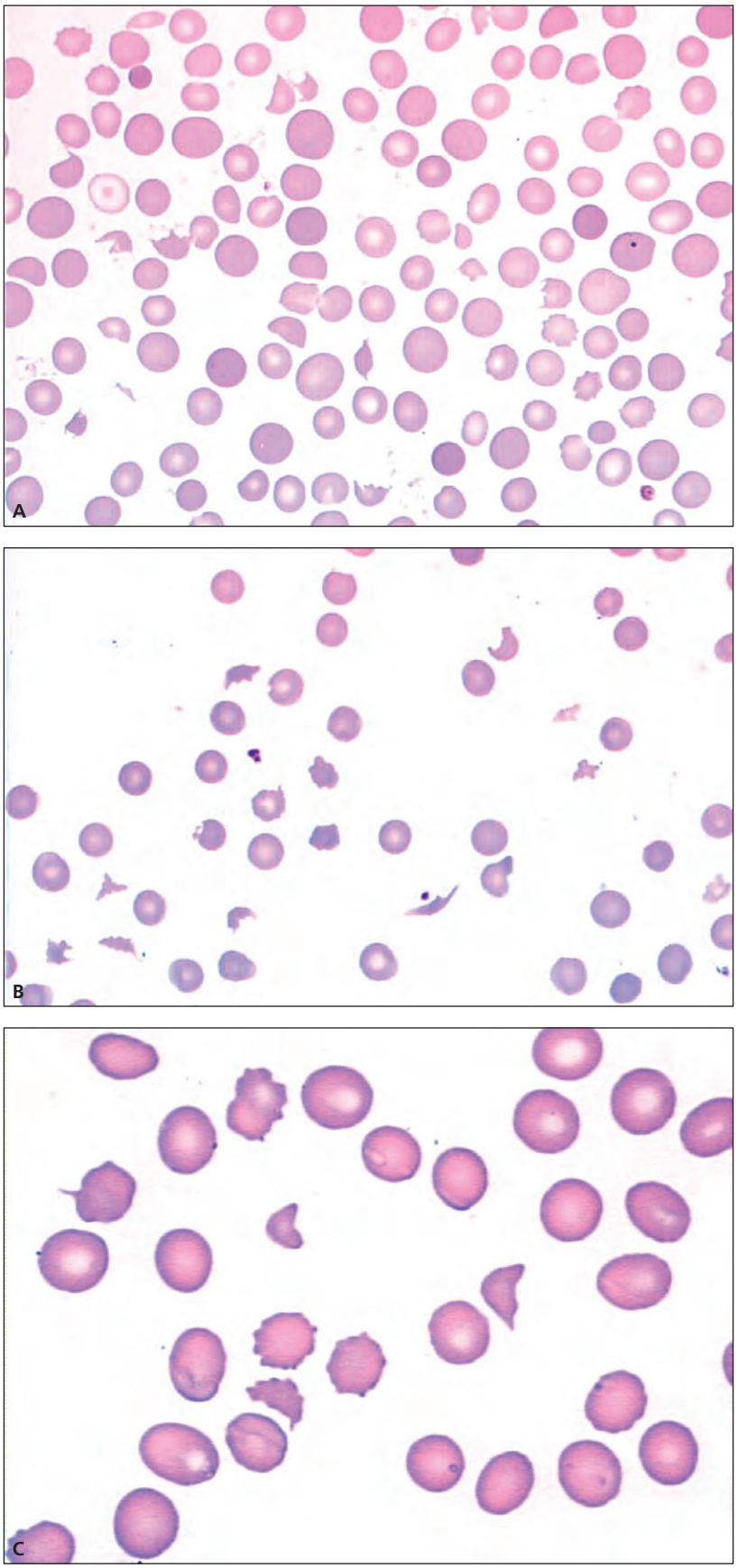



**Fig. 2.39** Schistocytes in peripheral blood films of three patients with thrombotic thrombocytopenic purpura. (A) There are numerous hyperchromic spherocytes of various sizes, helmet cells and schistocytes that are quite variable in shape, as are also various irregularly contracted cells similar in outline to small echinocytes. Top left, a microspherocyte, which represents the final phase of schistocyte production by red cell fragmentation. A hyperchromic macrocyte on the right contains a Howell-Jolly body and, like various other cells, has a polychromatic tinge. (B) Numerous triangular schistocytes, helmet cells (top right and in the centre, adjacent to the top edge) and microspherocytes (bottom right). (C) These schistocytes are triangular with irregular edges. There are some irregularly contracted cells lacking an area of central pallor and with their outline deformed by small spicules. (Images A and B courtesy of J.F. Lesesve.)

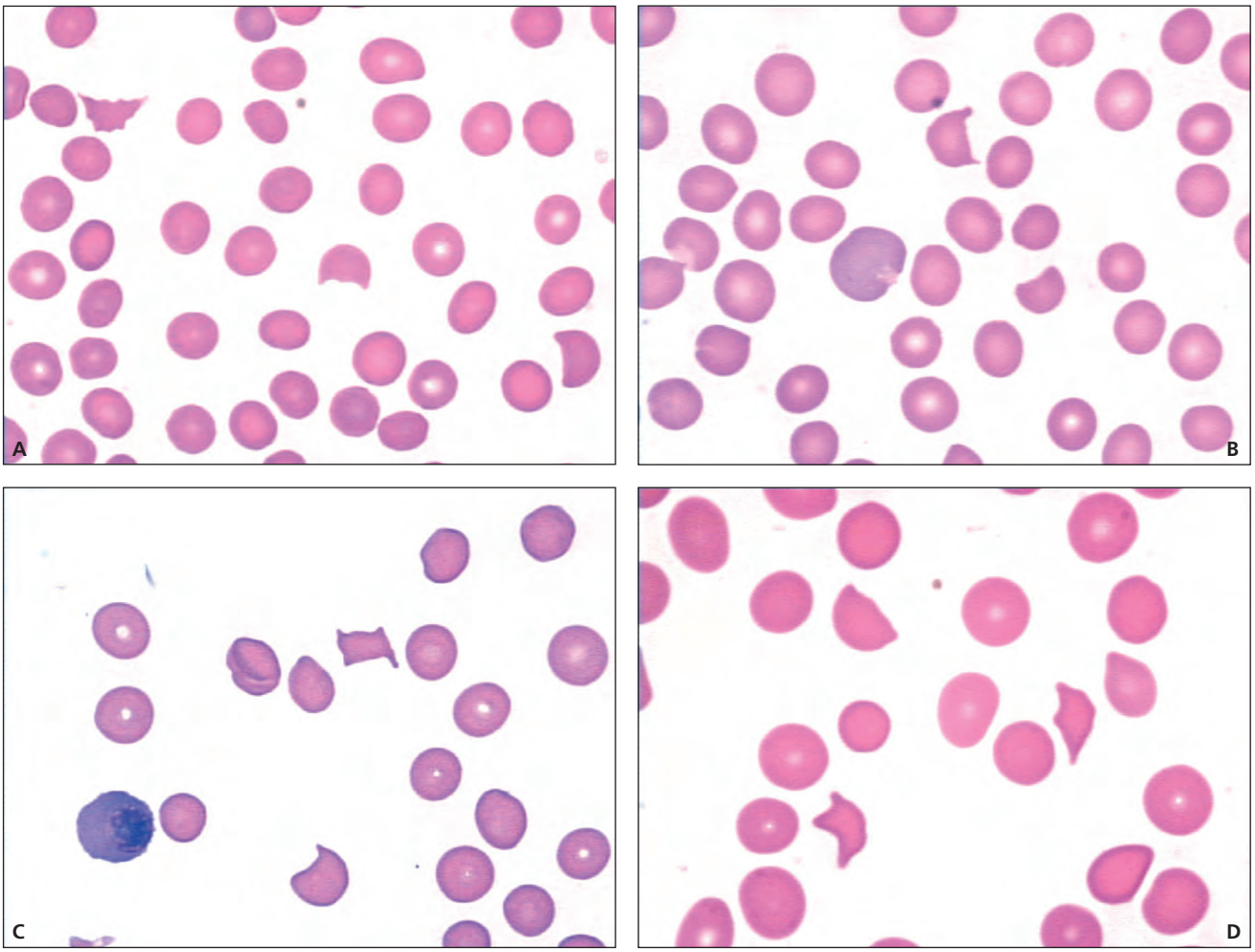



**Fig. 2.40** Schistocytes in patients with thrombotic microangiopathy. (A) From a patient with bone marrow metastases from carcinoma of the breast. Top left, a triangular schistocyte; in the centre and on the right, two typical helmet cells. (B) In the same film, centre top, a keratocyte, bottom left, a giant polychromatic cell and on the right, a helmet cell. Both keratocytes and helmet cells result from amputation of small cytoplasmic fragments at the level of endothelium involved in thrombus formation. (C) From a patient with bone marrow metastases from carcinoma of the lung. There are schistocytes and irregularly contracted cells; on the left, a polychromatic erythroblast. (D) Thrombotic microangiopathy in a patient who has had an allogeneic haemopoietic stem cell transplant. There are schistocytes, a helmet cell and hyperchromic microspherocytes.

### Keratocytes

These 'horned' cells have two lateral horns that surround a usually short concave segment of membrane (Fig. 2.40). They have the same significance as helmet cells, even though the fragment that has been detached is smaller. Less often there is a larger number of horns, but generally even numbers. Keratocytes must be included in the schistocyte count.

Although keratocytes and bite cells[91] can be morphologically identical, the mechanism responsible for the damage differs. The keratocyte is the result of intravascular mechanical damage in thrombotic microangiopathies and in relation to cardiovascular prostheses. Bessis has reproduced them experimentally by making microincisions in the erythrocyte membrane with a laser, which is followed by fusion of the two parts of the membrane with loss of small fragments.[49] Bite cells, on the other hand, are formed when the spleen removes Heinz bodies formed by oxidative damage

### Erythrocyte fragments*

Assigning, as appears opportune, the term schistocyte to red cells that were originally normal but have been damaged by

*Translator's comment: It should be noted that not all experts make a distinction between a schistocyte and a fragment, regarding these terms as synonymous with the underlying cause to be determined. Similarly, not all experts make a distinction between morphologically identical keratocytes and bite cells, again considering that once the morphological abnormality has been recognised, the cause must be determined.

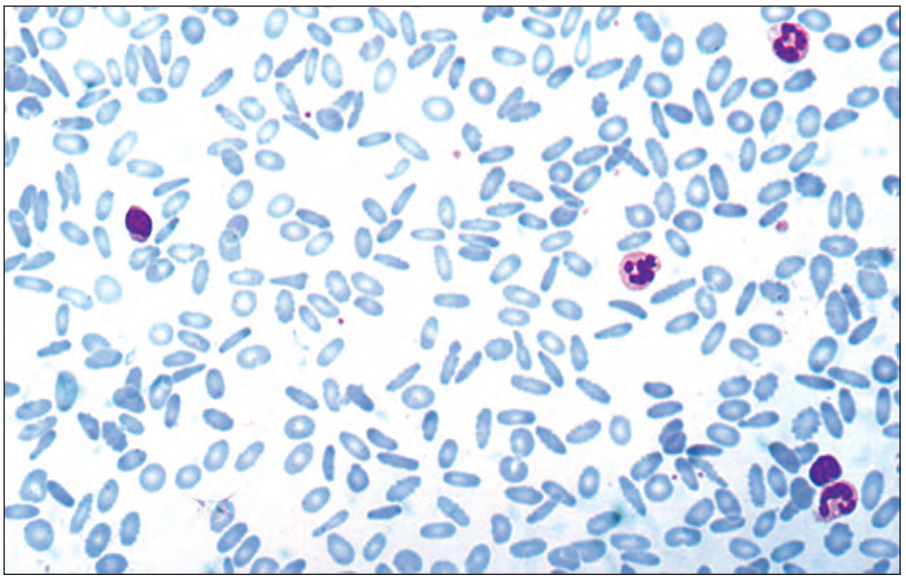


**Fig. 2.41** Hereditary elliptocytosis; peripheral blood film. Many red cells have the shape of a very long and thin cigar: these are the cells that are appropriately designated elliptocytes. There is a lower proportion of more rounded ovalocytes. There are no normal shaped cells.

extrinsic intravascular forces, one can also recognise erythrocyte fragments of various shapes, that are released into the circulation, or formed in the circulation, in conditions in which there is a serious disturbance of erythropoiesis (abnormal mitoses and maturation, karyorrhexis, intramedullary apoptosis in megaloblastic anaemia and beta thalassaemia major) (see, for example, Figs 2.30C and 2.32B) or serious abnormality of the bone marrow microenvironment, as in patients with myelofibrosis or with bone marrow metastases, in whom however the occurrence of a true TTP is not rare.[92] Fragmentation can also occur in the circulation when there is an intrinsic abnormality of the red cells, either inherited (pyropoikilocytosis) or acquired (autoimmune haemolytic anaemia). Physiological microlesions occur in the spleen, in reticulocytes, which are 'pitted' to eliminate residual organelles and haemosiderin granules: this could account for the very small number of erythrocyte fragments that can be observed in normal subjects.

Certain automated haematology analysers can provide an 'alarm' or 'flag' indicating erythrocyte fragmentation or can produce a count of fragmented erythrocytes based on their size and optical density. These methods do not have the level of specificity of microscopic observation, but they can be useful for indicating the presence of the anomaly and for monitoring the number of fragments over time.

### Dacrocytes

These are asymmetrical red cells, as if pinched off by tweezers, having the shape of a teardrop (hence also called teardrop cells), a drop or a pear, with a pointed extremity, resembling a tail of variable length (see, for example, Figs 2.17, 2.38 and 2.44B). They occur in largest numbers in bone marrow fibrosis, as in primary myelofibrosis, osteopetrosis or bone marrow metastases. The classical concept of the tail being formed by stretching as the cell exits from a restricted and rigid medullary space does not have any experimental confirmation. Dacrocytes are found also in the blood films of patients with iron deficiency, pernicious anaemia and renal insufficiency, and as a spreading artefact.

### Elliptocytes/ovalocytes

Some authors use these terms as synonyms to refer to erythrocytes that are elongated, elliptical or like small cigars with blunt rounded ends (Figs 2.32B and 2.41). Sometimes the term elliptocyte is reserved for cells in which the lesser diameter is very small and the two sides are parallel, while the designation ovalocyte, considered more generic, is used for cells with more curvilinear sides and cells with an elongated oval shape. Particularly long thin elliptocytes are also referred to as 'pencil cells'.

This anomaly is most marked in hereditary elliptocytosis, a group of genetically determined conditions with reported frequency varying from 1 per 1000 to 1 per 5000, commoner in those of African or Mediterranean origin, probably because of selection linked to the resistance to malaria conferred by the defect. The underlying molecular abnormality can be quantitative or qualitative, affecting cytoskeletal proteins (spectrin, protein 4.1R or glycophorin C).[93] Most cases of hereditary elliptocytosis are clinically silent and are diagnosed after incidental examination of a blood film in which the proportion of elliptical red cells is above 25%. Elliptocytes are normocytic and normochromic with a normal MCV and osmotic fragility. Only in a minority of cases, around 10%, is the morphological abnormality associated with reduced red cell survival, with chronic

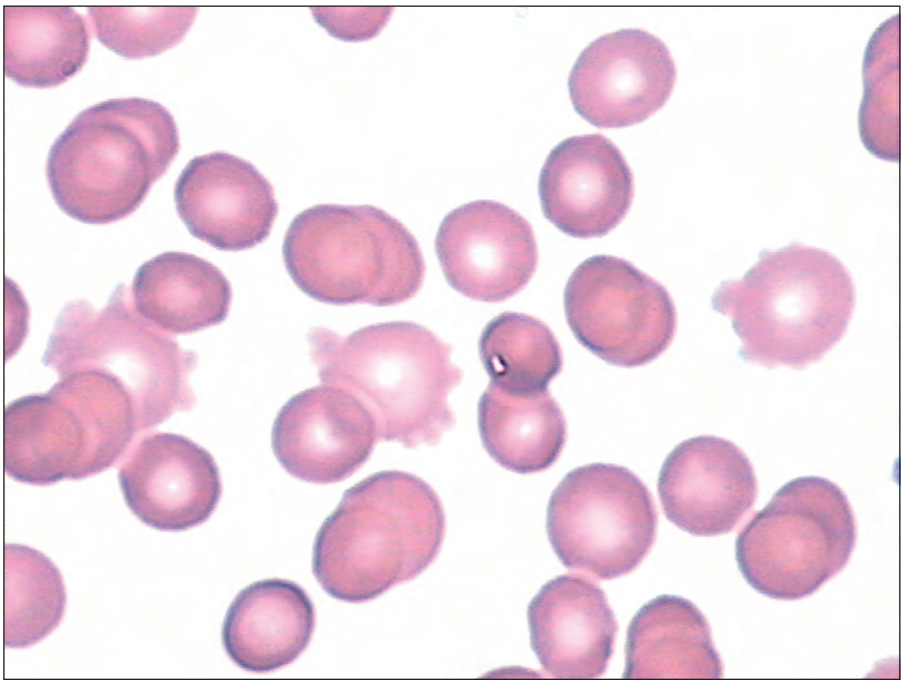


**Fig. 2.42** Blebbed erythrocytes in a peripheral blood film. This appearance of the red cell outline, with budding of ill-defined borders, which can evoke the explosion of a red cell, is seen in patients with extensive recent burns.

haemolysis, which is usually moderate, and with the presence of extra morphological abnormalities such as poikilocytes and fragments.

Hereditary pyropoikilocytosis is a variant of hereditary elliptocytosis, characterised by a severe haemolytic anaemia present from early infancy, with the blood film showing poikilocytosis, fragments and microspherocytes; cell size is very variable but is usually much reduced (MCV 25–75 fl) and there is increased osmotic fragility. These anomalies are typically increased by heat (thermal instability). Other variants include spherocytic hereditary elliptocytosis, distinguished by the simultaneous presence in the blood film of spherocytes with an oval outline (sphero-ovalocytes) and spherocytes (see Fig. 2.26), and the above-mentioned South-East Asian ovalocytosis, or stomatocytic elliptocytosis, in which the blood film shows both very large macro-ovalocytes and stomatocytes, which have the particular feature of a pale stoma of irregular shape or V or Y shaped, or sometimes double.[61]

Elliptocytosis is also sometimes found in hepatic and renal disease, in myelofibrosis, in thalassaemia and in iron deficiency anaemia (see Fig. 2.20). Elliptocytes can also be seen in small numbers in well-made blood films from normal subjects. Macro-ovalocytes, characterised by large size and an oval outline, are typical of megaloblastic erythropoiesis (see Fig. 2.13A).

The formation of elliptocytes can occur as an artefact during the spreading of the film (pseudoelliptocytosis), being seen mainly in the tail: in these cases the longest axes of the cell are always turned in the same direction (while genuine elliptocytes are randomly arranged).

### Budding erythrocytes

These bizarre, hyperchromic, lobulated erythrocytes with surface protrusions, resembling the shape of popcorn, are typically seen in severe burns[94] (Fig. 2.42), but sometimes also in myelodysplasia, in megaloblastosis and as an artefact in the blood of healthy subjects.

## Erythrocyte inclusions

### Basophilic stippling

Basophilic stippling, also known as punctate basophilia, indicates the presence in red cells of numerous, usually delicate, granules of a dark blue colour, corresponding to precipitated ribosomes, and thus composed mainly of RNA, visible on dried, fixed and panoptically stained films (Figs 2.43 and 2.44). It is a quite common finding and does not have any specific diagnostic significance. It is one of the three ways in which the residual cytoplasmic basophilia, so characteristic of immature erythroblasts, can be manifest in more mature cells, the others being as polychromasia and as the granulofilamentous material of reticulocytes. Basophilic stippling, however, unlike the other two forms, does not have the significance of regeneration, but rather is attributable to anomalous structure or composition of ribosomes or a defect in the enzymatic mechanism required for their catabolism; their precipitation and aggregation is thus favoured,

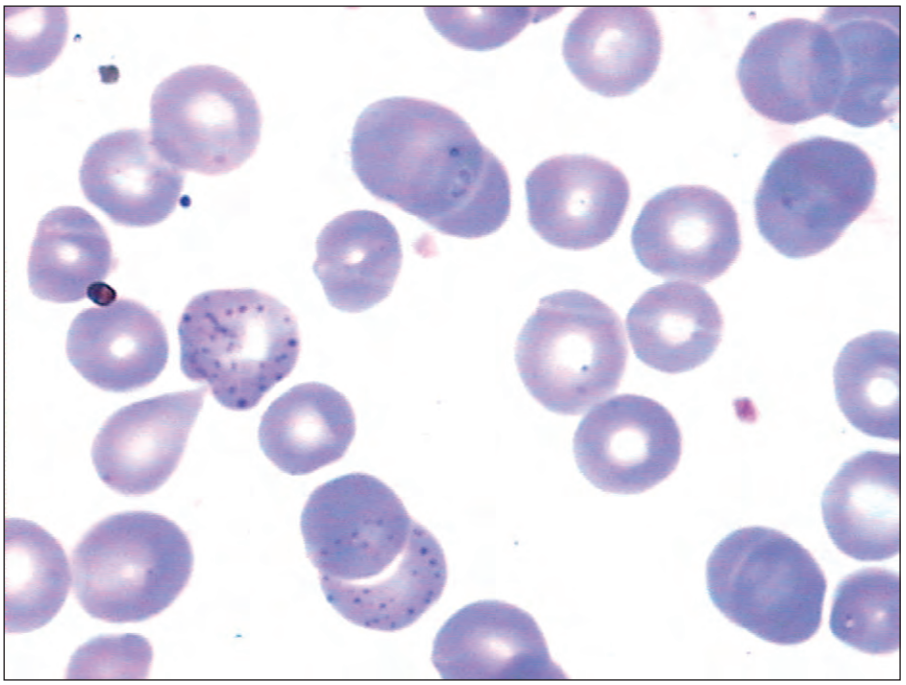


**Fig. 2.43** Basophilic stippling in a patient with lead poisoning; peripheral blood film. In the context of a normocytic population, there are red cells with fine bluish granules, of variable size and spread throughout the cell; some are delicate and others, close to the cell membrane, are coarser. This picture results from the massing together of precipitated ribosomes and does not have the same significance as reticulocytosis that is demonstrated on supravital stains. On the left, a dacrocyte, without any specific significance.

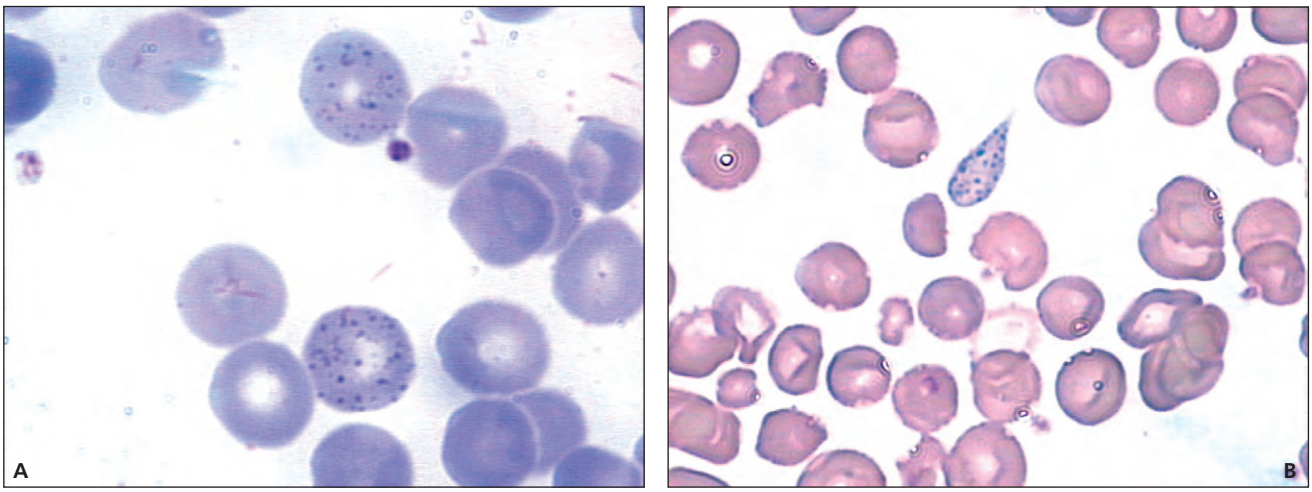



**Fig. 2.44** Basophilic stippling; peripheral blood film. (A) Two red cells with striking basophilic stippling in a patient with congenital pyrimidine 5′-nucleotidase deficiency. (B) Polychromatic dacrocyte with basophilic stippling in a patient with refractory anaemia. The red cell morphology is not well preserved in this part of the film so that other alterations, such as the ghost cell beneath the dacrocyte, cannot be considered informative.

*in vivo*, prior to fixation. In general, this ribosomal defect is associated with abnormalities in synthesis of haemoglobin.

The most obvious basophilic stippling is seen in lead poisoning, in which the granules are particularly large, and in other heavy metal poisoning (zinc, arsenic, silver, mercury) and in thalassaemia of all types. Among the microcytic anaemias, the presence of basophilic stippling, although not a constant observation, favours a diagnosis of beta thalassaemia (particularly in Mediterranean populations). Basophilic stippling is seen also in haemolytic anaemia, in myelodysplasia and in megaloblastosis. It is particularly obvious in the rare subjects with pyrimidine 5′-nucleotidase deficiency, which manifests itself clinically as a

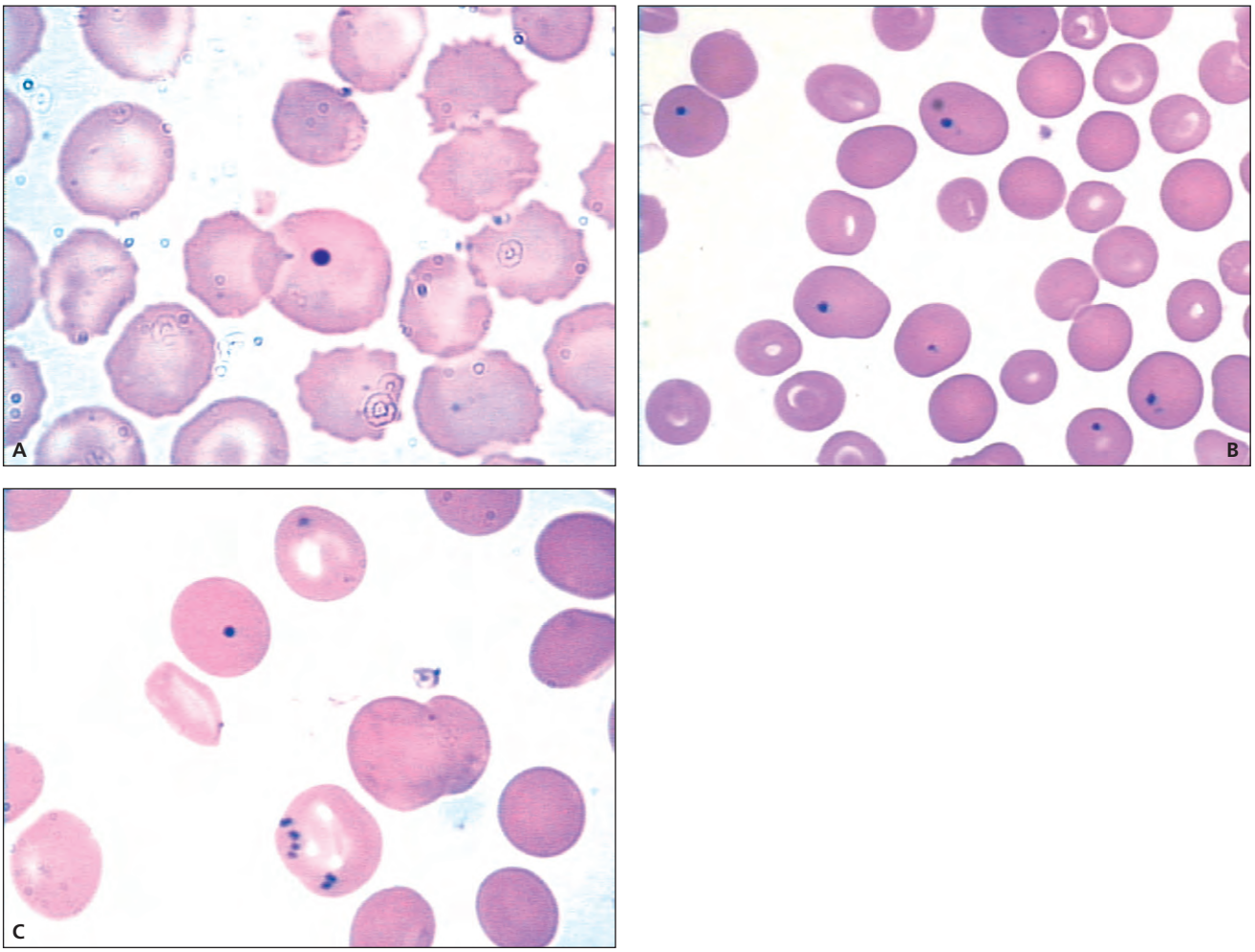



**Fig. 2.45** Howell-Jolly bodies; peripheral blood film. (A) From a patient with refractory anaemia with ring sideroblasts. The single large, spherical inclusion in the cytoplasm of the central macrocyte is a Howell-Jolly body, the residuum of a chromosome lost during a mitotic division. It is blackish violet or dark purple, different from the paler blue of basophilic stippling. The staining characteristics result from the higher content of acidic protein in nuclear DNA, which gives some affinity for eosin, in contrast to RNA, which is stained only with methylene blue. There are target cells and other cells similar to echinocytes without a central pale area. (B) Five red cells, among which the larger ones contain a single Howell-Jolly body. Along the central meridian, the ovoid macrocyte at the top has an inclusion above a Howell-Jolly body that is out of focus and perhaps is an extraneous deposit. From a patient with megaloblastic anaemia. (C) Myelodysplasia-related acute myeloid leukaemia during treatment. This image shows the morphological differences between Howell-Jolly bodies (top left) and two Pappenheimer bodies (bottom centre): the latter are smaller, less regular, often multiple and, as in this case, may be clustered.

generally mild non-spherocytic chronic haemolytic anaemia. The enzyme that is lacking is specific for diphosphorylation of nucleotides that originate with the catabolism of cellular RNA. Its hereditary deficiency, or its inhibition by lead, slows the degradation of RNA molecules and leads to their aggregation.

### Howell-Jolly bodies

These are basophilic spherical inclusions, sometimes called just Jolly bodies, visible in films fixed and stained panoptically; they are rounded, dark, blackish-blue, isolated, usually single or, less often, multiple, of variable size but generally small, with a dense compact structure, situated towards the periphery of the red cell but not in contact with the cell membrane (Fig. 2.45). They are remnants of nuclear chromatin (DNA), detached from chromosomes during cellular division.

They are a relatively frequent and not very specific erythroid abnormality, seen particularly in the presence of dyserythropoiesis (maximally in megaloblastic anaemia) and in conditions of accelerated erythropoiesis, such as acute haemolytic anaemia. They are seen more often after splenectomy and in functionally hyposplenic states, such as coeliac disease and sickle cell disease, in which the spleen's capacity to remove nuclear remnants has

been lost. They and other red cell inclusions must be distinguished from particles of dirt, stain precipitates and platelets superimposed on erythrocytes.

### Pappenheimer bodies

These are dense, dark blue, basophilic inclusions, smaller than Howell-Jolly bodies and differing from them in not being perfectly rounded; sometimes their outline is angulated; they are present in small numbers, isolated or, often, in small groups disposed asymmetrically in a restricted part of the cytoplasm (in contrast to the regular dispersion of basophilic stippling) (Fig. 2.46). They correspond to the siderosomes that are visible by electron microscopy, which are masses of haemosiderin together with ribosomes and residual degenerating mitochondria. They stain azure blue with the Perls reaction (Fig. 2.46B and C).

Pappenheimer bodies are seen particularly in congenital sideroblastic anaemia, often in the context of a dimorphic red cell population, in myelodysplasia with significant dyserythropoiesis, in haemolytic anaemia, in splenectomised patients and in conditions of iron overload.

### Cabot rings

These are curvilinear erythrocyte inclusions, which form a closed circle or oval, sometimes in a figure of eight formation (Figs 2.47, 2.48 and 2.49). The colour varies from red to purple, paler than a Howell-Jolly body; the colour depends also on the specific panoptic stain used. They are often associated with fine

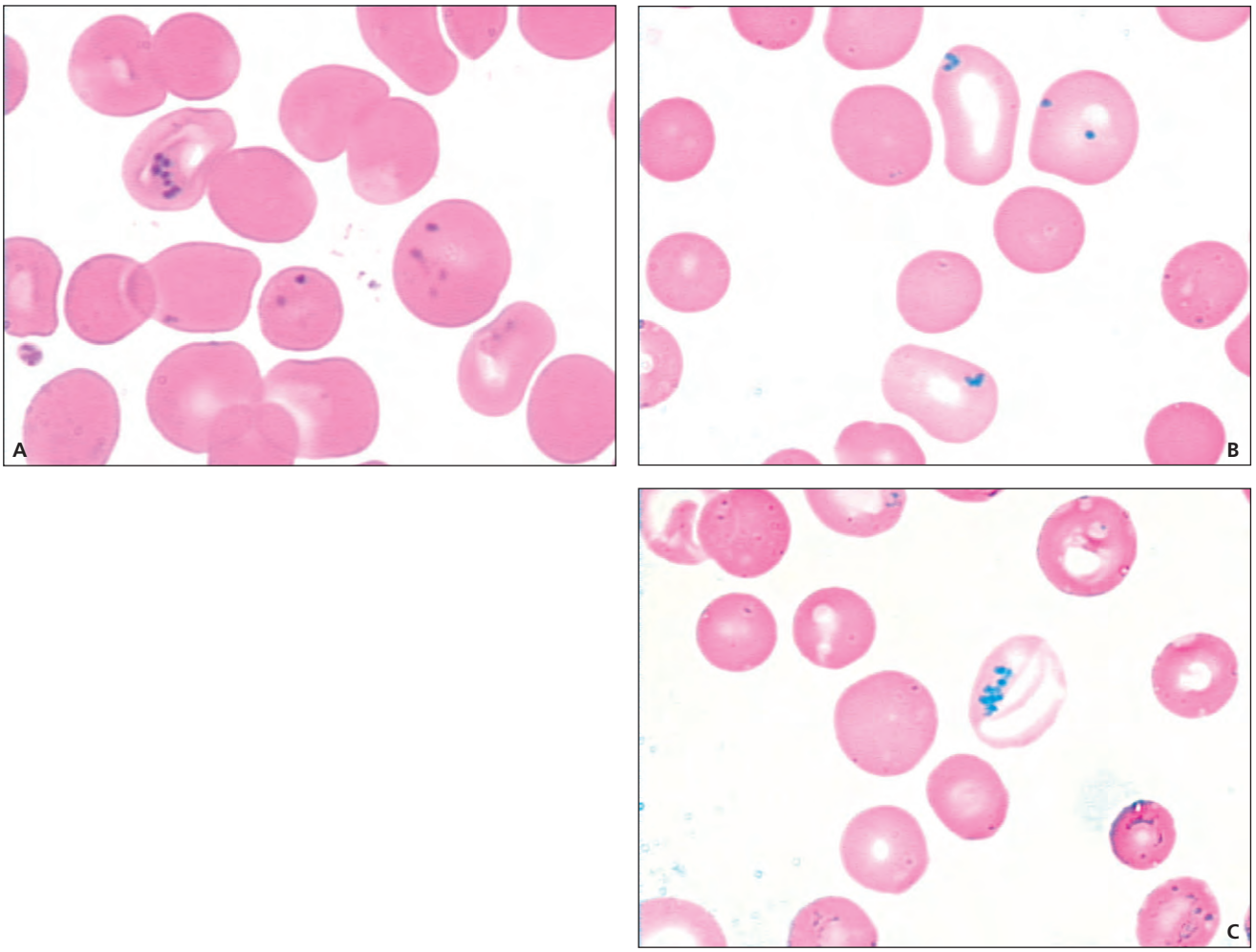



**Fig. 2.46** Pappenheimer bodies in circulating red cells from a multiply transfused patient with severe anaemia resulting from bone marrow hypoplasia with an associated haemophagocytic syndrome. (A) There are multiple granules differing from each other in size and shape. (B) Perls stain. Pappenheimer bodies are siderosomes, as is shown by their blue colour when stained with potassium ferrocyanide. (C) Perls stain. In a poorly haemoglobinised cell there are very typical erythrocyte siderosomes, these being clusters of granules that stain positively for iron and are composed of masses of haemosiderin admixed with ribosomes and the residues of mitochondria.

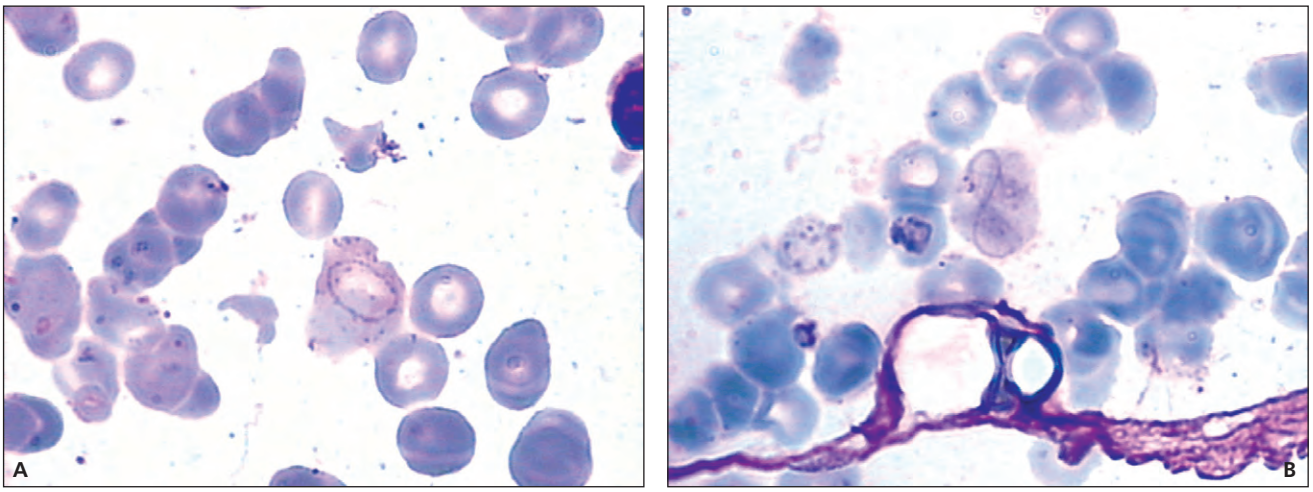



**Fig. 2.47** Cabot rings, from the bone marrow aspirate of a patient with therapy-related acute myeloid leukaemia following administration of chemotherapy for carcinoma of the breast. (A) Cabot ring, visible in a deformed macrocyte, identifiable from its characteristic circular form and crimson colour, paler than the staining of a Howell-Jolly body. Like the fine reddish azurophilic granules that accompany it, it is the residuum of the filamentous structure of the mitotic spindle. (B) A large red cell containing a Cabot ring twisted into the form of a figure of eight, together with numerous fine azurophilic granules. A smaller erythrocyte, on the left, also shows a complete ring. The preparation is otherwise not really evaluable for red cell morphology because of the density of the cells, which has facilitated rouleaux formation, and because of the presence of crushed cells.

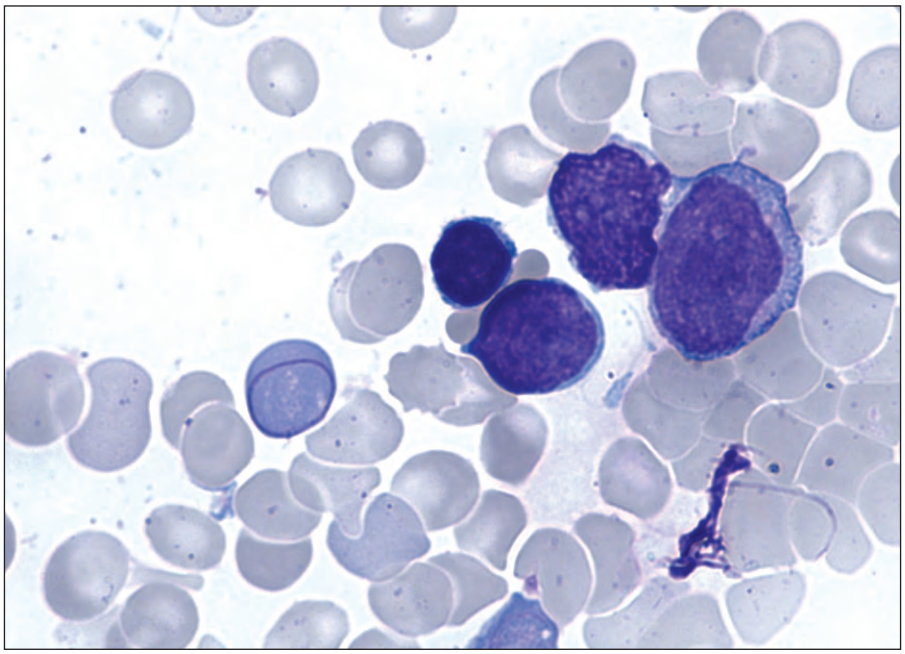


**Fig. 2.48** Peripheral blood film from a young patient with acute lymphoblastic leukaemia who has relapsed following allogeneic haemopoietic stem cell transplantation. A thick Cabot ring stands out from the bluish background of a polychromatic erythrocyte. A somewhat similar cell with a less distinct ring has been cut off at the lower margin of the image. Adjacent to a small lymphocyte with chromatin condensed into dark clumps are three blast cells, which vary with regard to size and nucleocytoplasmic ratio. That on the right is larger and has abundant basophilic cytoplasm containing small vacuoles: the chromatin is delicate and homogeneous and at least one nucleolus is apparent.

granules, variable in number, situation and size, with the same staining characteristics. Cabot rings and the granules that accompany them are the remnants of the mitotic spindle. They are seen after splenectomy, in megaloblastic anaemia, in acute haemolytic anaemia, in the extramedullary haemopoiesis of primary myelofibrosis and in myelodysplasia with marked dyserythropoiesis.

### Intra-erythrocyte crystals

In homozygotes for haemoglobin C, dense haemoglobin crystals can form within red cells; they are rhomboidal, tetragonal or rod-shaped, often causing distortion of the cellular outline and projecting from the cell (Fig. 2.50). This phenomenon can also be present in compound heterozygotes for haemoglobins S and C, in which the characteristic crystals deform the cells and

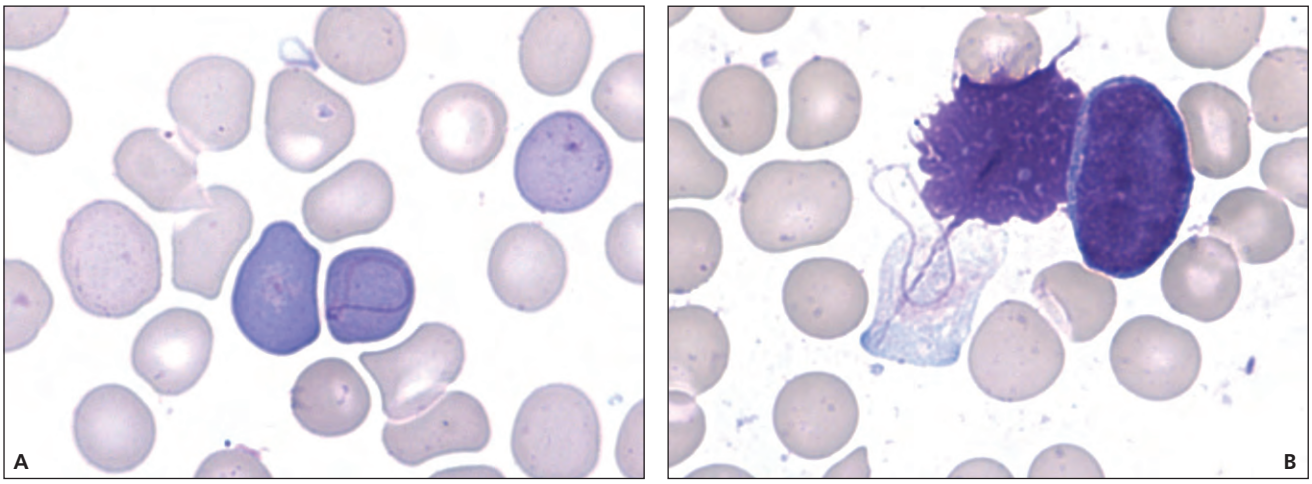



**Fig. 2.49** Peripheral blood film, same patient as in Fig. 2.48. (A) In the centre, two polychromatic erythrocytes contain fine azurophilic granules: in the cell on the right, a large Cabot ring is clearly visible, folded to form a small secondary loop. A pale macrocyte on the left has very fine granules and a circular shadow that represents the remains of a degenerating Cabot ring. Another polychromatic erythrocyte, full of granules, is seen on the right. (B) Superimposed on the cytoplasm of a damaged cell is a long Cabot ring. On the right, a lymphoblast.

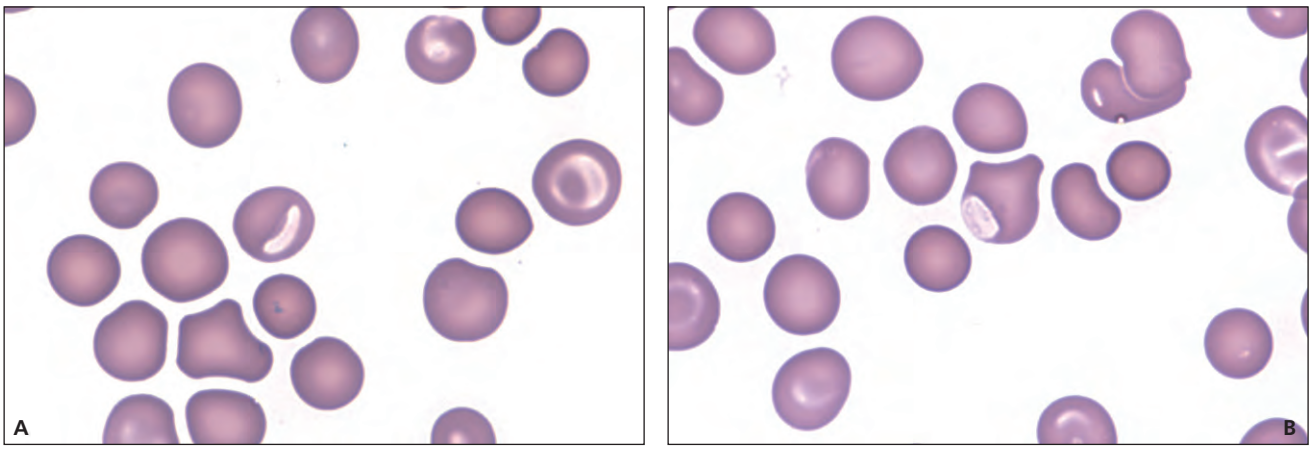



**Fig. 2.50** Haemoglobin C homozygote, peripheral blood film. (A) A typical crystalline formation of haemoglobin C is visible within a clearly defined area of central pallor. (B) In this deformed red cell, a crystal of haemoglobin C is large and almost rectangular.

are accompanied by sickle cells.[95] These crystals are homogeneous and dark and terminate in a blunt pyramid. In compound heterozygotes the cells that contain one or more crystals undergo a typical type of deformation and are known as SC poikilocytes[96]: they are small cells resembling sickle cells in being hyperchromic, but also show rectilinear and angulated outlines. Their frequency increases markedly after splenectomy.

### Haemoglobin H inclusions

These are formed by precipitation of tetramers of the beta chain of haemoglobin, which is present in excess over the alpha chain in haemoglobin H disease (deletion of three of the four alpha chain genes). They are visible much more rarely in alpha thalassaemia with deletion of only one or two alpha genes. They are not easily identifiable as inclusions with panoptic stains of fixed cells, where they sometimes appear as echinocytes with thick spines seen in three dimensions (Fig. 2.51). With supravital preparations (brilliant cresyl blue or new methylene blue), they are seen as rounded bluish inclusions distributed regularly through the cell in relatively high numbers (from 20 to 50 per cell). The erythrocyte thus has a 'golf-ball' appearance. The red cells of patients with haemoglobin H disease are microcytic (MCV $<70$ fl).

Haemoglobin H inclusions are better seen with supravital staining after prolonged incubation (2 hours at 37°C). In these preparations, they must be distinguished from reticulocytes (which contain darker granulofilamentous material, sometimes in a network, with a less uniform distribution and areas of

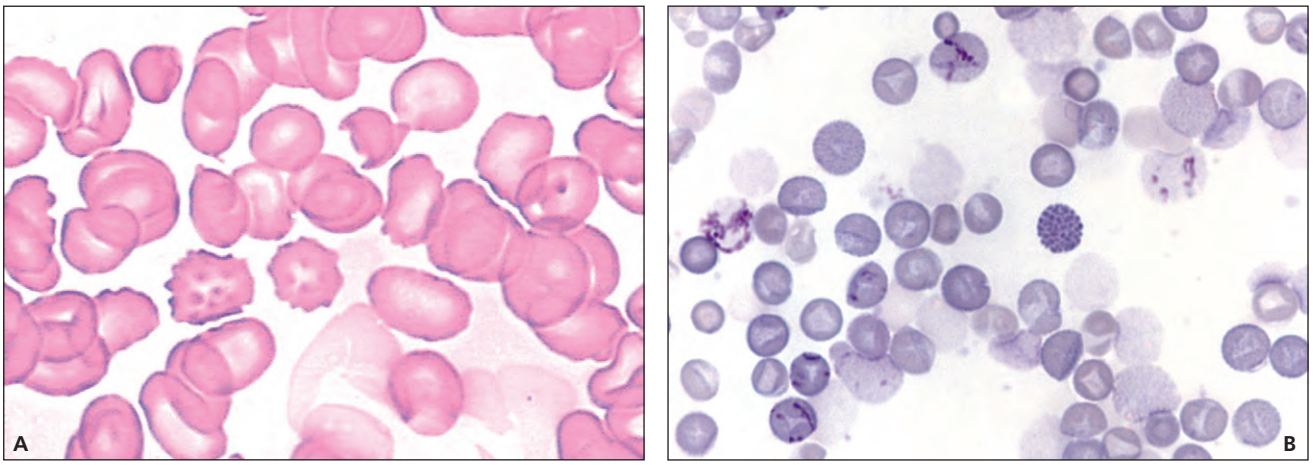



**Fig. 2.51** Peripheral blood film from a patient with haemoglobin H disease and the clinical picture of thalassaemia intermedia. (A) In a May-Grünwald-Giemsa-stained film there are two red cells with blunt projections and several other poikilocytes. (B) New methylene blue stain. A cell with a golf-ball appearance resulting from precipitation of haemoglobin H is apparent near the centre of the image. The intracellular distribution of the precipitates is homogeneous and this permits a distinction from reticulocytes, which are visible at the top and on the left of the image.

greater density) and from Howell-Jolly bodies (usually single, rarely double).

### Intra-erythrocytic micro-organisms

In malaria, one can see intra-erythrocyte plasmodia at various stages of maturation, from the early ring-form trophozoites to schizonts containing numerous small merozoites, and finally gametocytes (Fig. 2.52). Babesia similarly can be seen within erythrocytes. Among bacterial infections, bartonellosis shows numerous rod-shaped organisms 1–2 μm in length within red cells.

For a detailed description of intra-erythrocytic micro-organisms, we refer the reader to textbooks and atlases dedicated to parasite morphology.[97]

### Heinz bodies

These are intra-erythrocytic inclusions formed of denatured haemoglobin, not visible with panoptic stains but made visible by supravital staining of unfixed blood (using methyl violet, new methylene blue or brilliant cresyl blue). Heinz bodies are irregular in shape and are located mainly in contact with the inner surface of the red cell membrane (Fig. 2.53). They are distinguishable from the inclusions of reticulocytes, which are dispersed through the cytoplasm, separate from the cell margin. On flow cytometry the masses of denatured precipitated haemoglobin can combine with fluorochromes and cause a factitious increase, often very marked, in the reticulocyte count.[58]

The presence of Heinz bodies leads to reduced red cell survival as a result of reduced deformability and increased membrane permeability. They form in red cells when there is an unstable haemoglobin, such as haemoglobin Zurich or haemoglobin H, in thalassaemia, in G6PD deficiency and in normal subjects in whom haemoglobin has been denatured by an oxidising chemical. Some unstable haemoglobins are associated with severe haemolysis, to the extent of their being designated 'congenital Heinz body haemolytic anaemias'. On fixed and stained films one can see (in the case of an unstable haemoglobin) anisopoikilocytosis and microspherocytes, together with bite cells, resulting from removal of Heinz bodies by the spleen, or (in the case of G6PD deficiency) blister cells.

## Abnormal distribution of red cells in the blood film

### Agglutination

Agglutinates are masses of red cells, varying in size and shape and with an irregular outline. They can contain few or numerous cells, and there is an apparent loss of the distinction between one cell and another (Fig. 2.54). Red cell agglutinates generally result from the presence of a cold agglutinin, usually IgM. They are often seen in mycoplasma infection, in influenza and in other viral infections, but also in lymphoproliferative and plasma cell disorders, in cold haemagglutinin disease and in paroxysmal cold haemoglobinuria. They are rare in warm autoimmune haemolytic anaemia.

Agglutinates, if massive, can be recognised macroscopically, without the need of a microscope, by running blood up and down the specimen tube. Agglutination must not be confused with increased rouleaux formation.

### Increased rouleaux formation

In rouleaux formation red cells line up, adhering to each other by their concave surfaces, so that they resemble a stack of coins (Fig. 2.55). By convention, a rouleau is composed of at least four cells in linear array. In smaller rouleaux, the area of central

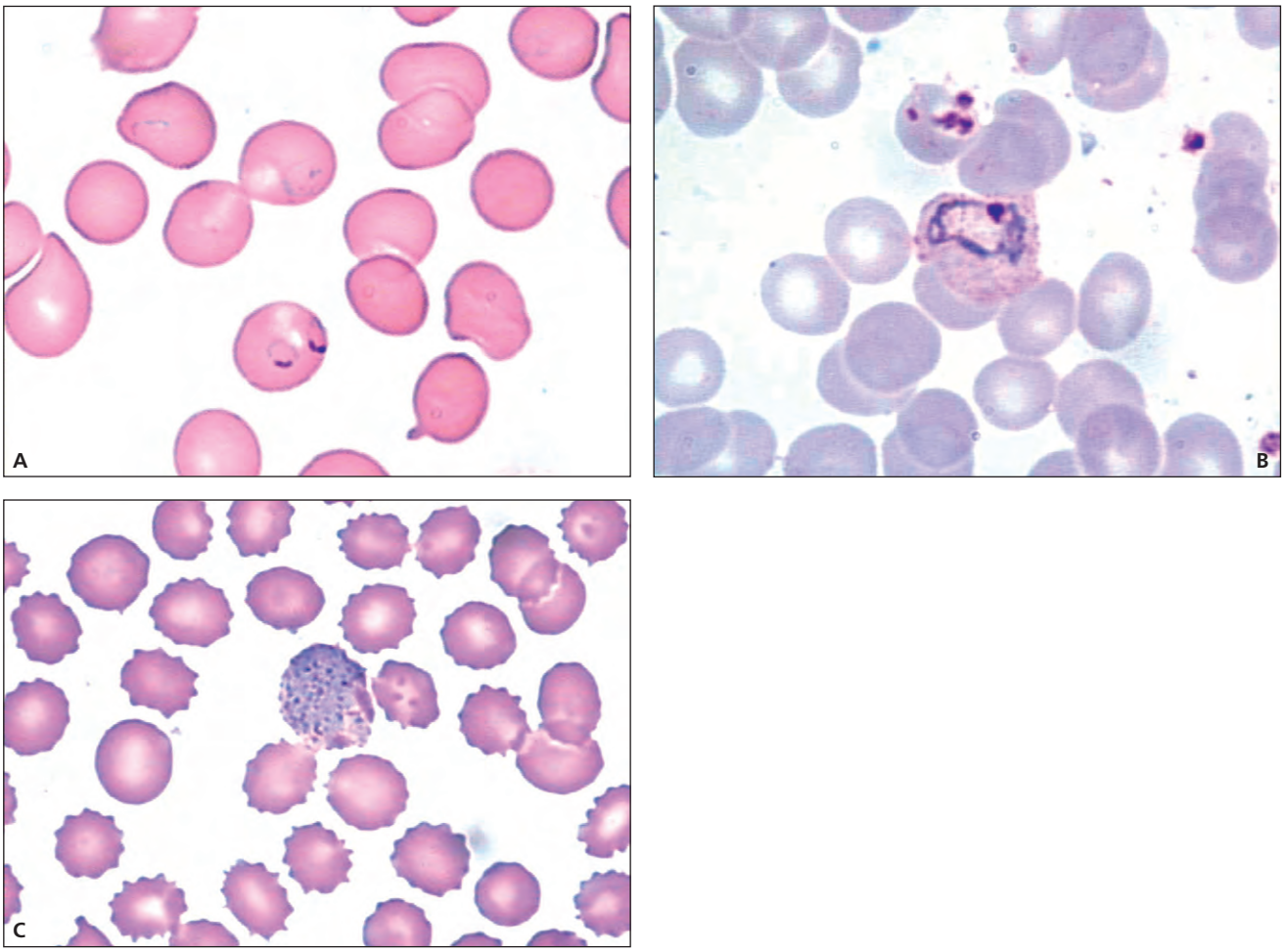



**Fig. 2.52** Erythrocyte inclusions that represent malaria parasites. (A) Peripheral blood. Ring trophozoites are recognisable in the red cell below the centre. Identification of the parasites as *Plasmodium falciparum* is suggested by the presence of multiple binucleated rings. (B) Mature malaria trophozoite in a degenerated macrocyte, found after a long search on a bone marrow film from a patient with fever of unknown origin; identification of the species was difficult. (C) Peripheral blood, *Plasmodium vivax*. The bluish structure containing gold-coloured granules is a maturing schizont. The red cells show echinocytic deformation, possibly artefactual.

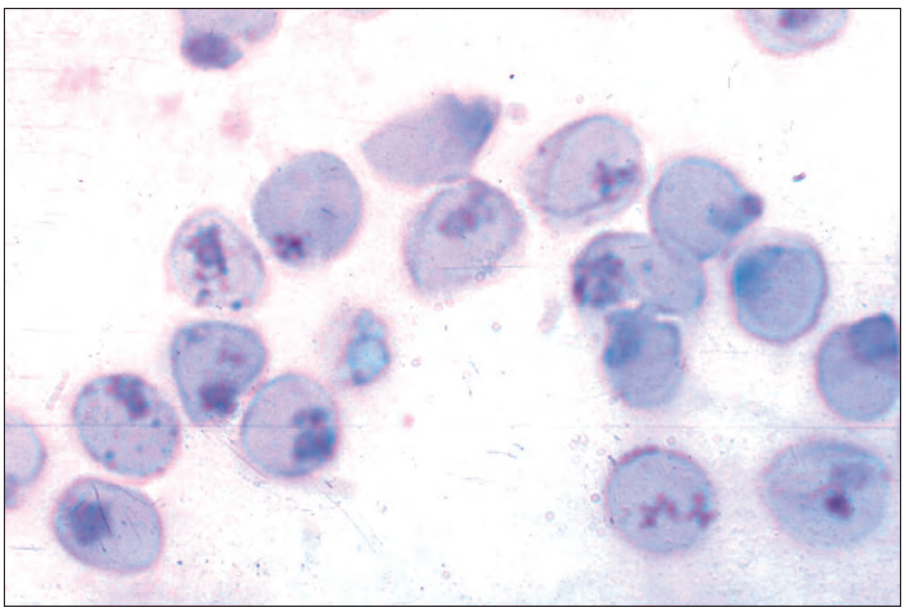


**Fig. 2.53** Peripheral blood film, vital stain with new methylene blue from a patient who was heterozygous for an unstable haemoglobin (haemoglobin Hammersmith). The Heinz bodies, formed from spontaneously denatured haemoglobin, are visible in all the cells as large irregular structures in contact with the inner surface of the red cell membrane. The appearance differs from that of the granulofilamentous material of reticulocytes both because of the shape of the inclusions and their peripheral location. These inclusions stain with fluorochromes and also with the vital stains used by automated instruments, leading to a falsely elevated reticulocyte count, approaching 100%.

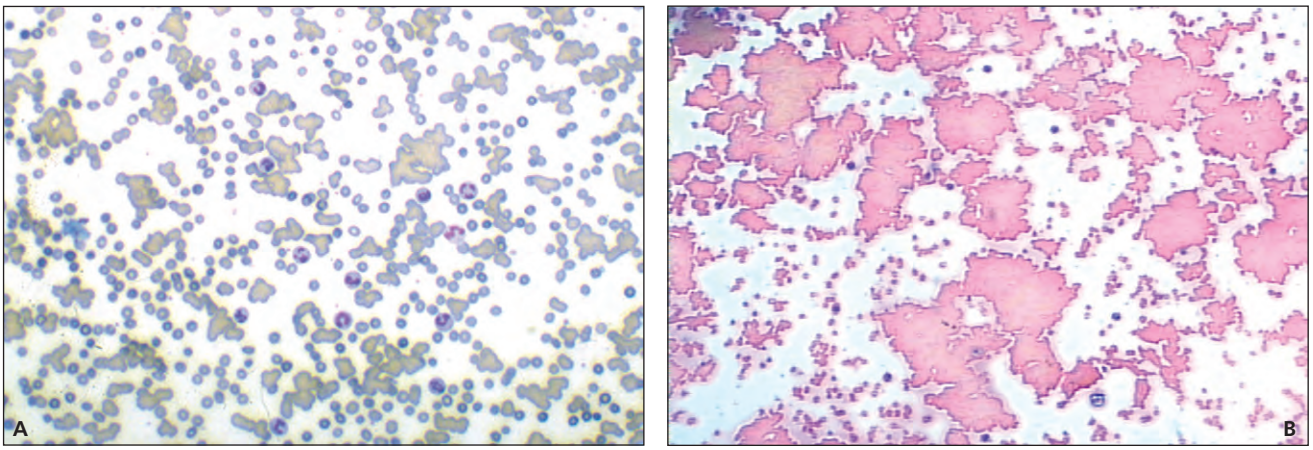



**Fig. 2.54** Red cell agglutination in a peripheral blood film. (A) From an asymptomatic subject with a cold agglutinin (titre 1:256). The red cells are agglutinated into small and medium-sized masses, from a few cells to a dozen or so, extending in all directions including forwards in three dimensions. In the larger clumps, the cells seem to lose their individuality and remain recognisable only at the edges of the agglutinates. The smaller elongated clumps are similar to rouleaux, but this appearance is lost when new layers of red cells adhere. This phenomenon led to a false increase in the MCH and MCHC (to 56 pg and 720 g/l). In cases such as this, in which many of the agglutinates are small, the overestimation of the MCV (112 fl) is very evident. Incubation of the EDTA-anticoagulated specimen at 37°C for an hour reverses the phenomenon, although not always completely. (B) Massive erythrocyte agglutination in a patient with cold haemagglutin disease. The red cells have formed very large agglutinates, which contain hundreds of cells that are no longer individually recognisable. The background staining is weakly pink as a result of dysproteinaemia. Erythrocyte morphology cannot be assessed. The only valid instrumental measurement in cases such as this is that of the haemoglobin concentration.

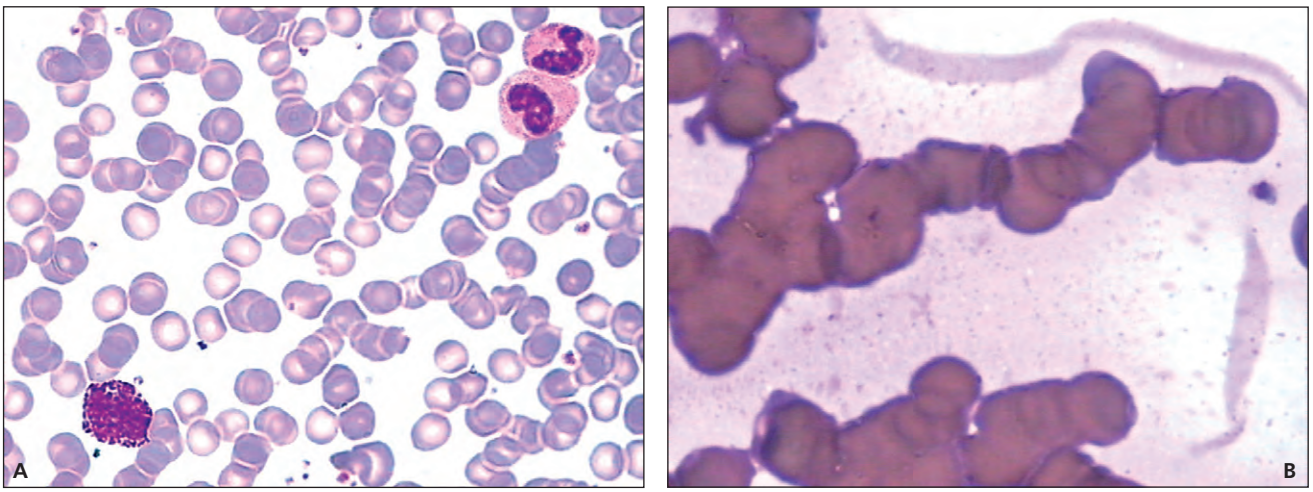



**Fig. 2.55** Rouleaux formation in a peripheral blood film. (A) The tendency of the red cells to form small elongated stacks of cells, partially superimposed on each other, is commoner in the thicker part of a blood film and also in bone marrow films. When this occurs, as in this case, in areas that are optimally spread, it is a good indicator of dysproteinaemia. Bottom left, there is a basophil with well-preserved morphology, in which the typical blackish-red granules are partly superimposed on the nucleus. Top right, two neutrophil band forms. (B) From a patient with multiple myeloma and a high level of paraprotein, massive rouleaux formation and abundant amorphous background material, which make evaluation of erythrocyte morphology impossible.

pallor is still apparent and, in comparison with agglutinates, the outlines of single cells are still visible. Rouleaux formation must be assessed in a thin part of the film where the distribution of erythrocytes should be uniform. In thicker areas, in fact, rouleaux formation is common but is artefactual as a result of overcrowding and the normal adhesiveness of erythrocytes. Increased rouleaux formation is the result of an abnormality of plasma proteins, either quantitative, qualitative or both, which leads to the surface of the red cells being coated by protein, annulling the normal repulsive forces between cells. It thus occurs with samples from patients with hyperproteinaemia or dysproteinaemia of various types: multiple myeloma or monoclonal gammopathy (paraproteinaemia), infections and chronic inflammatory and neoplastic disorders.

## Artefacts affecting erythrocyte morphology

Numerous cytomorphological alterations of red cells, in some cases very similar to those already described as indicators of a pathological process, can be caused by lack of skill in making or examining a blood film – by slow drying in a humid environment, by prolonged storage of the specimen before the film is made or by observing an inadequate area of the film. This type of artefact, which can often be distinguished from a genuine abnormality because it is present in only part of the film, can include:

- The lack of an area of central pallor (particularly towards the tail of the film) creating pseudo-spherocytosis;
- Target cell morphology;
- The presence of small intracytoplasmic vacuoles;
- The presence of echinocytes;
- The presence of stomatocytes.

## Other anomalies

### Erythroblasts in the peripheral blood

Orthochromatic, polychromatic and, rarely, basophilic erythroblasts (e.g. Figs 2.56 and 2.57) appear in peripheral blood films in a range of conditions, variously associated with:

1. A strong regenerative drive in severe anaemia when the bone marrow is normal and strongly stimulated;
2. Increased and dysplastic bone marrow erythropoiesis;
3. An abnormality of the marrow-blood interface when erythroblasts can emerge into the blood without signifying regeneration or compensation;
4. Extramedullary haemopoiesis of all types when blood cells are formed within the liver or the spleen.

By these various mechanisms, erythroblasts may be seen in the peripheral blood in severe acute or chronic haemolytic anaemia, including haemolytic disease of the newborn due to fetomaternal isoimmunisation; in premature neonates; following massive haemorrhage; in haemolytic and painful crises in sickle cell anaemia; in regeneration after aplasia; in megaloblastosis and the more severe forms of thalassaemia; in multiple trauma with numerous fractures and after orthopaedic interventions or thoracotomy; in primary and secondary myelofibrosis and other myeloproliferative neoplasms; in bone marrow metastases of non-haemopoietic neoplasms (associated with what was once called myelophthisic anaemia) and in extramedullary haemopoiesis.

With the exception of those resulting from compensatory or regenerative mechanisms, circulating erythroblasts are often dysmorphic and may be accompanied by atypical and immature leucocytes, particularly granulocyte precursors (leucoerythroblastic picture), granulocytes with anomalous nuclear segmentation, abnormal platelets, dysmorphic megakaryocytes and bare megakaryocyte nuclei, blast cells and, very rarely,

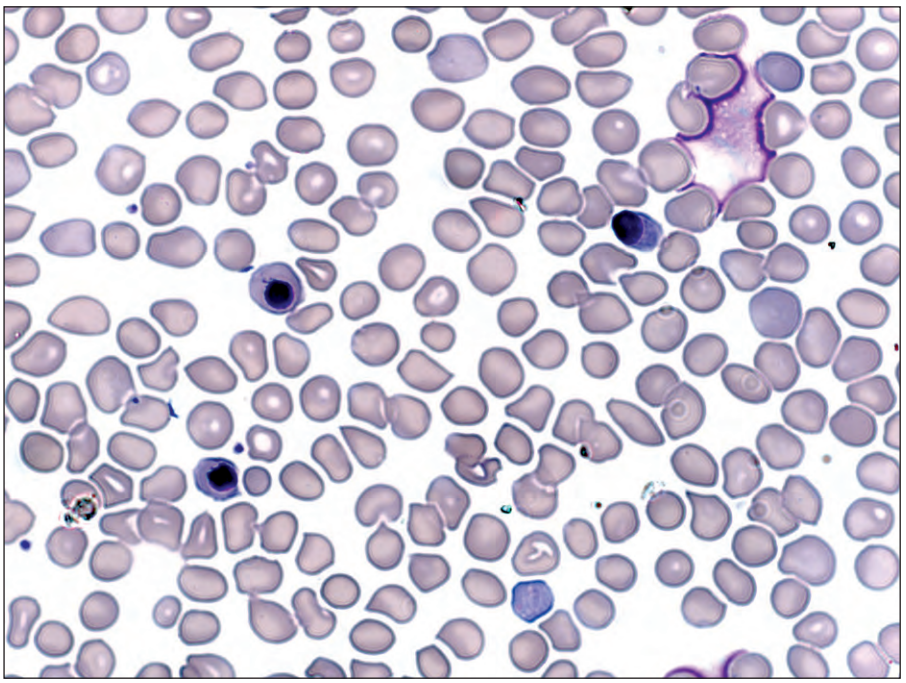


**Fig. 2.56** Cord blood film from a premature neonate. The red cells are large and easily deformable, with increased anisocytosis; there is little apparent central pallor because of the increased thickness of the cells and the less than ideal film. There are three erythroblasts with nuclei that are composed of a single dense chromatin mass. In the two on the left, the cytoplasm is orthochromatic in comparison with the erythrocytes, but the erythroblast on the right, with an eccentric nucleus, retains a polychromatic tinge: the small size of the cell and the homogeneous structure of the chromatin avoid any possible confusion with a plasma cell. At the bottom, towards the centre, a polychromatic erythrocyte.

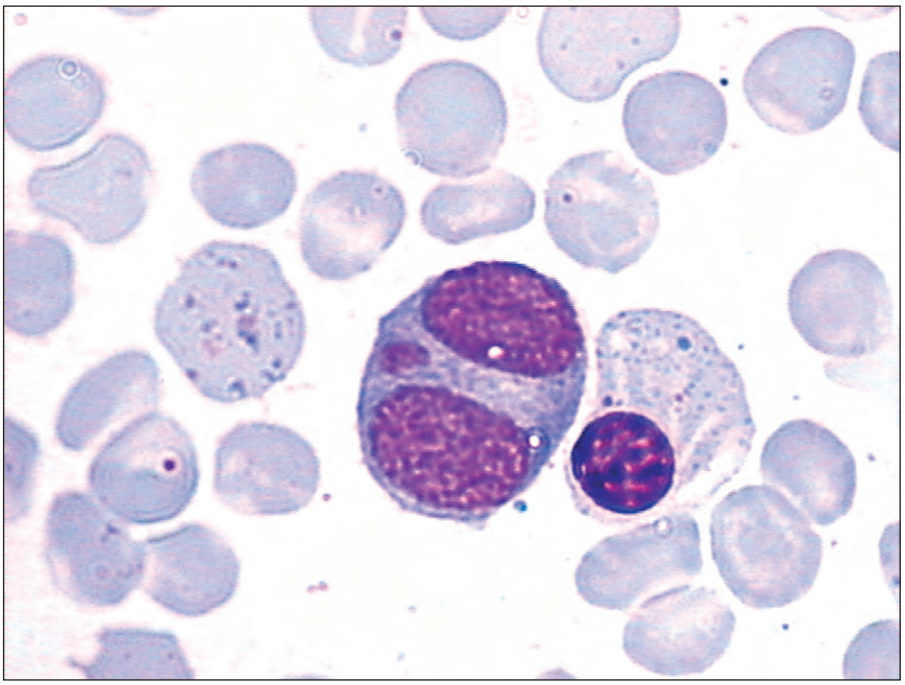


**Fig. 2.57** Erythroblast and binucleated blast cell in the peripheral blood; same case as Fig. 2.49. The large binucleated cell, with a large inclusion of the same reddish-purple colour as the nuclear chromatin, is a lymphoid blast. On the right, a dyserythropoietic erythroblast with orthochromatic cytoplasm, loaded with granules that are sometimes azurophilic and sometimes basophilic (as in the macrocyte on the left), with linear non-haemoglobinised areas and a rounded nucleus with chromatin clumps still separated by pale spaces.

non-haemopoietic malignant cells. Mature erythrocytes may show prominent anisopoikilocytosis and bizarre morphology with Howell-Jolly bodies, Cabot rings, polychromasia, dacrocytes and basophilic stippling. These aberrations are more prominent if the spleen is absent or hypofunctional.

Erythroblasts can also enter the circulation in certain paraphysiological states (as in neonates, particularly if premature or if there has been asphyxia)[98] and in severely hypoxic states that can result from cardiac or pulmonary disorders. The persistence of erythroblasts in the circulation has been found to have adverse prognostic significance in neonates,[99] in sepsis and in large case series of hospitalised patients, in particular being an indicator of reduced survival.[100,101] The unfavourable prognostic significance of circulating erythroblasts has also been confirmed in the course of haemopoietic stem cell transplantation[102] and in multiorgan failure.[103]

The most recent automated haematology analysers are able to provide, using various technological approaches, accurate and reproducible counts of circulating erythroblasts as part of the differential leucocyte count[18] (see Report 2.5). The availability of an automated count will certainly increase the diagnostic value of this measurement in a manner analogous to what has happened with reticulocyte counts.[104]

### Erythrophagocytosis in the peripheral blood

The presence of morphologically recognisable red cells within the cytoplasm of circulating nucleated cells (Fig. 2.58) is a rare phenomenon, which can occur with leukaemic myeloid blast cells, particularly if there is monocytic differentiation, and in association with t(8;16). Erythrophagocytosis by neutrophils has been described in paroxysmal cold haemoglobinuria,[61,105] myelodysplasia, sickle cell anaemia and other types of haemolytic anaemia.[106]

## Other red cell measurements

### MCHC

The MCHC depends on the relationship between the Hb and the Hct and provides an estimate of the concentration of haemoglobin within red cells. It is calculated from the Hb (in g/l) and the Hct (in l/l) using the formula MCHC = Hb/(Hct × 10), and is normally around 310–350 g/l, with a certain heterogeneity in the reference ranges reported in the literature. After the introduction of second-generation Coulter counters able to both directly determine erythrocyte size from impulse height by impedance technology, and also to calculate the Hct as the product of the RBC and MCV, the MCHC lost its diagnostic value.[107] A number of authors indicated in a series of letters to the *British Medical Journal*, published on several occasions during 1971 under the significant title *Epitaph for the MCHC*, the frequent observation of a normal MCHC in the presence of markedly hypochromic erythrocytes, underlining that hypochromia on the blood film was not the result of a real reduction in the intracellular haemoglobin concentration but merely an optical effect resulting from the reduced thickness of

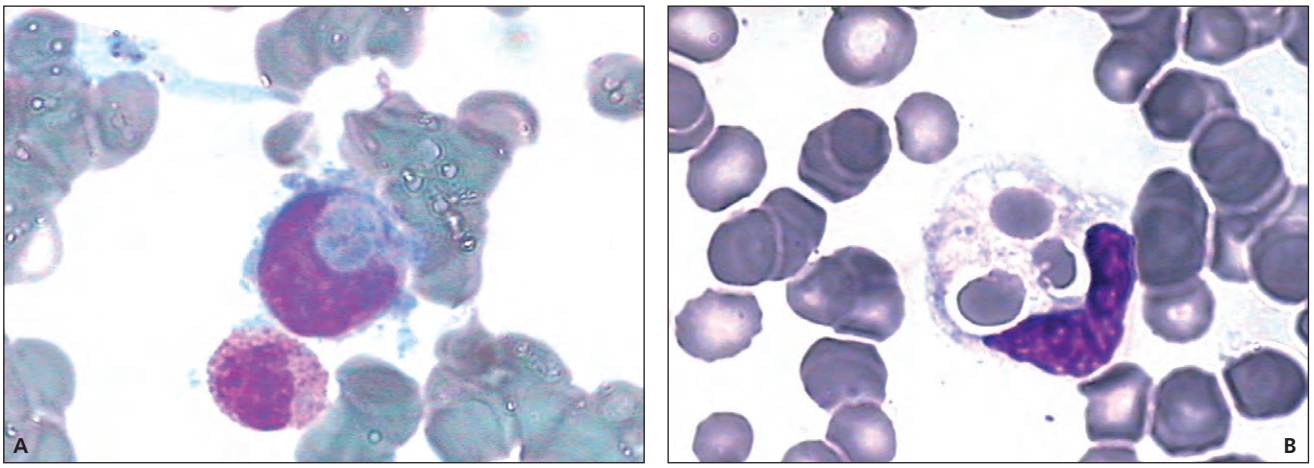



**Fig. 2.58** Erythrophagocytosis in the circulating blood. (A) In the peripheral blood film of this patient with sepsis, phagocytosis of red cells by a mononuclear cell, probably a monocyte. (B) Another rare example, with three still recognisable erythrocytes in the cytoplasm of a cell, the flattened nucleus of which has a chromatin pattern similar to that of a neutrophil.

the microcytic red cell.[108–110] The overestimation of the Hct by centrifugation had until then caused a spurious reduction in the MCHC. This artefact had been useful for diagnosis and disease classification, making the measurement more variable and sensitive to changes in erythrocyte composition caused particularly by iron deficiency. The MCHC, measured by impedance methods, had become more constant, so that it varied little from an average value of around 330 ± 20 g/l. This measurement was abnormal in less than 1% of samples analysed: in two thirds of such cases it was reduced as a result of severe iron deficiency anaemia.[111] A reduced MCHC with automated analysers is not, for example, valid as a means of detecting latent iron deficiency in blood donors: a fall is manifest only when there is a significant iron deficiency anaemia, when the Hb falls below 90 g/l.[29]

An increase of the MCHC above 350 g/l, however, is of value in screening for hereditary spherocytosis,[71] for the dehydrated variant of hereditary stomatocytosis (xerocytosis)[80] and for artefacts that may suggest the presence of haemolysis or cold agglutinins. The stability of the MCHC on automated analysers has rendered it useful for internal quality control: alteration of the MCHC most often indicates a loss of accuracy of one or more of the measurements from which it is derived, permitting continuous control of the measurements made in the red cell analytical channel and the haemoglobin channel.[112,113]

The CHCM, initially specific for the laser system of the ADVIA, is now available, based on various other technologies, on other instruments. It is an expression of the average intraerythrocytic haemoglobin concentration based on direct measurements of the cytoplasmic density of individual cells, employing the Mie theory; it can be compared, sample by sample, with the MCHC, calculated conventionally from the relationship between Hb and Hct: any discrepancy between the two figures is a clear signal of either malfunction of the instrument or an anomalous specimen.

### MCH

The MCH, obtained on electronic systems from the relationship between the Hb and the RBC, does not provide any additional information above what has been obtained from the indices already discussed. It is calculated from the Hb (in g/l) and the RBC ($\times 10^{12}$/l) using the formula MCH = Hb/RBC. The MCH varies in a linear manner in relation to the MCV, and is thus an indirect measurement of red cell volume. Its value in healthy adults and in patients with normocytic anaemia is generally between 27 and 33 pg.

Automated measurements of red cell indices can be altered in particular cases, not only by pathological changes in red cells but also by specific anomalies of blood specimens. The detection of these anomalous results is important both to avoid sending out an erroneous report and because sometimes the analytical anomaly permits the detection of conditions that are still clinically silent. The observation of indices that are too high or too low, sometimes with unrealistic, absurd or improbable values, is a typical occurrence. Thus, anomalies of the specimen can lead to a paradoxical increase of the MCHC and MCH, which can be the result of an erroneously high measurement of Hb and/or erroneously low measurement of the RBC. In the majority of instances the responsible mechanism is interference with the colorimetric measurement of Hb as a result of plasma turbidity; this can be the result of marked leucocytosis, incomplete haemolysis of red cells in the haemoglobin channel (as with haemoglobin C), hyperlipidaemia due to either disease or parenteral nutrition, a paraprotein (particularly IgM) or, rarely, marked polyclonal hypergammaglobulinaemia in a patient with a chronic inflammatory condition. When the red cells lyse *in vitro*, within the specimen tube, the MCHC and MCH are falsely elevated as a result of a factitious reduction in the RBC caused by the haemolysis. The same can occur in cases of *in vivo* intravascular haemolysis, as in ABO-incompatible transfusions,

in which the plasma still contains the haemoglobin liberated from the cells, which is measured together with the haemoglobin that was still contained in red cells. A similar phenomenon can be seen following an infusion *in vivo* of blood substitute based on haemoglobin solutions (haemoglobin-based oxygen carrier [HBOC]). The presence of erythrocyte agglutination, as in cold antibody-mediated autoimmune haemolytic anaemia (see Report 2.1) or, less often, from warm autoantibodies (see Report 2.6), causes paradoxical alterations of MCH and MCHC, with values that can be greater than 40 or 50, this often being associated with a factitious increase in the MCV (spurious macrocytosis).[114,115] The larger agglutinates, in fact, are excluded from the count as they are above the upper threshold for red cells and the RBC is thus falsely lowered, while the smaller agglutinates are classified as giant macrocytes. In these conditions, the only valid measurement for assessing the severity of the anaemia is the Hb (in g/l). This anomaly also has a clear effect on the indices of dispersion, the RDW and HDW (haemoglobin distribution width), and causes a characteristic alteration of the histograms and cytograms.

The CHCM, obtained from two angle measurements on individual red cells, is not subject to inaccuracy relating to turbidity or free plasma haemoglobin. The difference between MCHC and CHCM can thus be an indicator of haemolysis, *in vitro* or *in vivo*, since the MCHC is based on measurement of all the haemoglobin in the circulation, including free haemoglobin, while the CHCM measurement is dependent only on the haemoglobin present within erythrocytes. Other than in quality control of the blood specimen, this double methodology can also be exploited by antidoping laboratories to provide evidence that athletes have used HBOC that favour oxygen transport.[116]

A false increase in the MCV (and MCH), generally with a reduction in the MCHC, can be caused by inadequate storage of the blood specimen, either for too long a period or at too high a temperature, or by increased osmolarity of the plasma resulting from hyperglycaemia or hypernatraemia, e.g. due to dehydration. Conversely, a spurious reduction of MCV can be the result of hyponatraemia, as in inappropriate secretion of antidiuretic hormone.[117]

### Histograms and indices of dispersion

The concept of morphology of anaemias can be extended nowadays to the reports of automated instruments. The morphological information that can be obtained by examination of the blood films is preceded and enriched by the clues that are obtained from the automated instrument histograms of erythrocyte and reticulocyte volume and haemoglobin concentration, and similarly from the two-dimensional cytograms that represent in a more elaborate manner the relationship between various measurements.[118]

The first red cell distribution curve used for diagnostic purposes was developed in 1920 by Price-Jones, using manual measurement of the diameter of red cells projected and outlined in pencil on a sheet of paper. The erythrocyte distribution curves thus obtained were usually unimodal and symmetrical and permitted the description of the increased erythrocyte diameter in pernicious anaemia and the reduced diameter after haemorrhage, the increased anisocytosis in the same situations and the changes that occurred during therapy of pernicious anaemia with liver extract, restoration of a normocytic population finally being achieved. About 40 years later, the technology used in automated instruments made possible, thanks to the rapid, precise and accurate measurement of cells in suspension, the clinical use of the distribution curve to evaluate serially, anomalies and modifications of erythropoiesis. The same electronic circuits used to distinguish cells from background noise and to classify various types of cells on the basis of their volume permit the construction of histograms of cell volume. These developments have enriched the diagnostic information provided by the MCV, permitting a quantitative estimate of anisocytosis and showing, for example, that the red cells produced in iron deficiency anaemia are characterised not only by a low MCV but also by a greater degree of heterogeneity of size than is seen in normal subjects and in heterozygotes for beta thalassaemia.[107] The new index of anisocytosis, the RDW, was introduced as the co-efficient of variation of erythrocyte volume, expressed as a percentage. This could be expected to mirror the evaluation of anisocytosis from a blood film, although specific studies correlating the RDW and the microscopic assessment have not yet been done. The reference interval of the RDW on automated instruments has an average value of around 13%, with an upper limit of normal around 15%. The differences between analytical methods and the algorithms used in calculation between various instruments and technologies has made the standardisation of this value difficult, so that results have to be related to the instrument from which they were obtained.

There are no physiological or pathological conditions characterised by reduced anisocytosis or an RDW less than normal. On the contrary, the increased anisocytosis of iron deficiency anaemia relates both to the intrinsic abnormality of iron deficient erythropoiesis and to the progressive loss of iron stores, and is generally reflected in an increased RDW. This is a good indicator of iron deficiency with a sensitivity above 80%; its specificity, however, is much less, in general barely above 50%. In other words, it is rare to see an established iron deficiency anaemia without an increased RDW, but only in a little more than half the cases in which one sees an increase in the RDW is there iron deficiency. Some authors have denied the value of the RDW as an early indicator of latent iron deficiency in subjects with a normal Hb, such as blood donors,[119] while others have emphasised its sensitivity and specificity in this context.[120] An increased RDW permits iron deficiency to be suspected in the absence of microcytosis in certain specified circumstances, such as in patients on maintenance haemodialysis,[121,122] in malabsorption syndromes[123] and in epidemiological studies, both in the paediatric age range[124] and in the elderly.[125,126]

In a proposal for a classification of anaemia based on the MCV and RDW, which express respectively the average volume

and the heterogeneity of size of the red cell population, each of the three categories identified by the MCV (micro-, normo- and macro-cytic) are divided into two further categories on the basis of the RDW (homogeneous with a normal RDW; heterogeneous with an increased RDW).[63] Anaemia caused by nutritional deficiency is always heterogeneous, while that due to hypoproliferation is always homogeneous, regardless of the MCV. The results obtained in attempts to confirm this hypothesis are discordant.[127] The specific context of microcytosis is the only one where clinical applicability of the RDW has been demonstrated: the RDW is in fact increased in iron deficiency and almost always normal, or increased only to a limited extent, in thalassaemia trait not complicated by iron deficiency, even in cases with marked microcytosis.[128]

An increased RDW is an efficient indicator of anisocytosis in other haemoglobinopathies.[129,130] In sickle cell disease, the RDW has a prognostic value: it is particularly increased in sickle cell anaemia and in compound heterozygosity for S/beta thalassaemia, correlating inversely with the Hb, with the MCV and with the percentage of haemoglobin F, and correlating directly with the reticulocyte count.[131,132] An increased RDW is associated with an increased MCHC in many patients with hereditary spherocytosis.[69,70]

In recent years the RDW, an indicator of heterogeneity of the size of red cells, has been identified as an independent indicator of the risk of death both in patients with cardiovascular disease and more generally in the adult population above the age of 45 years.[133] A recent study has associated an increased HDW with the risk of carotid arteriosclerosis in subjects with hypertension.[134]

The HDW, initially determined optically with the use of a laser, measures heterogeneity in the haemoglobin concentration between individual red cells and correlates, in a linear manner, with anisochromasia apparent in blood films. The HDW is expressed in g/dl as the standard deviation of the CHCM: its reference interval is usually cited as 2.2–3.2 g/dl. This measurement is on average higher in iron deficiency than in thalassaemia trait, but with the difference being not more than 10% and the discriminating ability being modest,[27,135] less than that of the RDW.[136] In 11 cases of hereditary ovalocytosis studied with the H*1, HDW and RDW were both increased and the average value of CHCM was slightly reduced (317 ± 19 g/l), thus differing from the typical increase of the MCHC and CHCM in hereditary spherocytosis.[137] In paroxysmal nocturnal haemoglobinuria, an increase of normochromic macrocytes has been observed together with an increase in both the indices of dispersion, the RDW and the HDW.[138]

### Hypochromic erythrocytes

On the ADVIA system, optical measurements at two different angles following the interruption of a laser beam by an individual red cell are translated into volume and density of each cell, these being plotted on a two-dimensional map.[139,140] In the representation of the data the cytogram is divided into nine zones separated by two horizontal lines that represent reference limits for cell volume, equal to 60 and 120 fl, and two vertical thresholds, representing reference limits for haemoglobin concentration, equal to 28 and 41 g/dl (Fig. 2.59). Red cells with normal size and haemoglobin concentration are found in the central sector, while the peripheral sectors are occupied by subpopulations with specific abnormalities of size, haemoglobin concentration or both. The signals produced are also displayed as two histograms, of size and haemoglobin concentration, with the scale of each variable being on the abscissa and the

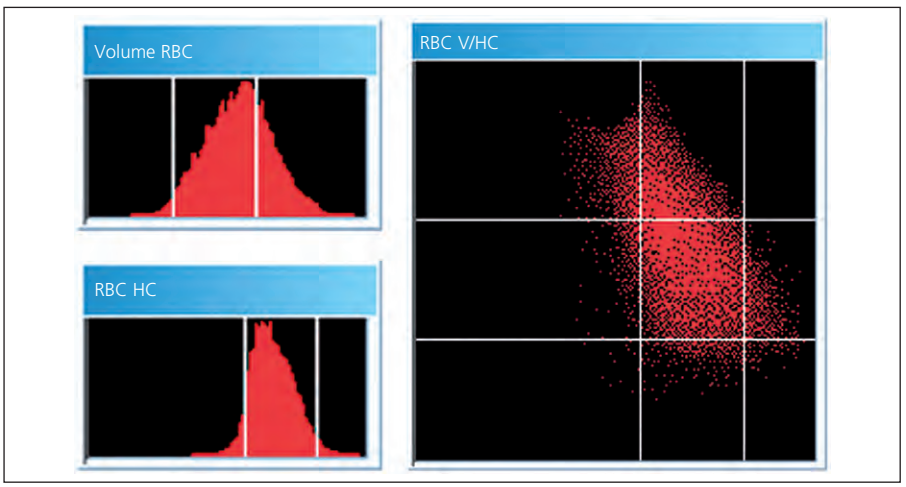



**Fig. 2.59** Histograms and two-dimensional cytogram of red cells with the ADVIA system from a patient with hepatic cirrhosis with haemorrhage from oesophageal varices. The histogram of red cell size, Volume RBC, is widened and asymmetrical, because of macrocytosis and anisocytosis: the red cells falling to the right of the upper threshold are classified as macrocytes. The histogram of erythrocyte haemoglobin concentration, RBC HC, is asymmetrical. The red cell cytogram, RBC V/HC, shows the distribution of red cells according to the two characteristics that have been analysed: here one sees numerous normochromic and hypochromic macrocytes.

frequency of events on the ordinate. Normal limits are shown on the histograms, permitting a visual recognition of the alteration in distribution. From the histograms one can observe:

- The central values of the distribution (MCV and CHCM), calculated from the average of all the impulses;
- The degree of dispersion of the distribution, either of cell size or cell haemoglobin concentration (RDW and HDW);
- The percentage of erythrocytes that fall beyond the various thresholds for volume and haemoglobin concentration, these being classified respectively as hypochromic, microcytic, hyperchromic and macrocytic;
- The descriptive alarms indicating morphological abnormalities of the erythrocytes.

The percentage of hypochromic erythrocytes, with a cellular concentration of haemoglobin (measured by the optical laser method) below 28 g/dl, is the best and earliest erythrocyte marker of depletion of iron stores (see Report 2.3). This measurement indicates the production of incompletely haemoglobinised erythrocytes because of a lack of iron of various origins and is a valid alternative to biochemical measurements for the early recognition of iron deficiency. In effect, the increase in hypochromic erythrocytes reflects a mismatch between the synthesis of new red cells and the molecules of iron that it has been possible to incorporate into haemoglobin during the preceding 100–120 days. Hypochromic red cells are produced when iron stores are exhausted, but also when there is a functional iron deficiency resulting from accelerated erythropoiesis. In particular, an increase in hypochromic cells has been confirmed as the most useful measurement for deciding when intravenous iron should be given to dialysed patients receiving erythropoietin or other erythropoiesis stimulating agents (ESA), with a sensitivity of 80%, only slightly improved by considering also measurement of the haemoglobin content of reticulocytes, the CHr.[141] The percentage of hypochromic erythrocytes and the CHr also permit the rapid recognition of iron deficiency in blood donors.[142] The percentage of hypochromic erythrocytes can also be increased as a result of inflammation, because of a functional iron deficiency resulting from reduced mobilisation of iron deposits, this correlating with an increased C-reactive protein.[143]

In haemodialysis patients, particularly those receiving ESA, iron-deficient erythropoiesis can develop, and must be identified and treated in order for the Hb to rise. The threshold value for the percentage of hypochromic erythrocytes below which erythropoiesis is iron deficient is around 5–10%.[141,144–146] European guidelines for haemodialysis patients require, in addition to ferritin and transferrin saturation, measurement of the percentage of hypochromic cells with a threshold value of above 10%, together with measurement of the CHr.[147]

The prevalence of hypochromic red cells, in comparison with the prevalence of microcytic red cells, is a characteristic that permits the classification of microcytic anaemia as due to iron deficiency, differentiating it from thalassaemia trait, in which the percentage of microcytes is consistently greater.[27] The micro:hypo ratio was found to be a useful criterion, permitting a diagnosis of iron deficiency when it was below a threshold value of 0.9 or 1.0. The utility of this value, subsequently incorporated into the instrument report, has been confirmed.[148,149] The greater prevalence of microcytes in relation to hypochromic cells has also been recognised in the more severe forms of thalassaemia (see Report 2.7).

The Abbott Sapphire system introduced, in the most recent version of the software, an analogous optical method for the determination of size and haemoglobinisation of erythrocytes. The Sysmex XE-5000 system provides a new measurement, the Hypo-He, which gives equivalent information to the evaluation of hypochromic cells by the ADVIA. It is based on a measurement of forward light scatter (which correlates with the erythrocyte haemoglobin concentration). An erythrocyte that contains less that 17 pg of haemoglobin is classified as Hypo-He. The threshold for recognising an actual or functional iron deficiency in a haemodialysis patient is a Hypo-He above 2.7%.[150,151] The LHD (low haemoglobin density) of the Beckman-Coulter system has been reported to have similar utility.[152]

### Hyperchromic red cells

Hyperchromic red cells are counted by the more advanced automated analysers as cells with a haemoglobin concentration above 41 g/dl. The percentage of these cells among the total red cell population is shown on a histogram of haemoglobin concentration and on a two-dimensional cytogram. These correspond to red cells with an increased haemoglobin concentration such as spherocytes, irregularly contracted cells and sickle cells.[153]

An increased percentage of hyperchromic cells provides an effective screening method for hereditary spherocytosis, of various degrees of severity, and similarly for other erythrocyte abnormalities resulting from a membrane defect.[71,154] Spherocytes are recognised as red cells with an haemoglobin concentration above the threshold and with a reduced volume, that is, as hyperchromic microcytes: the histograms and cytograms are highly characteristic with a peculiar tail to the right of the curve of cellular haemoglobin concentration, and an increase in the measurements reflecting haemoglobinisation (MCHC, CHCM, HDW)[72,73,155,156] (see Report 2.4). These abnormalities are more evident in non-splenectomised patients; they are sufficiently characteristic to be able to replace less sensitive methods such as the determination of osmotic fragility and can serve as the initial screening test for spherocytosis.[157]

### Fragmentation alarm and count of red cell fragments

Various instruments are able, by combining measurements of volume and light refraction, to separate platelets from red cell fragments (avoiding thus a factitious elevation of the platelet count), and can therefore indicate the presence of red cell fragments, and in the case of some instruments, count them. In the ADVIA system, red cell fragments are recognised in the platelet channel as elements with a volume of less than 30 fl (measured by low angle laser light scatter at 2–3°) and with a different

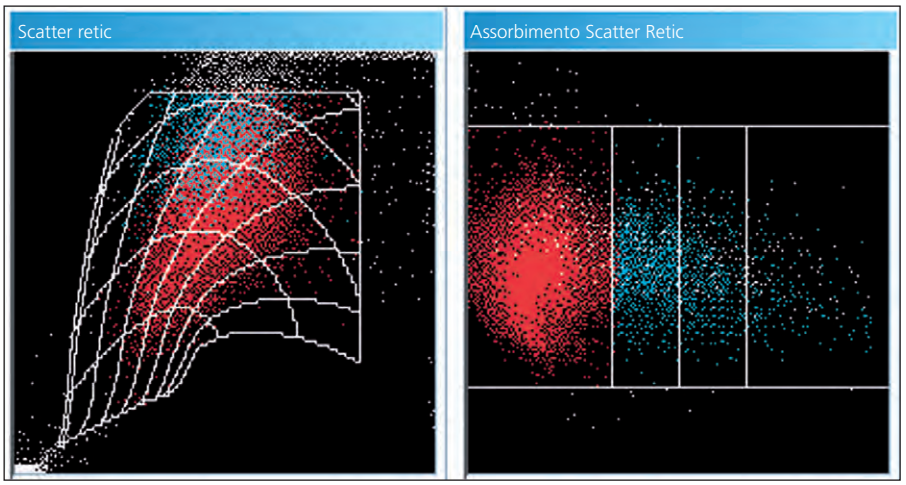



**Fig. 2.60** Cytograms obtained from the reticulocyte channel of the ADVIA system, after staining of the granulofilamentous material with oxazine 750. In the Assorbimento Scatter Retic scatter plot, the vertical thresholds separate the reticulocytes, in blue, from the rest of the red cells, and divide them into three populations with low, medium and high RNA content. The IRF parameter is obtained from the sum of reticulocytes in the latter two categories. In the Scatter retic cytogram it is evident that the reticulocytes, in blue, are larger and more hypochromic that the mature erythrocyte population.

density from that of platelets (light scatter at a higher angle of 5–15°). The Sysmex XE counts fragments (FRC%) in a specific area of the cytogram in the reticulocyte channel, as non-fluorescent particles with low intensity forward scatter.[158–160]

According to published studies, these methods for counting red cell fragments are sensitive, but not very specific in comparison with a microscopic count of schistocytes, inasmuch as all red cell fragments, regardless of their origin and morphological features, are included in the automated measurement. The principal use then resides in the high negative predictive value of instrumental results, which is very useful for screening (low risk of false negatives), while the recognition of fragments by the automated instrument always requires microscopic confirmation.[86,161,162]

### Immature reticulocyte fraction

On average, reticulocytes spend about 24 hours in the circulation, during which time they undergo maturation, with gradual modification of morphological, biochemical and functional characteristics.[53] In 1931 Heilmeyer distinguished, by supravital staining, five classes of reticulocyte with decreasing quantities of granulofilamentous material corresponding to the morphological stages of maturation of the reticulocyte.[163] Reticulocytes corresponding to class 0 (nucleated), I and II of Heilmeyer's classification are rare in the peripheral blood of healthy subjects and patients with hyporegenerative anaemia. They are increased in conditions of intense erythropoietic stimulation, as in haemolytic anaemia (stress reticulocytes). These cells, released prematurely into the circulating blood, are large and very rich in RNA.[164] They can be recognised in blood films stained by panoptic methods as polychromatic erythrocytes.[64]

Automated instruments that are able to count reticulocytes include an evaluation of their maturity, based on staining with a fluorochrome or a supravital stain (Fig. 2.60). The method is not standardised and each instrument uses its own definition, criteria for separation from more mature forms and reference values.[165] The term 'immature reticulocyte fraction (IRF)' is the one that is most widely used, at least in part because it is comparable between different methods. Despite this lack of standardisation, the IRF has found interesting clinical applications. An increased IRF indicates a recent acceleration of erythroid maturation, caused by an abrupt massive erythropoietin stimulus, leading to the skipping of one or two mitotic divisions and the early release of reticulocytes into the circulation. It thus represents, with a high sensitivity and much earlier than the reticulocyte count, the current state of erythropoietic activity, giving extra and earlier information than the reticulocyte count.[166] One of the principal clinical indications of the IRF is in the evaluation of bone marrow recovery after haemopoietic stem cell transplantation[167,168] or after chemotherapy.[165]

In blood specimens from patients with malaria, plasmodia are counted in part with reticulocytes, causing a factitious elevation in the IRF.[169] This artefact can be useful in monitoring parasitaemia. The IRF, finally, can be falsely elevated in specimens containing leukaemic blast cells: this underlines the importance of an accurate interpretation of alarms indicating anomalies of red cell distribution in cytograms.[170]

### Reticulocyte indices

Reticulocytes have an average volume (MCVr) about 20% higher than that of red cells (MCV).[10,50] Their average haemoglobin concentration (designated CHCMr in the ADVIA

system) is lower than the MCHC of the entire erythrocyte population. The average haemoglobin content in picograms (CHr), calculated as the product of the volume and haemoglobin concentration of single reticulocytes, is similar to, or slightly higher than, that of mature red cells (MCH) (Fig. 2.60).

The average size of reticulocytes is increased in macrocytic anaemia, in particular that attributable to ineffective erythropoiesis.[171] The MCVr was initially incorporated into algorithms for the identification of erythropoietin doping in athletes,[172] but its utility has not been confirmed. The CHCMr can be an early marker of iron deficiency, but it is less used than the count of hypochromic cells. The reticulocyte measurement that has received most attention both in scientific and clinical fields is the reticulocyte haemoglobin content (CHr). This provides an indirect but effective measurement of the availability of iron for erythropoiesis in the few days necessary for the formation of the reticulocytes present in the circulation, thus adding qualitative information to the quantitative data of the reticulocyte count and the IRF.

Measurement of the reticulocyte haemoglobin content has become established in clinical use as an indicator of functional iron deficiency, in which, in the course of ESA therapy, a disequilibrium develops between the need for iron for erythropoiesis and its availability, since the macrophage iron stores cannot be mobilised with sufficient rapidity, made worse by the negative influence of inflammatory cytokines. The threshold value of CHr below which erythropoiesis is iron deficient and there is insufficient haemoglobinisation of erythrocytes has been suggested by various authors to be between 23 and 29 pg. The value of 28 pg corresponds to the 2.5 percentile of the normal distribution.[145,173] Both European[147] and American[174,175] guidelines suggest a value of 29 pg. Recent studies suggest a higher threshold level, e.g. 31.2 pg, offers a better combination of sensitivity and specificity.[150] The utility of the CHr has been demonstrated in patients with a lack of adequate iron for erythropoiesis, both functional deficiency, resulting from administration of ESA for patients with renal insufficiency on dialysis,[176–178] and actual deficiency resulting from depletion of iron stores,[179,180] when it is a sensitive and specific indicator of exhaustion of bone marrow stores.[181,182] In normal conditions, the CHr is in general slightly higher than the haemoglobin concentration of mature red cells measured in the reticulocyte channel (CH) and the CHr/CH is greater than one. When the reticulocytes are less haemoglobinised, there is an inversion of this ratio, with the CHr being lower than the CH: this can indicate the recent development of iron deficiency,[176] but also the presence of an inflammatory condition.[145] The construction of a matrix of four sectors which compares the CHr as an indicator of the recent efficacy of haemoglobinisation of new reticulocytes with soluble transferrin receptor/ferritin ratio as an indicator of iron stores, permits the distinction, in an elegant manner, of cases of genuine iron deficiency anaemia from functional iron deficiency and anaemia of chronic disease.[145] The CHr, not being influenced by inflammatory states, has been found to be useful for the diagnosis of iron deficiency anaemia and for distinguishing it from anaemia of chronic disease.[183] However, this value increases very rapidly, after only 48 hours, when iron deficiency anaemia is treated with intravenous iron.[184]

The CHr correlates well with the percentage of hypochromic cells and, according to some studies, has superior sensitivity and diagnostic efficacy.[141] The percentage of hypochromic cells and the CHr are considered complementary for interpreting disorders of erythropoiesis in relation to iron[185]: hypochromic erythrocytes, in effect, provide data that reflect the availability of iron during recent weeks or months, while the CHr is influenced particularly by recent alterations in iron availability. The use of these two erythrocyte measurements is conceptually similar to the use of the percentage of $HbA_{1c}$ and the blood glucose concentration in diabetic patients,[145] CH (and $HbA_{1c}$) indicating a long-term situation and the CHr (or blood glucose) the acute development of functional iron deficiency (or adequacy of recent diabetic control).

Other instruments provide functional reticulocyte measurements. The Sysmex XE provides a measurement, designated Ret-He and expressed in picograms, which indicates the average value of the haemoglobin content of reticulocytes. The Ret-He is calculated mathematically from the values of ret-Y and rbc-Y (the average of forward light scatter in the individual histograms, proportional respectively to dimensions and haemoglobin content). The Ret-He is the average of the distribution of values of haemoglobin content in individual reticulocytes. This measurement has a diagnostic efficacy analogous to that of the CHr, with a lower threshold of 30.6 pg, for the diagnosis of iron deficiency in dialysed patients[150] or of 27.2 pg in paediatric or adult populations.[173,184,186] Both the percentage of hypochromic cells and the Ret-He are altered by the administration of ESA.[187]

### Automated counting of erythroblasts

A count of circulating erythroblasts has recently become available on automated instruments (see Report 2.5). Instruments recognise these cells using fluorochromes and optical methods of discrimination, with an analytical sensitivity limited to one or two erythroblasts per 100 leucocytes.[18]

The availability of a precise count as part of the routine FBC permits an evaluation of the presence and quantity of circulating erythroblasts in various clinical circumstances, such as in thalassaemia patients on transfusion regimes, and also their use as a prognostic indicator in neonates, including premature neonates.[95,96,101–104]

## The presence of alterations in leucocytes, platelets or both

The recognition of quantitative and qualitative anomalies of cells of other lineages in the peripheral blood is, naturally, a fundamental element in the diagnosis of anaemia.

The presence of thrombocytopenia, thrombocytosis, neutropenia, leucocytosis and alterations of various subpopulations of leucocytes leads to the consideration of a considerable range

of pathological conditions, often clonal, that affect the haemopoietic marrow (myelodysplasia, leukaemia, aplasia, neoplastic infiltration). Hypersplenism can also cause pancytopenia. Evans syndrome, the association of autoimmune haemolytic anaemia and autoimmune thrombocytopenia, may be recognised.[188] The finding of blast cells or other immature cells in the blood film also raises the suspicion of serious disease that may require examination on a bone marrow aspirate.

## In depth diagnosis: the morphology of bone marrow erythropoiesis

The diagnosis of the majority of cases of anaemia does not require examination of a bone marrow aspirate, but is based on tests that evaluate specific pathogenetic mechanisms that may be responsible for the reduced or abnormal production or survival of red cells (Table 2.1). Examination of bone marrow films, however, can give fundamental information, particularly in:

**Table 2.1** Laboratory investigations to confirm and further elucidate the diagnosis of anaemia based on the measurement of haemoglobin concentration (g/l), MCV (fl), reticulocytes and peripheral blood film morphology

1. Erythrocyte distribution: unconjugated bilirubin, LDH, free plasma haemoglobin, haptoglobin, haemopexin, serum methaemalbumin; urobilin, urobilinogen, haemoglobin or haemosiderin in the urine; survival of radiolabelled red cells; plasma ADAMTS13 for microangiopathies
2. Iron balance: serum ferritin, serum iron, iron-binding capacity of plasma, plasma transferrin saturation, soluble transferrin receptor, evaluation of bone marrow iron stores with a Prussian blue stain, zinc protoporphyrin, erythrocyte ferritin, cardiac and hepatic magnetic resonance imaging (MRI)
3. Megaloblastosis: cytology of the bone marrow aspirate, serum vitamin $B_{12}$, serum and red cell folate, plasma homocysteine, serum and urinary methylmalonic acid, holotranscobalamin, Schilling test (not currently available), serum gastrin, pH of gastric juice, anti-intrinsic factor and antiparietal cell antibodies, gastroduodenoscopy with biopsy
4. Structural erythrocyte alterations: osmotic fragility, also after incubation for 24 hours as 37°C, red cell eosin-5-maleimide (EMA) binding by flow cytometry, acidified glycerol lysis time, cryohaemolysis, spontaneous autohaemolysis; analysis of proteins of the membrane and the cytoskeleton; Ham acid lysis test and glucose lysis test, flow cytometric evaluation of CD55, CD58 and CD59 (erythrocytes and neutrophils) for paroxysmal nocturnal haemoglobinuria
5. Investigation of haemoglobin: quantification of haemoglobin $A_2$ and haemoglobin F by HPLC, haemoglobin and globin chain electrophoresis, isoelectric focusing, sickle test, sickle solubility test, testing for an unstable haemoglobin, investigation for Heinz bodies and haemoglobin H inclusions after supravital staining, methaemoglobin, oxygen dissociation curve
6. Investigation of red cell enzymes: Heinz body test, screening test for G6PD deficiency and tests for other erythrocyte enzymes, glutathione stability tests, methaemoglobinaemia and sulphhaemoglobinaemia
7. Immunohaematology: direct and indirect antiglobulin test with titre, specificity and thermal range of any antibodies; cold agglutinins, Donath-Landsteiner test, agglutination and lysis of enzyme-treated cells
8. Cytogenetic analysis; DNA analysis (for specific indications and for prenatal diagnosis)

- Megaloblastic anaemias, revealing the characteristic morphological features and helping in the differential diagnosis of clonal disorders;
- Normocytic anaemias with a normal or clearly reduced reticulocyte count, without an obvious cause, particularly if associated with alterations in white cells or platelets;
- Autoimmune haemolytic anaemia, looking for an associated neoplastic condition;
- The presence of unexplained alterations in leucocytes, platelets or both, or in the presence of a leucoerythroblastic blood film.

It is therefore useful to describe in this chapter the cytological features of the bone marrow in various types of anaemia, while clonal neoplasms such as myelodysplasia, leukaemia and myeloproliferative neoplasms will be dealt with in chapters specifically dedicated to them.

The bone marrow aspirate in an anaemic patient may show trilineage hypoplasia or selective hypoplasia of the erythroid lineage, or increased erythropoiesis as in haemolytic anaemia or when there is ineffective erythropoiesis. The latter, in which anaemia is associated with erythroid hyperplasia in the bone marrow and yet the reticulocyte count is not increased, is recognised in severe thalassaemic conditions, in megaloblastosis and in the myelodysplastic syndromes, associated with characteristic morphological changes in erythropoiesis and, in certain of these conditions, also in other lineages.

Perls Prussian blue stain is routinely employed for the evaluation of iron stores and for the detection of atypical accumulation of iron in erythroid precursors. Haemosiderin deposits in macrophages stain blue and are usually quantified with a gradation from 0 (absent) to +1 (reduced), +2 (normal), +3 (increased), +4 (greatly increased); or alternatively with a more detailed division into seven grades (from 0 to +6).[189,190] Iron stores can only be evaluated in bone marrow films containing fragments, with a minimum of seven fragments being necessary for optimal sensitivity[191]: an aspirate that lacks fragments is not evaluable for iron stores. Observation is first done at low power, assessing individual fragments, and then at a higher power to identify the macrophages with their blue-tinged cytoplasm (Fig. 2.61). The rest of the bone marrow film must also be evaluated, and a description given of any abnormal sideroblasts – those showing increased siderotic granulation or a perinuclear ring of granules (ring sideroblasts)[192] (Fig. 2.62).

### Microcytic and inflammatory anaemias

A bone marrow aspirate is not necessary for the detection of iron deficiency. A bone marrow aspiration may, however, be performed in patients with established iron deficiency anaemia either because of an error in the diagnostic protocol or in the context of another concomitant abnormality or a suspected haematological disorder, in which case the aspirate will appear mildly hypercellular, with mild or moderate erythroid hyperplasia. The significant observation is micronormoblastic maturation (Fig. 2.63). The erythroid precursors are smaller than

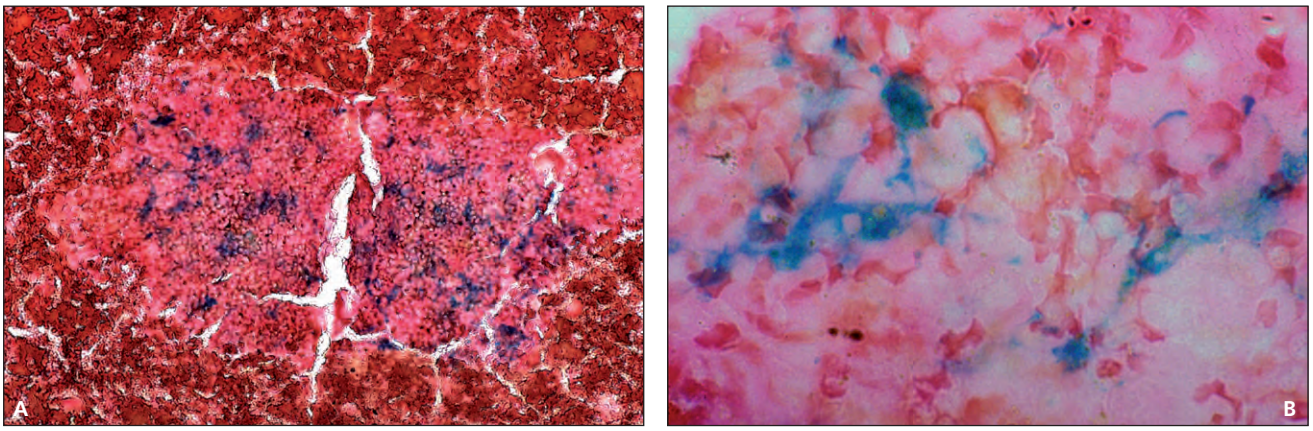



**Fig. 2.61** Perls stain for the demonstration of iron in a bone marrow film. (A) Bone marrow fragment with iron stores well represented. The intense blue staining, standing out from the background red (attributable to the eosin counterstain), shows clearly where ferric ferrocyanide has precipitated. (B) The plentiful cytoplasm of the macrophages, with diffuse blue staining, is apparent. The Perls stain is based on the liberation, by hydrochloric acid, of the $Fe^{3+}$ ions that are part of the intracellular haemosiderin: these react with potassium ferrocyanide forming the blue precipitate, designated ferric ferrocyanide or Prussian blue.

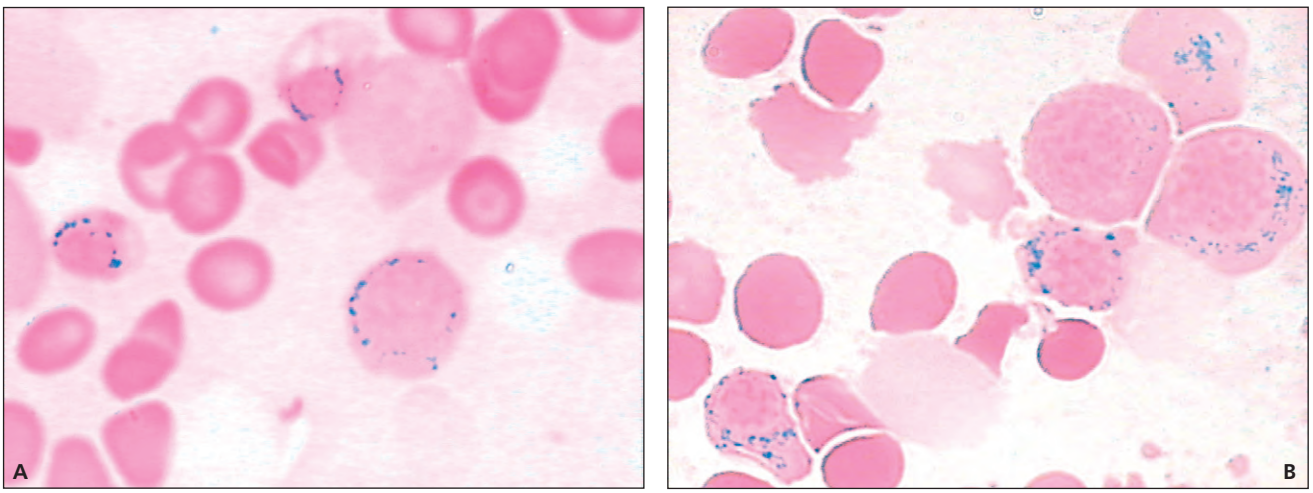



**Fig. 2.62** Bone marrow film, Perls stain. (A) Ring sideroblasts at various stages of maturation, as is shown by the variation in nuclear size. Erythroblasts are classified as ring sideroblasts, according to the WHO classification of myelodysplastic syndromes, when there are five or more blue-staining siderotic granules, surrounding at least a third of the nuclear circumference. (B) Four ring sideroblasts, laden with siderotic granules that are arranged in a ring around the nucleus. The largest erythroblast, on the right, is probably at the basophilic erythroblast or proerythroblast stage of maturation. The large cell without a nucleus, higher up, is a siderocyte. From a patient with refractory anaemia with ring sideroblasts.

normal, with a clear deviation to the right of the maturation curve: the predominant population is that of polychromatic and orthochromatic erythroblasts with scanty cytoplasm, pale, pink or greyish, often with irregular cytoplasmic margins, in which the block in synthesis of haemoglobin retards definitive maturation to reticulocytes. Mitotic figures are more frequent than normal (Fig. 2.64). This picture, associated with microcytosis and hypochromia of the circulating erythrocytes, reflects the slowing of cytoplasmic maturation and the increased number of cellular divisions. Dysmorphism may be apparent, but is generally not marked, and affects particularly the nucleus with distortion and nuclear protrusions, karyorrhexis and internuclear chromatin bridges.[193–195] These signs of dyserythropoiesis are non-specific and their presence should not lead to a suspicion of myelodysplasia.

Investigation of bone marrow macrophage iron by means of a Perls stain shows a total absence or marked reduction of stainable material: macrophages containing iron are not seen and erythroblasts with visible iron granules are very rare or totally absent. However, a completely negative Perls reaction in bone

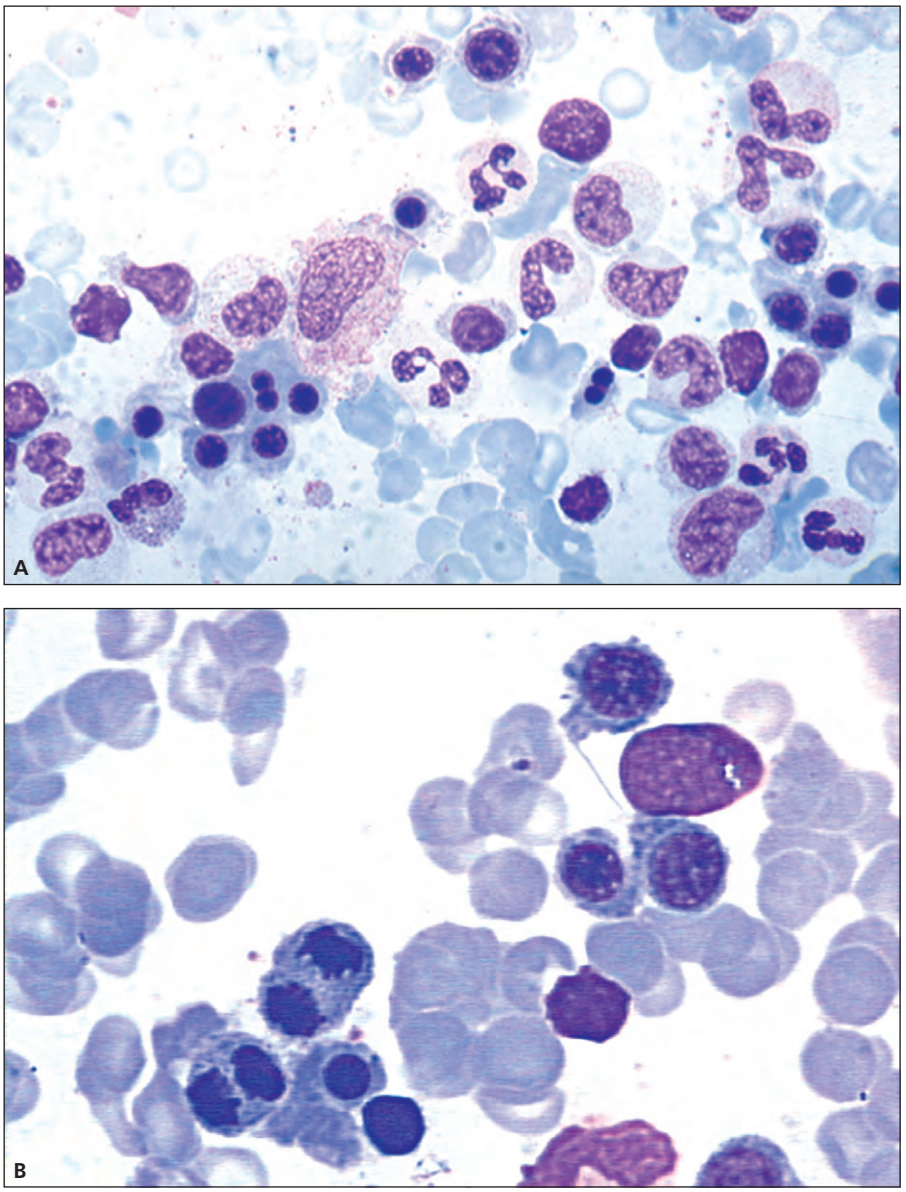



**Fig. 2.63** Micronormoblastic maturation in iron deficiency, staging bone marrow aspirate from a patient with lymphoma and severe iron deficiency anaemia not yet being treated (Hb 72 g/l, MCV 61 fl). (A) In this microscopic field there are various precursors and mature cells of neutrophil lineage, an eosinophil and some lymphocytes, the latter recognisable from their scanty cytoplasm, their eccentric nuclei and the characteristic condensation of the chromatin into irregular clumps. In addition, there are numerous small erythroblasts, mainly orthochromatic and polychromatic. Their nuclei are small and round. Two orthochromatic erythroblasts are binucleated: dyserythropoietic features of this type are common in severe iron deficiency and indicate the lack of specificity of morphological signs or dyserythropoiesis in relation to the diagnosis of myelodysplastic syndrome. (B) On the left, two almost orthochromatic erythroblasts, in anaphase, adjacent to a third erythroblast with a completely condensed nucleus. Mitotic figures at such an advanced stage of maturation are not common in normal bone marrows. Top right, two basophilic erythroblasts appear to be joined by an internuclear filament.

marrow films is not definitive proof that iron deficiency is the only pathological process operating[196]: for example, latent iron deficiency is not rare in women with a haematological neoplasm, even in conditions in which one would expect increased iron stores, such as myelodysplasia. It is also necessary to remember the possibility that the patient may have already started iron therapy at the time when the bone marrow aspiration was done; particularly with intravenous iron therapy, one may see large bluish granules of iron-containing molecules in bone marrow macrophages. Furthermore, initiation of therapy may increase erythroid hyperplasia. This possibility must always be considered in order to avoid serious diagnostic

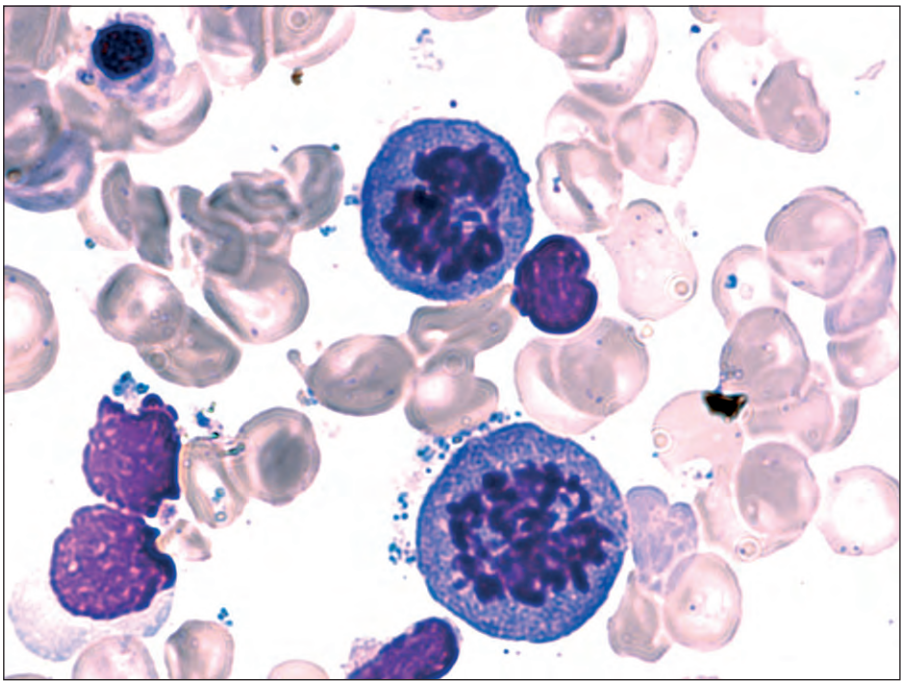


**Fig. 2.64** From a bone marrow aspirate performed to investigate possible metastasis from a disseminated and bleeding adenocarcinoma of the colon. Two erythroblasts in mitosis; top left, an orthochromatic microerythroblast. The erythrocytes are hypochromic.

errors, particularly when considering the differential diagnosis with myelodysplasia (in which, however, it is rare to have marked microcytosis). It is important to emphasise again that assessment of bone marrow iron stores is no longer part of the diagnostic pathway in iron deficiency anaemia. A bone marrow aspirate with a Perls stain is, however, indispensable if there is a suspicion of myelodysplasia, in order to identify the presence of ring sideroblasts. As well as being essential for the diagnosis of refractory anaemia with ring sideroblasts, these abnormal forms are also the characteristic abnormality of a heterogeneous group of rare congenital sideroblastic anaemias, resulting from a defect that leads to accumulation of ferritin and haemosiderin within mitochondria.[197] In the congenital form, diagnosed particularly in children but also, when mild, later in life, there is bone marrow erythroid hyperplasia and sideroblasts, often ring sideroblasts at all stages of maturation, constitute from 15% to 20% of the erythroid population. There can also be megaloblastoid features because of a concomitant folate deficiency or a reduced number of sideroblasts when there is a superimposed iron deficiency. The circulating progeny of the abnormal sideroblasts are microcytic and hypochromic, often with marked anisocytosis, anisochromasia and dimorphism; the blood film also shows erythrocytes containing Pappenheimer bodies (corresponding to the siderocytes seen on a Perls stain).

In anaemia associated with chronic inflammatory diseases, the bone marrow picture is extremely heterogeneous. Cellularity can be normal or somewhat reduced, with moderate dyserythropoiesis and increased macrophages, sometimes with prominent haemophagocytosis or erythrophagocytosis. Storage iron is in general well represented, while sideroblasts are reduced or absent. The underlying condition, however, can have a marked influence on the cytomorphological profile of haemopoiesis, e.g. leading to neutrophilia with granulocytic hyperplasia (see Chapter 11).

In the bone marrow aspirate of subjects with thalassaemia heterozygosity, again examined for unrelated reasons, there is usually, depending on the severity of the defect, moderate erythroid hyperplasia with micronormoblastic erythropoiesis (Fig. 2.65). Similarly, depending on the severity of the ineffective erythropoiesis with intramedullary destruction of erythroblasts, there are variable numbers of macrophages with features of activation, haemophagocytosis, erythrophagocytosis and an accumulation of unrecognisable cellular debris and dark yellow or gold material that is birefringent of polarised light, corresponding to the positively staining haemosiderin seen on a Perls stain. These appearances are marked in thalassaemia intermedia and Cooley anaemia (thalassaemia major), in which the micronormoblastic erythroid hyperplasia is so massive that it produces skeletal deformity. Particularly in beta thalassaemia major, erythroid hyperplasia is extreme and ineffective,[198] as a result of the intramedullary death of erythroblasts laden with alpha chain inclusions, the alpha chains being unable to find beta chains with which to combine to form a haemoglobin molecule. Dyserythropoiesis shows characteristic features (Figs 2.66 and 2.67): micronormoblasts with scanty pale cytoplasm, lacking in haemoglobin, with basophilic stippling and nuclear

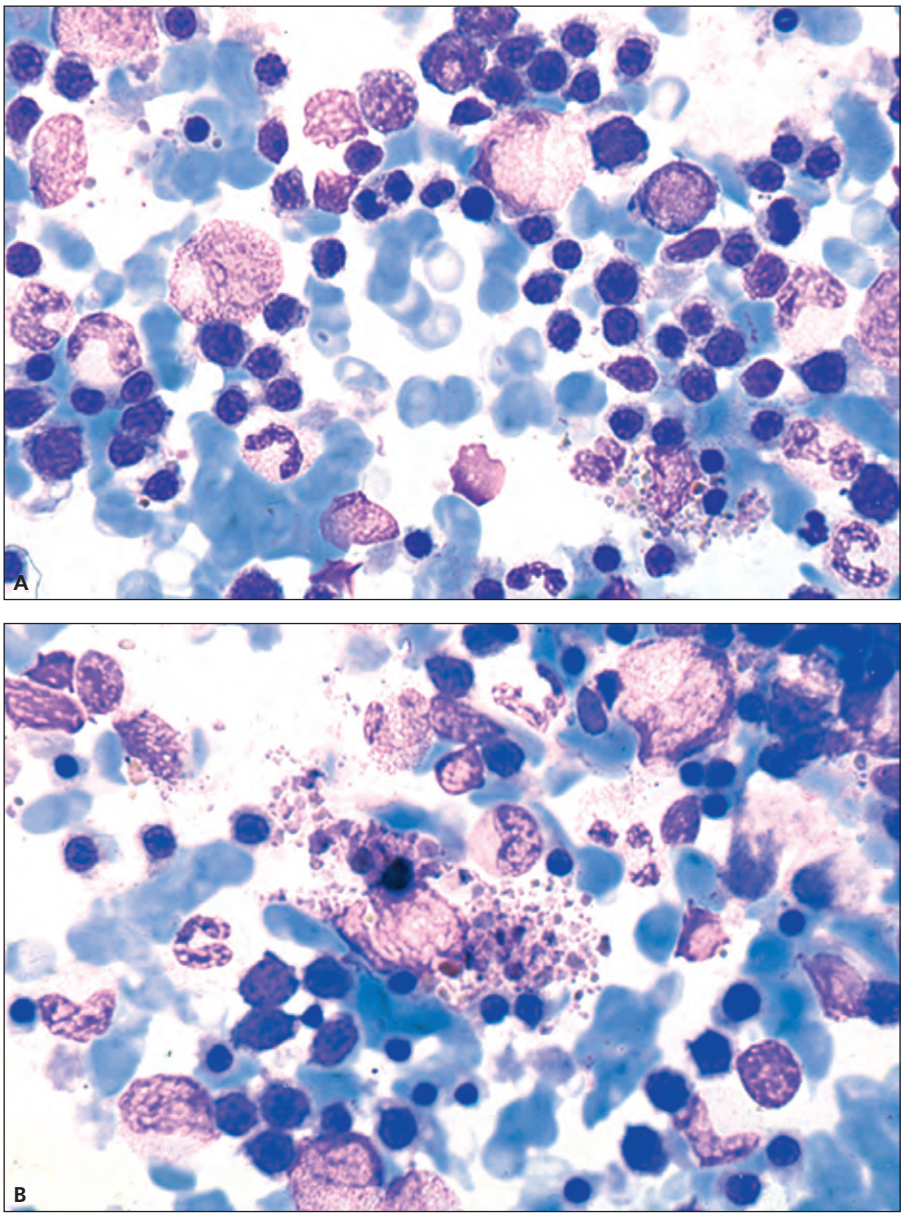



**Fig. 2.65** Microerythroblasts and an increase of iron-laden macrophages in the bone marrow aspirate of a patient with Hodgkin lymphoma and beta thalassaemia trait, having a staging bone marrow examination. (A) Conspicuous erythroid hyperplasia with right shift of the maturation curve, with mainly small orthochromatic and polychromatic erythroblasts. Bottom right, there is a macrophage with plentiful cytoplasm distended by large gold granules that represent masses of haemosiderin. (B) The large central macrophage contains, in addition to gold granules, the remains of nuclei that indicate the presence of moderately ineffective erythropoiesis in subjects with thalassaemia minor.

anomalies.[199] The existence of a genetic abnormality must be suspected in all such cases in order to avoid erroneous diagnostic conclusions, hypothesising an acquired erythroid anomaly (e.g. dyserythropoiesis as a feature of myelodysplasia). Bone marrow iron stores are markedly increased and the Perls stain shows iron-laden macrophages containing phagocytosed erythroblasts, testimony to the intramedullary death that is occurring.[200]

## Megaloblastic anaemia

Megaloblastic anaemia as a result of vitamin $B_{12}$ or folate deficiency is a potentially very serious condition, which is manifest in well-established cases as macrocytic anaemia with the presence of anisopoikilocytosis and red cell fragments in the peripheral blood film, together with leucopenia, thrombocytopenia and hypersegmented neutrophils.[201] Bone marrow preparations in megaloblastic anaemia are characterised by increased

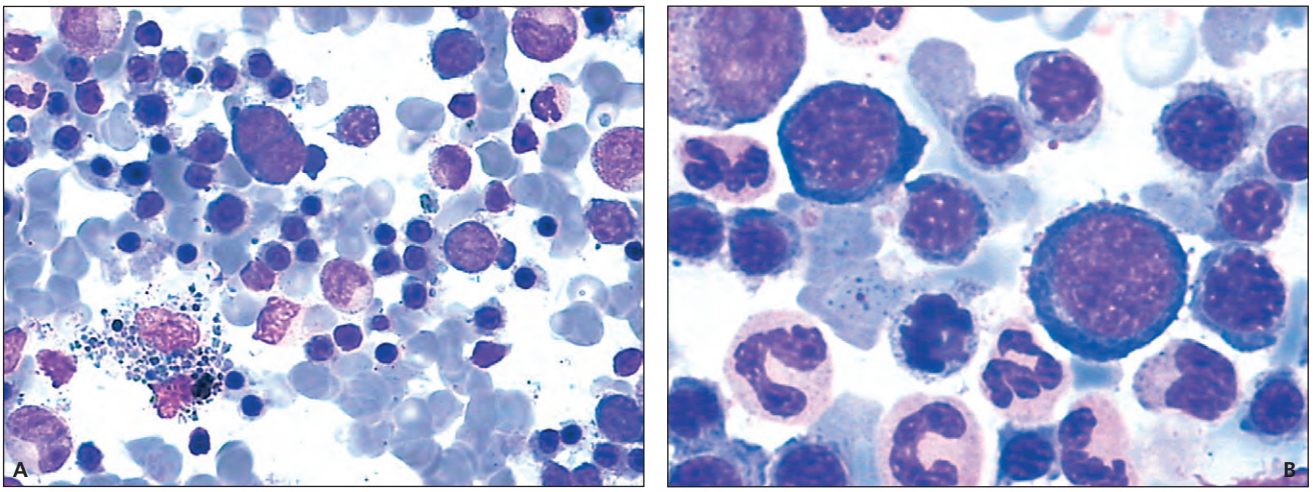



**Fig. 2.66** Bone marrow aspirate in thalassaemia intermedia. (A) Marked erythroid hyperplasia with erythroblasts in all stages of maturation. The polychromatic and orthochromatic erythroblasts are small. Centre top, a binucleated erythroblast. On the right edge, immediately below a promyelocyte, a normal appearing basophilic erythroblast. Bottom left, a macrophage with ample cytoplasm distended by cellular debris. (B) From another patient with thalassaemia intermedia, erythroid hyperplasia with moderate dysplasia. The largest cell is a proerythroblast, recognisable from its voluminous nucleus with chromatin showing small areas of condensation; two nucleoli are apparent; the cytoplasm is intensely basophilic, with the exception of the pale arcoplasm. The periphery of the cytoplasm shows small nail-like protrusions. Top left, a basophilic erythroblast with deep blue cytoplasm and nuclear chromatin in blocks, joined by a cytoplasmic bridge to a similar but smaller cell. The other erythroblasts are almost all polychromatic. An erythroblast just below the centre has reformed its nucleus badly after mitosis and seems to contain a discontinuous intertwining of chromosomes.

cellularity, often marked, with a relative increase in the number and size of the erythroid precursors (designated since the time of Ehrlich by the term 'megaloblast'). These show, even at low power, typical and often very striking morphological abnormalities, with left shift of the maturation curve, an increase in cells of morphologically more immature stages and an abundance of large precursors with abundant, intensely basophilic cytoplasm so that the whole marrow has a bluish appearance (Fig. 2.68). Characteristic dysmorphism is also apparent in the granulocytic and megakaryocytic lineages. The cellular abnormalities are the result firstly of a defect in the synthesis of DNA, which slows duplication of nuclear material and impedes the normal mitotic sequence, while cytoplasmic maturation and haemoglobin synthesis continue unimpeded, and secondly to the ineffectiveness of myelopoiesis, with obvious apoptosis, nuclear degeneration and intramedullary cell death.

At high power the characteristic abnormalities of basophilic precursors (promegaloblasts and basophilic megaloblasts) include increased volume, abundant cytoplasm in comparison with the amount of nuclear material (unusual at this stage of erythropoiesis) and, above all, abnormal chromatin structure, this being dispersed, less prone to form small condensed chromatin clumps than normoblastic precursors (Fig. 2.69). The chromatin filaments intertwine, forming a very fine, delicate network, like small evenly sized beads (a pearly nucleus according to French authors, sieve-like according to Anglo-Saxon authors). The cytoplasm is abundant and intensely basophilic, with very obvious pale arcoplasm and non-staining stipples indicating hypertrophic mitochondria. Similar anomalies are seen in the basophilic megaloblasts, in which chromatin condensation remains incomplete.

If the morphology of the most immature precursors leaves some diagnostic uncertainty because of their resemblance to the corresponding stages in normoblastic erythropoiesis, the recognition of megaloblastosis becomes progressively easier and more certain with successive phases of erythropoiesis. The cardinal morphological feature of megaloblastosis, the asynchrony between nuclear and cytoplasmic development in haemopoietic precursors, finds its maximal expression in polychromatic and orthochromatic erythroblasts (Fig. 2.70). In these later cells, the failure to complete the sequence of maturation is particularly apparent. The nucleus, with its inability to synthesise nucleic acids normally, maintains a fine reticular chromatin pattern and appears much less mature that the abundant cytoplasm, in which the synthesis of haemoglobin continues, not being impeded by the vitamin deficiency. The maximum diagnostic value resides in the polychromatic megaloblasts, which are gigantic with an enormous amount of cytoplasm, which is now almost completely haemoglobinised, and a large nucleus with its immature dispersed chromatin, maintaining structural characteristics similar to those of the nuclei of promegaloblasts. In other polychromatic megaloblasts and even more in orthochromatic megaloblasts, the nuclei start to show chromatin condensation and pyknosis, with marked atypia and bizarre morphology (Fig. 2.71): distortion, pyknosis, nuclear fragmentation and formation of multiple Howell-Jolly bodies; sometimes the nuclei

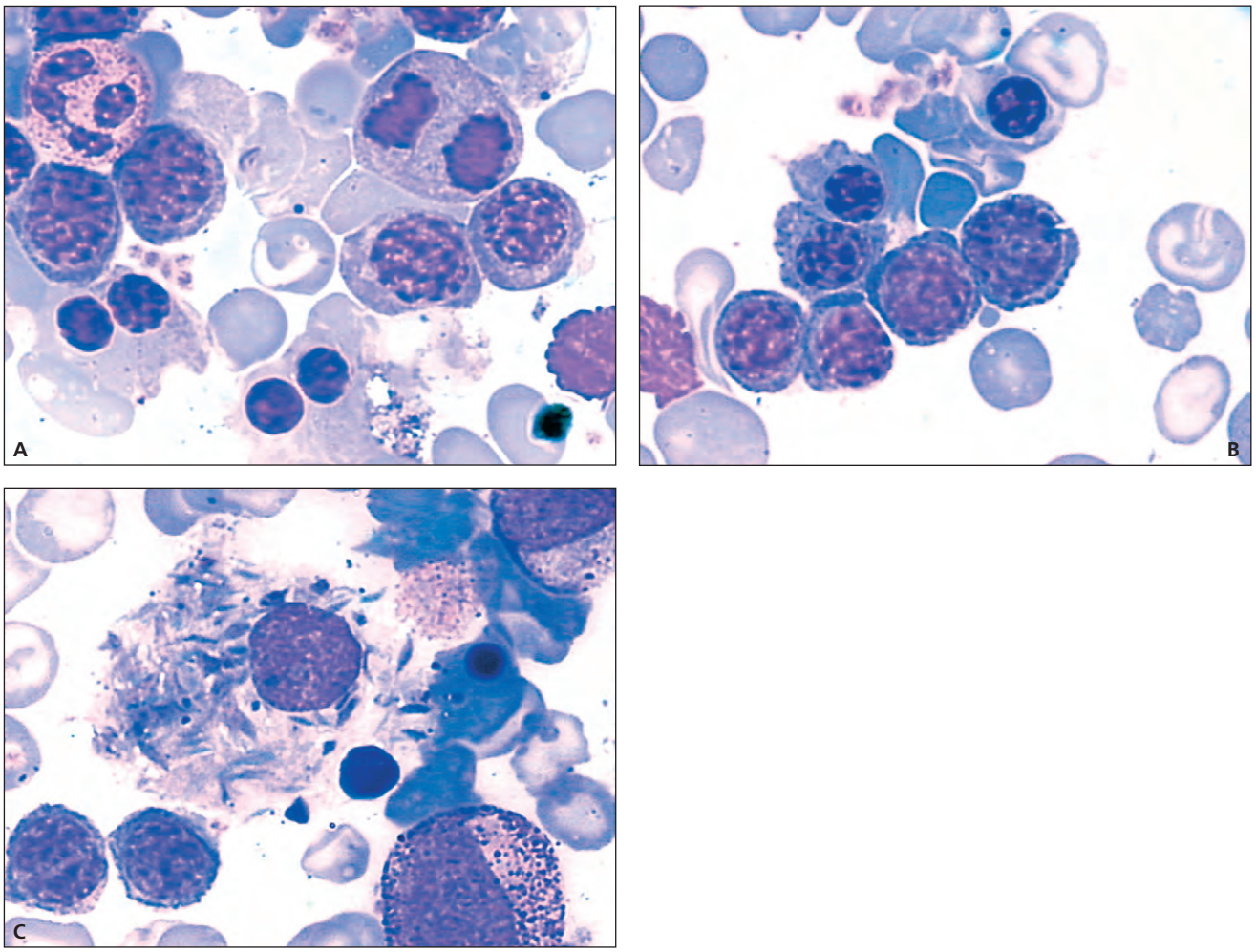



**Fig. 2.67** Bone marrow film from a patient with beta thalassaemia major. As in the patients illustrated in previous figures, the bone marrow examination was done in the course of diagnostic investigations that did not relate to the thalassaemia. The features of dyserythropoiesis with erythroid hyperplasia are very marked. (A) At the sides of the image, two pairs of basophilic erythroblasts with chromatin condensed into blocks. At the top, an erythroblast in telophase, with the chromosomes grouped at the two poles; in the cytoplasm there are filamentous structures that suggest degenerating fibres of the mitotic spindle. Bottom left, there are two binucleated orthochromatic erythroblasts. In the centre, a deformed red cell contains a Howell-Jolly body. (B) A small group of four basophilic erythroblasts, a polychromatic erythroblast and two orthochromatic erythroblasts with asynchronous maturation. (C) A macrophage with a very eccentric nucleus, with a spongy chromatin structure, in which a pale nucleolus can be seen. The cytoplasm, plentiful and well defined, contains numerous paracrystalline rhomboidal inclusions, formed from lipid derived from the degraded membranes of bone marrow cells. Cells of this type are designated pseudo-Gaucher cells: their presence, more common in patients with myeloproliferative neoplasms, indicates the severity of the ineffective haemopoiesis.

fail to divide and are thus hyperdiploid, with large dark blocks of chromatin resembling lumps of coal.

Multinuclearity and defect of cell division are common and heterogeneous, with the presence in the same cell of dysmorphic nuclei and nuclear fragments of various sizes, as if terminal enucleation in megaloblasts is by means of karyorrhexis and lysis. Some cells have small pyknotic nuclei with non-homogeneous pale cytoplasm, due to incomplete haemoglobinisation. There are numerous mitotic figures, fixed in a variety of shapes, often present in polychromatic erythroblasts, which in normoblastic erythropoiesis are no longer capable of cell division: fragments of chromosomes become detached and seem to wander around in the ample grey-rose cytoplasm. Cytogenetic analysis may show increased chromosomal fragility, leading to a risk of diagnostic error with a diagnosis of a clonal disorder being considered.[202]

The morphology of the various stages of maturation in megaloblastic erythropoiesis is very heterogeneous, so that together with cells that are frankly megaloblastic there are precursors with conservation of more normal maturation, and with all the intermediate stages between the two extremes also being present.

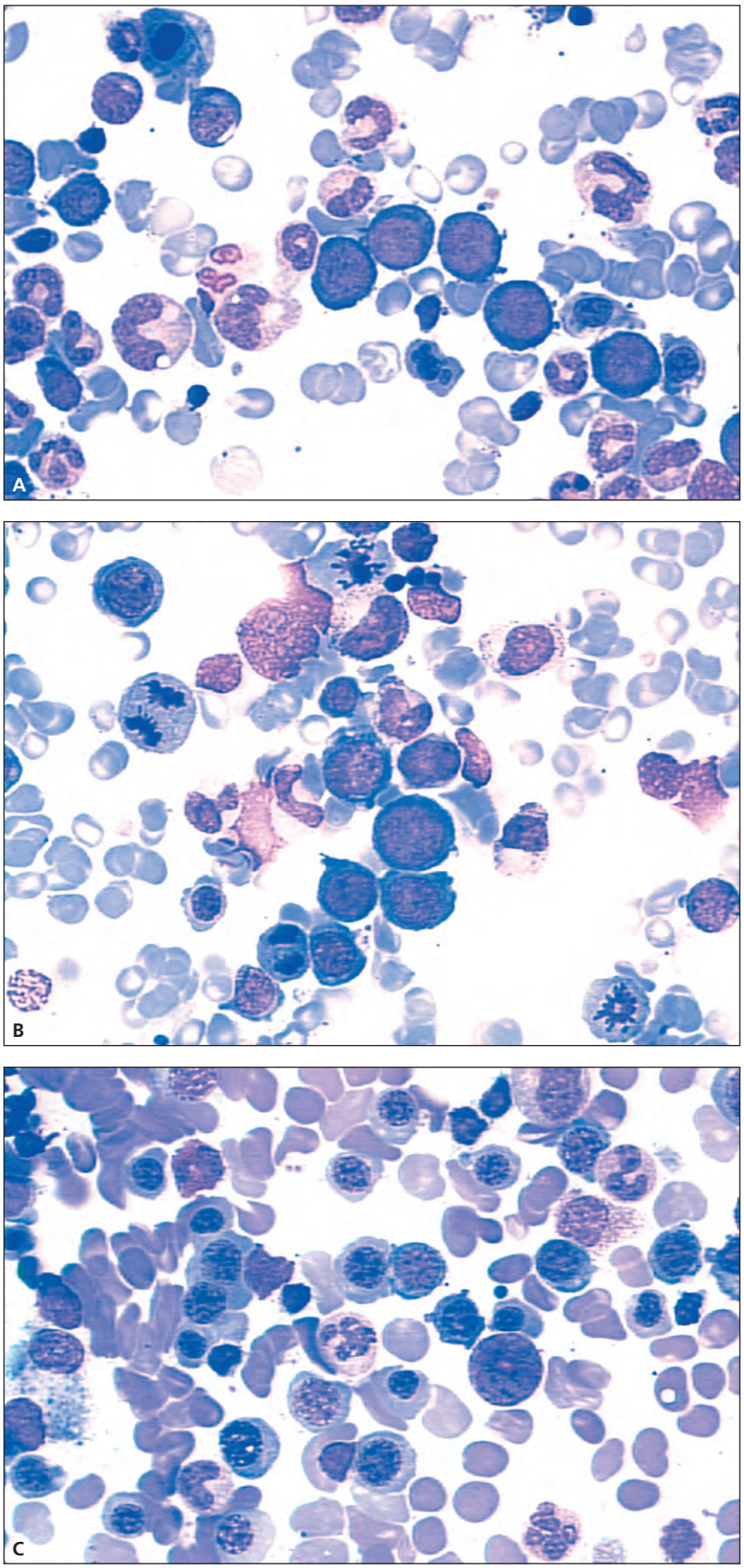



**Fig. 2.68** Bone marrow aspirate, megaloblastic anaemia. At medium power, the preparation is seen to be cellular, with a good representation of both erythroid and granulocytic series. (A) From the centre towards the right, five proerythroblasts with plentiful basophilic cytoplasm, which in some instances is forming short pseudopodia, and with nuclei that are finely granular (the chromatin pattern of a promegaloblast), round, centrally placed and almost never abutting the internal surface of the cytoplasmic membrane. When these very early erythroid precursors are numerous, the bone marrow film acquires the appearance of a 'blue bone marrow'. Close to the proerythroblasts are various polychromatic erythroblasts, which have more obvious features of megaloblastosis: there is marked nucleocytoplasmic asynchrony and nuclear dysmorphism, the nucleus often being multilobed. The granulocytic series shows gigantism of metamyelocytes and neutrophil band forms and a tendency to hypersegmentation of the rare mature forms (bottom left corner). To the right of this five-lobed neutrophil there is a macrocyte with a Howell-Jolly body and defective haemoglobinisation. (B) Adjacent to several promegaloblasts, there are basophilic megaloblasts, top left and low in the centre, in which the cytoplasm has already lost its original intense basophilia, becoming blue-grey because of the initiation of haemoglobin synthesis; the nuclei are still immature with small regularly distributed chromatin clumps (asynchrony). In addition, mitotic figures are seen in erythroblasts with polychromatic, sometimes almost orthochromatic, cytoplasm: an early metaphase, centre top; a more advanced metaphase, bottom right; towards the left, two cells in anaphase, with two groups of chromosomes migrating towards the poles. (C) Numerous erythroid cells in intermediate stages of maturation, with cytoplasm that is mainly polychromatic and quite variable chromatin condensation. These cells show the greatest evidence of the nucleocytoplasmic asynchrony that characterises the dysplasia of megaloblastic erythropoiesis. In the centre of the image, above a hypersegmented mature neutrophil, a polychromatic megaloblast still joined to, or superimposed on, a basophilic megaloblast. There is another hypersegmented neutrophil bottom right, two cells of eosinophil lineage (a myelocyte on the right and on the left a mature eosinophil) and a macrophage, adjacent to the left edge, recognisable from its oval spongy nucleus.

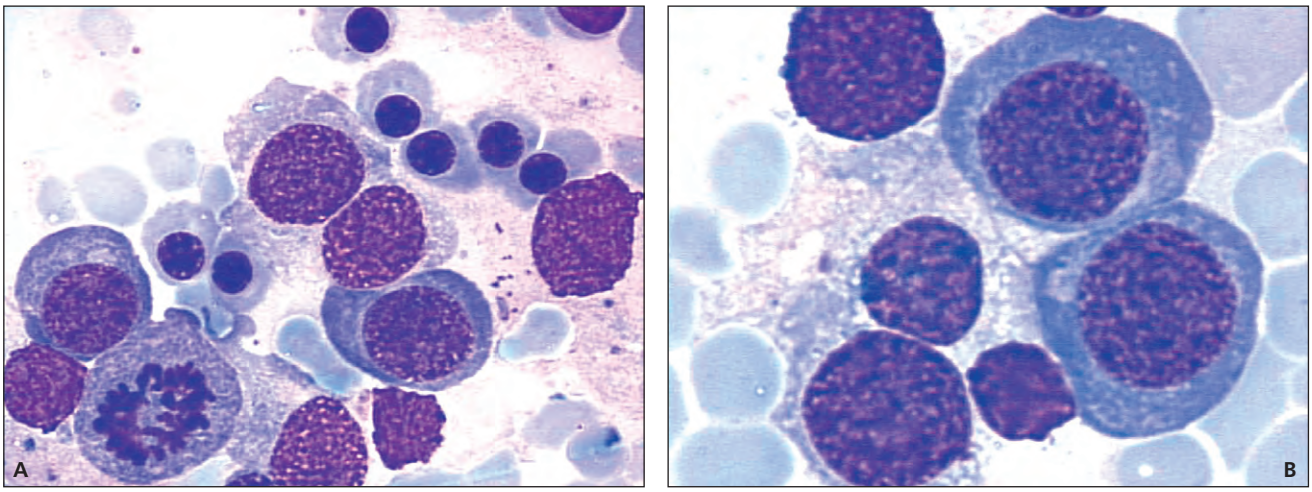



**Fig. 2.69** Megaloblastic anaemia, bone marrow aspirate. (A) The two cells with plentiful basophilic cytoplasm are promegaloblasts. The large oval nuclei have chromatin condensed into fine or coarse beads, with a distinct nuclear membrane, as if sketched with charcoal. A nucleolus, surrounded by a circle of condensed chromatin, is more obvious in the cell on the right. The intense basophilia results from the presence of numerous ribosomes, responsible for the future synthesis of globin chains. A pale thin area of arcoplasm is seen in the cell on the right, while a more compact Golgi complex that appears to be attached to the nucleus is seen in the cell on the left; the cytoplasm of the latter appears as if embroidered with beads and pale threads (the negative images of mitochondria and endoplasmic reticulum). In the centre, a giant binucleated polychromatic megaloblast: while the frayed cytoplasm is still greyish, the nuclei are much more immature, having a granular structure with small areas of chromatin condensation alternating with pale spaces. A cell with similar cytoplasm, bottom left, is in mitosis. The other seven smaller erythroblasts are almost orthochromatic with more or less condensed nuclei. (B) Another two basophilic erythroblasts with a granular chromatin structure without nucleoli. The nuclei are round and, although slightly eccentric, do not touch the cell membrane. The intensely blue cytoplasm appears perforated by white dots that represent hypertrophied mitochondria and by the paranuclear clear area of the Golgi apparatus. The thin filament that joins these two large cells seems to arise from the nuclear structure. At the bottom, a binucleated polychromatic megaloblast with very asymmetric nuclei.

The mature red cells show marked anisopoikilocytosis, with fragments, dysmorphic microcytes and very large oval anucleate cells (macro-ovalocytes) (see Figs 2.12 and 2.13), with frequent Howell-Jolly bodies, basophilic stippling, polychromasia and, less often, Cabot rings and the related reddish azurophilic granules representing remnants of the mitotic spindle.

The cytological features of megaloblastosis are not confined to the erythroid lineage, as one might suppose from the terms 'megaloblastic anaemia' or 'pernicious anaemia' (Fig. 2.72). Megaloblastosis is in fact a panmyelosis, which affects all actively proliferating haemopoietic cells (and not only them: morphological abnormalities of the same type, with an immature nucleus and nucleocytoplasmic asynchrony, are also observed, e.g. in regenerating epithelial cells of the gastrointestinal tract). In the granulocytic series, the two characteristic cytological abnormalities are gigantism of the marrow precursors and hypersegmentation of the nuclei of the mature granulocytes (Fig. 2.73). The increased size is apparent at all stages of maturation, but becomes very obvious in the metamyelocytes, which are another of the stigmata of megaloblastosis. These have a very large horseshoe-shaped nucleus, which sometimes has a very bizarre shape, set in ample pale cytoplasm, which appears mature, without residual patches of basophilia. In addition to their striking size, granulocytes have an altered chromatin structure, with a delicate intricate network of filaments in the earlier forms and delayed condensation, irregular and partial, in the myelocytes and their progeny. The increase in nuclear lobes in the mature neutrophils, to five, six or even more, is better seen in peripheral blood films, in which there is often also neutropenia; however, also in marrow preparations one can observe that nuclear segmentation is increased, with some hypersegmented cells, which in the absence of megaloblastosis are quite infrequent (Fig. 2.74). All the above-mentioned abnormalities are also apparent in the eosinophil lineage (large precursors and, in the peripheral blood, mature cells with three or more nuclear lobes) and in the monocyte lineage (in which the chromatin is paler than normal, with a delicate pattern, as if swollen, and sometimes nuclei with bizarre and convoluted shapes).

Abnormalities in megakaryocytes are seen in advanced megaloblastosis and are accompanied by thrombocytopenia. Megakaryocytes may be gigantic with ample mature cytoplasm and very hyperdiploid nuclei with an increased number of lobes (Fig. 2.75). There are also immature forms with nucleocytoplasmic asynchrony.

The marrow in megaloblastic anaemia contains macrophages laden with all sorts of debris, their role being the disposal of the abundant cellular detritus that results from massive intramedullary cell death (Fig. 2.76).

The morphological hallmark of well-established megaloblastic anaemia is thus, in summary, very markedly ineffective myelopoiesis with dysmorphism. Remembering that this well-developed picture is not nowadays so common, it is of greatest

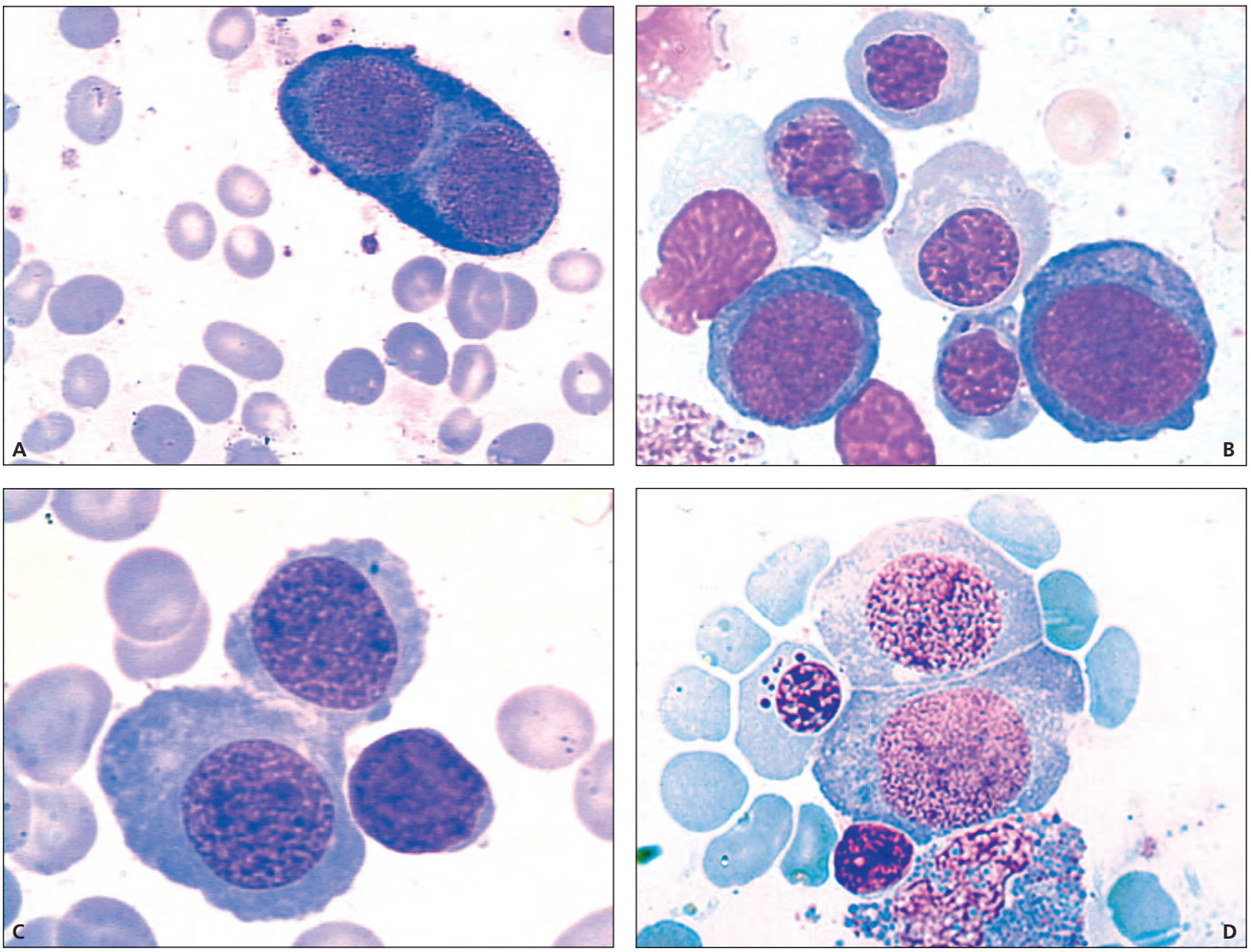



**Fig. 2.70** Bone marrow aspirates from patients with pernicious anaemia: diagnostic value of nucleocytoplasmic asynchrony. (A) The giant cell is a binucleated megaloblast, almost at the end of a mitotic division. In both the nuclei the chromatin is extremely fine, pearly or granular. Nucleoli can be barely distinguished. The nuclear membrane is not very distinct, not clearly outlined as is usual in these cells. (B) Two promegaloblasts with intensely basophilic cytoplasm, arcoplasm and whitish stippling indicating hypertrophied mitochondria. One notes the fine granularity of the chromatin, the oval shape of the nuclei clearly outlined by the continuous nuclear membrane and the presence of multiple, not clearly outlined, nucleoli. The diagnostic cell in this field is the polychromatic cell in the centre – striking evidence of the asynchrony of megaloblastic erythropoiesis, which has a nucleus appropriate for a basophilic erythroblast but ample cytoplasm, which is verging on orthochromasia. (C) Two large polychromatic megaloblasts. Their nuclei are very similar: granular chromatin with minimal areas of condensation, a very obvious nuclear membrane and no visible nucleoli. The haemoglobinised cytoplasm varies in amount. These are the cells, together with giant metamyelocytes and hypersegmented neutrophils, that must be identified to make a reliable diagnosis of megaloblastic erythropoiesis. (D) In a different patient the features of nucleocytoplasmic asynchrony are even more marked, being most evident in the polychromatic megaloblasts, such as the two large cells in the centre. The lower of these, in particular, has an extremely immature nucleus, identical because of the presence of nucleoli and pearly chromatin, to that of a promegaloblast. An orthochromatic megaloblast on the left has a nucleus that is not yet pyknotic, with alternating pale spaces and dark chromatin clumps, and numerous Howell-Jolly bodies of various sizes.

importance that the haematologist is very familiar with the features so that erroneous interpretation as severe clonal myelodysplasia or, in the not uncommon cases in which there are numerous immature precursors and a percentage of myeloblasts that cannot be ignored, as an acute myeloid leukaemia with erythroid predominance (erythroleukaemia). Such diagnostic errors can obviously have devastating consequences, given the type of treatment that may be given and the consequences that this may have.

Another not uncommon source of error comes from the observation of an incomplete picture, resulting from the co-existence of another nutritional deficiency, in particular iron deficiency, or from the common practice of patients taking multivitamin preparations before the initiation of the diagnostic process, thus producing partial regeneration of a variable proportion of haemopoietic cells, with a return of these to normal, from the point of view of maturation and morphology. These occurrences can easily alter not only the typical bone marrow

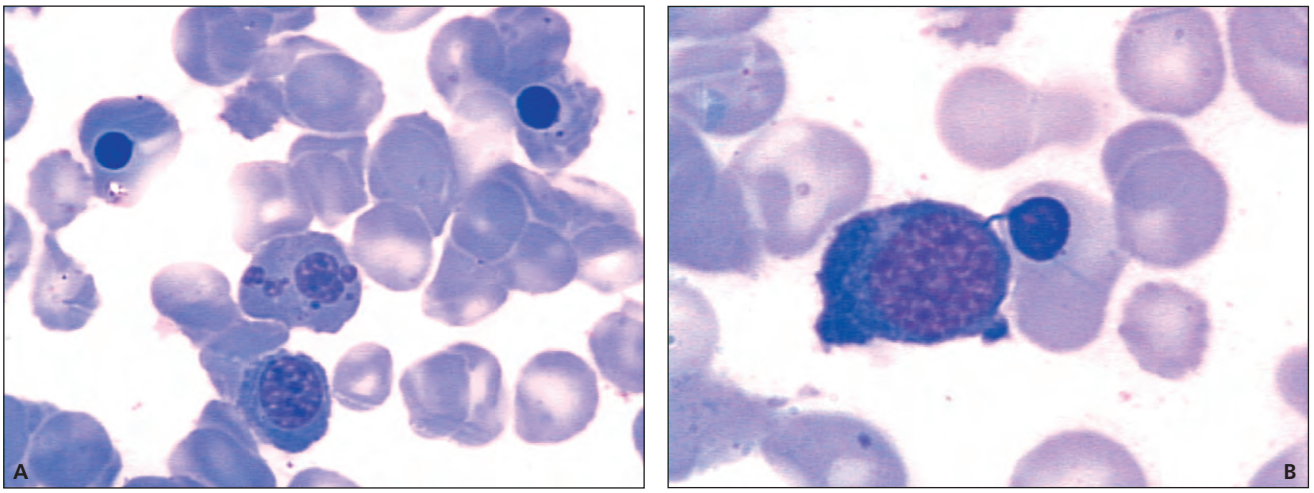



**Fig. 2.71** Bone marrow aspirates from patients with pernicious anaemia: bizarre erythroid maturation. (A) At the bottom in the centre, a small basophilic erythroblast with moderate nucleocytoplasmic asynchrony. Slightly higher, an almost orthochromatic erythroblast with the nucleus showing karyorrhexis, with nuclear projections and detached nuclear fragments that still contain small pale areas. In the two top corners, two orthochromatic erythroblasts with plentiful cytoplasm, in which the nucleus is now a small sphere ready for extrusion. (B) Paradoxical asynchrony: the two erythroblasts appear joined by a solid internuclear bridge, which demonstrates that they have been generated at the same time by a single mitotic division, yet their differentiation is quite different. The cell on the left is a basophilic megaloblast, with the nucleus showing small chromatin blocks separated by paler spaces and the cytoplasm being intensely basophilic with blebs. The cell on the right, however, has matured to the extent of becoming an orthochromatic erythroblast, with chromatin condensed into a blackish rounded mass.

picture, but also the appearance of mature erythrocytes, with a reduction or disappearance of macrocytosis and, in the latter case, with the presence of a reticulocyte response. Iron deficiency associated with a deficiency of vitamin $B_{12}$ and/or folic acid must be thought about particularly when a more or less typical megaloblastic marrow is discovered during investigation of a normocytic, or even slightly microcytic, anaemia. Furthermore, vitamin therapy can produce striking erythroid regeneration with reticulocytosis and the rapid disappearance of megaloblastic precursors, and can unmask a pre-existing iron deficiency, which then compromises the therapeutic response and leads to the appearance of microcytes in the circulation. In these cases, the RDW and automated instrument histograms can give valuable information that elucidates the diagnosis.

Finally, the characteristic features of megaloblastic erythropoiesis can be observed to a greater or lesser extent in patients treated with anticancer drugs (Fig. 2.77) and in some cases of myelodysplasia.

## Hyper-regenerative anaemia

In the haemolytic anaemias, congenital or acquired, characterised by reduced red cell survival, a bone marrow aspirate is generally not necessary for the diagnosis, but may be performed to exclude associated pathology, particularly when there has been a sudden fall in the Hb with reticulocytopenia (aplastic crisis). The most frequent findings in these patients, regardless of the aetiology and pathogenesis, is erythroid hyperplasia with a left shift of the maturation curve associated with dyserythropoiesis (nuclear dysmorphism, chromatin bridges and Howell-Jolly bodies, which may be multiple) (Fig. 2.78). Sometimes there are features of ineffective erythropoiesis (increased erythrophagocytic macrophages, which are often numerous, particularly when there is haemolysis) and megaloblastoid features resulting from a secondary folate deficiency (which can aggravate the anaemia), resulting in turn from accelerated erythropoiesis. These alterations are more prominent when there is acute haemolysis with a marked reticulocytosis. Iron stores are typically increased, particularly when there has been a protracted evolution, as a result of iron retained by the macrophages after haemolysis; but it is not exceptional to find iron depletion, particularly in young women.

Aplastic crises in haemolytic anaemia are in general associated with a selective erythroblastopenia, which is usually viral in origin (see below). This presents acutely, particularly in hereditary spherocytosis and in sickle cell anaemia. In both these instances, another possible cause of a sudden arrest of erythropoiesis is a megaloblastic crisis, resulting from severe folate depletion.

If one excludes these episodes, paroxysmal nocturnal haemoglobinuria is the only haemolytic condition in which there are signs of peripheral haemolysis associated with a hypocellular marrow.

## Bone marrow aplasia and hypoplasia

Among the conditions responsible for an 'empty marrow', both in the aspirate and the trephine biopsy, is aplastic anaemia (Fig. 2.79); this condition is characterised by an extreme reduction in haemopoietic cells, which are often reduced to a few somewhat megaloblastoid erythroblasts. In Fanconi anaemia, an inherited syndrome with chromosomal instability, macrocytosis

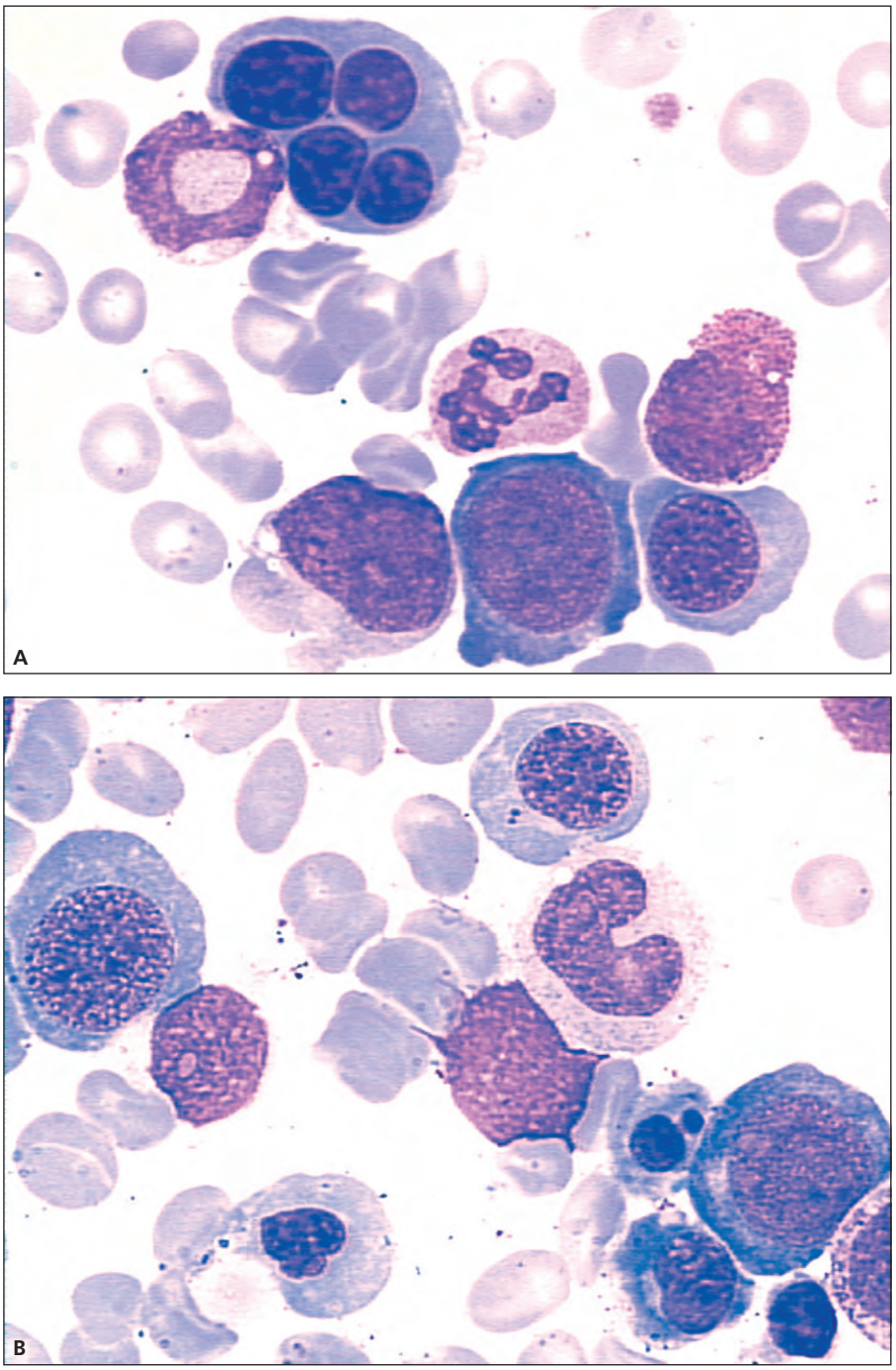



**Fig. 2.72** Megaloblastic anaemia; bone marrow aspirate. In this patient one sees all the most typical abnormalities. (A) In this field: (1) a promegaloblast with cytoplasmic projections and a round clearly outlined nucleus with numerous nucleoli; (2) on its right, a polychromatic megaloblast with nucleocytoplasmic asynchrony; (3) a neutrophil granulocyte with a hypersegmented nucleus; (4) on its right, an eosinophil with two or possibly three nuclear lobes obscured by granules; (5) a giant polychromatic erythroblast with four nuclei that differ in size and shape. On the bottom edge, to the left of the promegaloblast, a large blast, from the angular nucleus and the heterogeneity of the chromatin and the nucleoli probably a myeloblast, with greyish agranular cytoplasm. Cells of this type, if present in small numbers, should not lead to an erroneous suspicion of myelodysplasia or erythroleukaemia. (B) Cells of the erythroid series are frankly megaloblastic, with a promegaloblast bottom right, two polychromatic megaloblasts in the centre at the top and on the left, three orthochromatic megaloblasts with striking nucleocytoplasmic asynchrony and, in two of these cells, irregular and fragmented nuclei. The giant neutrophil metamyelocyte at 2 o'clock is important for the cytological confirmation of megaloblastosis; it has a horseshoe-shaped nucleus and chromatin that is less condensed than normal for this stage of maturation.

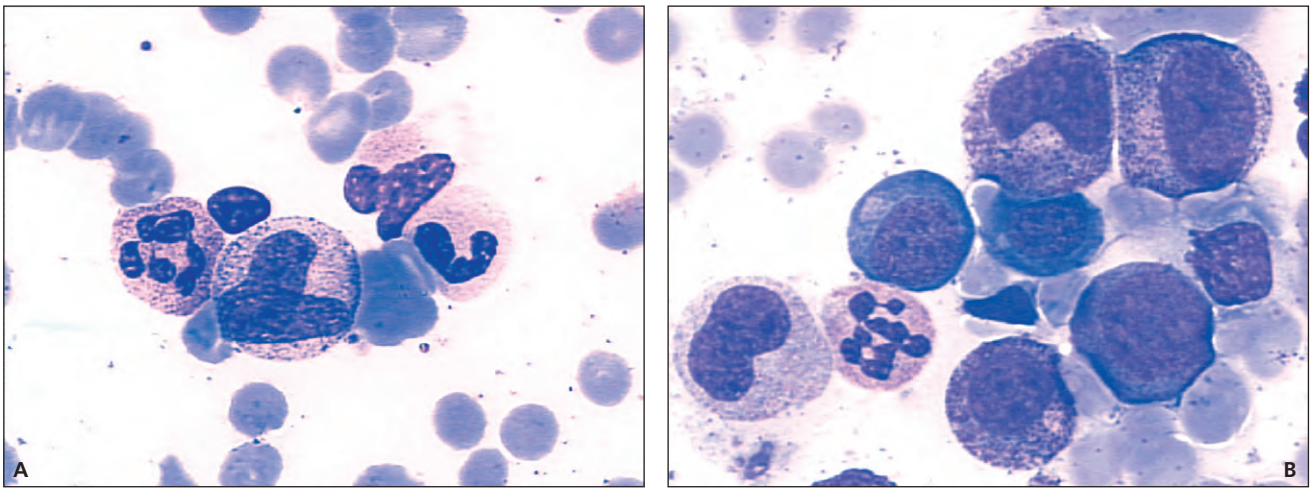



**Fig. 2.73** Megaloblastosis affecting the granulocytic series; bone marrow aspirate. (A) A giant metamyelocyte in the centre, with pale nuclear chromatin and peripheral cytoplasmic basophilia. On its left, a hypersegmented neutrophil. (B) From the same preparation, surrounding two erythroid precursors are numerous cells of neutrophil lineage at various stages of maturation. From left to right: (1) a hypogranular metamyelocyte with pale nuclear chromatin; (2) a mature neutrophil with eight nuclear lobes (a rare finding in the bone marrow); (3) at the bottom, a neutrophil myelocyte of normal size, heavily granulated with the background cytoplasm being still basophilic with a pale Golgi zone; (4) at the top, a giant granular neutrophil metamyelocyte; (5) immediately to the right, a late promyelocyte, rich in dark red specific granules on a basophilic background, with a large nucleolus and a conspicuous Golgi zone; (6) bottom right, a less mature promyelocyte, with delicate chromatin, nucleoli, intensely basophilic cytoplasm and fine granules.

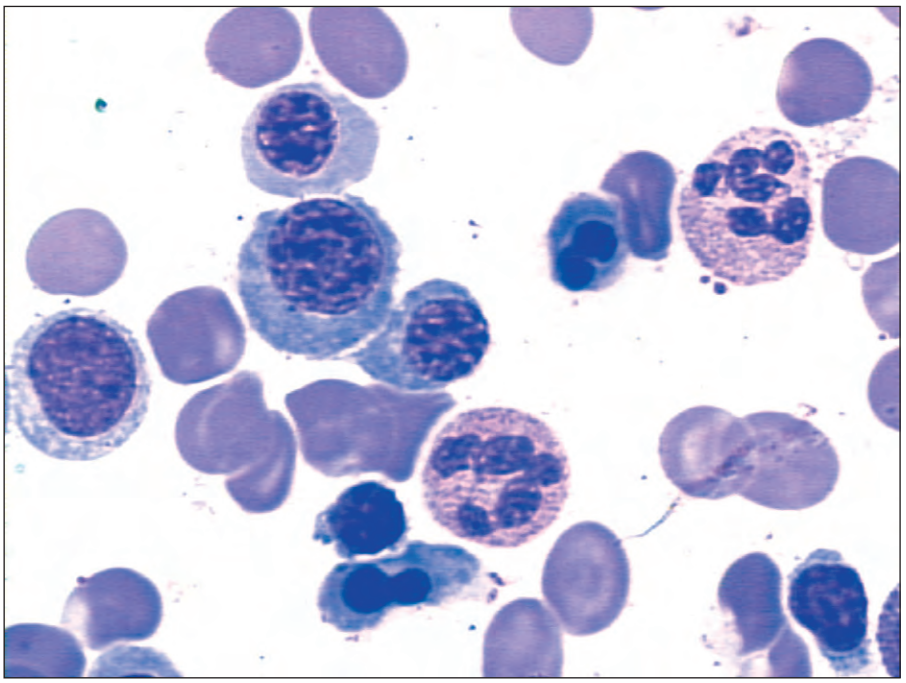


**Fig. 2.74** This microscopic field shows the morphological heterogeneity of megaloblastic anaemia. One sees neutrophil granulocytes with hypersegmented nuclei: the chromatin structure of the numerous nuclear lobes is less pyknotic than normal, while the granules and the cytoplasmic morphology are normal. In the centre of the image, two basophilic megaloblasts are dissimilar in their dimensions, while their nuclear morphology and their cytoplasmic staining are practically identical. At the top, a polychromatic megaloblast with an immature nucleus. Another two erythroblasts have abnormal bilobed nuclei, with chromatin that is completely condensed but cytoplasm that is still orthochromatic. The cell on the left edge is difficult to classify; it may be a blast or a very immature megaloblast. Two lymphocytes, at the bottom in the centre and in the bottom right corner, complete the cytological picture.

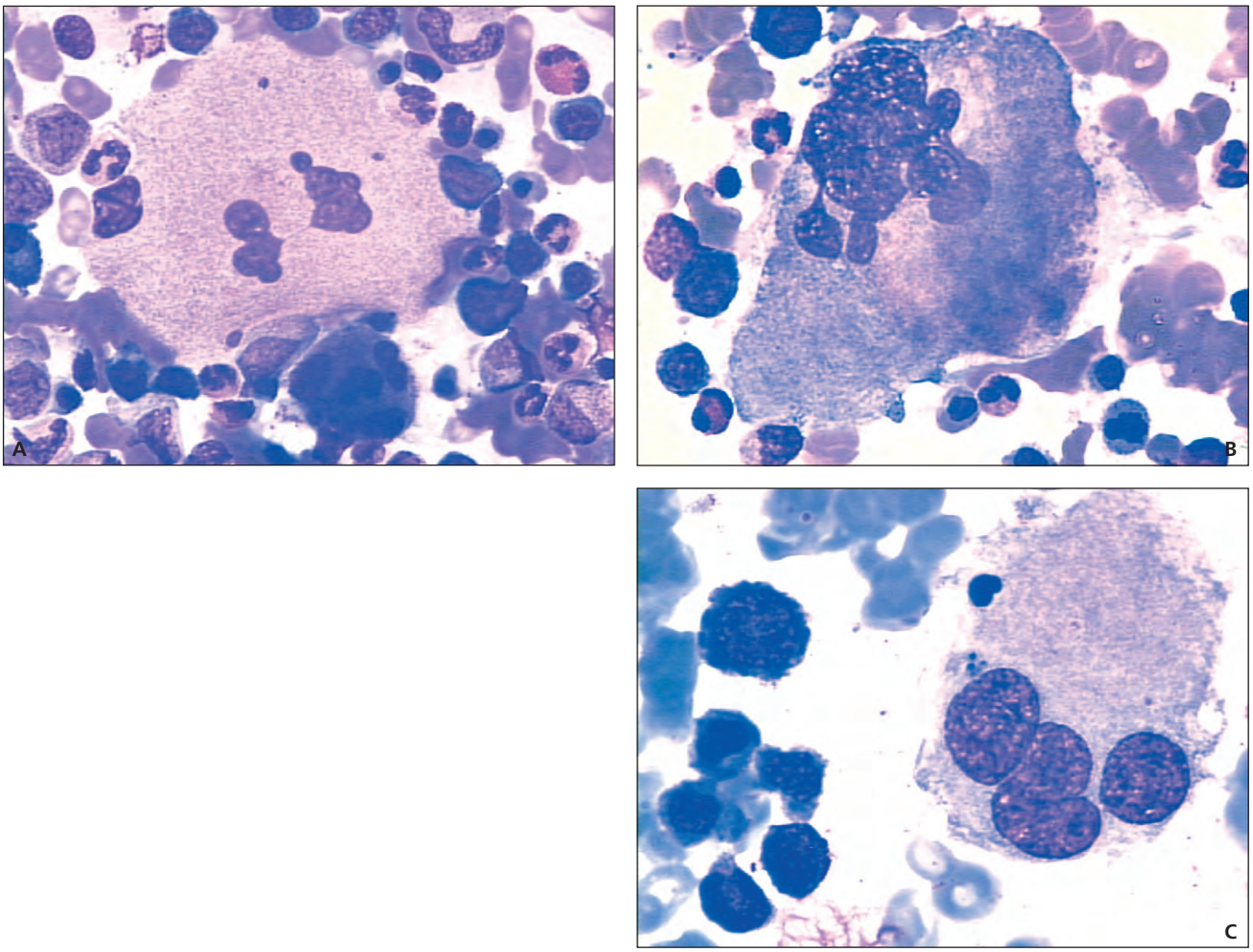



**Fig. 2.75** Dysmorphism of megakaryocytes and their precursors in megaloblastic anaemia; bone marrow aspirate. (A) Among granulocytic precursors with features of gigantism (top right) and erythroid precursors at various stages of maturation, one observes a giant megakaryocyte with a vast amount of mature cytoplasm and a multilobated nucleus, composed mainly of two halves joined by a filament. In contact with the left edge of its cytoplasmic membrane is a third component of the nucleus, detached from the others, with denser chromatin. Immediately beneath this giant megakaryocyte is another much smaller megakaryocyte, probably becoming apoptotic before it has completed its maturation, with a nucleus composed of numerous pyknotic lobes and cytoplasm that is still in part basophilic. (B) Giant megakaryocyte with a hyperdiploid nucleus and heterogeneous cytoplasm with areas of basophilia, paler areas and evident abnormal platelet formation on the right edge. (C) This megakaryocyte precursor is dysplastic, with marked nucleocytoplasmic asynchrony. The cytoplasm is mature with fine granules, but only rare areas of platelet demarcation. The nucleus is formed of four large lobes, perhaps in part separated, with the spongy chromatin, showing little condensation, of a promegakaryocyte, visible but not very distinct nucleoli and a clearly outlined nuclear membrane.

and a tendency to pancytopenia, the bone marrow aspirate shows variable cellularity, sometimes total aplasia with signs of dyserythropoiesis such as nucleocytoplasmic asynchrony, multinucleated erythroblasts and nuclear fragmentation. The blast percentage is important, because of the possible evolution to acute myeloid leukaemia. Acquired aplastic anaemia, resulting from toxic, viral or autoimmune damage to haemopoietic stem cells and progenitors, is subdivided into three levels of severity, based on the severity of peripheral cytopenia. Bone marrow cellularity is reduced as the result of a reduction in all three haemopoietic lineages. Isolated small groups of haemopoietic precursors may persist, together with empty particles occupied by fat cells, macrophages, endothelial cells, plasma cells, lymphocytes and mast cells. An estimation of cellularity in terms of the percentage of the marrow cavity occupied by haemopoietic cells requires a trephine biopsy and histological assessment.

Pure erythroid hypoplasia (pure red cell aplasia [PRCA]) is characterised by a hyporegenerative normocytic normochromic anaemia, with reticulocytopenia. The arrest of erythropoiesis can result from a defect intrinsic to erythroid progenitors or be secondary, e.g. to immunological factors, when PRCA is associated with thymoma or, more frequently, resulting from an infection by parvovirus B19. Primary infection by this small DNA

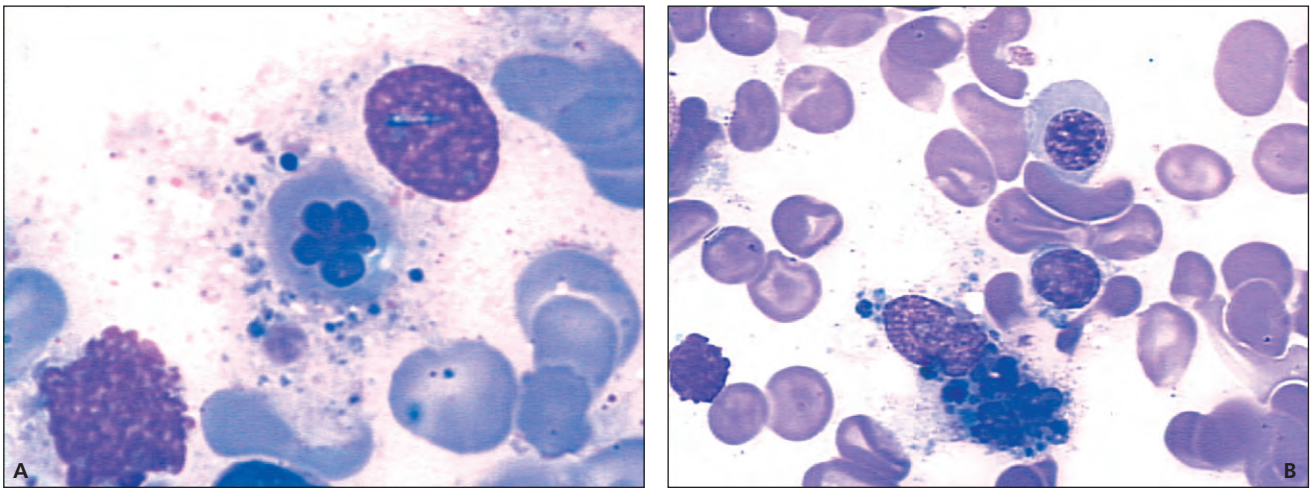



**Fig. 2.76** Bone marrow aspirate: examples of ineffective myelopoiesis in megaloblastic anaemia. (A) A macrophage, in which the nucleus with spongy chromatin is clearly visible top right, has within its cytoplasm the residues of numerous cells, a platelet and an orthochromatic erythroblast with a multilobated flower-shaped nucleus. (B) At the top towards the right, an erythroblast showing typical asynchrony: the slightly ovoid nucleus still has structure with chromatin clumps separated by paler spaces, while the cytoplasm is in the late polychromatic stage of maturation. Lower down, a macrophage with a spongy nucleus, with a pale stamped out nucleolus and plentiful cytoplasm with indefinite margins, packed with homogenised pyknotic cellular debris.

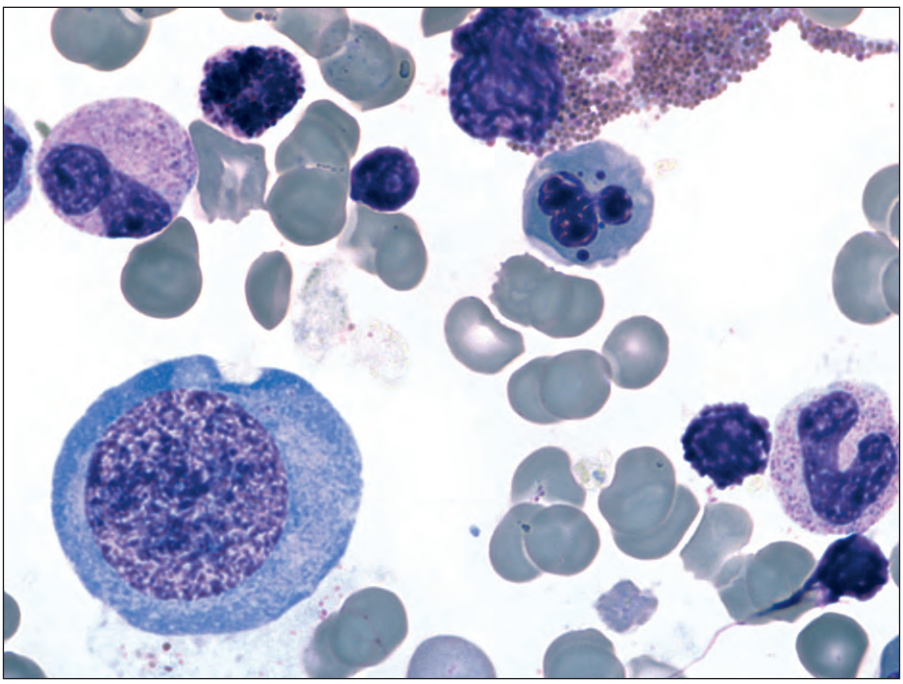


**Fig. 2.77** Bone marrow aspirate from a patient who has had an autologous stem cell transplant for non-Hodgkin lymphoma. The erythroid series shows megaloblastic features: the basophilic erythroblast at the bottom has a nucleus with chromatin condensed into small pearl-like clumps separated by numerous paler areas; the orthochromatic erythroblast, top right beneath the flattened cytoplasm of a damaged eosinophil, has a nucleus showing karyorrhexis, with asymmetric lobes and large Howell-Jolly bodies. There are also two neutrophil band forms and, close to the upper edge, a small basophil.

virus in healthy individuals is generally asymptomatic or associated with erythema infectiosum (fifth disease), although sometimes with minor haematological manifestation persisting for several days. The virus specifically infects erythroid precursors, binding to the P antigen (globoside) of the membrane and inhibiting the development of BFU-E and CFU-E.[203] The replication of virus within erythroid progenitors leads to a brief, transitory suppression of erythropoiesis which, in healthy subjects, causes only a slight fall of 20–30 g/l in the Hb, with a brief disappearance of erythroid precursors from the bone marrow aspirate by the tenth day.[204] The erythroid tropism and its inhibitory effect on erythropoiesis are most evident clinically in patients with chronic haemolytic anaemia, such as hereditary spherocytosis and sickle cell disease,[56,57] in which erythropoiesis is already hyperactive and red cell survival is short: infection is manifest as a transient aplastic crisis with reticulocytopenia (not

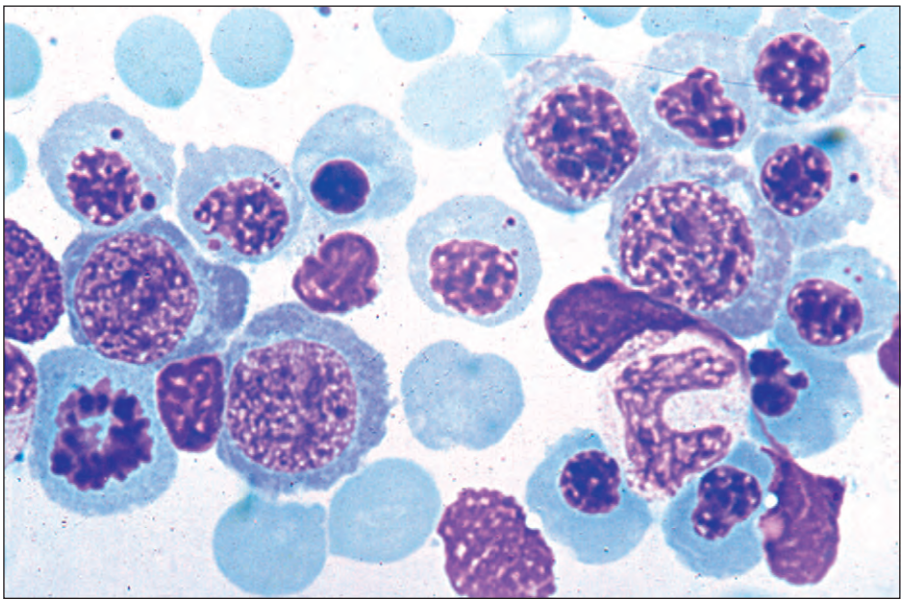


**Fig. 2.78** Bone marrow aspirate from a patient with cold antibody-mediated autoimmune haemolytic anaemia. Erythroid hyperplasia with marked dyserythropoiesis, non-specifically linked in this case to a strong erythropoietin-mediated proliferative drive. There are three basophilic erythroblasts with megaloblastic features (retarded nuclear maturation but cytoplasm that is partially haemoglobinised) and various polychromatic erythroblasts with Howell-Jolly bodies. Only two nuclei have fully condensed chromatin. Bottom left, an almost orthochromatic erythroblast arrested in metaphase.

to be confused with a haemolytic crisis, which is associated with further erythroid expansion and reticulocytosis). Aplastic crises, which can be severe, depending on the underlying haematological condition, occur only once during a person's life, since permanent immunity develops. In immunocompromised subjects (post-transplant, immunosuppressive therapy, congenital immune deficiency, HIV infection), on the other hand, the lack of an adequate immune response favours chronic infection with chronic inhibition of erythropoiesis and persistent hyporegenerative anaemia.[205]

The bone marrow aspirate in aplastic crisis or PRCA shows a characteristic almost complete absence of erythroid precursors, from the basophilic erythroblast to the orthochromatic erythroblast (Figs 2.80 and 2.81). The presence of giant proerythroblasts is pathognomonic for parvovirus B19 infection; these may be present in small groups appearing almost syncytial. They have pale nuclear chromatin and giant 'nucleoli', which in fact are viral inclusions, and ample basophilic cytoplasm, which can show marked vacuolation and peripheral pseudopod-like protrusions; sometimes there is a tendency to appear megaloblastoid. They are sometimes designated 'lantern cells' because of the very pale chromatin with enhancement of the nuclear membrane that is apparent on biopsy specimens. Sometimes these characteristic cells are lacking; when they are present, it is important not to confuse them with leukaemic blast cells or metastatic cells. The overall picture is that of a total block in erythroid differentiation and maturation, in contrast to the total normality, both quantitative and morphological, of the granulocytic and megakaryocytic lineages. There are no cytological feature of dysplasia and no increase in blast cells: these are important criteria for making a distinction from a haematological neoplasm such as myelodysplasia. There may be an increase in lymphocytes, plasma cells and mast cells with formation of lymphoid aggregates. Iron stores are in general preserved or increased. The bone marrow picture naturally can vary from case to case when parvovirus infection supervenes in an immunodepressed patient in whom a haematological disorder has been treated with radiochemotherapy or haemopoietic stem cell transplantation. A definitive diagnosis can be made by polymerase chain reaction (PCR) for viral DNA.

In rare cases, infection with parvovirus B19 has been reported as causing bone marrow necrosis or marked dyserythropoiesis with erythroid hyperplasia, multinuclearity, internuclear chromatin bridges and intranuclear inclusions representing viral particles, sufficient to suggest an erroneous diagnosis of myelodysplasia or congenital dyserythropoietic anaemia.[206,207]

### Virus-associated haemophagocytic syndrome

Haemophagocytosis is defined as a condition in which, for reasons that are not specific, the bone marrow macrophages show signs of increased phagocytic activity, ingesting erythrocytes, leucocytes and platelets, and their bone marrow precursors. This macrophage activation is seen in various pathological processes, such as haemolytic anaemia, ineffective erythropoiesis, infections and malignant tumours. A haemophagocytic syndrome, also known as haemophagocytic lymphohistiocytosis, is a severe relatively uncommon hyperinflammatory clinical state, resulting from the production of cytokines by an immune

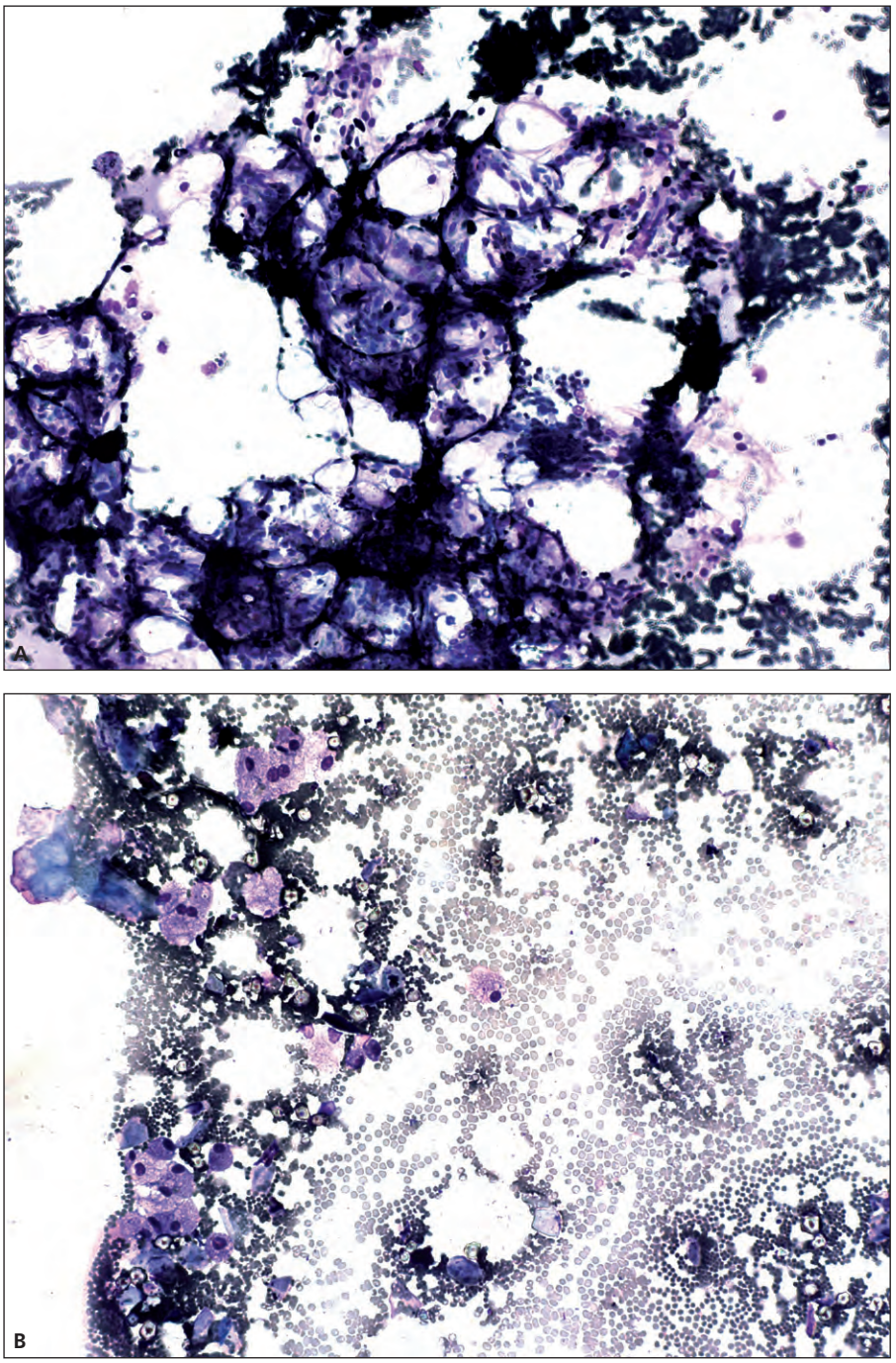



**Fig. 2.79** Bone marrow aspirate showing markedly reduced cellularity in patients with aplastic anaemia. (A) Aplastic anaemia. The bone marrow fragments are empty. There are no haemopoietic precursors, all the cells present being stromal cells. (B) Fanconi anaemia. The marrow appears markedly hypocellular. There are no fragments and all the cells seen in the tail of the film are foamy macrophages with oval, sometimes bilobed, nuclei.

system that is strongly stimulated but is not effective in its response. The morphological picture is characterised by multilineage cytopenia, bone marrow hypocellularity, histiocytic hyperplasia and macrophages showing marked haemophagocytosis (Figs 2.82 and 2.83). This picture is seen particularly, but not only, in children with infections of various types but often viral; parvovirus B19 has been recently recognised as one possible aetiological agent.

## Bone marrow necrosis

In some anaemias, such as sickle cell anaemia or that due to severe widespread infection or inflammation, and also in

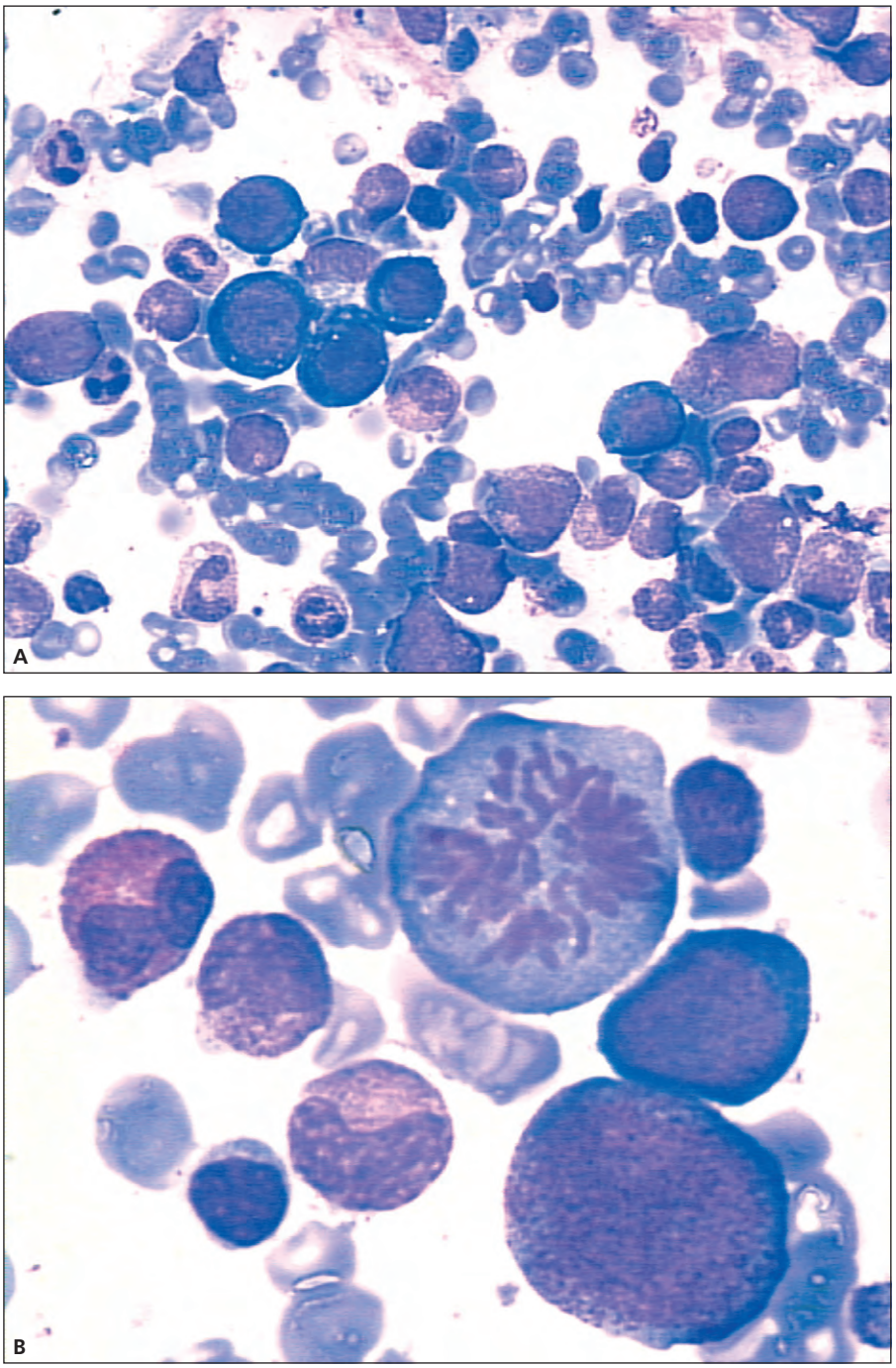



**Fig. 2.80** Bone marrow aspirate from a patient with pure red cell aplasia. The patient had trilineage cytopenia and severe anaemia (Hb 54 g/l) three weeks after autologous haemopoietic stem cell transplantation for non-Hodgkin lymphoma; treatment with G-CSF was being given. (A) In a cellular preparation the only erythroid precursors are five proerythroblasts nuclei that have finely reticular chromatin, and ample cytoplasm that is intensely basophilic with some vacuoles. Granulocytic precursors are, however, plentiful and normal maturation is occurring. Reticulocytes were 0.01%. The diagnosis of parvovirus B19 infection was confirmed by PCR. (B) Anticlockwise from the bottom right corner: an early neutrophil promyelocyte with numerous azurophilic granules and a large nucleus with fine chromatin; a proerythroblast; a lymphocyte; an erythroid cell in mitosis with basophilic cytoplasm and long distinct chromosomes; an eosinophil band form; another lymphocyte and, in the centre, two neutrophil metamyelocytes.

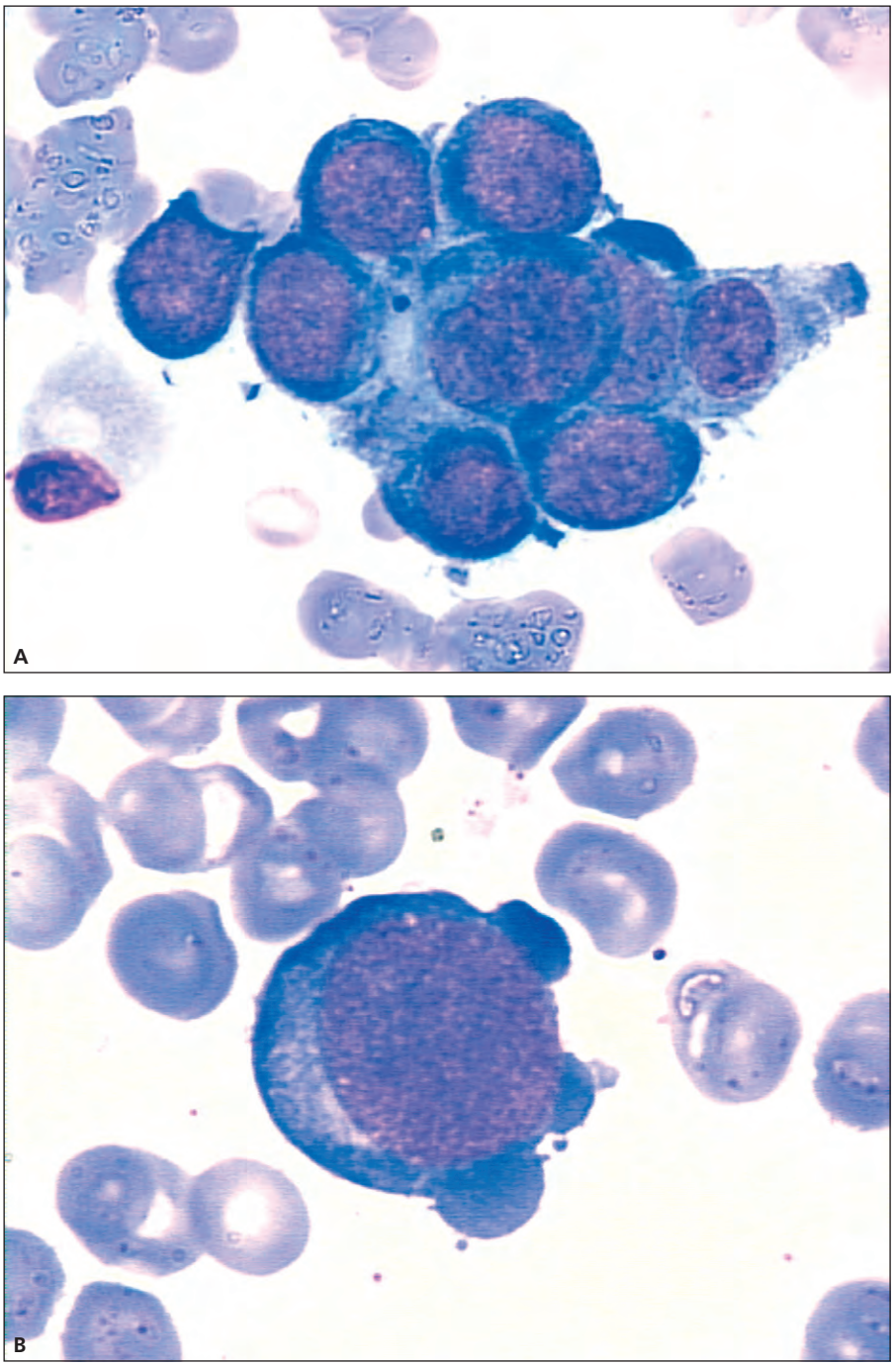



**Fig. 2.81** Bone marrow aspirate from another patient with parvovirus B19 infection and pure red cell aplasia. (A) Nine proerythroblasts with megaloblastoid nuclei form a compact mass, but their cytoplasmic limits can be distinguished. The largest cell, in the centre, is tetraploid. There are no differentiating erythroid precursors. (B) Giant proerythroblast with very fine, granular, homogeneous nuclear chromatin, in which indistinct nucleoli can be discerned. The cytoplasm is intensely basophilic with plentiful arcoplasm and three large pseudopodia. Cells of this type are described as 'lantern cells' when they are seen in histological sections.

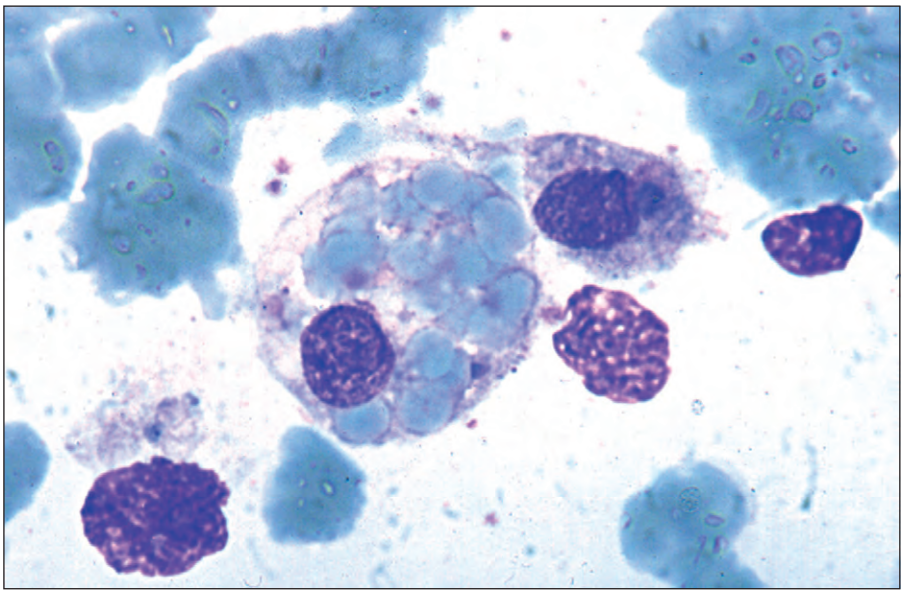


**Fig. 2.82** Haemophagocytic syndrome in an intensive care ward patient with sepsis and pancytopenia. The cytoplasm of a macrophage is packed with well-preserved red cells. This appearance suggests macrophage activation resulting from hyperstimulation by cytokines, with inability to degrade and digest the intracellular contents.

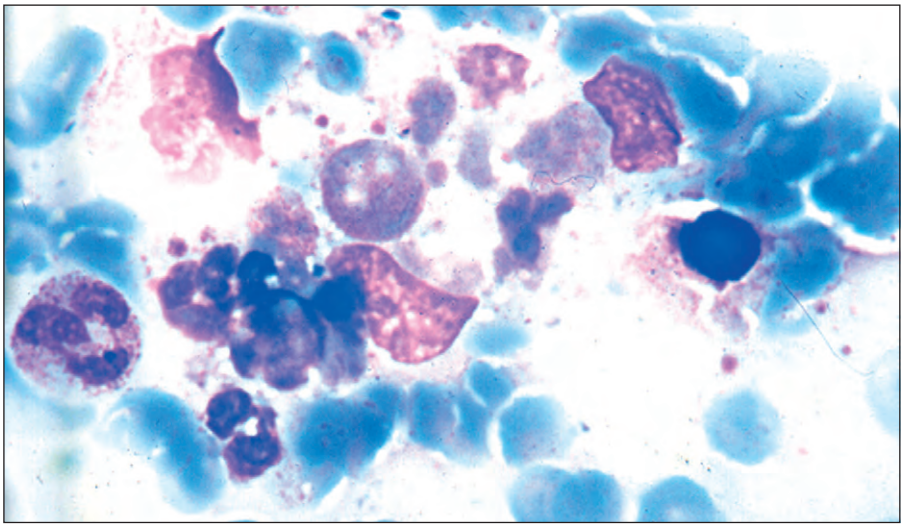


**Fig. 2.83** Macrophage activation in a patient with parvovirus B19 infection. This macrophage has a nucleus with an unusual angulated outline, but with the normal spongy chromatin texture; its very ample cytoplasm, indefinite at the edges, is packed with cellular debris of various types, including nuclear residues, platelets and erythrocytes in various stages of degeneration.

leukaemia, lymphoma, and bone marrow metastases, it is possible to observe the features of bone marrow necrosis in bone marrow films.[208–210]

In these cases, the film shows abundant background amorphous pinkish material that represents necrotic cells. Any remaining recognisable cells in these areas show distortion and damage, such as pyknotic nuclei, cytoplasmic shrinkage and blurring of the cellular margins; finally, the remnants of cells are transformed into structureless masses, the recognisable cells remaining being stromal elements and reactive cells such as plasma cells and mast cells (Fig. 2.84).

## Gelatinous transformation

In anorexia nervosa and cachectic states, such as those due to carcinoma, AIDS or chronic infection, the bone marrow may show gelatinous transformation. There is loss of haemopoietic cells and fat cells, which are replaced by amorphous pink, slightly granular material that represents mucopolysaccharide.

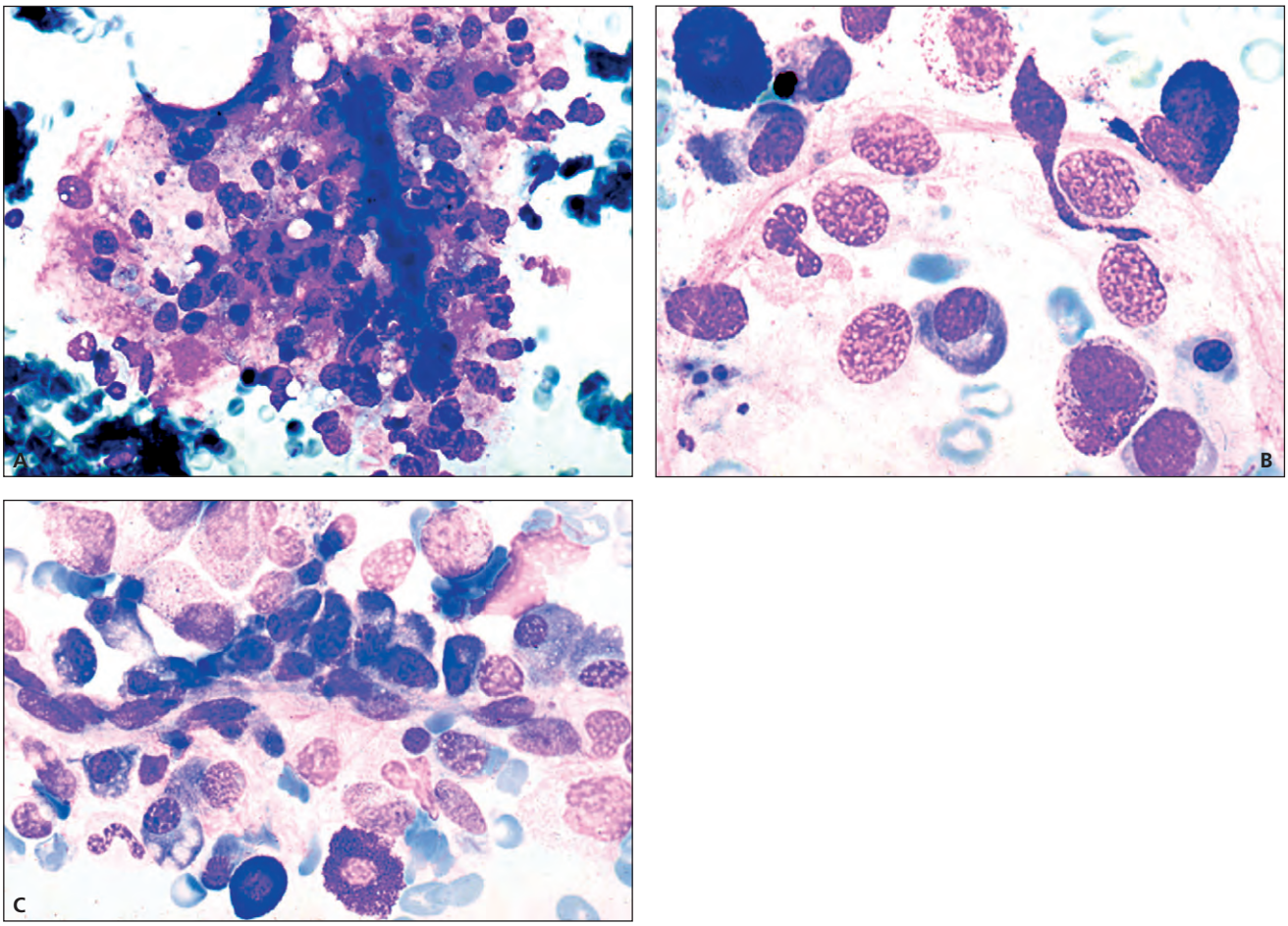



**Fig. 2.84** Bone marrow aspirate with features of inflammation. (A) In this small fragment, a meshwork incorporating lymphoid and stromal elements represents fibrinoid necrosis. (B) Stromal and inflammatory cells in the bone marrow aspirate of a patient with sepsis. The image is transversed by an arc of pseudofibrous material actually representing cytoplasm torn apart by aspiration, but still joined to the nuclei of sinusoidal endothelial cells, the oval spongy nuclei of which are apparent. In the top corners are two mast cells with rhomboidal cytoplasm packed with blue-black granules, leaving the central nucleus still visible. A third fusiform mast cell has a long cytoplasmic tail which appears to be insinuating itself between the nuclei of the endothelial cells. (C) Reactive cells are crowded around a well-preserved medullary sinusoid. The endothelial cells are lined up along the median axis. There are numerous plasma cells, two mast cells at the bottom and various granulocyte precursors at the top. The recognition of sinusoids in bone marrow aspirates is not rare in protracted inflammatory conditions associated with inflammation and fibrosis of the bone marrow.

## Congenital dyserythropoietic anaemia

Peripheral blood and bone marrow morphology is fundamental for the diagnosis of congenital dyserythropoietic anaemia (CDA). Confirmation is by genetic analysis, which shows specific abnormalities, and by means of demonstration of ultrastructural or membrane protein abnormalities.[211–213]

In the peripheral blood, the erythrocytes are often macrocytic in CDA type I and normocytic in CDA type II, with poikilocytes in all types (ovalocytes, microspherocytes, dacrocytes and irregularly contracted cells), erythrocytes with basophilic stippling and dysmorphic erythrocytes. In CDA type I, one also sees large, irregularly shaped macrocytes, which recall the cells of megaloblastosis. In CDA type III, the anaemia is macrocytic with a small number of giant erythrocytes.

The bone marrow is very cellular in all forms of CDA (Fig. 2.85), with erythroid hyperplasia and a much reduced myeloid:erythroid ratio, between 0.2 and 1.[214] Dyserythropoiesis is marked, with prominent signs of ineffective erythropoiesis. The most common anomalies are the presence of binucleate erythroblasts (typical of type II), multinuclearity and incompletely separated nuclei, karyorrhexis, anomalies of chromatin structure and internuclear chromatin bridges (particularly in type I). Nuclear abnormalities are more prominent in polychromatic and orthochromatic erythroblasts. In type I CDA, there are large polyploid erythroblasts and the chromatin structure tends to be megaloblastoid. In type II, the erythroblasts are multinucleated and gigantic, with megaloblastic chromatin, karyorrhexis and cytoplasmic inclusions. Particularly in type II, there are pseudo-Gaucher cells containing birefringent needle-shaped material. The association of dyserythropoiesis and ring sideroblasts is not common but has been described as a specific variant of congenital dyserythropoietic anaemia (Fig. 2.86).[215]

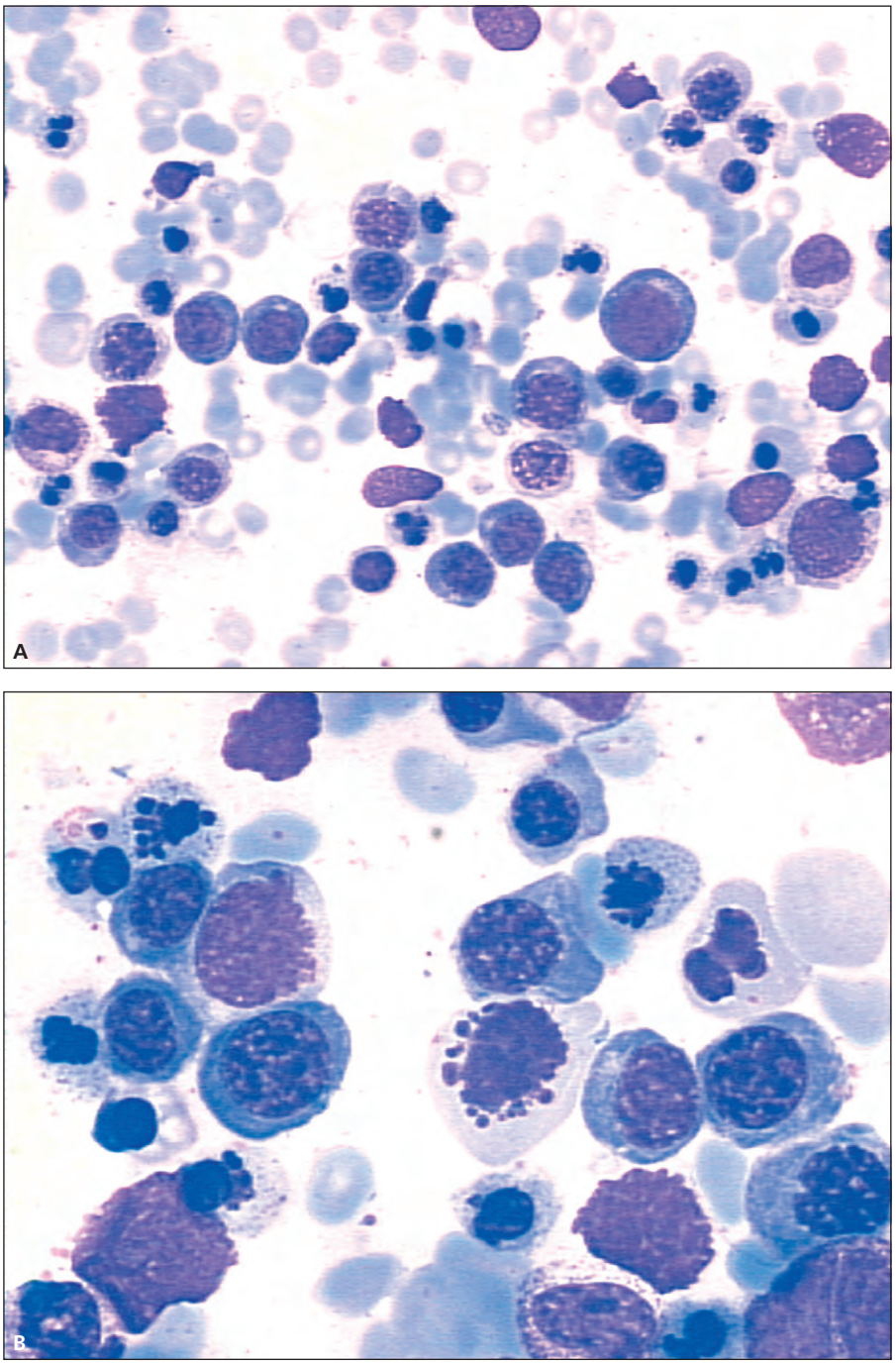



**Fig. 2.85** Congenital dyserythropoietic anaemia (CDA). The bone marrow aspirate is cellular as a result of erythroid hyperplasia; there is marked dyserythropoiesis with signs of ineffective erythropoiesis. From a patient with type II CDA. (A) There are numerous erythroblasts in all stages of maturation. The large proerythroblast on the right appears megaloblastoid because of the delicacy of the chromatin network. In the basophilic and early polychromatic erythroblasts, asynchronous maturation prevails, with delay and irregularity in chromatin condensation in relation to the well-preserved haemoglobinisation of the cytoplasm. In the later polychromatic erythroblasts and in the numerous orthochromatic erythroblasts, the morphological abnormality is even more marked, particularly with regard to the nuclei in which the chromatin is condensing into multilobed pyknotic masses. (B) The nuclear anomalies of the orthochromatic and polychromatic erythroblasts are very evident. While the morphology of the basophilic and early polychromatic erythroblasts is generally preserved, the marked abnormality of chromatin condensation is affecting almost all the nuclei of the more mature cells, with fragmentation, efflorescence ('flower formation'), binuclearity (as a result of endomitosis), quite numerous Howell-Jolly bodies appearing like rosary beads around the nucleus, and arrested and anomalous mitoses. Some orthochromatic erythroblasts also show basophilic stippling.

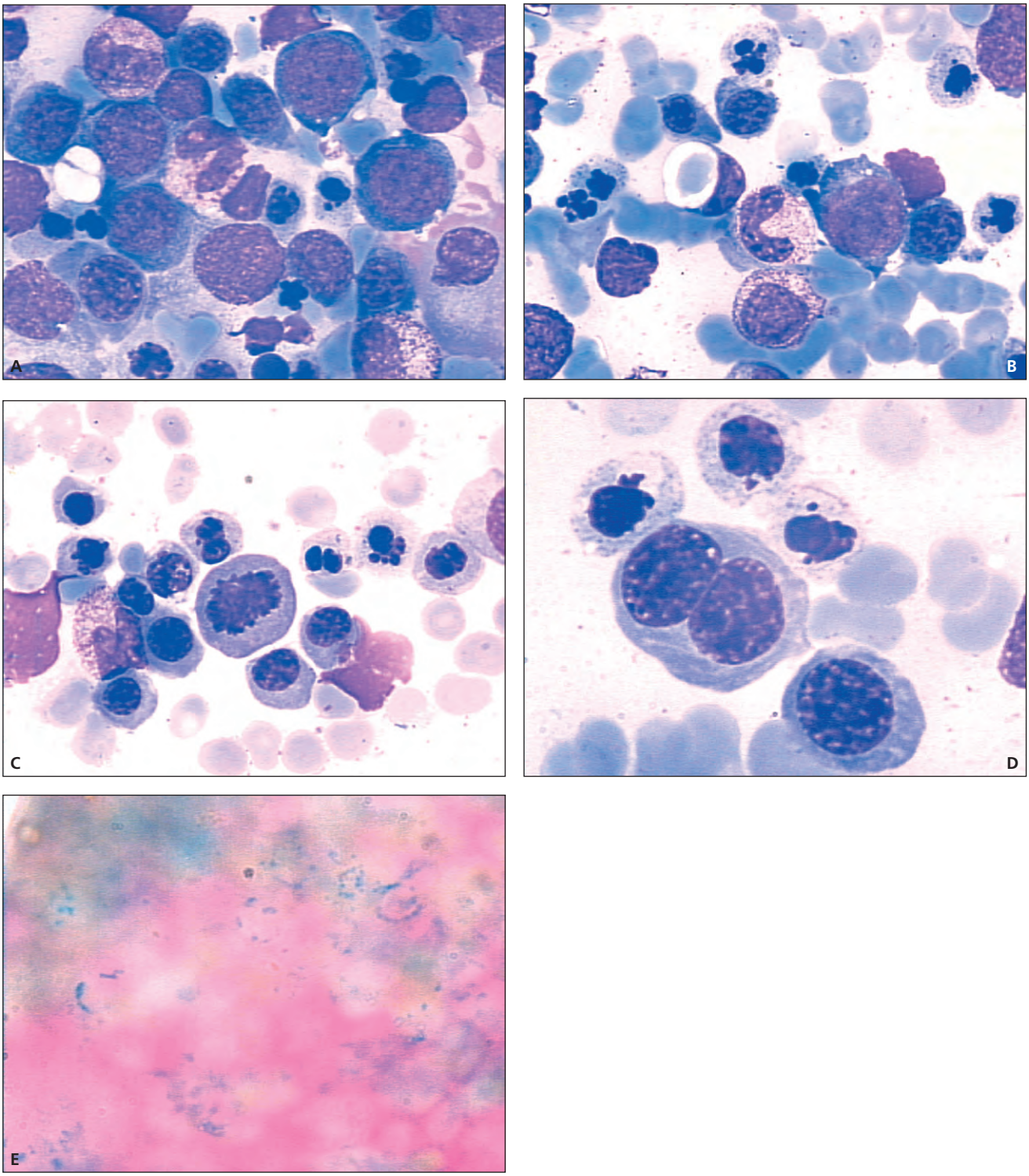
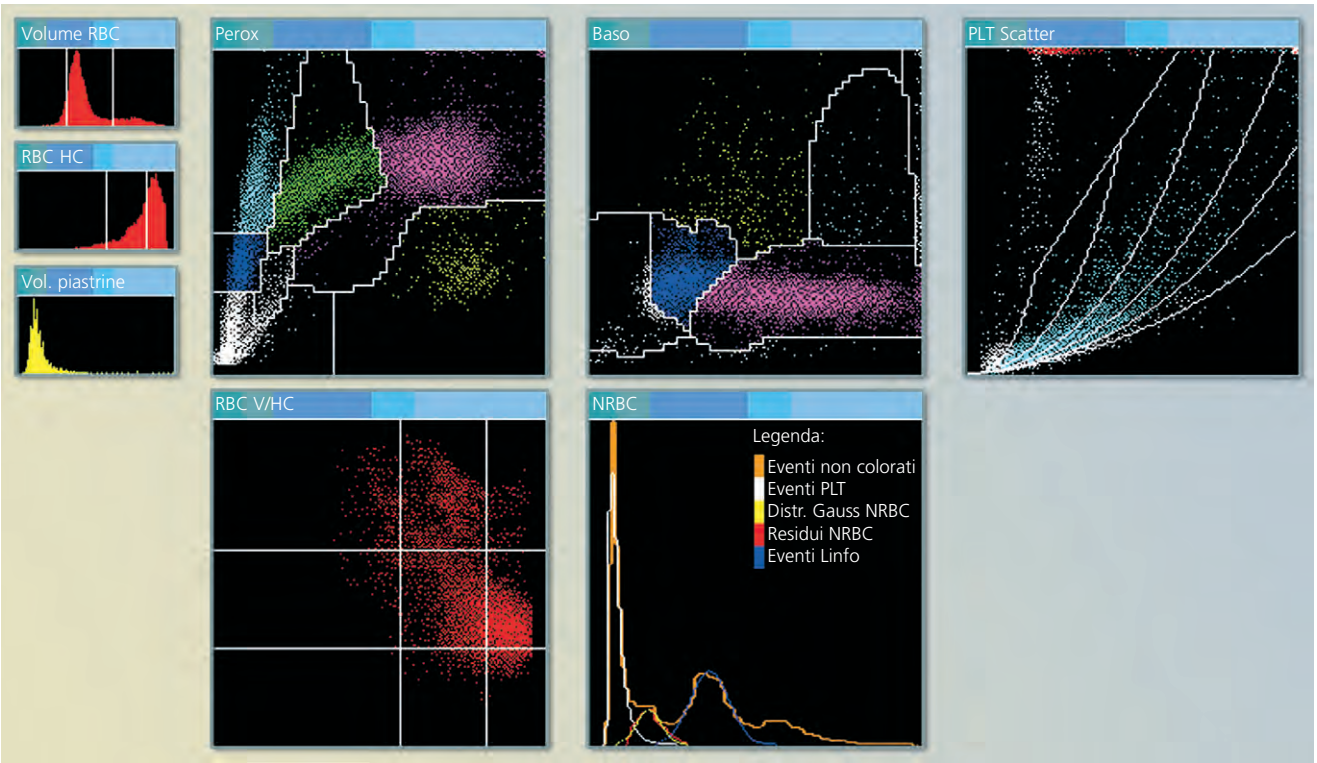



**Fig. 2.86** Bone marrow aspirate from another young patient with CDA (type II), with mental retardation and multiple congenital abnormalities. (A) Marked erythroid hyperplasia with mainly megaloblastoid proerythroblasts and basophilic erythroblasts. Bottom right, a plasma cell with plentiful hypertrophic cytoplasm extending upwards into flaming cytoplasmic fragments. (B) A proerythroblast with reticular chromatin, some basophilic and polychromatic erythroblasts and five orthochromatic erythroblasts with nuclei fragmenting into pyknotic clumps still joined to form curious flower-like shapes. Basophilic stippling is very apparent in some of the more mature cells. In the centre, to the left of a metamyelocyte, a mononuclear cell with cytoplasm occupied by a giant vacuole containing a phagocytosed erythrocyte. (C) The polychromatic erythroblasts show mild retardation of nuclear maturation and, in the cell in the centre, an arrested mitosis; the orthochromatic erythroblasts have fragmented nuclei and pink cytoplasm. (D) A large binucleated erythroblast, with the chromatin pattern of a basophilic erythroblast but with polychromatic cytoplasm; in the cytoplasm there is a negative image of cytoplasmic filaments, which seem to link this cell to another erythroblast below and to the right of it. At the top, three orthochromatic erythroblasts with drumstick-like nuclear appendages, defective haemoglobinisation and basophilic stippling. The cell on the right contains a residual filament of the mitotic spindle. (E) A Perls stain shows that the majority of the more mature dysplastic erythroblasts in this patient are ring sideroblasts.

## REPORT 2.1 Cold autoimmune haemolytic anaemia during the course of infectious mononucleosis



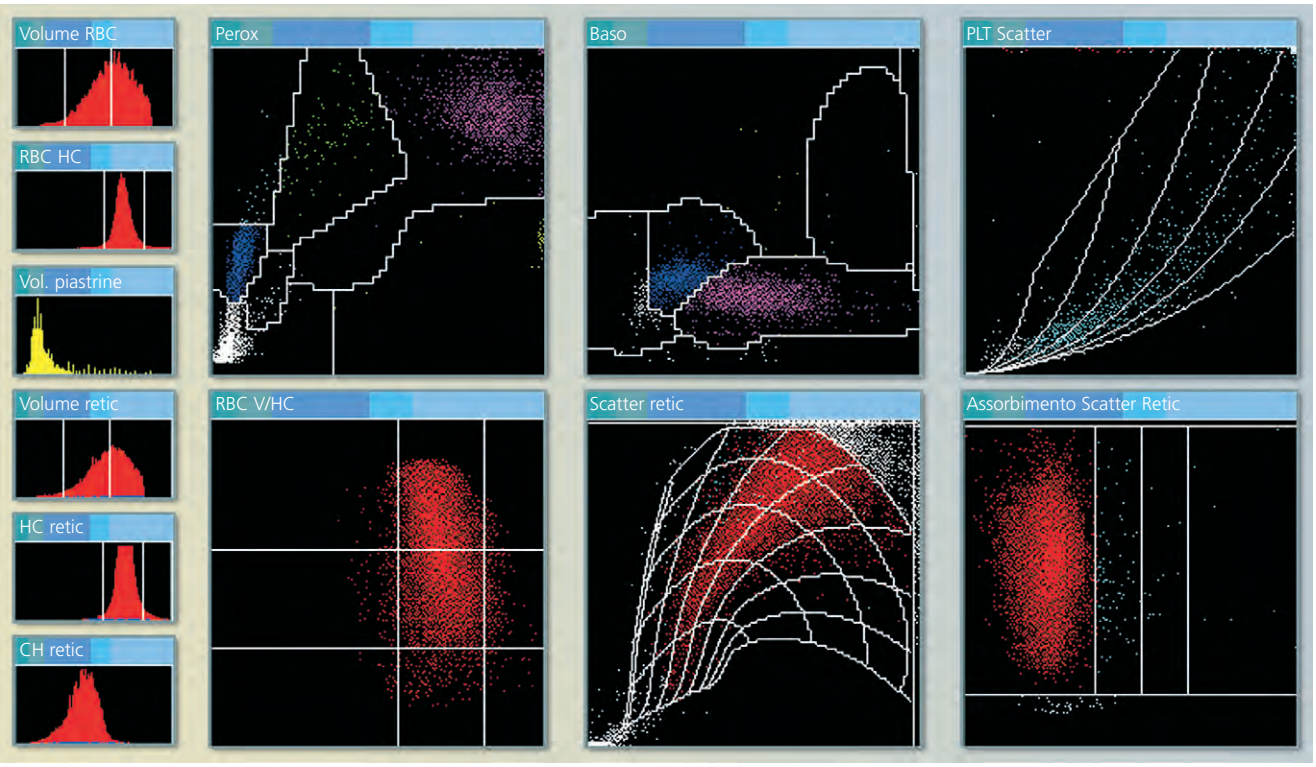

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 60 | g/l | * |
| RBC | 1.08 | $\times 10^{12}$/l | * |
| HCT | 0.094 | l/l | * |
| MCV | 86.9 | fl | * |
| MCH | 55.3 | pg | * |
| MCHC | 637 | g/l | * |
| CHCM | 409 | g/l | CHCM-CE |
| RDW | 32.4 | % | * |
| HDW | 5.09 | g/dl | * Anisocytosis +++ |
| HYPO | 3.1 | % | |
| HYPER | 56.8 | % | Hyperchromia +++ |
| MICRO | 6.2 | % | Microcytosis + |
| MACRO | 14.0 | % | Macrocytosis +++ |
| RBC fragments | 0.02 | $\times 10^{12}$/l | |
| RBC ghosts | 0.03 | $\times 10^{12}$/l | |
| RET | 12.39 | % | * |
| RET | 133.8 | $\times 10^{9}$/l | * |
| CHr | 34.6 | pg | |
| IRF (M + H) | 35.6 | % | |
| PLT | 353 | $\times 10^{9}$/l | PLT-ORN |
| MPV | 8.3 | fl | MPV |
| PDW | 42.6 | % | |
| Large platelets | 4 | $\times 10^{9}$/l | |
| MPC | 21 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 12.72 | | * |
| WBC Perox | 13.63 | | * |
| NEUT | 6.57 | 48.2 | * |
| LYMPH | 2.57 | 18.8 | * |
| MONO | 2.17 | 16.0 | * |
| EOS | 0.42 | 3.1 | * |
| BASO | 0.33 | 2.4 | * |
| LUC | 1.57 | 11.5 | * Atypical +++ |
| NRBC | 0.97 | 7.1/100 WBC | * NRBC + |
| LI | 2.19 | | |
| MPXI | 2.0 | | |
| MN | | 52.1 | |
| PMN | | 46.3 | |
| BLASTS | | 1.7 | Blasts + |
| Perox HPX | | 2.8 | |
| Perox SAT | | 0.6 | |

Severe normocytic anaemia with abnormal values for MCH, MCHC and, to a lesser extent, CHCM: the difference has caused errors in the CHCM-CE (indicating lack of correlation). The red cell volume histogram is deformed by a flattened shoulder on the right. The red cell haemoglobin concentration histogram shows that almost all the red cell population is hyperchromic; in the red cell V/HC cytogram the majority of signals are normocytic and hyperchromic and at the top there is a cloud of normochromic macrocytes. There is marked reticulocytosis, both relative and absolute, with an increase of the IRF. Red cell ghosts, which are recognised in the system as events detected in the platelet channel but with a refraction index of less than 1.35, are increased and are visible on the platelet cytogram as a vertical strip to the left of the sector. The sample/system alarm PLT-ORN (platelet origin noise) indicates that there are an increased number of events in the noise area of the platelet cytogram, more than 2% of the total signals. The distribution of the leucocyte population in the Perox channel has a pointed spindle shape, which is higher in the LUC area. There is a slight increase in the basophil count as a result of signals above the horizontal threshold.

**Morphology.** Differential count: neutrophils 48%, eosinophils 3%, basophils 0.5%; lymphocytes 21%, activated and atypical lymphocytes 15.5%, monocytes 12%; erythroblasts 4 per 100 WBC. Massive red cell agglutination. Immunophenotyping of the lymphocytes has shown that 79% are CD3 positive with a CD4/CD8 ratio of 0.79 with 9% activated B cells (CD19 positive, CD56 positive). The massive cold antibody-mediated red cell agglutination has caused in this case not only haemolysis and severe anaemia but also a paradoxical increase in the MCHC and MCH. The extreme hyperchromia is the result of antibody damage to non-agglutinated erythrocytes. The Blast flag (+) has been activated because the signals counted as blast cells in the Baso channel are little more that 1.5% (with LUC >4.5%): a cautious approach that guarantees slide review in cases of leukaemia with lymphoblasts with dense chromatin.

## REPORT 2.2 Megaloblastic anaemia at diagnosis



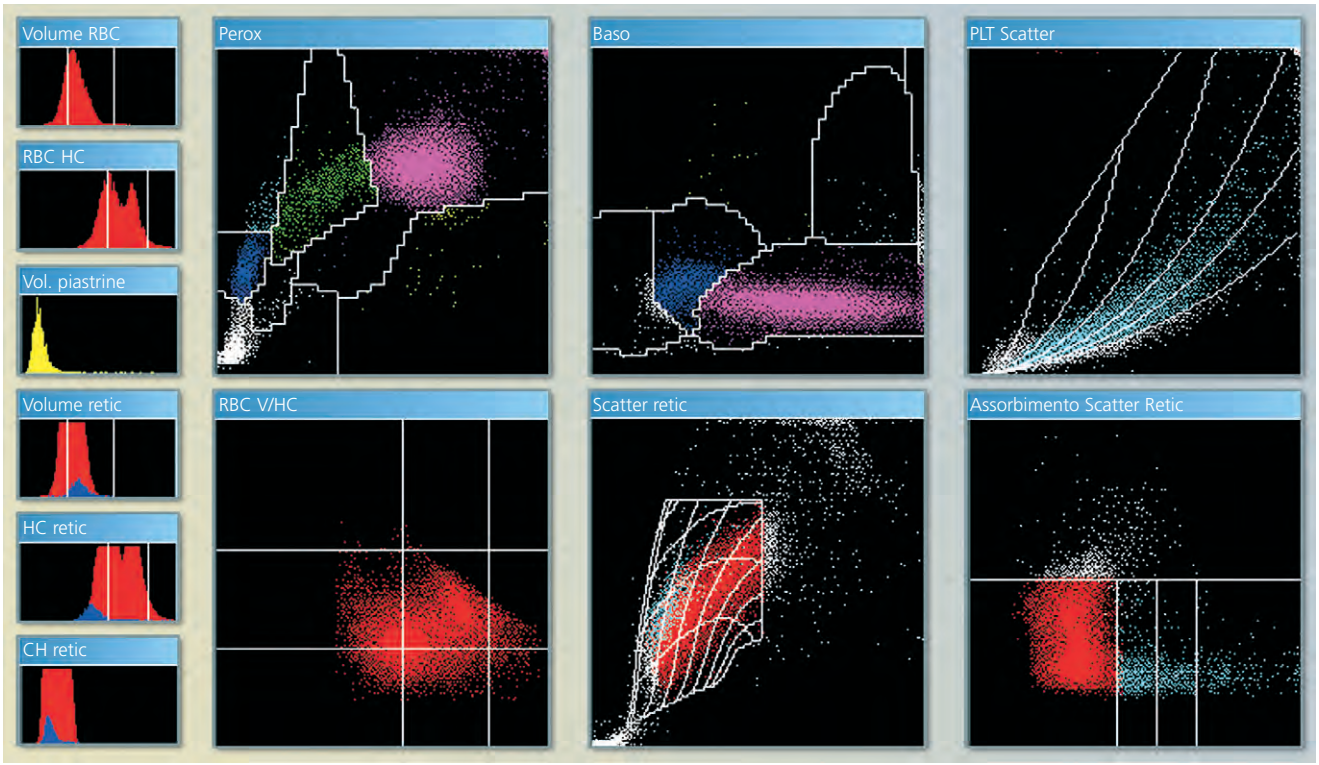

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 47 | g/l | |
| RBC | 1.12 | $\times 10^{12}$/l | |
| HCT | 0.14 | l/l | |
| MCV | 122.0 | fl | |
| MCH | 41.4 | pg | |
| MCHC | 340 | g/l | |
| CHCM | 345 | g/l | |
| RDW | 22.6 | % | Anisocytosis +++ |
| HDW | 2.86 | g/dl | |
| HYPO | 1.7 | % | |
| HYPER | 1.7 | % | Macrocytosis +++ |
| MICRO | 1.9 | % | |
| MACRO | 52.8 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 0.89 | % | |
| RET | 10.0 | $\times 10^{9}$/l | |
| CHr | 41.7 | pg | |
| IRF (M + H) | 8.7 | % | |
| PLT | 83 | $\times 10^{9}$/l | |
| MPV | 11.5 | fl | |
| PDW | 53 | % | |
| Large platelets | 7 | $\times 10^{9}$/l | |
| MPC | 24 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 2.71 | | * |
| WBC Perox | 2.75 | | * |
| NEUT | 1.42 | 52.5 | * |
| LYMPH | 1.10 | 40.7 | * |
| MONO | 0.10 | 3.8 | * |
| EOS | 0.03 | 1.2 | * |
| BASO | 0.01 | 0.4 | * |
| LUC | 0.04 | 1.3 | * |
| NRBC | 0.24* | 8.8/100 WBC | * NRBC + |
| LI | 1.72 | | * |
| MPXI | 27.2 | | |
| MN | 44.1 | | |
| PMN | 54.3 | | |
| BLASTS | | 3.5 | |
| Perox HPX | | 2.3 | |
| Perox SAT | | 2.1 | |

Very severe anaemia with increased MCV and RDW. The histogram of RBC size is considerably expanded at the base, indicating heterogeneity of size with a large proportion of macrocytes, which appear, however, to be normochromic. The two-dimensional V/HC red cell cytogram has the appearance of a ball of fire launched towards the top. Reticulocytes are not numerous. There is leucopenia, neutropenia, monocytopenia and thrombocytopenia: the neutrophil population in the Perox cytogram is shifted to the right with an increased MPXI. The lobularity index is reduced.

**Morphology.** The white cell differential count does not deviate from the instrumental count. Erythroblasts are present, 5 per 100 WBC. Neutrophils are increased in size with 4% having more than six nuclear lobes: this appearance explains the increased peroxidase content. The reduced LI, on the other hand, is attributed to the reduction of condensation of nuclear chromatin. The red cell series is dysmorphic with the blood film showing extreme anisocytosis, gigantic macrocytes, poikilocytes, small fragments and Howell-Jolly bodies. There is an NRBC flag and microscopy confirms the presence of quite dysmorphic orthochromatic erythroblasts. The presence of thrombocytopenia is usual in cases of severe megaloblastosis: the platelets are heterogeneous with giant forms with a dense granulomere.

## REPORT 2.3 Acute severe rectal bleeding as the initial symptom of carcinoma of the rectum, after the emergency transfusion of one unit of packed red cells



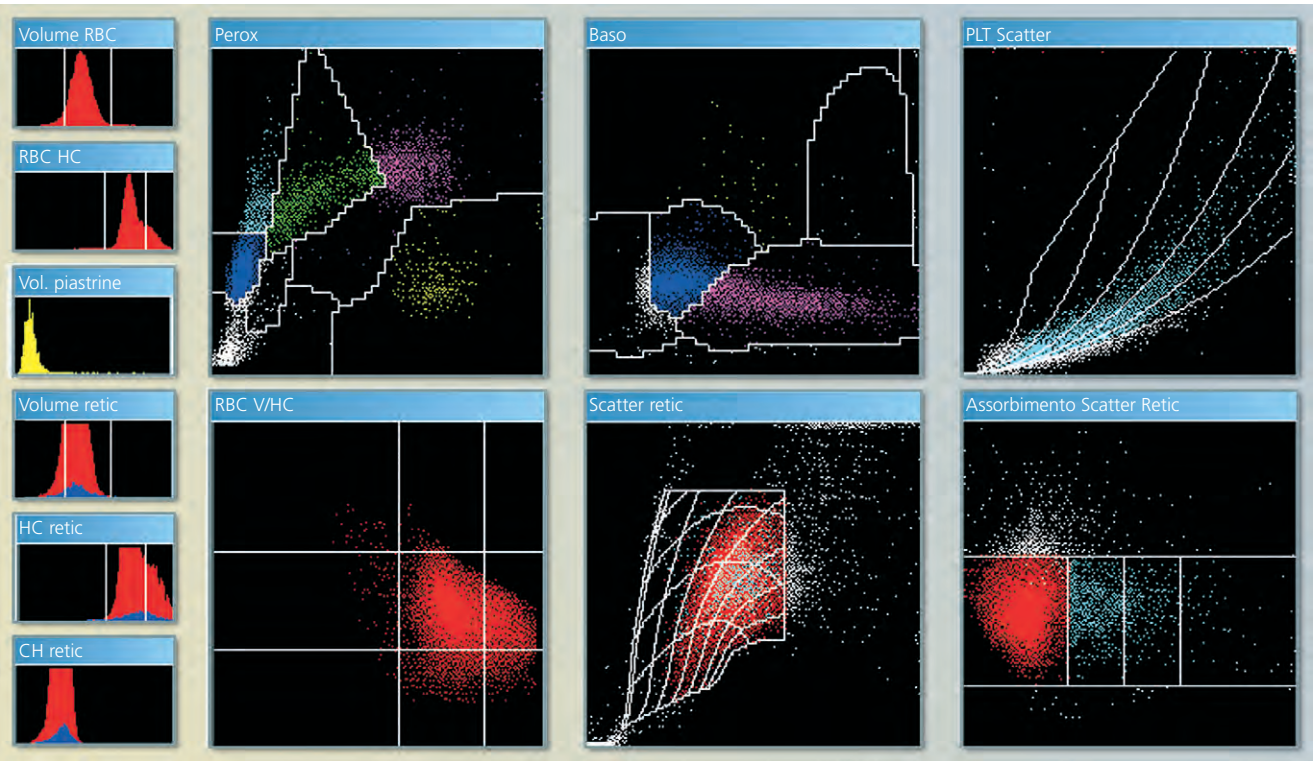

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 54 | g/l | |
| RBC | 2.45 | $\times 10^{12}$/l | |
| HCT | 0.173 | l/l | |
| MCV | 70.5 | fl | |
| MCH | 22.1 | pg | |
| MCHC | 313 | g/l | |
| CHCM | 317 | g/l | |
| RDW | 18.5 | % | Anisocytosis ++ |
| HDW | 4.98 | g/dl | Anisochromasia +++ |
| HYPO | 26.3 | % | Hypochromia +++ |
| HYPER | 2.1 | % | |
| MICRO | 21.5 | % | Microcytosis +++ |
| MACRO | 0.1 | % | |
| RBC fragments | 0.06 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 3.65 | % | |
| RET | 89.4 | $\times 10^{9}$/l | |
| CHr | 18.4 | pg | |
| IRF (M + H) | 14.5 | % | |
| PLT | 685 | $\times 10^{9}$/l | |
| MPV | 6.9 | fl | |
| PDW | 39.3 | % | |
| Large platelets | 2 | $\times 10^{9}$/l | |
| MPC | 29 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 21.72 | | |
| WBC Perox | 21.65 | | |
| NEUT | 19.37 | 89.2 | |
| LYMPH | 1.41 | 6.5 | |
| MONO | 0.72 | 3.3 | |
| EOS | 0.08 | 0.4 | |
| BASO | 0.02 | 0.1 | |
| LUC | 0.,12 | 0.5 | |
| NRBC | 0.,00 | 0.0/100 WBC | |
| LI | 2.23 | | |
| MPXI | −5.8 | | |
| MN | | 11.3 | |
| PMN | | 87.4 | |
| BLASTS | | 0.3 | |
| Perox HPX | | 3.1 | |
| Perox SAT | | 0.2 | |

The original red cell population is hypochromic and microcytic. There is a prevalence of hypochromic cells and the micro:hypo ratio is 0.82, typical of iron deficiency. Like the MCV, however, this has been influenced by the presence of normocytic transfused cells, easily seen on the red cell cytogram and the red cell HC histogram. There is a moderate reticulocytosis, as a response to severe acute anaemia, and this has been favoured also by a certain amount of iron received with the transfusion. The reticulocytes, however, remain markedly hypochromic and a part of the original red cell population, as can be seen both from the two-dimensional uncorrected Scatter Retic map, and from the Absorbance Scatter Retic graphical representation. There is a marked neutrophil leucocytosis with optimal distribution shown on both the cytograms. There is a reactive thrombocytosis.

**Morphology.** The differential white cell count is the same as the instrument count, without dysmorphism of abnormal cells. The red cell series shows marked anisocytosis, microcytosis and hypochromia, with dimorphism caused by a minor population of normocytic normochromic red cells. There is moderate poikilocytosis with some cells showing basophilic stippling and rare dacrocytes. The iron deficiency, responsible for the initial hypochromia and microcytosis, is the result of blood loss of long duration, unrecognised by the patient. The small size of the reticulocytes indicates that erythropoiesis has been stimulated by acute haemorrhage, but the iron deficiency has not been overcome.

## REPORT 2.4 Hereditary spherocytosis in a small patient, three years of age, with splenomegaly and fever (with a subsequent diagnosis of cytomegalovirus infection)



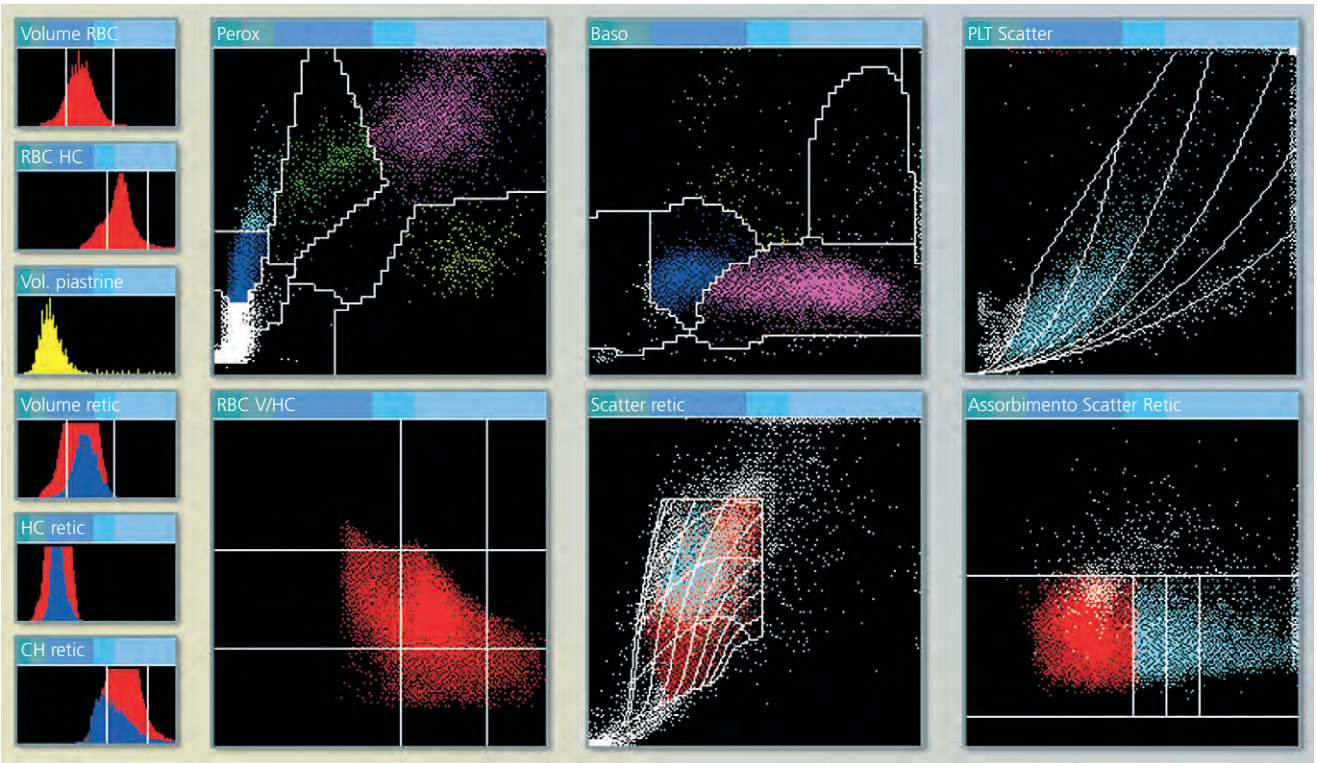

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 75 | g/l | * |
| RBC | 2.55 | $\times 10^{12}$/l | * |
| HCT | 0.21 | l/l | * |
| MCV | 82.2 | fl | * |
| MCH | 29.3 | pg | * |
| MCHC | 356 | g/l | * |
| CHCM | 378 | g/l | * |
| RDW | 16.3 | % | * Anisocytosis + |
| HDW | 3.73 | g/dl | * Anisochromasia + |
| HYPO | 0.4 | % | |
| HYPER | 18.4 | % | Hyperchromia +++ |
| MICRO | 4.8 | % | Microcytosis + |
| MACRO | 0.3 | % | |
| RBC fragments | 0.06 | $\times 10^{12}$/l | |
| RBC ghosts | 0.03 | $\times 10^{12}$/l | |
| RET | 3.75 | % | |
| RET | 95.6 | $\times 10^{9}$/l | |
| CHr | 29.8 | pg | |
| IRF (M + H) | 27.6 | % | |
| PLT | 791 | $\times 10^{9}$/l | |
| MPV | 6.4 | fl | |
| PDW | 39.7 | % | |
| Large platelets | 0 | $\times 10^{9}$/l | |
| MPC | 20 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 9.88 | | * |
| WBC Perox | 9.07 | | |
| NEUT | 1.12 | 11.3 | * |
| LYMPH | 7.17 | 72.5 | * |
| MONO | 0.84 | 8.5 | * |
| EOS | 0.27 | 2.7 | * |
| BASO | 0.04 | 0.4 | * |
| LUC | 0.45 | 4.5 | * |
| NRBC | 0.00 | 0.0 | * |
| LI | 1.95 | | |
| MPXI | −9.9 | | |
| MN | | 22.8 | |
| PMN | | 75.2 | |
| BLASTS | | 3.3 | Blasts + |
| Perox HPX | | 0.7 | |
| Perox SAT | | 0.5 | |

Marked lymphocytosis with moderate neutropenia: the distribution of leucocyte subtypes is typical on both cytograms, with good positioning of the thresholds. There is a weak blast flag resulting from the presence of elements to the left of channel 8 in the Baso cytogram, between 1.5% and 5.0%, associated with >4.5% of LUC. There is a severe regenerative anaemia with a normal MCV for the age. The MCHC is increased and the directly measured CHCM is even higher. The red cell volume histogram is broadened with a tail in the zone of volume <60 fl. The red cell population appears compact with a shoulder on the HC histogram in the appropriate direction, in the zone of red cells with a haemoglobin concentration of >410 g/l. On the red cell cytogram, hyperchromic cells are increased to above 18%; they are mainly normocytic but in part microcytic.

**Morphology.** Neutrophil granulocytes 12%, eosinophils 3%, basophils 0.5%, lymphocytes 66%, large lymphocytes (sometimes with basophilic cytoplasm and non-condensed chromatin) 10.5%, monocytes 8%. The red cell series is characterised by the presence of numerous hyperchromic spherocytes, with a reduced diameter in relation to the non-spherocytic red cells; they constitute 20% of the red cells. Some red cells show basophilic stippling, others a weakly polychromatic tinge. Reticulocytes are moderately increased, as happens in this form of congenital haemolytic anaemia resulting from a membrane defect.

## REPORT 2.5 Painful crisis in sickle cell disease (compound heterozygosity for haemoglobin S and beta thalassaemia) in a 14-year-old boy who has been periodically transfused



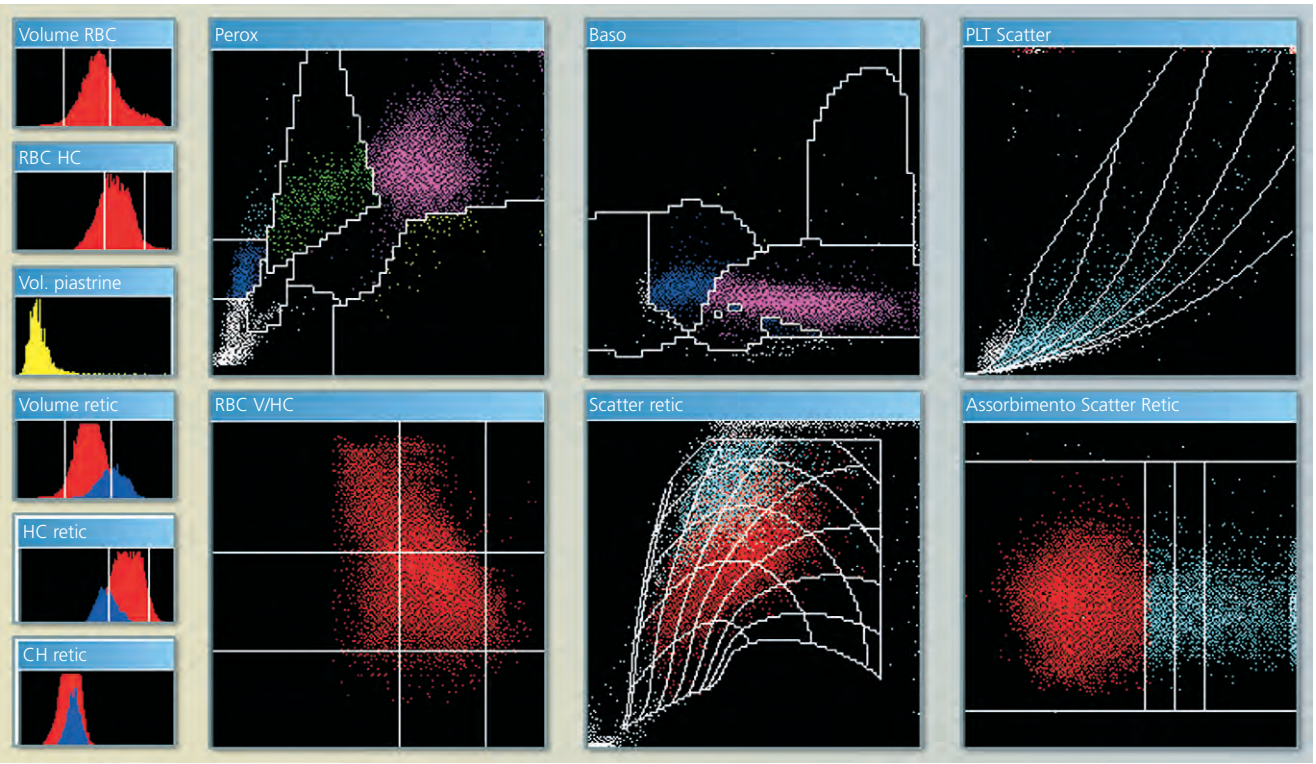

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 80.6 | g/l | |
| RBC | 3.57 | $\times 10^{12}$/l | |
| HCT | 0.282 | l/l | |
| MCV | 79.0 | fl | |
| MCH | 24.2 | pg | |
| MCHC | 307 | g/l | |
| CHCM | 319 | g/l | |
| RDW | 21.1 | % | Anisocytosis ++ |
| HDW | 4.63 | g/dl | Anisochromasia +++ |
| HYPO | 19.2 | % | Hypochromia +++ |
| HYPER | 2.6 | % | |
| MICRO | 12.1 | % | Microcytosis +++ |
| MACRO | 0.3 | % | |
| RBC fragments | 0.11 | $\times 10^{12}$/l | RBC fragments + |
| RBC ghosts | 0.03 | $\times 10^{12}$/l | |
| RET | 15.6 | % | |
| RET | 556.6 | $\times 10^{9}$/l | |
| CHr | 25.5 | pg | |
| IRF (M + H) | 51.7 | % | |
| PLT | 494 | $\times 10^{9}$/l | * PLT aggregate + |
| MPV | 13.5 | fl | * |
| PDW | 52.8 | | |
| Large platelets | 9 | $\times 10^{9}$/l | |
| MPC | 21 | | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 8.03 | | WBC-CE |
| WBC Perox | 12.05 | | PX-NO, PX-NV |
| NEUT | 3.69 | 46.0 | * |
| LYMPH | 2.68 | 33.4 | * |
| MONO | 0.58 | 7.2 | * |
| EOS | 0.38 | 4.7 | * |
| BASO | 0.16 | 2.0 | * |
| LUC | 0.54 | 6.8 | * Atyp + |
| NRBC | 7.84 | 97.7/100 WBC | * NRBC +, N-RBC |
| LI | 2.35 | | |
| MPXI | 2.8 | | |
| MN | | 47.3 | |
| PMN | | 48.9 | |
| BLASTS | | 1.0 | |
| Perox HPX | | 2.9 | |
| Perox SAT | | 0.6 | |

Marked anaemia with microcytosis and two populations of red cell apparent on the red cell cytogram, reflecting the co-existence of pathological cells and transfused cells. The red cell volume and HC histograms are broadened and asymmetrical. There is marked reticulocytosis with a very striking increase in the IRF. The numerous hypochromic cells are mainly reticulocytes and newly formed erythrocytes. The system indicates the presence of numerous red cell fragments and numerous erythroblasts, which are visible also in the leucocyte cytogram. The leucocyte count in the Perox channel is an overestimate, which has been indicated by a correlation flag (CE). The platelet count and the MPV are increased and highlighted with asterisks and an indication of platelet aggregates adjacent to the 'noise' area in the Perox cytogram.

**Morphology.** Neutrophil granulocytes 52.5%, eosinophils 5.5%, basophils 0%, lymphocytes 36%, monocytes 6%. Large platelets are present but not platelet aggregates. The red cells show anisopoikilocytosis with microcytosis, hypochromic red cells, target cells, polychromasia, basophilic stippling, Howell-Jolly bodies, contracted and sickled cells, microspherocytes and red cell fragments. The irregularly contracted cells and the sickle cells cannot be completely converted into spheres, and thus the accuracy of the counts of the erythrocyte subpopulations is reduced. Erythroblasts are very numerous (64 per 100 leucocytes); they are mainly orthochromatic erythroblasts with pyknotic nuclei, with smaller numbers of polychromatic erythroblasts.

## REPORT 2.6 Warm autoimmmune haemolytic anaemia in a patient with HIV infection



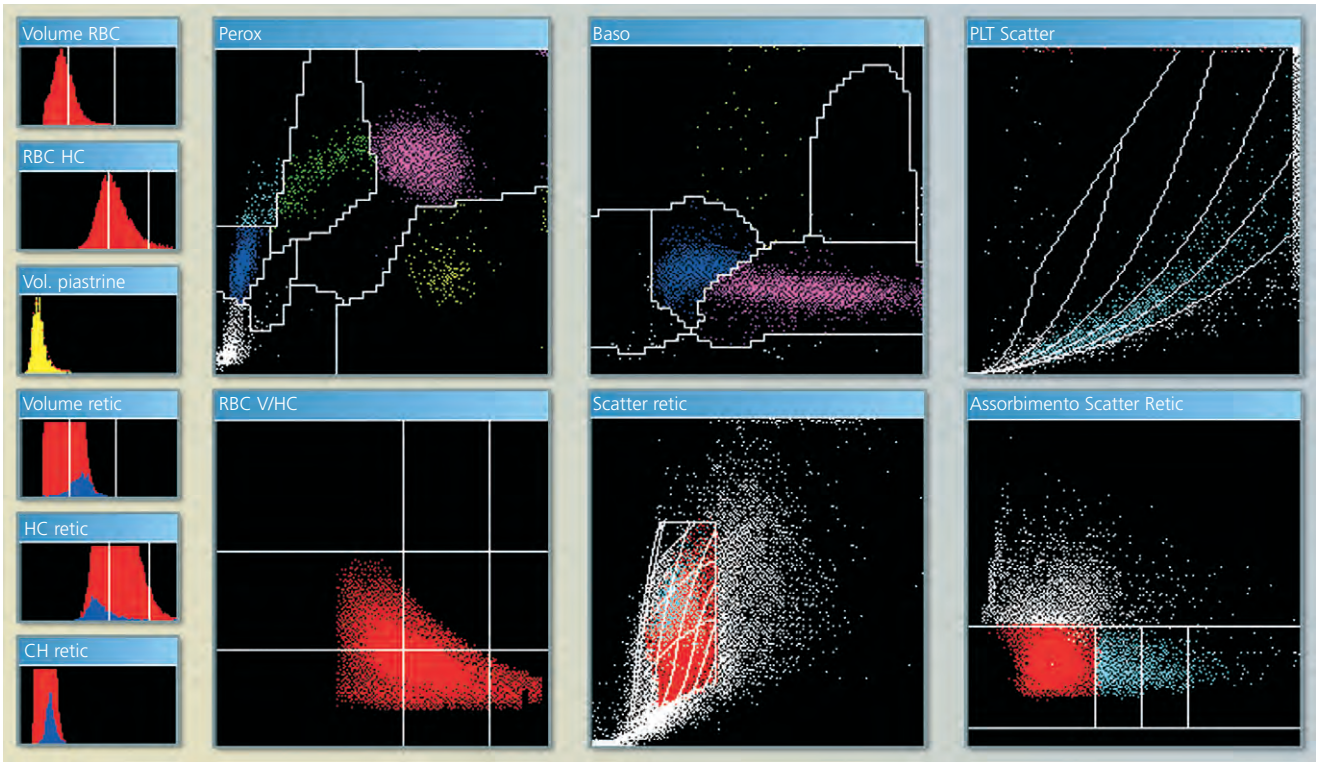

| Parameter | Value | Unit | Flags |
|---|---|---|---|
| HGB | 56 | g/l | |
| RBC | 1.58 | $\times 10^{12}$/l | |
| HCT | 0.177 | l/l | |
| MCV | 112.1 | fl | |
| MCH | 35.3 | pg | |
| MCHC | 315 | g/l | |
| CHCM | 315 | g/l | |
| RDW | 23.1 | % | Anisocytosis +++ |
| HDW | 4.82 | g/dl | Anisochromasia +++ |
| HYPO | 24.2 | % | Hypochromia +++ |
| HYPER | 1.6 | % | |
| MICRO | 1.2 | % | |
| MACRO | 30.4 | % | Macrocytosis +++ |
| RBC fragments | 0.02 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 17.05 | % | * |
| RET | 269.3 | $\times 10^{9}$/l | * |
| CHr | 33.9 | pg | * |
| IRF (M + H) | 36.7 | % | |
| PLT | 261 | $\times 10^{9}$/l | |
| MPV | 9.2 | fl | |
| PDW | 47.8 | % | |
| Large platelets | 3 | $\times 10^{9}$/l | |
| MPC | 22 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 7.91 | | * |
| WBC Perox | 7.52 | | * |
| NEUT | 6.39 | 90.8 | * |
| LYMPH | 0.88 | 11.1 | * |
| MONO | 0.47 | 6.0 | * |
| EOS | 0.09 | 1.2 | * |
| BASO | 0.01 | 0.1 | * |
| LUC | 0.07 | 0.8 | * |
| NRBC | 0.22 | 2.8/100 WBC | * NRBC + |
| LI | 2.25 | | |
| MPXI | −4.1 | | |
| MN | | 11.3 | |
| PMN | | 88.5 | |
| BLASTS | | 0.7 | |
| Perox HPX | | 0.2 | |
| Perox SAT | | 0.3 | |

The red cell population in this patient with severe anaemia is constituted mainly of hypochromic macrocytes. Many of these, as can be seen from the Scatter Retic cytogram, are reticulocytes, but the macrocytosis could be in part a result of previous antiretroviral therapy. The immature reticulocyte fraction is greatly increased, underlining the strongly regenerative nature of this haemolytic anaemia: in the histograms the reticulocytes appear, as one expects, larger and more hypochromic than the mature red cells. The differential count is distinguished by marked lymphopenia.

**Morphology.** The differential count on the blood film confirms the instrumental count and also shows the presence of macrocytosis and anisopoikilocytosis. There are some spherocytes and occasional orthochromatic erythroblasts. There are no signs of agglutination and the MCH and MCHC are normal, indicating that there is no free haemoglobin in the plasma and thus that the haemolysis is mainly extravascular.

## REPORT 2.7 Moderate anaemia with a high red cell count and marked microcytosis in a patient with haemoglobin H disease




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 95 | g/l | * |
| RBC | 6.44 | $\times 10^{12}$/l | * |
| HCT | 0.352 | l/l | * |
| MCV | 54.6 | fl | * |
| MCH | 14.7 | pg | * |
| MCHC | 270 | g/l | * |
| CHCM | 302 | g/l | * CHCM-CE |
| RDW | 24.0 | % | * Anisocytosis +++ |
| HDW | 5.04 | g/dl | * Anisochromasia +++ |
| HYPO | 36.6 | % | * Hypocromia +++ |
| HYPER | 3.0 | % | |
| MICRO | 67.8 | % | * Microcytosis +++ |
| MACRO | 0.0 | % | |
| RBC fragments | 0.78 | $\times 10^{12}$/l | RBC fragments |
| RBC ghosts | 0.03 | $\times 10^{12}$/l | |
| RET | 2.84 | % | |
| RET | 183.0 | $\times 10^{9}$/l | |
| CHr | 19.1 | pg | |
| IRF (M + H) | 6.6 | % | |
| PLT | 195 | $\times 10^{9}$/l | |
| MPV | 6.4 | fl | |
| PDW | 46.2 | % | |
| Large platelets | 4 | $\times 10^{9}$/l | |
| MPC | 23 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 7.08 | | |
| WBC Perox | 6.75 | | |
| NEUT | 4.00 | 56.5 | |
| LYMPH | 2.27 | 32.1 | |
| MONO | 0.34 | 4.8 | |
| EOS | 0.23 | 3.2 | |
| BASO | 0.06 | 0.8 | |
| LUC | 0.18 | 2.5 | |
| NRBC | 0.00 | 0.0 | |
| LI | 2.9 | | |
| MPXI | −6.6 | | |
| MN | | 36.9 | |
| PMN | | 58.6 | |
| BLASTS | | 1.1 | |
| Perox HPX | | 0.4 | |
| Perox SAT | | 0.2 | |

Red cells classified as microcytic are more numerous than those classified as hypochromic; the micro:hypo ratio is 1.85, characteristic of microcytosis due to thalassaemia. There is an increase of the indices that indicate anisocytosis and anisochromasia. The value of the (calculated) MCHC is an underestimate in relation to the (directly measured) CHCM: this discrepancy has led to a flag for poor correlation, indicated by the asterisks beside the relevant red cell measurements. The calculated Hb [HBG = CHCM × RBC] is higher (106 g/l) than that measured colorimetrically. On the red cell V/HC cytogram, the red cell population forms a triangle extending its point towards the right into the quadrant of hyperchromic microcytes: these are actually erythrocyte fragments, the increase of which is well measured by the system, being visualised in the area to the right in the platelet cytogram. The reticulocytes are increased as a percentage and even more as an absolute count, in relation to the increased number of red cells. Erythroblasts are not present and neither the leucocyte count nor the differential count shows any anomaly or discrepancy.

**Morphology.** The leucocyte differential count is superimposable on the instrument count. There is microcytosis with anisocytosis, anisochromasia and, particularly, poikilocytosis, with the presence of target cells, bizarre forms, fragments and microspherocytes. The morphological heterogeneity of these red cells reflects ineffective haemopoiesis, with intramedullary destruction of erythroblasts and peripheral haemolysis caused by the accumulation in the cytoplasm of beta globin chains that cannot be utilised because of the lack of alpha chains resulting from the deletion of three of the four alpha genes.

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CHAPTER 3

# Myelodysplastic Syndromes

## Definition

The myelodysplastic syndromes (MDS) are a heterogeneous and complex group of clonal haematological neoplasms arising from a haemopoietic stem cell, characterised by an alteration in haemopoiesis which becomes ineffective as the result of an irreversible defect in cellular differentiation together with increased apoptosis. They are characterised by:

1. Peripheral cytopenia involving one or more lineages, with normal or increased bone marrow cellularity in the majority of cases;
2. Morphological dysplasia in at least 10% of cells in at least one haemopoietic lineage;
3. Blast cells less than 20% in the blood and marrow;
4. Frequent cytogenetic and molecular genetic abnormalities;
5. Significant although variable risk of evolution to acute leukaemia.

The diagnosis of MDS is based on a quantitative evaluation of the peripheral blood and bone marrow using basic haematological techniques (Table 3.1). The use of additional diagnostic methods is, in some cases, a powerful tool for further elucidation of the diagnosis.

## General clinical and haematological features

The MDS are haematological neoplasms of the elderly with a slight male predominance. The median age at diagnosis is 70 years. The incidence is reported as 3–8 new diagnoses per 100 000 individuals per year, but rises in the population above the age of 70 years to more than 20–35 per 100 000/ per year.[1–3] Recently, in the United States an annual incidence of 75 new diagnoses per 100 000 in a population above the age of 65 years has been reported.[4] The onset is often insidious.[5] The clinical symptoms results from the cytopenias: the most frequent – fatigue, pallor and asthenia – are the result of anaemia, and are present to a variable extent in around 85% of patients at diagnosis. Around 45% of patients have pancytopenia, while a lower proportion have selective thrombocytopenia or leucopenia. It is uncommon, at disease onset, to find lymphadenopathy, splenomegaly, hepatomegaly or mucocutaneous haemorrhage. In every case series there are a significant number of totally asymptomatic patients in whom the diagnosis is an incidental one.

In *de novo* MDS, environmental factors, ethnic factors, familial propensity to haematological neoplasia or exposure to toxic agents such as cigarette smoke, benzene, organic solvents and pesticides may be aetiological factors.[6] By definition, patients with a previous history of exposure to chemotherapy or irradiation are excluded. For this reason, a careful history of previous disease and previous or current pharmacological treatment must be obtained in patients in whom MDS is suspected.

The molecular mechanisms responsible for neoplastic transformation are still in large part unknown. According to the currently best accepted molecular pathogenetic model, MDS is the result of a series of genetic and epigenetic alterations that occur in a very immature haemopoietic stem cell: this process gives rise to an 'MDS clone' characterised by altered differentiation mechanisms and increased susceptibility to apoptosis; over time the clone acquires an advantage over normal immature stem cells, leading to peripheral cytopenias and an increase of blast cells.[7] The majority of mutations that have been recognised in patients with MDS lead to a loss of function, rather than increased expression, of the genes involved.[8]

The natural history of MDS, in the absence of treatment, is characterised by progressive worsening of the ineffective haemopoiesis, bone marrow failure and cytopenia. The incidence of evolution to acute myeloid leukaemia (AML) is increased and to a large extent can be related to the percentage of blast cells present at diagnosis.[9] Patients with refractory anaemia, with or without ring sideroblasts, often have an indolent clinical course with a very low risk of leukaemic evolution. It has, however, been demonstrated that in the great majority of patients, death is, for other reasons, intrinsic to the disease, rather than resulting from acute leukaemic evolution.[10,11] The heterogeneity of MDS leads to heterogeneity in the therapeutic options, selected on the basis of an accurate prognostic classification.[12] There are a variety of such classifications applicable to MDS patients at

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**Table 3.1** Haematology laboratory: diagnostic criteria for the myelodysplastic syndromes

1. **Quantitative anomalies = peripheral cytopenia (pan-, bi-, mono-)**
   - Anaemia (Hb < 100 g/l):
     - MCV normal (70%), increased (30%), reduced (rare)
     - RDW almost always increased
     - Abnormality of the volume distribution curve (dimorphism)
     - Reticulocytes normal or slightly increased
     - Increase in the immature reticulocyte fraction (rich in RNA)
     - Rarely pseudoreticulocytosis
   - Leucopenia:
     - Neutropenia (neutrophils $<1.8 \times 10^9$/l, more recently $<0.8 \times 10^9$/l)
     - Rarely eosinophilia and/or basophilia
     - Monocytes always $<1 \times 10^9$/l
   - Thrombocytopenia (platelet count $<100 \times 10^9$/l):
     - MPV normal or increased
     - PDW always increased
2. **Ineffective haemopoiesis**
   - Bone marrow cellularity normal or increased, reduced in 10%
   - Morphological features: haemophagocytic macrophages
3. **Presence of a proportion of cells (minimum 10%) showing dysplastic features specific for each lineage**
4. **Presence and percentage of blast cells, granular or agranular, in the peripheral blood and bone marrow**
5. **Monocytes in the peripheral blood and the bone marrow, in the blood always $<1 \times 10^9$/l**
6. **Bone marrow iron**
   - Assessment of quantity
   - Ring sideroblasts
7. **Complementary investigations**
   - Cytogenetic and molecular genetic testing
   - Histological and immunohistochemical investigations
   - Flow cytometric immunophenotyping

diagnosis (providing a prognostic score): the most used is the 'International Prognostic Scoring System (IPSS)' (Table 3.2)[13] based on the analysis of an international cohort of 800 patients with *de novo* MDS or chronic myelomonocytic leukaemia (CMML) who were treated only symptomatically, so that the course of the disease reflects only the natural history of the illness. Taking into account the independent statistical weight of three biological characteristics of the neoplasm at the time of diagnosis (number of cytopenias in the peripheral blood, percentage of blast cells in the bone marrow and cytogenetic profile), a model has been constructed in which patients are stratified into four prognostic categories, two lower risk (low risk and intermediate 1) and two higher risk (intermediate 2 and high risk). The median survival has been estimated at 5.7 and 3.5 year respectively for the two lower risk categories and at 1.2 and 0.4 years respectively for the two higher risk categories. Considering the possible evolution of MDS, an early diagnosis is desirable so that the most appropriate therapeutic regime can be adopted from the beginning of the illness. Various other groups have proposed prognostic stratification schemes that are sometimes an improvement on the already notable efficacy of the IPSS.[14] The International Working Group for Prognosis in MDS proposed a revision, the IPSS-R, which identifies five prognostic categories rather than four, maintaining as fundamental criteria the blast percentage, the cytopenia and the cytogenetic risk groups.[15] The threshold for the definition of neutropenia has, however, been lowered to $0.8 \times 10^9$/l. The system designated MPSS includes in the scoring system the age, leucocytosis and performance status.[16] In particular, the inclusion of World Health Organization (WHO) subgroups can

**Table 3.2** International Prognostic Scoring System – Revised (IPSS-R) for stratification of myelodysplastic syndrome patients at diagnosis: prognostic and cytogenetic risk categories[15]

| Prognostic risk category | Total points | Median survival (years) | Time to leukaemic evolution in 25% of cases (years) |
|---|---|---|---|
| Very low | ≤1.5 | 8.8 | Not reached |
| Low | >1.5–3 | 5.3 | 10.8 |
| Intermediate | >3–4.5 | 3.0 | 3.2 |
| High | >4.5–6 | 1.6 | 1.4 |
| Very high | >6 | 0.8 | 0.73 |

| | Points for each variable | | | | | | |
|---|---|---|---|---|---|---|---|
| Prognostic variable | 0 | 0.5 | 1 | 1.5 | 2 | 3 | 4 |
| Bone marrow blasts (%) | ≤2 | – | >2–<5 | – | 5–10 | >10 | – |
| Cytogenetic risk | Very favourable | – | Favourable | – | Intermediate | Unfavourable | Very unfavourable |
| Haemoglobin (g/dl) | ≥10 | – | 8–<10 | <8 | – | – | – |
| Platelets ($\times 10^9$/l) | ≥100 | 50–<100 | <50 | – | – | – | – |
| Neutrophils ($\times 10^9$/l) | ≥0.8 | <0.8 | – | – | – | – | – |

| Prognostic risk | Cytogenetic anomaly |
|---|---|
| Very favourable | -Y, del(11q) |
| Favourable | Normal, del(5q), del(12p), del(20q), two other abnormalities with del(5q) |
| Intermediate | del(7q), +8, +19, i(17q), other single or double clonal abnormalities |
| Unfavourable | 7, inv(3)/t(3q)/del(3q), two abnormalities with -7/del(7q), complex karyotype with three alterations |
| Very unfavourable | Complex karyotype with more than three alterations |

increase the clinical utility of the IPSS.[17,18] The WHO category, the degree of anaemia and the requirement for transfusion support are further variables that correlate significantly with survival in patients with MDS, and thus a new valid prognostic system has been developed, designated the WHO-based Prognostic Score System, or WPSS.[19,20] Finally, other unfavourable prognostic features that have been identified include advanced age, the degree of dysplasia (unilineage or multilineage), the presence and degree of abnormality of the distribution of haemopoietic precursors in histological sections ('abnormal localisation of immature precursors [ALIP]'), the presence and degree of medullary fibrosis and *in vitro* growth characteristics that resemble those of leukaemia.

## Evolution of the classification of the myelodysplastic syndromes

In 1900 von Leube described for the first time the syndrome of 'leukanaemia', characterised by the co-existence of a pernicious anaemia and leukaemia, caused in his opinion by a simultaneous alteration of both erythroid and myeloid cells in the bone marrow, which was different from that in leukaemia. In the years that followed, this condition was generally grouped with 'idiopathic anaemia' or 'atypical leukaemia'[21–23] and was designated and classified in a great variety of ways (Table 3.3). In 1976, the FAB (French-American-British) co-operative group, in their proposals for a new classification of acute lymphoid and acute non-lymphoid leukaemias, described in detail two particular haematological pictures, 'refractory anaemia with excess of blasts' and 'chronic myelomonocytic leukaemia', grouping them together under the heading of 'dysmyelopoietic syndromes' and excluding them from the definition of acute leukaemia.[24] In 1982, the same FAB group published a more complete proposal for the classification of this group of disorders, typified by peripheral cytopenia, changing the designation to 'myelodysplastic syndromes'[25]; they included three new groups and redefined, with more precision, the five entities now identified on the basis of specific morphological criteria, both quantitative and qualitative: refractory anaemia (RA), refractory anaemia with ring sideroblasts (RARS), refractory anaemia with excess of blasts (RAEB), refractory anaemia with excess of blasts in transformation (RAEB-t) and CMML. The FAB criteria for classification, which continue to be the basis of more recent classifications, are based on:

1. Peripheral cytopenia involving one or more haemopoietic lineages;
2. Ineffective haemopoiesis, shown by the association of peripheral cytopenia and a hypercellular bone marrow;
3. Morphological dysplasia affecting one, two or three lineages;
4. The percentage of blasts in the blood and marrow;
5. The number of monocytes.

**Table 3.3** Evolution of the taxonomy of myelodysplastic syndrome from 1900 to 1982[12]

| Year | Designation | Author |
|---|---|---|
| 1900 | Leukanaemia | von Leube W (Klin Wschr 1900, 37:851) |
| 1938 | Refractory anaemia | Rhoads CP, Barker WH (JAMA 1938, 110:794) |
| 1939 | Erythraemia with immature cells | Israels MCG (J Pathol Bacteriol 1939, 48:209) |
| 1939 | Subleukaemic myelosis | Reifenstein GH (Am J Med Soc 1939, 197:215) |
| 1940 | Pseudo-acrestica anaemia | Wilkinson J (1940, cited in Dacie and White, 1949) |
| 1946 | Atypical pernicious anaemia | Foy H, Kondi A, Murray JF (J Pathol Bacteriol 1946, 58:157) |
| 1949 | Preleukaemic anaemia | Hamilton-Peterson JL (Acta Haematol 1949, 2:309) |
| 1949 | Pseudo aplastic anaemia | Dacie JV, White JC (J Clin Pathol 1949, 2:1) |
| 1951 | Erythraemic myelosis | Emery JL (J Pathol Bacteriol 1951, 63:395) |
| 1953 | Preleukaemia | Block M, Jacobson LO, Bethard WF (JAMA 1953, 152:1018) |
| 1954 | Chronic erythraemic myelosis | Martin WJ, Bayrd ED (Blood 1954, 9:321) |
| 1956 | Refractory anaemia with ring sideroblasts | Bjorkman SE (Blood 1956, 1:250) |
| 1958 | Refractory sideroblastic anaemia | Heilmeyer L, et al. (Folia haematologica 1958, 2: 49) |
| 1959 | Refractory normoblastic anaemia | Dacie JV, et al. (Br J Haematol 1959, 5:56) |
| 1963 | Smouldering acute leukaemia | Rheingold JJ, et al. (N Engl J Med 1963, 268:812) |
| 1968 | Idiopathic ineffective erythropoiesis | Waltuch G, et al. (Ann Intern Med 1968, 68:1005) |
| 1970 | Primary acquired refractory sideroblastic anaemia | Hines JD, Grasso LA (Semin Hematol 1970, 7:86) |
| 1971 | Refractory idiopathic sideroblastic anaemia | Kushner JP, et al. (Medicine 1971, 50:139) |
| 1975 | Hypoplastic acute myeloid leukaemia | Beard ME, et al. (Br J Haematol 1975, 31:167) |
| 1976 | Refractory anaemia with excess of blasts | Dreyfus B (Nov Rev Fr Hematol-Blood Cells 1976, 17:33) |
| 1978 | Haemopoietic dysplasia | Linman JW, Bagby GC (Cancer 1978, 42:854) |
| 1979 | Subacute myeloid leukaemia | Cohen JR, et al. (Am J Med 1979, 66:959) |
| 1980 | Dysmyelopoietic syndrome | Streuli RA, et al. (Blood 1980, 55:636) |
| 1982 | Myelodysplastic syndrome | Bennett JM, et al. (Br J Haematol 1982, 51:189) |

With this classification it has been possible to correlate subtypes of MDS with other variables that have been found to be very useful for prognostic stratification and for choice of therapy, such as cytogenetics, molecular genetics, the pattern of distribution of cells in the bone marrow, the results of *in vitro* culture, cell kinetic studies and the ploidy of megakaryocytes.

Notwithstanding the evident advantages of the FAB classification in terms of accuracy of diagnosis and the ability to compare different series of cases, there remained some doubts with regard both to details of interpretation (the exact definition of a blast with granules) and of a more general character (a myeloproliferative component in MDS). These difficulties carried the risk of errors in differential diagnosis between other important haemopoietic disorders, such as acute leukaemia and the myeloproliferative syndromes.[26] Since the publication of the FAB 1982 article, more than 15 000 scientific articles on diverse diagnostic and prognostic aspects of MDS have been published: morphology remains a keystone in the diagnosis of MDS, even if it is currently impossible to reach a unanimous opinion in this group of disorders, particularly when the disease is in its early stages.[27] From a strictly morphological point of view there are two principal diagnostic problems: agreed criteria for identifying a cell as dysplastic and the specificity of dysplasia for a diagnosis of MDS.

The diagnostic classification now in use is that proposed by the working group of the WHO in 2008 in a volume[28] which expanded and updated the first edition of 2001.[29] The important changes relating to the WHO classification of MDS, in comparison with the FAB classification, are:

- The threshold of blast cells necessary to make a diagnosis of acute leukaemia has been reduced to 20% on a myelogram done on 500 nucleated cells;
- A new group of myeloid neoplasms has been recognised, the myelodysplastic/myeloproliferative neoplasms (MDS/MPN), among which is included CMML;
- Seven distinct entities have been recognised within the MDS group, in comparison with the preceding five;
- Precise morphological criteria have been proposed, both quantitative and qualitative, for the definition of morphological dysplasia, with the aim of harmonising microscopic assessment with reference to the minimal criteria necessary for an unequivocal recognition of dysplasia: in particular, to recognise dysplasia within a specified lineage in the bone marrow it is necessary that dysplastic features be present in at least 10% of erythroid precursors (not taking account of mature erythrocytes) and/or 10% of granulocytic cells (in this case also including mature cells) out of a count of at least 200 cells of each lineage and/or in a minimum of 10% of megakaryocytes, at least 30 of these having been evaluated.

With regard to specificity, certain problems remain:

- Already in the 2001 WHO publication, groups of haematological neoplasms with dysplasia had been recognised that nevertheless were not classified as MDS;
- It is noted that low numbers of dysplastic erythroid, granulocytic or megakaryocytic cells can be recognised in the bone marrow of healthy subjects[30];
- Dysplastic aspects of bone marrow cells, such as asynchrony of proliferation and differentiation of single cells, and disturbance of the normal marrow architecture are frequent in all situations of chronic inflammation and protracted irritation of the marrow;
- Non-clonal morphological dysplasia can cause many problems in differential diagnosis, especially when only one lineage (generally erythroid) is abnormal: it should also be emphasised that even with trilineage dysplasia the possibility of a transient reactive condition cannot be excluded.

Cytogenetic and molecular genetic analysis provide a useful supplement to the diagnostic process. Flow cytometric immunophenotyping can also offer support for a diagnosis when it demonstrates aberrant expression of antigens normally associated with a particular maturation stage: however, these observations must be interpreted with great care, always remembering the morphological and clinical context. A basic immunophenotypic panel for identifying myelodysplasia has recently been proposed.[31] In MDS all these aspects are occurring in a primary clonal context.

Excluded from the MDS classification are all those disorders that are not primarily haemopoietic but result from constitutional chromosomal abnormalities, although they do have signs of myelodysplasia and an increased risk of development of leukaemia – such as Down syndrome, Turner syndrome, Klinefelter syndrome, Bloom syndrome, ataxia telangiectasia and dyskeratosis congenita. As already noted, not all haematological neoplasms that show morphological dysplasia are MDS: myelodysplastic aspects can be recognised in cases that, based on other criteria, are classified as MDS/MPN, AML with myelodysplasia-related changes or therapy-related AML.[32]

Specific morphological, clinical and cytogenetic criteria in general permit a rapid diagnosis, but conclusions can be uncertain and problematical in the less obvious forms of MDS, e.g. cases with hypocellularity or bone marrow fibrosis,[33] that lack diagnostic cytogenetic or molecular genetic abnormalities. Patients with subtle morphological changes, haematological parameters at the limit of normal or only slightly abnormal, in the absence of other non-morphological markers, must be kept under observation, until there is clearer evidence of the nature of the condition: such cases have been described in the literature as 'idiopathic cytopenia of undetermined significance (ICUS)' when there is persistent cytopenia without dysplasia or diagnostic cytogenetic abnormalities and as 'idiopathic dysplasia of uncertain significance (IDUS)' when, on the contrary, there is dysplasia or abnormality of the red cells indices, such as persistent unexplained macrocytosis in the absence of cytopenia.[34] The first of these, ICUS, which was proposed by the International Working Group on Morphology of MDS (IWGM-MDS), was specifically excluded from the MDS category in the 2008 WHO

classification; similarly, cases of IDUS do not meet the WHO criteria for an MDS diagnosis.

## Diagnostic criteria: peripheral blood

In the majority of cases, consideration of the blood count and the blood film features shows quantitative alterations and morphological peculiarities in the three haemopoietic lineages that provide firm diagnostic indications.

### Blood count

The threshold values recommended by the WHO group for the recognition of cytopenia in suspected MDS are the same as those proposed for risk stratification in the IPSS prognostic scoring system:

- Haemoglobin concentration less than 100 g/l;
- Neutrophils less than $1.8 \times 10^9$/l;
- Platelets less than $100 \times 10^9$/l.

Some of these values have been revised in the new proposals for prognostic stratification, e.g. the neutrophil threshold being altered to $0.8 \times 10^9$/l. In any event, these are not indispensable requirements for diagnosis in cases with marked morphological dysplasia or a significant cytogenetic abnormality. Numerous studies, moreover, have demonstrated the usefulness of new automated instrument measurements for characterising the cellular atypia in MDS.[35]

Anaemia, though quite often apparent at presentation, can be very variable. It can be normocytic and normochromic or macrocytic: the rare observation of a microcytic normochromic population, in the absence of concomitant conditions such as iron deficiency or thalassaemia, is generally indicative of a rare variant with formation of haemoglobin H associated with marked dyserythropoiesis. The mean cell volume (MCV) is normal or increased, while the erythrocyte distribution width (RDW) is almost always increased, indicating disorganised erythropoiesis. In RARS, automated instruments show marked anisopoikilocytosis, with moderate macrocytosis and an increase in hypochromic cells.[36]

At diagnosis the number of white cells is generally normal or reduced: around half of patients have an absolute neutropenia.[37] Absolute monocytopenia is quite uncommon, while there is often a relative monocytosis, reflecting the concomitant neutropenia. The presence in the circulation of granulocyte precursors or blast cells is rare and indicative of higher risk MDS. Basophilia is rare and is generally associated with severe dysplasia. Morphological alterations in dysplastic neutrophils can be revealed by automated counters, there being specific reproducible deviations from normal of measurements of impedance, light scattering, fluorescence and peroxidase activity.[38–42]

The platelet count can be normal. In about 25% of patients there is thrombocytopenia of variable severity present at diagnosis; however, it is not often present as the sole cytopenia at diagnosis of *de novo* MDS. In a minority of cases there is thrombocytosis, in general associated with macrocytosis and a specific cytogenetic abnormality, del(5q) or 5q-. The combination of thrombocytosis with an increase in ring sideroblasts identifies a MDS/MPN that is often associated with the *JAK2* V617F mutation.[43] Automated measurements of mean platelet volume (MPV) can be normal or increased, particularly in patients with marked dysmegakaryopoiesis and unfavourable cytogenetic abnormalities, in whom the increase is particularly in the immature platelet fraction (IPF).[44,45] The platelet distribution width (PDW) is usually increased, indicating the heterogeneity of the population, often also evident in the blood film which shows very large platelets together with microplatelets. The mean platelet component (MPC) measures the optical density of platelets; it falls after activation or when there is a defect in granule formation, as can occur in MDS.[46]

The reticulocyte count can be normal, reduced or, rarely, somewhat increased. In patients at diagnosis of MDS the reticulocytes, analysed by double angle light scatter, are larger and have a reduced haemoglobin content in comparison with normal reticulocytes.[47] Abnormalities in the pattern of reticulocyte maturation have also been described with a reduction in more mature reticulocytes – those with low fluorescence due to a low RNA content,[48] and a resultant increase in the proportion of immature reticulocytes with strong fluorescence.[36,49,50] In monitoring patients with refractory anaemia being treated with recombinant erythropoietin, the fraction of immature reticulocytes has a positive predictive value for identifying those who will respond to treatment. In rare cases, one can observe a misleading, quite marked increase in the reticulocyte count (pseudo-reticulocytosis), caused not by accelerated production of erythrocytes but by persistence of the granulofilamentous material in mature red cells for the duration of their life span in the circulation, as a result of an alteration in the normal processes of RNA degradation.[51] On the other hand, true reticulocytosis is observed, rarely, when MDS is associated with autoimmune haemolytic anaemia.[52]

### Morphology of dysplasia

The microscopic observation of nucleated cells, erythrocytes and platelets in a fixed and stained blood film is fundamental – in association with observation of the bone marrow aspirate – for the recognition of those morphological criteria on which modern systems of classification of MDS are based (Table 3.4). In general, dysplastic features affect the cell line that shows cytopenia, but this is not always so: for example, there may be thrombocytopenia without anaemia but associated with dyserythropoiesis and minimal morphological alterations in megakaryocytes. In patients in whom leucopenia is marked, it can be very useful to make a blood film after enrichment of the white cells, using a drop of blood taken from the interface between the red cells and the plasma (i.e. from the buffy coat) after centrifugation of the specimen tube.

**Table 3.4** Peripheral blood morphology in haemopoietic dysplasia

**Erythroid series**
- Shape:
  - Anisocytosis
  - Dimorfism
  - Poikilocytosis: red cell fragments, dacrocytes, ovalocytes, stomatocytes
  - Target cells
- Colour:
  - Anisochromasia
  - Polychromasia
- Erythrocyte inclusions:
  - Basophilic stippling (RNA)
  - Howell-Jolly bodies (DNA)
  - Pappenheimer bodies (siderosomes)
- Circulating erythroblasts (dyserythropoietic):
  - Bi- or multi-nuclearity
  - Asynchrony and megaloblastosis
  - Nuclear fragmentation and bizarre nuclei

*Neutrophil granulocytes*
- Nucleus:
  - Lobularity:
    Hyposegmentation (pseudo-Pelger)
    Hypersegmentation
  - Chromatin density:
    Increased – pyknotic (pseudo-Pelger)
    Reduced – spongy
  - Howell-Jolly-like bodies
- Cytoplasm:
  - Anomalies of granulation:
    Reduction or absence
    Increased
    Irregular distribution
    Deficiency of peroxidase (or of other enzymes)
  - Vacuoles
  - Areas of basophilia, Döhle bodies
- Tendency to morphological homogeneity in individual cases:
  - Type I dysplasia (small, hyposegmented)
  - Type II dysplasia (large, variable nuclear segmentation)

*Other leucocytes*
- Eosinophils and basophils:
  - Anomalies of nuclear morphology
  - Anomalies of granulation
- Immature granulocytes (dysmorphic) and/or blasts (agranular, granular)
- Monocytes:
  - Anomalies of nuclear morphology
  - Anomalies of granulation and of the cytoplasm

**Platelet series**
- Size:
  - Increased variation in size (platelet anisocytosis)
  - Large platelets
  - Giant platelets
  - Small platelets
- Morphology:
  - Reduction or absence of granules (grey platelets)
  - Central dense concentration of granules:
    Hyperchromic (resembling lymphocytes)
- Tattered pieces of megakaryocyte cytoplasm (unseparated platelets)
- Micromegakaryocytes
- Bare megakaryocyte nuclei

## The erythroid series

When observing a peripheral blood film it is possible to see one or more of the following morphological anomalies in the red cells (Figs 3.1 through to 3.6): anisocytosis, poikilocytosis with the presence of dacrocytes, red cell fragments, ovalocytes, target cells and stomatocytes; anisochromasia or polychromasia; basophilic stippling, nuclear remnants (Howell-Jolly bodies) or Pappenheimer bodies. Sometimes one observes two red cell populations, one of which is usually normal, while the other, being a direct expression of the anomalous neoplastic clone, is microcytic or macrocytic. It is not rare to see a variable proportion of erythroid precursors, in general polychromatic or orthochromatic erythroblasts, but occasionally basophilic erythroblasts, with the morphological stigmata of dyserythropoiesis (Figs 3.4 and 3.5): some erythroblasts may be binucleated or multinucleated with megaloblastic features or bizarre nuclei.

## The granulocytic series

Dysgranulopoiesis can be exceedingly variable, from absent to severe, and can involve the nucleus or the cytoplasm (Figs 3.5 through to 3.20). The morphological alterations in neutrophil nuclei that have a high degree of specificity for MDS are represented by abnormalities in lobulation and in chromatin density:
- The absence of segmentation with dense chromatin, almost pyknotic and forming large clumps (pseudo-Pelger appearance);
- Hyposegmentation with bilobed nuclei, or bizarre shapes, with sessile nodules or pedunculated attachments, peanut shaped or, rarely, ring forms;
- Increased number of lobes, with chromatin density reduced, increased or normal.

Less specific nuclear abnormalities include the presence of band and ring forms of mature neutrophils, testimony to the loss of capacity for segmentation of the neoplastic clone, and the presence along the edge of the nucleus of numerous nuclear projections, either thin or short and stubby, or of a shape that, in male subjects, simulates the drumstick projections that are normal in women (pseudo-Barr bodies of pseudo-drumsticks).[53]

In the cytoplasm one may observe an alteration in granule content, with the presence of neutrophils that appear hypogranular, agranular or, rarely, hypergranular. Evaluation of granularity can be done only on films that are optimally stained: granules, in fact, are the morphological characteristic whose visualisation is most easily compromised by an inadequate staining technique, whether this is manual or automated. A reduction to two thirds of the normal granule content of a neutrophil has been recently proposed as a morphological criterion for the identification of dysplastic neutrophils.[54] Abnormality of granule formation with coalescence to form giant granules of a pseudo-Chédiak-Higashi type is observed less often in *de novo* MDS, while these can be a feature of AML associated with t(8;21).

Usually, morphological alterations present in a characteristic and constant manner in individual patients: in some the

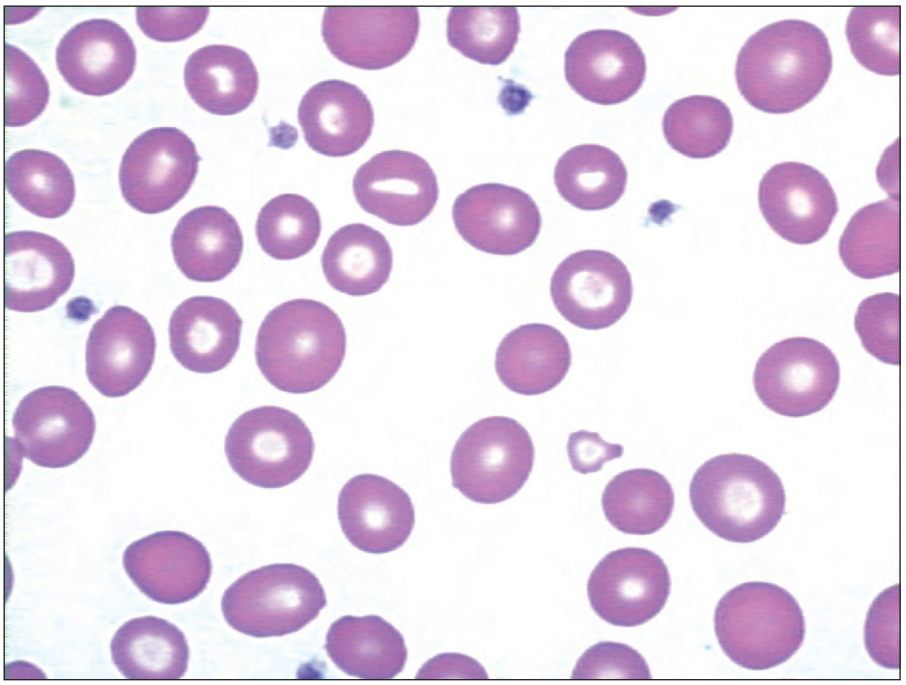


**Fig. 3.1** Erythrocyte polymorphism with anisocytosis and poikilocytosis in the peripheral blood film of a patient with refractory anaemia. Some of the red cells are normocytic with a well-preserved area of central pallor, while other cells have reduced or absent central pallor, appearing spherocytic. Other erythrocytes are hyperchromic macrocytes. Top centre, a stomatocyte and, lower down, a small hypochromic dacrocyte. The visible platelets are hyperchromatic.

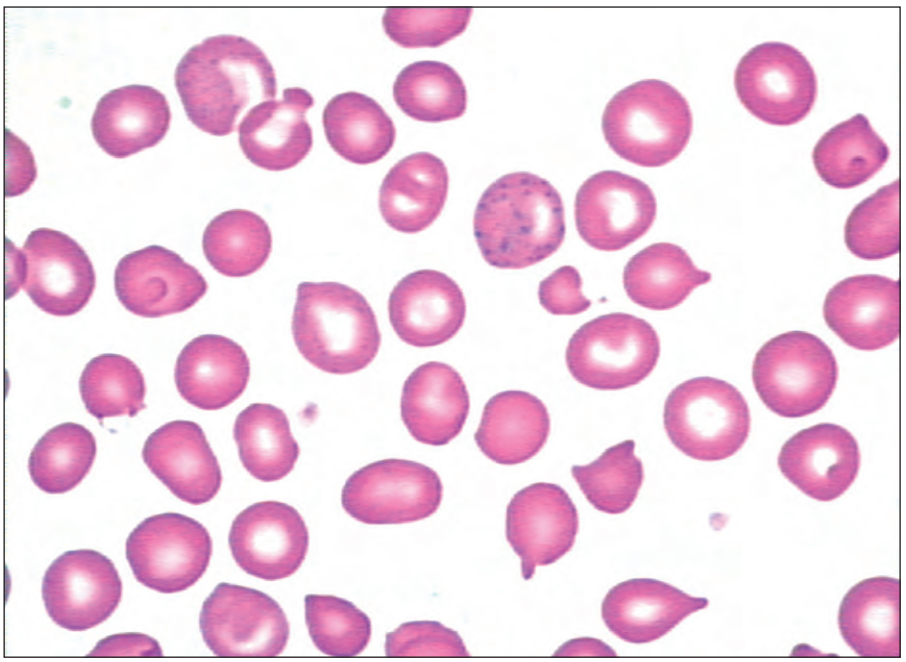


**Fig. 3.2** Anisocytosis and poikilocytosis in a patient with refractory anaemia with excess of blasts type 1 (RAEB-1) associated with bone marrow fibrosis. In the context of normocytic cells, there are some large erythrocytes showing basophilic stippling, particularly evident in the cell just to the right and above the centre. Some cells have a teardrop shape because of a single polar extension, while at least two microcytes resemble keratocytes, with two or three spicules that deform the outline. There is also a hyperchromic red cell fragment. The intracellular distribution of haemoglobin is sometime irregular with the pale area being off centre and semilunar.

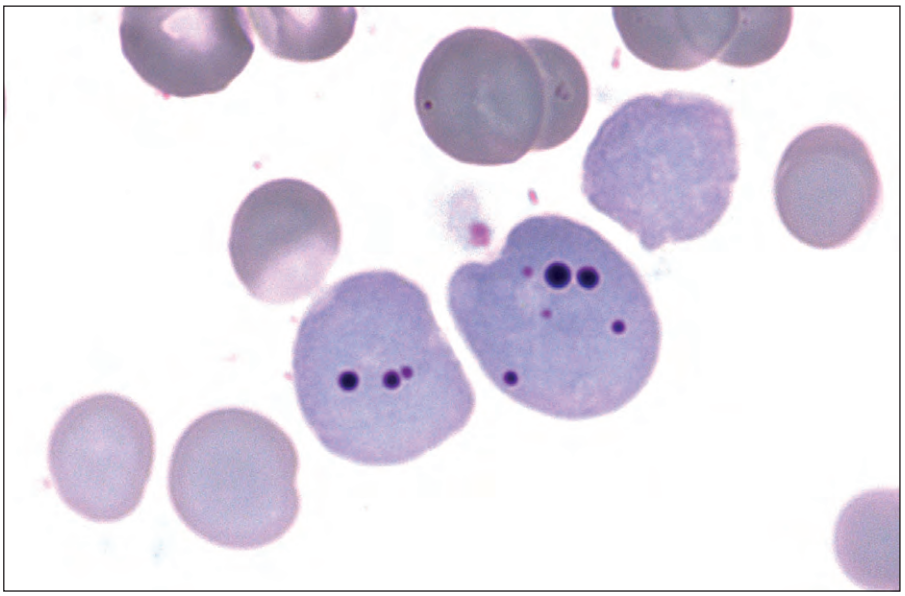


**Fig. 3.3** Multiple Howell-Jolly bodies of variable size within two large polychromatic cells. In this quite thin area of the film, the cell outlines are deformed by projections and there is also a platelet with a small eccentric chromomere. These remnants of nuclear material, corresponding to chromosomes or chromosomal fragments lost in the course of a previous mitosis, are spherical and dark blackish-violet with clear outlines. In the erythrocyte on the right, there are also two smaller azurophilic granules (paler violet), corresponding to remnants of the mitotic spindle.

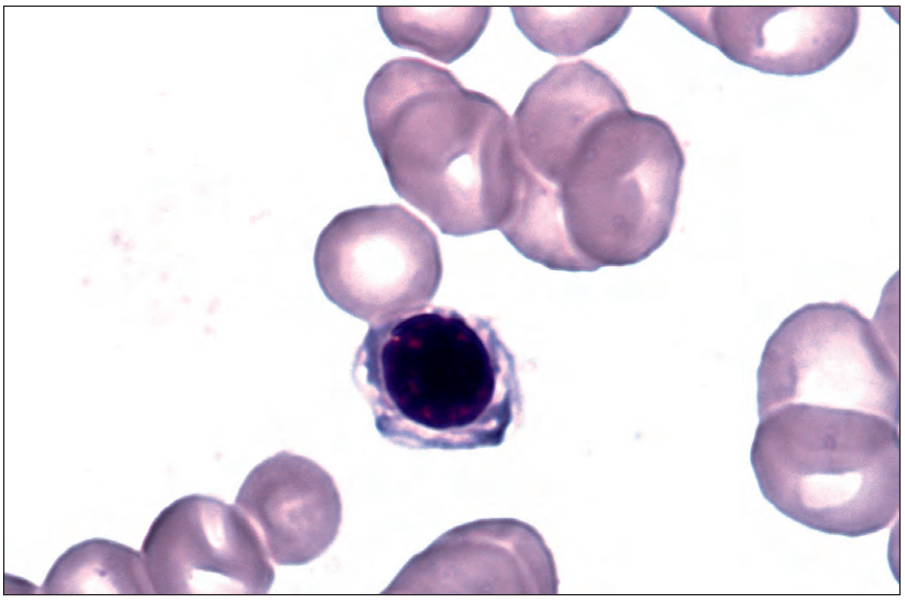


**Fig. 3.4** The presence of orthochromatic and polychromatic erythroblasts in the blood film is not rare in the forms of myelodysplasia in which the haemoglobin concentration is reduced and erythropoiesis is active but ineffective, and also in myelodysplasia associated with bone marrow fibrosis. In this small polychromatic erythroblast the scanty greyish cytoplasm shows large empty linear areas, indicating defective haemoglobinisation and irregular distribution of the remaining cytoplasmic organelles. The nucleus, almost completely condensed, continues to occupy too large a proportion of this small cell.

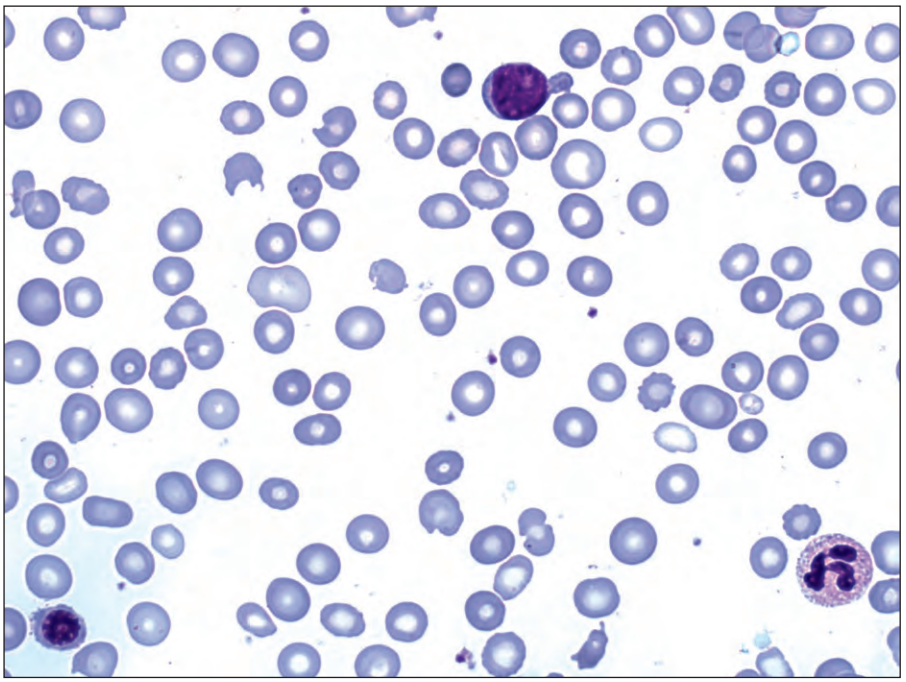


**Fig. 3.5** Peripheral blood film of a patient with RAEB-2. The red cell population shows anisopoikilocytosis and a tendency to dimorphism because of the presence of a significant proportion of smaller hypochromic erythrocytes. There are also a significant proportion of heterogeneous hyperchromic poikilocytes, in which the features of red cell fragmentation are present in the form of acutely angled spicules and discontinuity of the cell membrane. Top centre, a blast cell, which at this power appears hypogranular, with scanty basophilic cytoplasm which forms a hand-mirror type of projection; the nucleus shows non-homogeneous chromatin and several nucleoli, one of which is prominent. In the left bottom corner, an orthochromatic erythroblast (with retarded nuclear maturation in relation to the full haemoglobinisation of the cytoplasm) and, in the right bottom corner, a neutrophil.

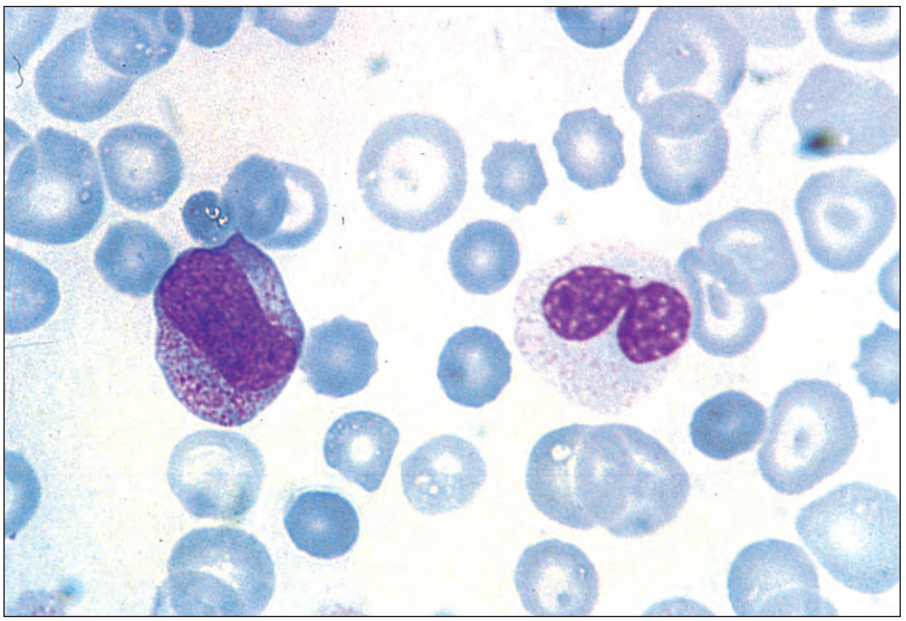


**Fig. 3.6** Dysgranulopoiesis; peripheral blood film of a patient with RAEB-1. On the right, a dysplastic neutrophil granulocyte which shows two of the most specific stigmata of dysgranulopoiesis. (1) The bilobed nucleus shows one of the two typical nuclear forms designated pseudo-Pelger; the chromatin is condensed into heterogeneous clumps leaving empty spaces between them; the bridge between the lobes is a short thin band rather than a filament. (2) Marked reduction in granulation, with fine granules scattered through weakly basophilic cytoplasm. On the left, a granular blast cell: its nucleus is almost rectangular, with an angular outline and some straight areas, finely condensed chromatin and some pale shadows of nucleoli; the basophilic cytoplasm does not have any paler areas and is completely but irregularly occupied by obvious azurophilic granules. The background red cells comprise a double population, with normal erythrocytes with a well-stained ring of haemoglobin and a small pale centre, sometimes showing echinocytic deformation, and hypochromic macrocytes, often showing target cell formation.

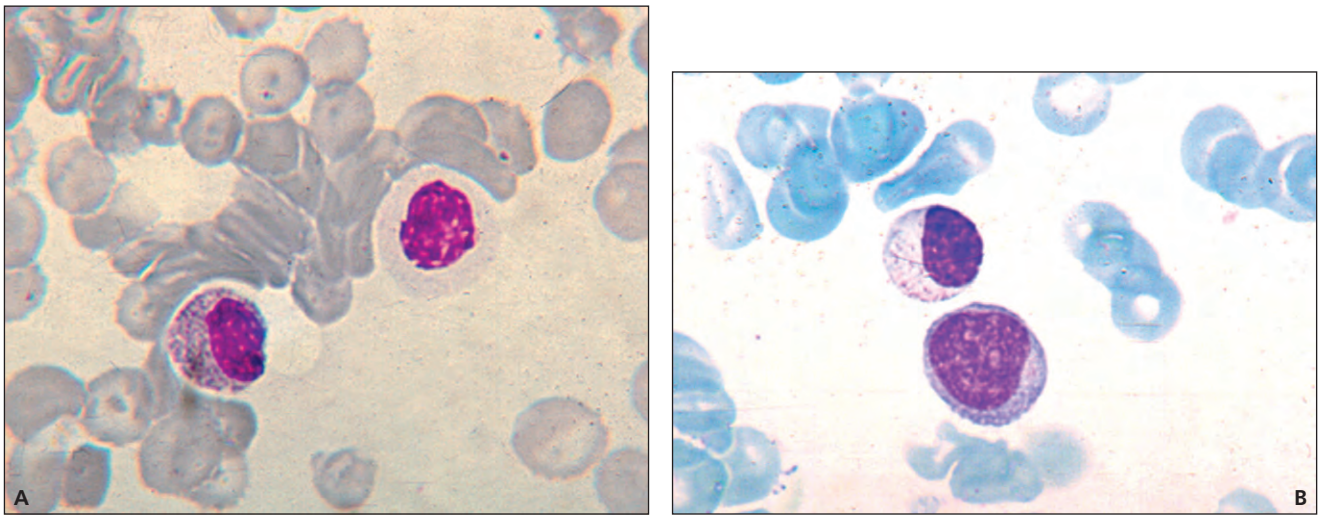



**Fig. 3.7** Dysgranulopoiesis in the peripheral blood: absence of nuclear segmentation (the second variant of the pseudo-Pelger anomaly) and aberrant granulation of mature granulocytes. (A) On the right, a well-differentiated neutrophil with greyish, completely agranular cytoplasm and a non-segmented nucleus, reduced to a single irregular central mass of dense, glassy-looking chromatin. The nuclear outline is irregular, appearing to be deformed by small projections and indentations. On the left, an eosinophil granulocyte with cytoplasm packed with very delicate opaque granules shows the same nuclear aberration. This appearance, with a total absence of nuclear lobation, is similar to that seen in the rare homozygotes for the congenital Pelger-Huët anomaly. (B) The neutrophil granulocyte at the top shows a non-lobed nucleus (pseudo-Pelger), which is eccentric with prune-coloured chromatin condensed, as usual, into alternating dark masses and small paler spaces. The cytoplasm is pink with a peripheral greyish, weakly basophilic area which contains, in its lower part, a few wine-red granules. At the bottom, a blast cell with homogeneous chromatin, with one clearly seen nucleolus and some paler areas that cannot be unequivocally identified as small nucleoli; the moderately basophilic cytoplasm shows a few fine azurophilic granules in the area of paranuclear pallor on the right.

neutrophils have a pseudo-Pelger type of nucleus, hyperdense chromatin and reduced cell size, while in others large neutrophils with hypersegmented nuclei prevail.

Vacuoles, areas of cytoplasmic basophilia and Döhle bodies (formed of small masses of ribosomes and membrane of the endoplasmic reticulum) are variably present, but do not have any diagnostic specificity, being also seen in bacterial infections and various types of inflammatory state. Neutrophils can be increased or decreased in size. From the point of view of cytochemistry, a partial or total loss of peroxidase activity, alkaline phosphatase activity or both is relatively frequent.

Sometimes, but not always, the presence of a particular morphological abnormality correlates with a specific cytogenetic or molecular genetic abnormality. The presence of non-segmented neutrophils with a characteristic condensation of the chromatin into large blocks separated by ample pale areas, evoking fragmentation of DNA, was not associated with a recurring cytogenetic abnormality.[55] However, the presence of neutrophils with pseudo-Pelger nuclei and vacuolated cytoplasm suggests the deletion of chromosome 17p and the tumour suppressor gene *TP53*.[56]

In MDS, the Arneth formula often shows a left shift, with immature granulocytes sometimes being present (Figs 3.15 through to 3.20); this is not, however, of prognostic significance.

Morphological alterations in eosinophils are less frequent and comprise anomalies in nuclear lobulation and in the quantity and structure of the granules (Fig. 3.18). Mature eosinophils can have dysmorphic feature that cannot be defined as specific for dysplasia: nuclear hypo- or hyper-segmentation (eosinophils normally being mainly bilobed); immaturity, abnormal staining characteristics, reduced numbers or irregular size and distribution of granules; vacuolisation, basophilia or the presence of a zone of agranular pale or basophilic cytoplasm. Minor degrees of these abnormalities can also be recognised in non-clonal, reactive eosinophilia and in idiopathic eosinophilia; clonal alterations in eosinophils, often morphologically more anomalous, are seen also in chronic eosinophilic leukaemia, in myeloid neoplasms associated with *FIP1L1-PDGFRA* or rearrangement of *PDGFRB*, and in acute myeloblastic and acute myelomonocytic leukaemia associated respectively with t(8;21) and abnormalities of chromosome 16.[57]

Dysplastic changes in basophils, affecting size, nuclear maturity and, particularly, the size, number and distribution of the granules (Fig. 3.19), are rare in MDS, while they are seen more frequently in chronic myeloid leukaemia, particularly in the accelerated phase, and in AML, particularly in association with t(6;9).[58,59] Basophils that have degranulated as a result of activation can be seen in urticaria, anaphylactic shock and postprandial hyperlipidaemia.[60]

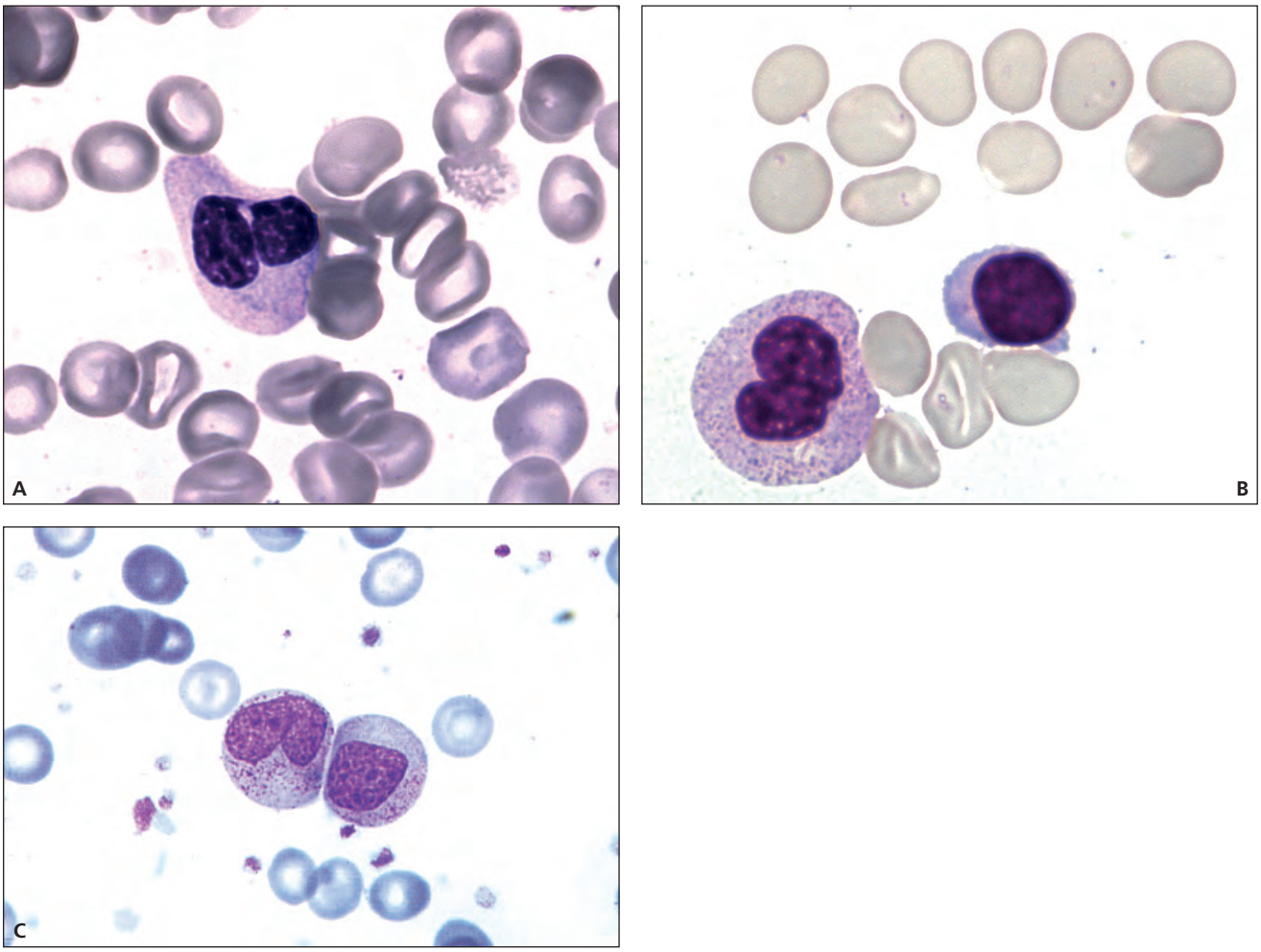



**Fig. 3.8** Dysgranulopoiesis in the peripheral blood: neutrophils with hypersegmented (pseudo-Pelger) nuclei and defective cytoplasmic granulation. (A) A mature neutrophil with a bilobed nucleus and pink cytoplasm with a greyish tinge of basophilia, in which no granules are visible. The chromatin is condensed into dark clumps. (B) On the left, a neutrophil with cytoplasm containing numerous very fine granules spread through the pale lilac cytoplasm. The nucleus has condensed, but paler chromatin with two lobes that appear to be superimposed on each other, so that the structure that joins them cannot be evaluated: it is possible to follow the structure and the outline of the lower lobe which crosses the lower part of the other lobe. On the right, a small mature lymphocyte with normal, weakly basophilic cytoplasm and a nucleus that shows a different type of nuclear condensation. (C) These two granulocyte in a peripheral blood film have non-segmented nuclei with pale spongy chromatin with small areas of condensation alternating regularly with delicate pale spaces. The nucleus of the cell on the left has two overlapping lobes, while that of the cell on the right is polygonal. The cytoplasm is basophilic with numerous irregularly distributed granules which stand out, because of their intense purple colour, from the background basophilia of the cytoplasm, leaving, however, large empty areas. The degree of dysplasia is such that it is difficult to determine the level of maturation of this cell, which could be either a neutrophil metamyelocyte or a dysplastic myelocyte. There are a number of hyperchromatic platelets, the largest of which, on the left, has a large eccentric chromomere which lets the agranular basophilia of the hyalomere be appreciated.

### Blast cells

The presence of blast cells in the circulation is a determining feature for classification according to the WHO criteria, and is indicative of an unfavourable prognosis. When present, blasts are generally small to medium in size with a high nucleocytoplasmic (N:C) ratio (Figs 3.6, 3.7, 3.12, 3.19 and 3.21): the nucleus is often central with one or two nucleoli and fine dispersed chromatin; the cytoplasm is relatively scanty and basophilic; primary (azurophilic) granules may be absent, scanty or, sometimes, more numerous. Patients who at presentation have a low percentage of circulating blasts, less than 1%, have a worse prognosis, even if they also have less than 5% blast cells in the bone marrow.[61,62] The recognition of Auer rods in the cytoplasm, either in circulating or bone marrow blast cells, carries an unfavourable prognosis and leads to classification as RAEB-2, regardless of the percentage of blast cells in the blood or marrow. A cytochemical stain for peroxidase and the study of specific immunophenotypic markers are useful, when

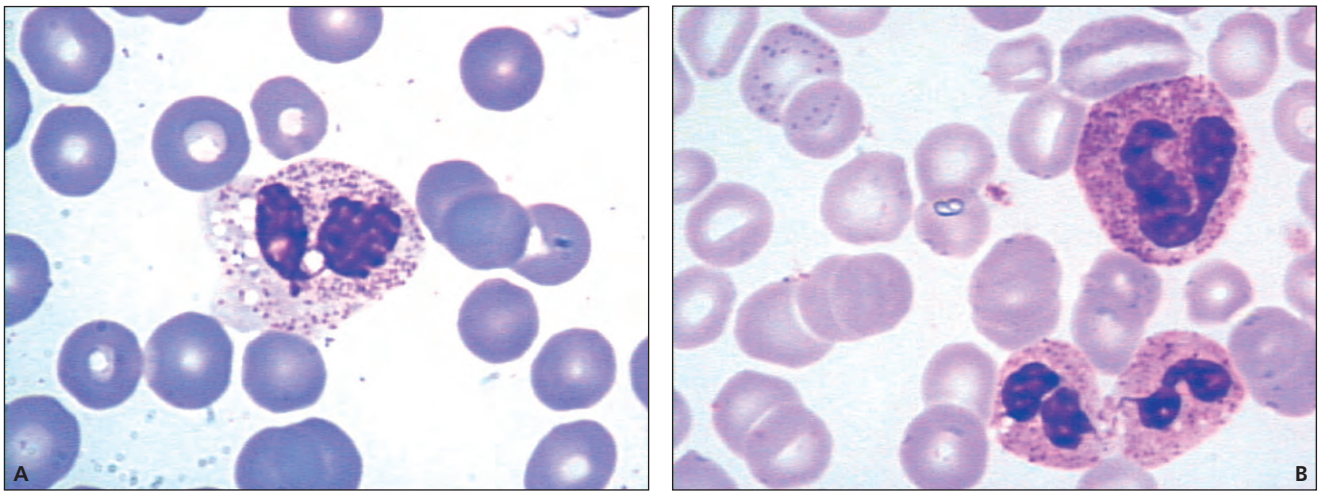



**Fig. 3.9** Dysgranulopoiesis in the peripheral blood: various types of alteration of nuclei and cytoplasm. (A) Neutrophil with a bilobed nucleus and chromatin markedly condensed into coarse blocks. The lobe on the right has an irregular outline composed of small circles and in three dimensions appears to be covered in knobs and composed of two nuclear segments that are fused or juxtaposed. A thin chromatin bridge adjacent to the vacuole in the centre of the cell joins it to the lobe on the left: from the latter there extends downwards a pendunculated appendage or drumstick. The grey-blue colour of the cytoplasm, attributable to residual basophilia, is another sign of neutrophil dysplasia when seen in mature neutrophils, particularly when associated with the even more significant reduction and irregular distribution of the granules; these are particularly reduced in the left part of the cell which is, however, rich in optically empty vacuoles of variable size. (B) A male patient. Top right, a neutrophil of increased size with nuclear lobes still joined by a thick band of chromatin. The cytoplasm is pink and hypergranular. The two neutrophils in the bottom right corner have more marked dysplasia, being very small with very dense chromatin and, in the case of the cell on the left, a bilobed (pseudo-Pelger) nucleus. In fact, careful observation discloses that they are actually still joined by a bridge of hypogranular cytoplasm through which runs a very fine chromatin filament, which starts from a small cone of chromatin on the right of the left-hand nucleus and runs upwards, in part almost vertically, to reach an analogous projection on the left lobe of the right-hand band neutrophil. In the top left corner is a macrocyte with basophilic stippling on which is superimposed, appearing almost transparent, a normal erythrocyte.

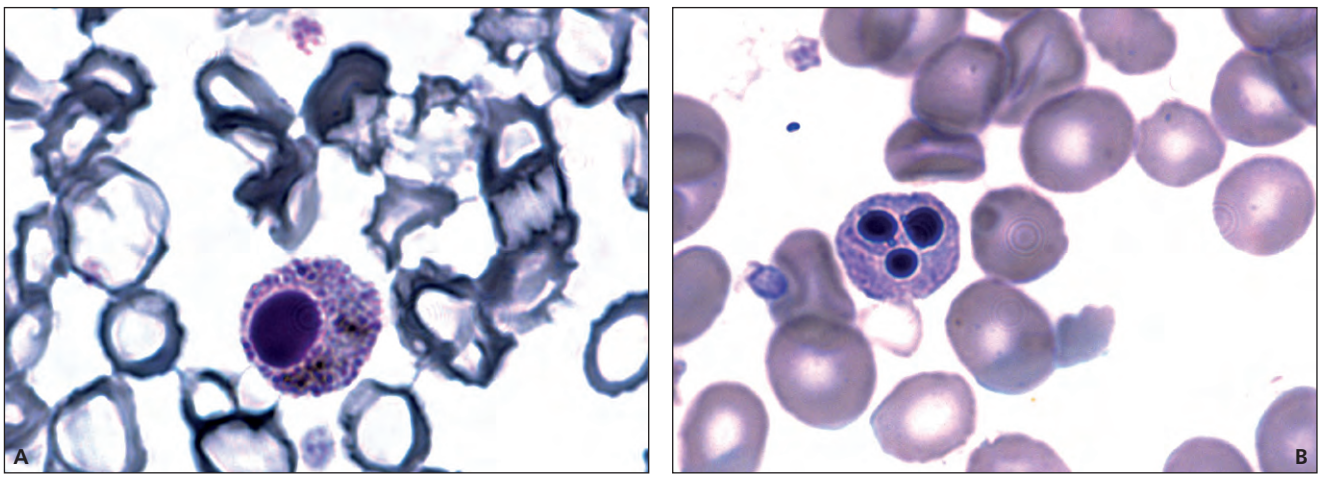



**Fig. 3.10** Apoptosis of neutrophil nuclei in the peripheral blood. This alteration can be the result of the blood specimen being stored for too long and should not be erroneously interpreted as a sign of dysplasia. (A) Pyknotic condensation of the nucleus with the chromatin reduced to an oval mass with a clear outline and no visible internal structure. A positive myeloperoxidase (MPO) reaction serves to confirm that the apoptotic cell is a neutrophil. (B) The nucleus of this necrobiotic neutrophil is fragmented and liquefied into three homogeneous blackish masses without any apparent connection between them. The effect of apoptosis on the cytoplasm is seen in the dark bluish colouration and the presence of an optically empty bubble, formed by membrane protrusion, that extends form the lower pole of the cell. The formation of bubbles or blisters arising from the cytoplasmic membrane of dying cells is known in biology as zeiosis.

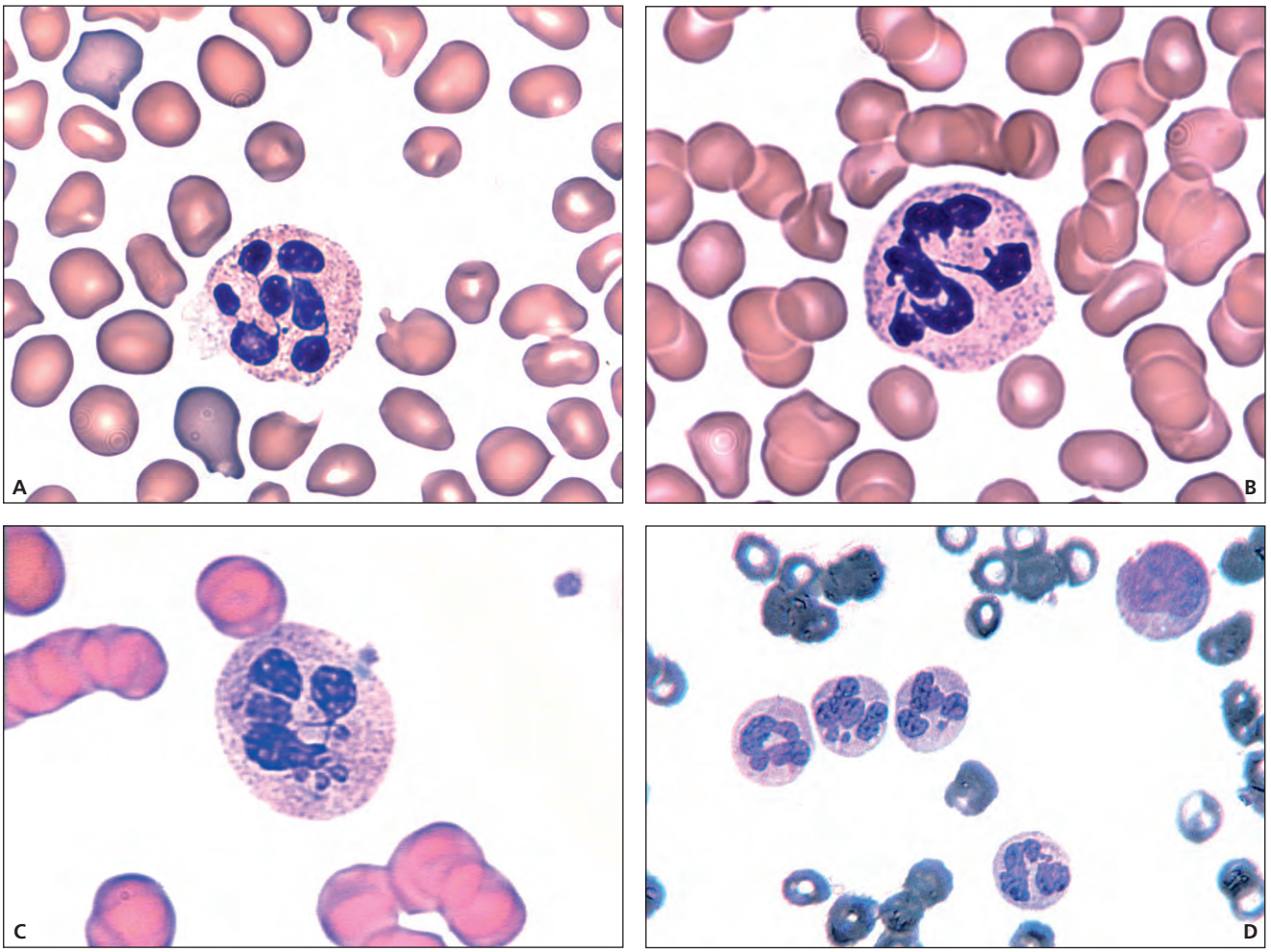



**Fig. 3.11** Dysgranulopoiesis of mature neutrophils: hypersegmentation with an increased number of nuclear lobes. When such a cell is increased in size, as is the case here, it is known as a macropolycyte. This appearance is less specific for myelodysplasia than hyposegmentation, but it can be recognised as a feature when it is associated with other alterations such as are seen in images (B–D). (A) A neutrophil of increased size with seven nuclear lobes as the only anomaly, in a female patient with refractory cytopenia with multilineage dysplasia (RCMD). The normal chromatin condensation maintains the typical dark violet colour with small pale areas. The lobes are oval and regular in shape and the filaments that unite them are thin and distinct. The cytoplasm is pink with a normal content of fine granules. There are two nuclear appendages, one in the form of a drumstick and the other thin and sessile. Cells such as this are also typical of megaloblastic anaemia and result from the tetraploidy of bone marrow precursors. In MDS they are often accompanied by bone marrow features of megaloblastoid erythropoiesis. The morphology of the red cells, which should be evaluated with caution in this quite thin area of the film, shows mild anisopoikilocytosis with two polychromatic erythrocytes and two teardrop cells. (B) Hypersegmented dysplastic neutrophils in a male patient with refractory anaemia. The separation between the numerous visible lobes is incomplete and irregular. There are numerous nuclear appendages, some of which resemble Barr bodies only partially, since the peduncle is very short, thick or absent. Cell size is not increased and the cytoplasm is pink and well granulated. (C) A very dysplastic neutrophil in the blood film of a male patient with RAEB-1. There are five nuclear lobes joined by thin bridges of variable length, and five nuclear appendages of pseudo-drumstick type, sometimes with a small pale area in the centre, which have almost the appearance of small abortive lobes. The cytoplasm is pink with some pale grey patches and very delicate granules that are barely visible. Top right, at 2 o'clock in the cell, is a basophilic Döhle body, close to which is an adherent platelet. (D) Neutrophils with hyperlobated nuclei and completely agranular cytoplasm. The two cells in the centre have pedunculated appendages of drumstick type. The image, though not of optimal quality, is a good example of severe dysplasia in RAEB-2. These cells were negative on an MPO stain, confirming the lack of granules and the functional deficit. In the top right corner, a blast cell with relatively dense chromatin, a nucleolus and fine azurophilic granules.

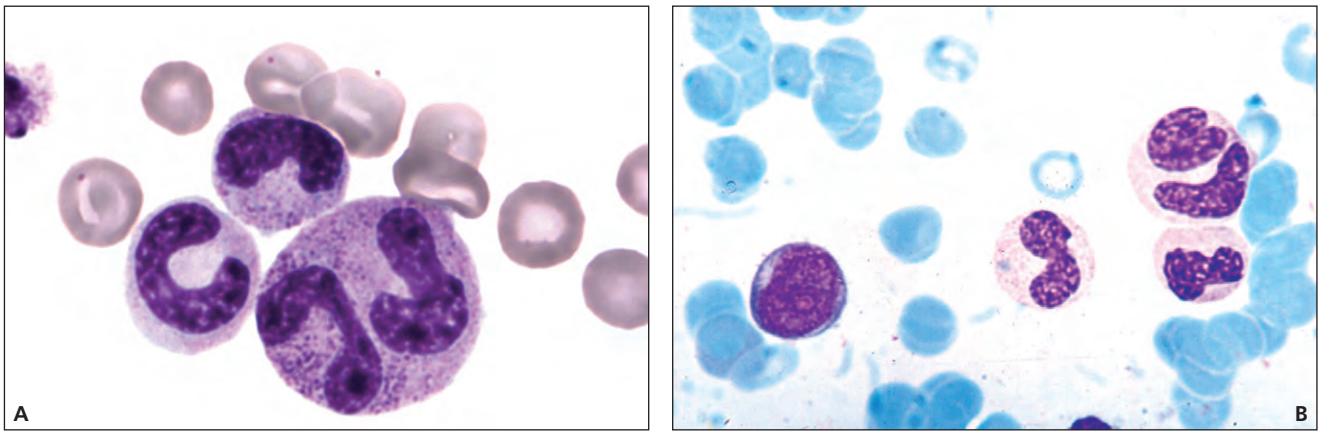



**Fig. 3.12** Dysgranulopoiesis in the peripheral blood: neutrophils with two nuclei. This phenomenon is fairly rare and is usually accompanied by other morphological indications of dysplasia. (A) Neutrophil band forms. The cell on the left has cytoplasm that is still weakly basophilic and has some fine, barely visible granules. That on the right is double normal size and has two nuclei, neither of which is segmented; it is the result of nuclear division that has not been followed by cytoplasmic division into daughter cells. The granules are clearly seen but irregularly distributed. (B) Three hyposegmented neutrophils, one of which is giant and has two nuclei. The chromatin in these cells is condensed into numerous small clumps with an increase in pale fissures, referred to as 'abnormal clumping'. On the left, an apparently agranular blast cell with granular chromatin and a prominent nucleolus; its scanty cytoplasm is basophilic with a pale paranuclear zone.

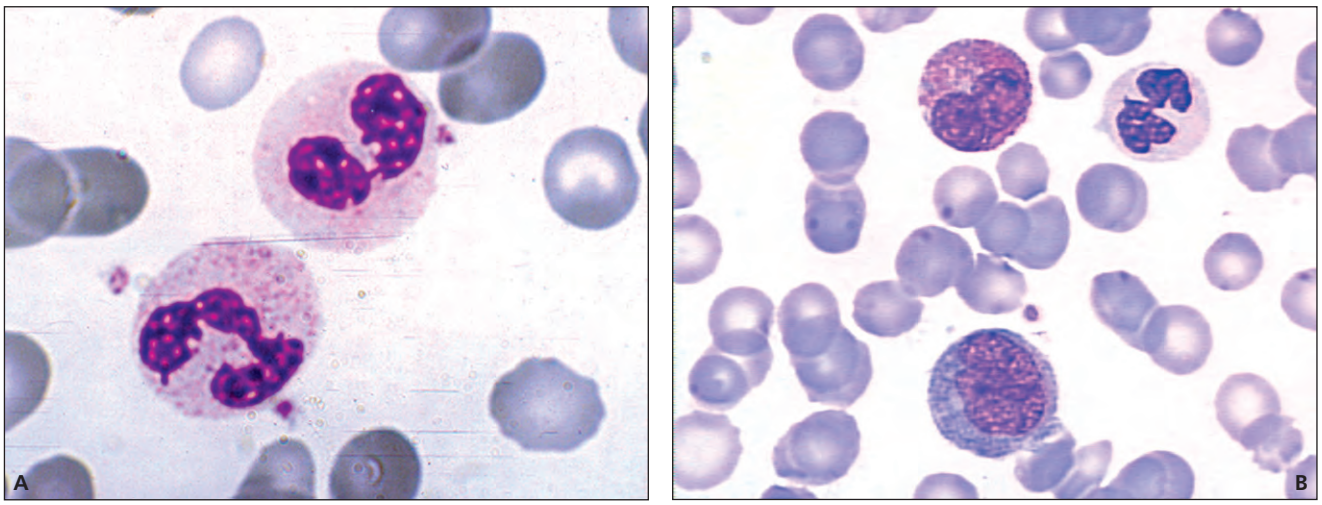



**Fig. 3.13** Absence of cytoplasmic granulation in mature neutrophils as a very specific peripheral blood feature of myelodysplasia. (A) The neutrophil at the top shows, in a single cell, the majority of the morphological features of dysplasia: the cytoplasm is bluish-pink and completely agranular, the nucleus is bilobed (pseudo-Pelger) and has chromatin condensed into very dark masses the colour of an aubergine and, as a non-specific additional feature, there is a short sessile nuclear appendage. The neutrophil lower down also has few granules, standing out from the rose-blue cytoplasm, and has two small nuclear appendages. (B) Top right, the neutrophil has anomalous and incomplete nuclear segmentation but, in particular, it is totally lacking specific granules; its cytoplasm appears completely empty. Beside it, in the centre, is an eosinophil with superimposed nuclear lobes and not much chromatin condensation; its blue cytoplasm is packed with orange granules. At the bottom is a monocyte, also characterised by a dysplastic nucleus, the chromatin of which is anomalous, spongy, like blown glass.

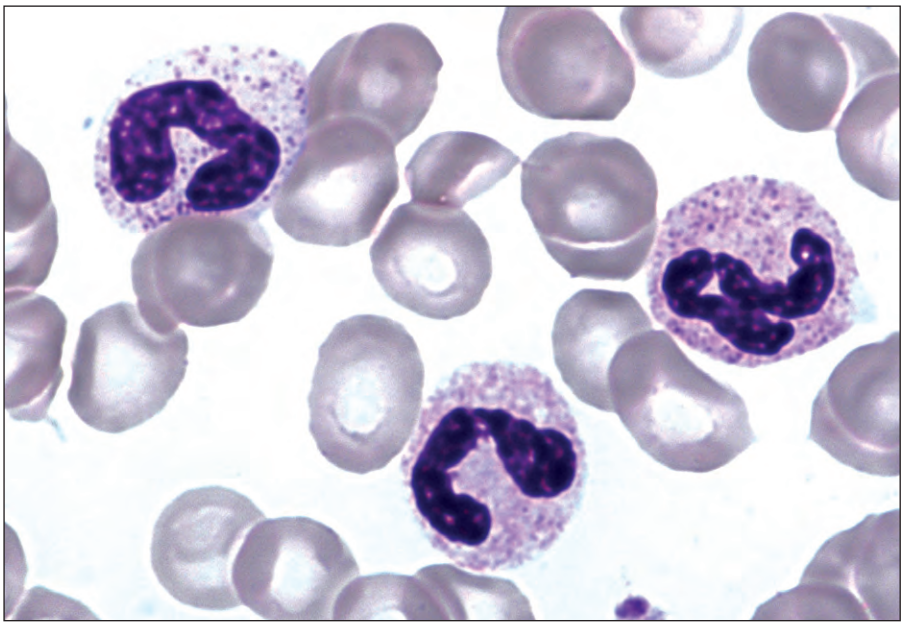


**Fig. 3.14** Dysplastic neutrophils in a buffy coat film from a patient with RAEB-1. The nuclei are hyposegmented, particularly in the cell on the left, even if the chromatin is not as compact as in a typical pseudo-Pelger cell. A basic criterion for dysplasia is hypogranularity: this is almost total in the cell at the bottom, which has finely vacuolated pink cytoplasm; the band cell, top left, is only partially lacking in granules but sufficiently so for it to be recognised as dysplastic (reduction of granules to <30% of the content in a normal neutrophil is the criterion proposed); its granules are clearly seen but they are reduced in number and irregularly distributed, leaving part of the weakly basophilic cytoplasm visible. The cell further to the right has cytoplasm that appears almost normal (a basophilic projection resembles, apart from the strange localisation, a Döhle body), but the nuclear morphology is dysplastic.

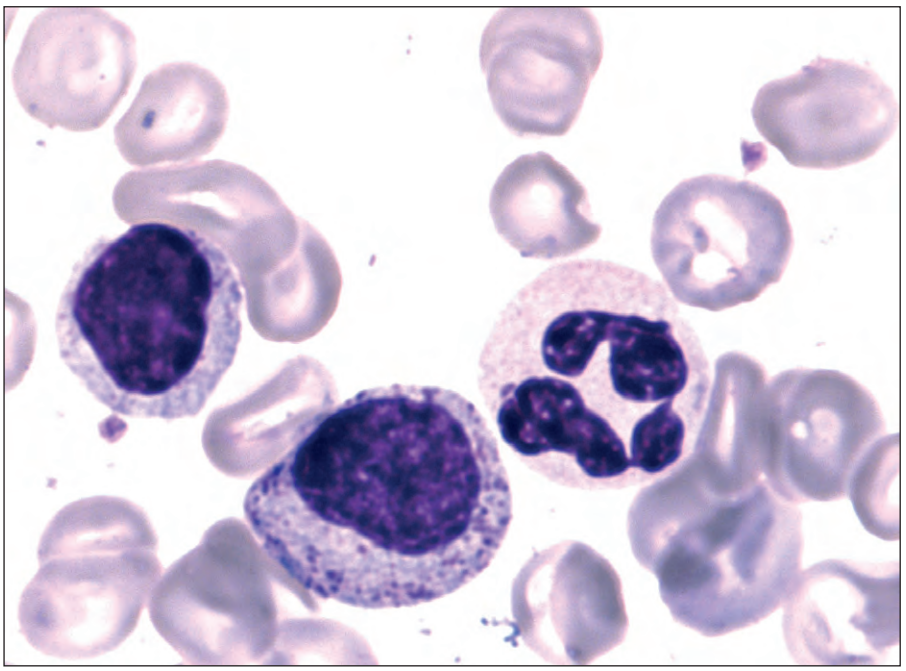


**Fig. 3.15** Granulocytic dysplasia in the peripheral blood. The mature neutrophil on the right has a well-segmented nucleus, with small non-specific nuclear appendages, and a Döhle body on the right, adjacent to the membrane (at 3 o'clock). Its cytoplasm is pink and its granulation is reduced to very fine dust-like granules, almost invisible; this is particularly obvious if a comparison is made with the myelocyte in the centre at the bottom, which has very conspicuous granules, though distributed in a disorderly manner. The cell on the left is certainly a preapoptotic myelocyte: it is very small and the chromatin appears liquefied, almost structureless (as is particularly evident if a comparison is made with the normal chromatin structure of the well-formed nucleus in the myelocyte in the centre); the cytoplasm is bluish-grey, lacking in homogeneity and without visible granules. A hyperchromatic platelet appears to have adhered to the inferior pole of this cell.

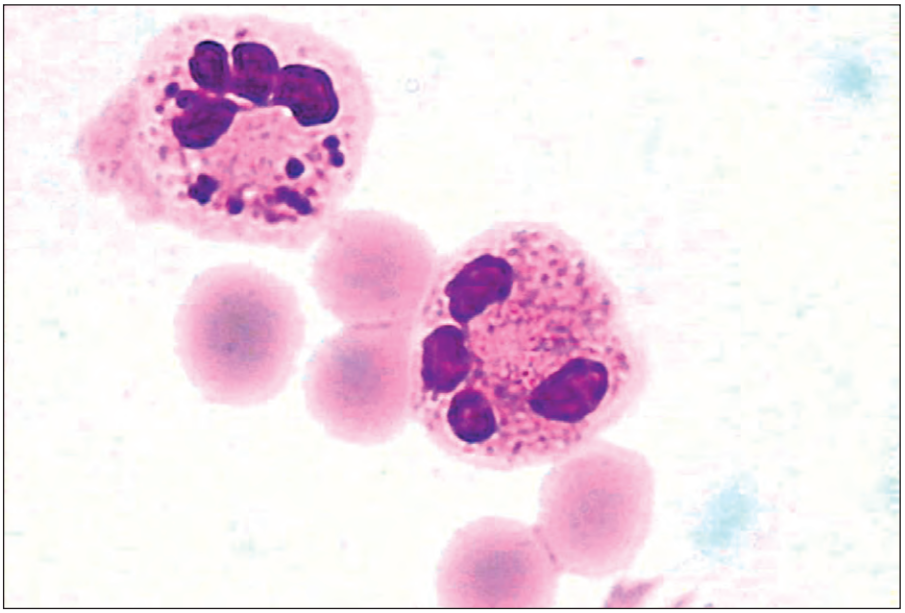


**Fig. 3.16** Preapoptosis and giant granules of pseudo-Chédiak-Higashi type in the peripheral blood film of a patient with RCMD. The cell top left has a nucleus composed of four oval lobes with pyknotic violaceous chromatin, in which the chromatin clumps are almost fused. Within the intensely pink cytoplasm are large, round, blackish structures, of the same colour as the nuclear lobes: these are masses of apoptotic chromatin detached from the fragmented nucleus (as seems to be demonstrated by the presence of a filament that joins some of them to the lobe on the left). Mixed with these, however, are paler granules, of abnormal size, sometimes coalesced, which are scattered through the cytoplasm. The left part of the cytoplasm, overflowing its natural circumference, is forming agranular pink projections or blebs, the phenomenon of zeiosis which has been described above. The neutrophil on the right has similar nuclear characteristics: lobes are composed of pyknotic liquified chromatin (with that on the right appearing to have lost its connection with the other lobes); the cytoplasm is rich in large granules, similar, even if smaller and more numerous, to those seen in the Chédiak-Higashi anomaly; this is most clearly seen in the upper part of the cell. Also in this cell, there is a thin, but clearly outlined, external part of the cytoplasm that appears agranular.

positive, for confirming the myeloid nature of blast cells, particularly when the blasts are agranular. In some cases, in fact, the blast cells appear small and undifferentiated, similar to lymphoblasts or stem cells, but are found to be positive not only for CD34 but also for antigens indicative of myeloid differentiation.

### The megakaryocyte/platelet lineage

In MDS, increased heterogeneity of platelet size is common (platelet anisocytosis), but one can also recognise dysmorphism (in itself not very specific for MDS) (Figs 3.22 through to 3.25). In some cases the blood film shows agranular (grey) platelets or hypogranular platelets, sometimes with a single large granule. Conversely, hyperchromatic or giant platelets may be observed, with a central concentration of dark granules, which can even resemble lymphocytes (Fig. 3.23C). Sometimes one finds in peripheral blood films fairly large strands of megakaryocyte cytoplasm, formed of numerous dysmorphic platelets that have not yet separated from each other (Fig. 3.24). The presence in the circulation of micromegakaryocytes (Fig. 3.25) or 'bare' megakaryocyte nuclei is specific for MDS and other dysplastic conditions, such as some MPN and AML: they are easier to find in buffy coat films. They are more frequent in high risk myelodysplasia and are associated with haemorrhagic phenomena.

## Diagnostic criteria: the bone marrow aspirate

The examination of a bone marrow aspirate in MDS (Table 3.5) most often shows increased cellularity (Fig. 3.26), with hyperplasia of the erythroid or granulocytic series, or both.[63] In 30–40% of cases, cellularity is normal (Fig. 3.27), while in about 10% of patients the bone marrow appears hypocellular. It is not rare in MDS to find non-specific alterations, such as increased lymphocytes, plasma cells or mast cells or, particularly, an increase in haemosiderin-laden macrophages with some haemophagocytosis[64] (see Fig. 3.127).

### The erythroid series

The most frequent appearance is of erythroid hyperplasia with ineffective erythropoiesis. Dyserythropoiesis is of variable severity (Figs 3.28 through to 3.50). Asynchrony of nuclear and cytoplasmic maturation is common, with nuclei having

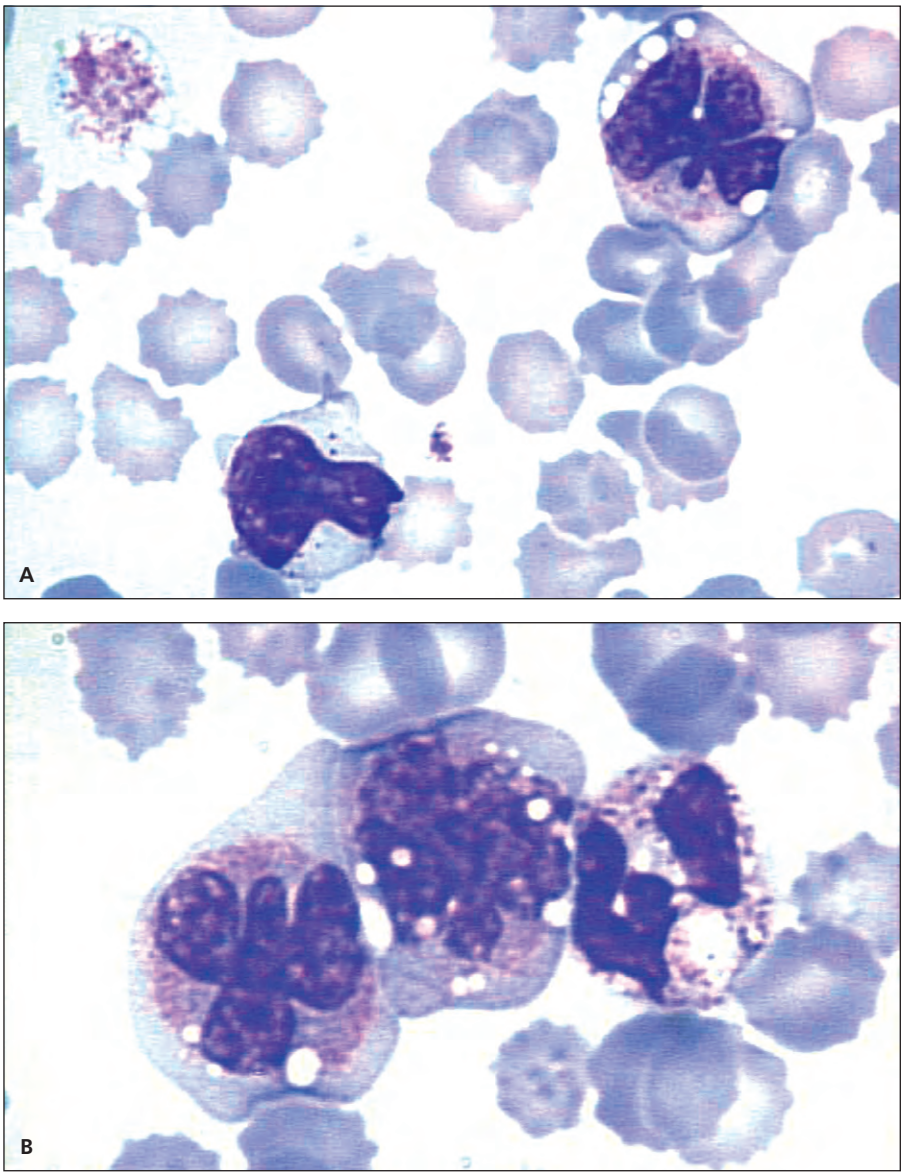



**Fig. 3.17** Severe neutrophil dysplasia in a patient with RAEB-1; peripheral blood film. (A) The strange cell in the top right corner is a dysplastic mature neutrophil. The nucleus is anomalously lobulated, resembling a clover leaf, with three lobes that have little structure to the chromatin, and a sessile appendage that is joined to a short central pivot. The cytoplasm is subdivided into two concentric zones: the internal zone contains very fine granules massed together, which impart a pink tinge to the cytoplasm and appear to be separated from the surrounding external part of the cytoplasm by a sort of internal pseudo-membrane. This outer zone is basophilic with enhanced basophilia at the periphery, and contains nine variably sized, rounded and optically empty vacuoles. The cell adjacent to the lower edge of the image is a large granular lymphocyte, in which a few lysosomal granules stand out clearly from the watery blue cytoplasm. In the top left corner is a giant platelet, larger than the erythrocytes, with a large well-stained chromomere and an external vacuolated sky-blue hyalomere. All the erythrocytes have been transformed into echinocytes. (B) The two neutrophils to the left and in the centre show even greater evidence of the morphological and structural aberrancy described for (A). Particularly in the cell on the left, the separation of the two zones of the cytoplasm is very clearly seen, as is the trilobed form of the nucleus, the shape of which resembles that of an American cactus. These cells show some necrobiotic features, but their morphology clearly shows their derivation from a pathological clone, differing from the neutrophil on the right, which shows minor dysmorphism, including vacuolation and intensely staining granules.

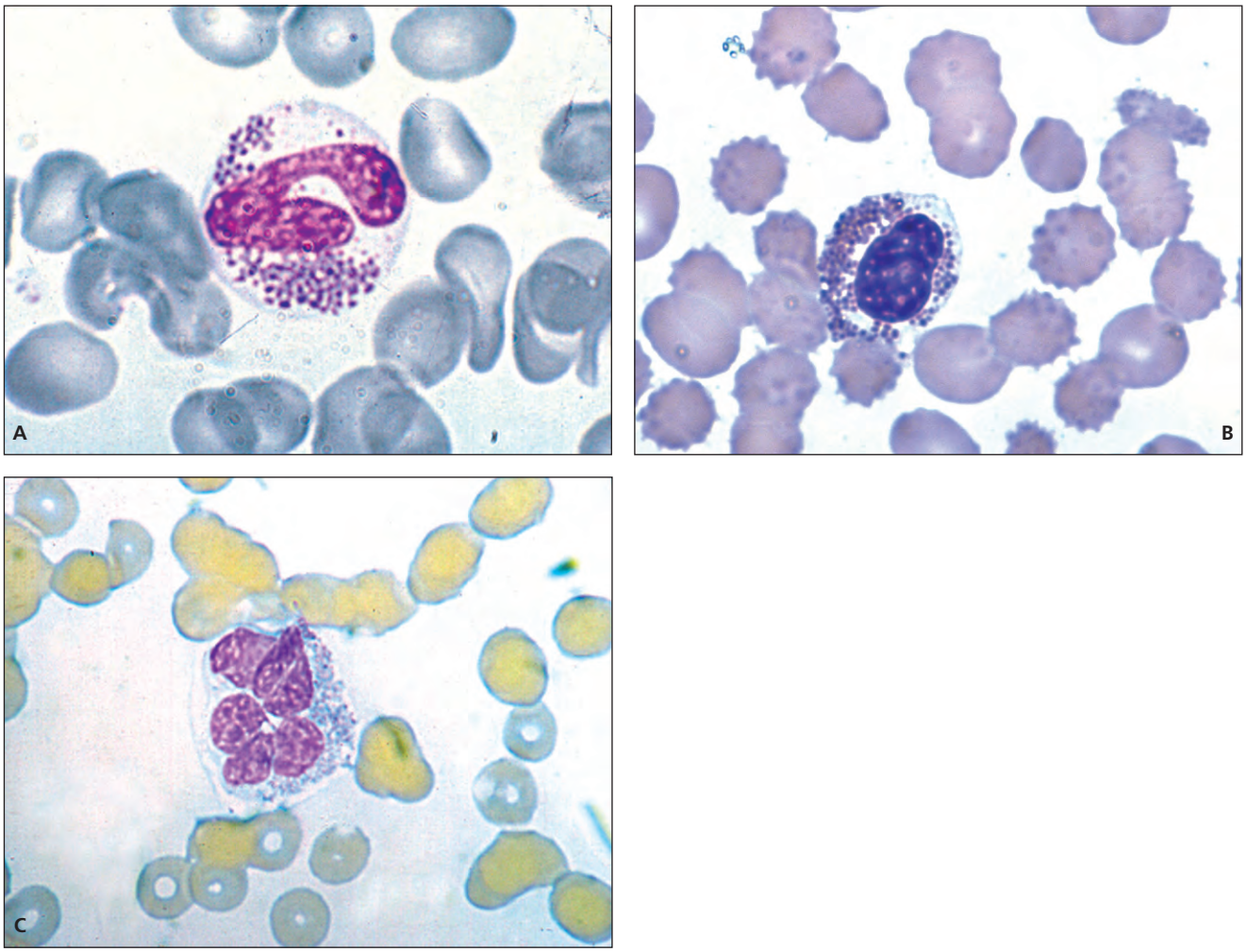



**Fig. 3.18** Dysmorphism and dysplasia of mature eosinophils in a peripheral blood film. (A) An eosinophil band form with partial hypogranularity. A little less than half of the cytoplasm is agranular; the residual granules, massed together in the lower portion and the left part of the cell, appear normal with regard to size, shape and colour. This appearance, with an anomalous quantity and distribution of granules is not specific for dysplasia: eosinophil hypogranularity, with areas of empty cytoplasm, can be a reactive phenomenon or be related to accelerated but effective production of eosinophils in various conditions – allergic, infective, parasitic or neoplastic. (B) Differing from the previous cell, this eosinophil shows the stigmata of dysplasia. The nucleus is irregularly oval, non-segmented, pseudo-Pelger, with strongly condensed chromatin. The cytoplasm remains weakly basophilic in the agranular part, indicating impaired maturation and the persistence of ribosomes; the granules are poorly distributed and are not uniform is size or staining characteristics. (C) This eosinophil has a bizarre nucleus with relatively pale chromatin and numerous small hyperchromatic granules crowded together along the right edge of the greyish cytoplasm.

chromatin that is still immature despite well-haemoglobinised cytoplasm or, more rarely, *vice versa*. Erythroblasts can be binucleate or multinucleate; nuclear dysmorphism is among the most characteristic features of dyserythropoiesis and consists of nuclear protrusions (lobulation), fragmentation or karyorrhexis, dumbbell-shaped nuclei, gigantism and pyknosis. Often there are anomalous mitotic figures, with detachment of one or more chromosomes from the mitotic spindle, these forming Howell-Jolly bodies. Another quite frequent dyserythropoietic feature is the presence of internuclear bridges which join two adjoining nuclei; these can be at the same stage of maturation or, when dysplasia is marked, can be at different stages; the bridges are composed of chromatin. They need to be distinguished from cytoplasmic bridges which are more common and show the persistence of fibres and microtubules of the mitotic spindle that have not yet dissolved; they may be seen even when erythropoiesis is normal and correspond simply to the conclusion of telophase.

Erythroblast cytoplasm can be vacuolated, with vacuoles being variable in size; they are not well defined, thus differing from vacuoles caused by toxins such as alcohol; the vacuoles may represent glycogen and be PAS positive. Sometimes the

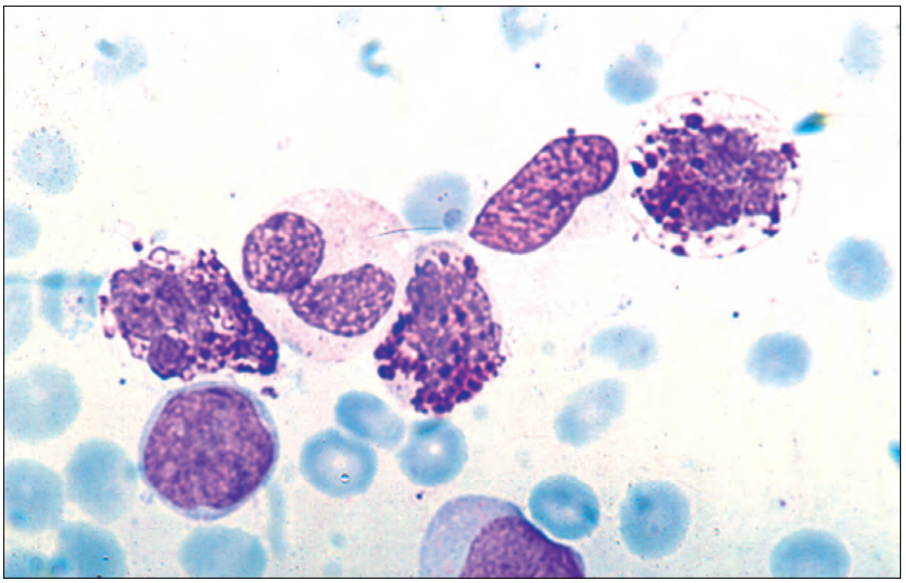


**Fig. 3.19** Basophil granulocyte series. There is no agreed definition of dysplasia for this lineage. Basophils can show various degrees of nuclear dysmorphism and, particularly, reduction and structural alteration of granules; however, these changes are non-specific and can be seen in non-neoplastic conditions such as allergic and inflammatory states and also, particularly, in myeloproliferative neoplasms (MPN) and acute myeloid leukaemia (AML) with a basophil component, often in association with specific cytogenetic abnormalities. Basophils can, however, be increased in number and show morphological alterations in some cases of MDS, as in this case of RAEB-2 and in the case shown in Fig. 3.123. In this image there are three mature basophils showing moderate hypogranularity; the nuclear lobes that are revealed appear more numerous than normal. In the basophil high on the right, in particular, there are few granules and they are gathered around the nucleus, which is in part hidden: the periphery of the cytoplasm, which appears swollen and spherical (probably because the cell is preaptotic), is in effect occupied by a circle of optically empty vacuoles. One also observes: a hypogranular neutrophil with two lobes that have spongy glass-like chromatin; an agranular blast cell with a fine homogeneous network of chromatin, nucleoli and scanty basophilic cytoplasm; and, cut off by the lower edge of the image, the top of a granular blast cell.

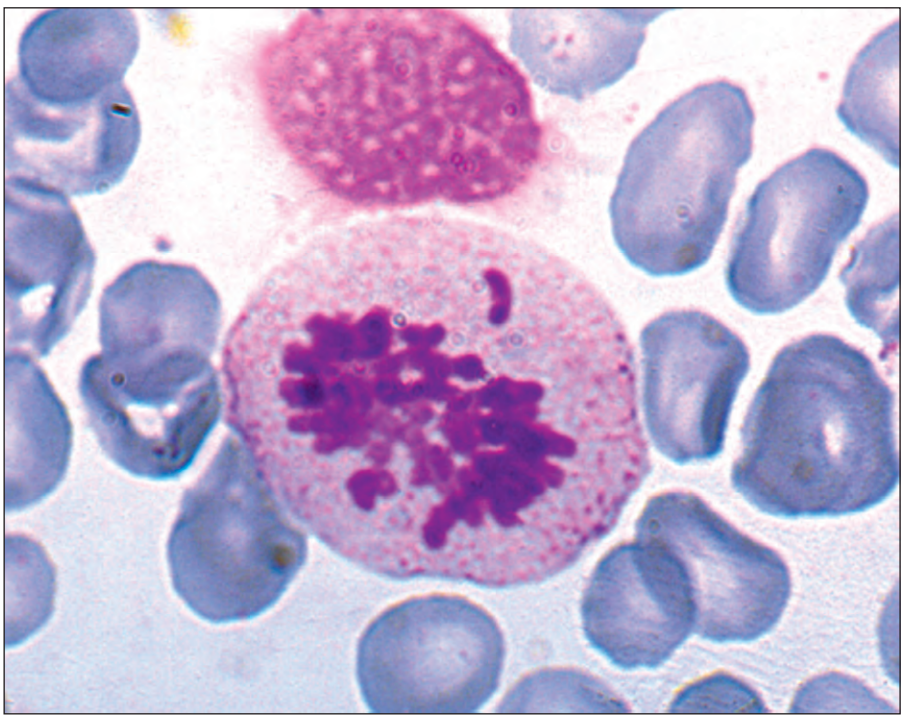


**Fig. 3.20** Granulocyte precursor in a peripheral blood film, blocked in a late stage of mitosis with the chromosomes gathered at two poles of the mitotic spindle; the cytoplasm is poorly granulated. One of the chromosomes, at the top, appears to have escaped from the normal orderly process of mitosis and is isolated and free of the mitotic spindle. This phenomenon can give rise to Howell-Jolly bodies similar to those that are seen when haemopoiesis is dysplastic and also in non-clonal dysmorphism such as that seen in HIV infection and other non-clonal conditions (see Figs 3.130 and 3.131).

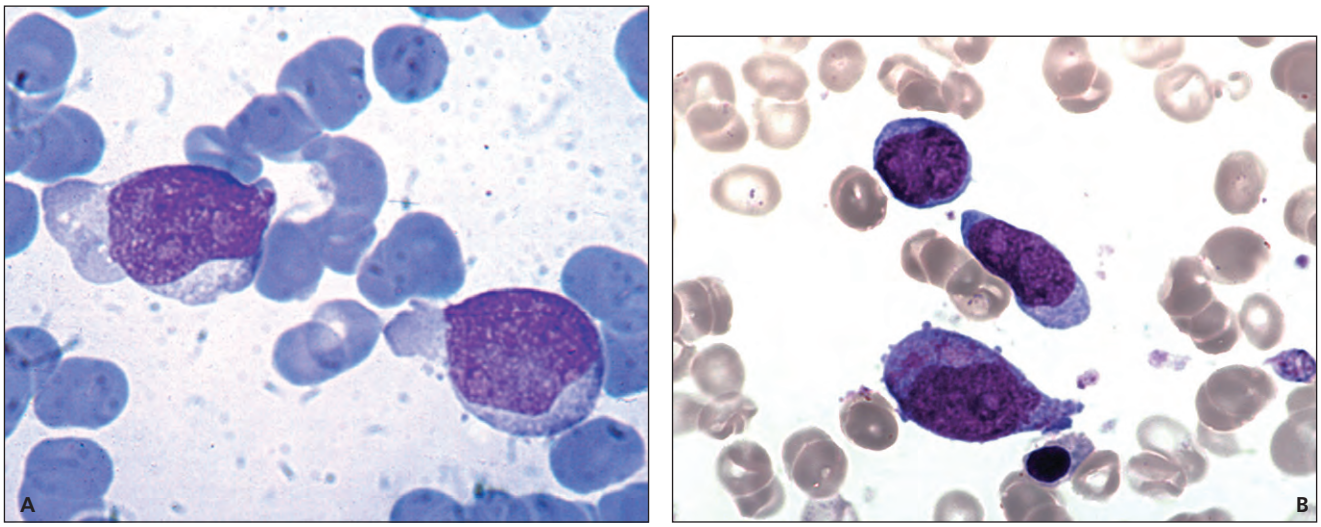



**Fig. 3.21** Blast cells in peripheral blood films of patients with MDS. In applying the 2008 WHO classification, the correct count of blast cells in a peripheral blood film is as fundamental as the count of bone marrow blasts. The peripheral blood count is usually easier because of what a previous generation of haematologists called the *hiatus leucaemicus*, the general lack in the blood of cells at an intermediate level of maturation, such as normal and dysplastic promyelocytes, which not uncommonly in the bone marrow lead to interpretative difficulties. (A) Two agranular blast cells with fairly plentiful cytoplasm elongated into projections in the direction in which the film has been spread; there is intense peripheral reinforcement of basophilia. (B) In the centre and at the top, two agranular blasts with non-homogeneous nuclear chromatin, several nucleoli and intensely basophilic cytoplasm. Lower down is a granular blast cell with more plentiful cytoplasm containing numerous azurophilic granules of variable size, perhaps in part coalescing. Immediately beneath, a small erythroblast with cytoplasm that is still polychromatic and a nucleus that is now completely pyknotic, homogeneous and almost black. On the right, a giant hyperchromatic platelet, blue-violet with some azurophilic granules.

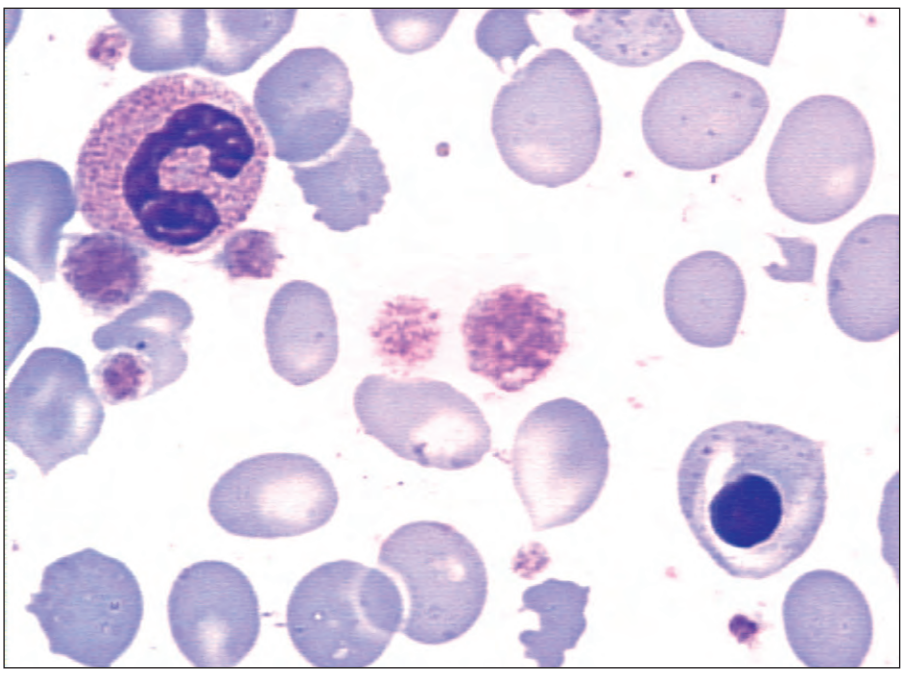


**Fig. 3.22** Multilineage dysplasia in a patient with RAEB-2; buffy coat film. In addition to a neutrophil band form with very dense chromatin and granular cytoplasm, there are platelets showing a range of alterations in size and morphology. The three platelets immediately beneath the neutrophil are large and dense and show, particularly in the giant platelet, a clear distinction between the thin basophilic agranular ragged hyalomere and the central chromomere, formed by a concentration of granules that are not individually distinguishable. In the centre of the image, two platelets that lack a hyalomere, one of which is giant – similar in size to an erythrocyte. In the bottom right corner, immediately below a dysplastic erythroblast (basophilic stippling and presence of a curvilinear haemoglobin-free area), is a small hyperchromatic platelet. The mature red cells in this field also show notable abnormalities such as the presence of hyperchromic red cell fragments, ill-formed dacrocytes and basophilic stippling.

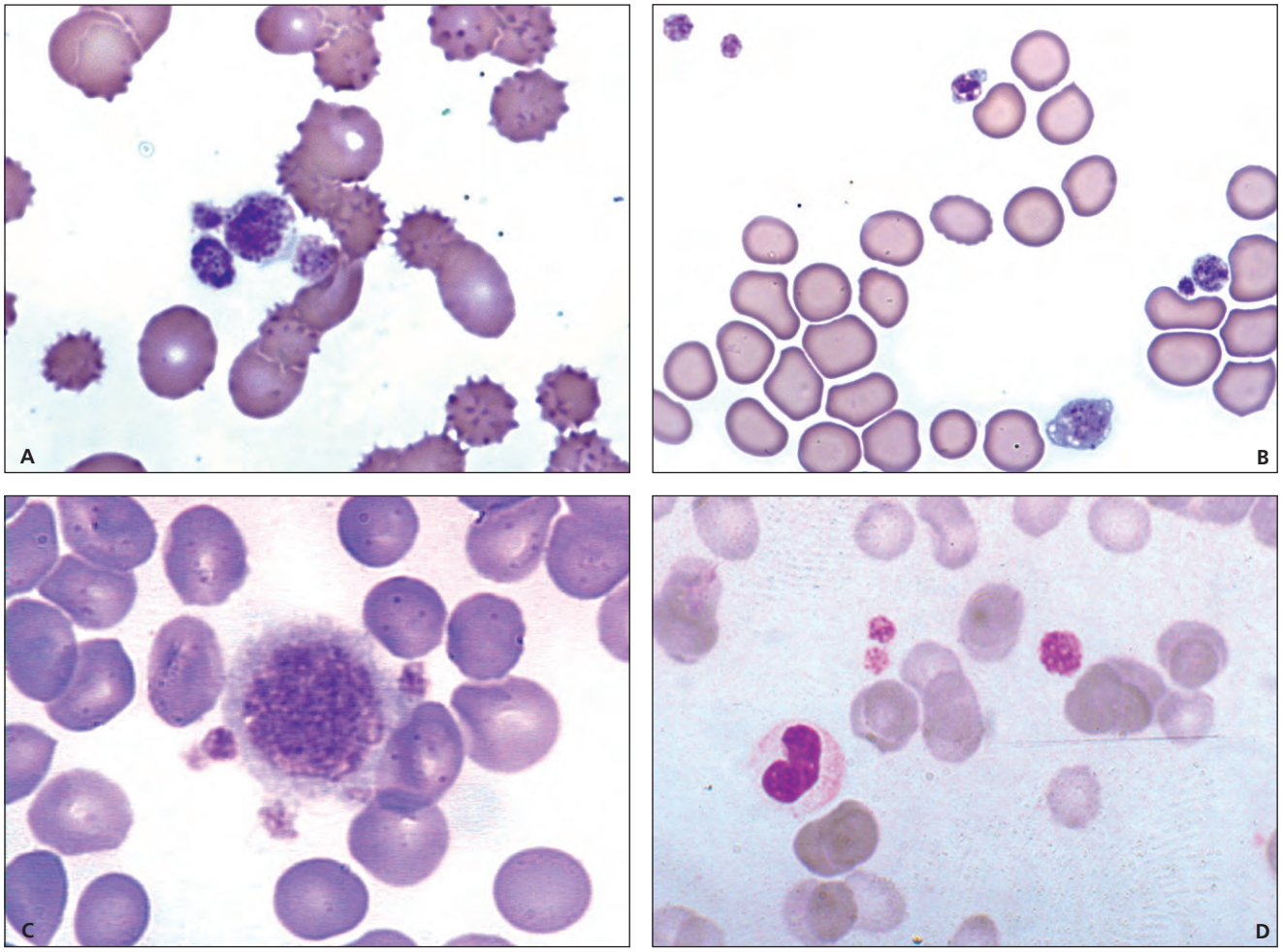



**Fig. 3.23** Platelet dysmorphism in patients with MDS. (A) Platelet anisocytosis, gigantism and hyperchromia. The largest platelet has a visible basophilic hyalomere on the right. Many of the red cells are echinocytic with numerous fine spicules, visible on all surfaces of the cell. (B) Marked platelet anisocytosis and hyperchromia in a patient with thrombocytopenia and unilineage dysplasia affecting the megakaryocyte lineage (platelets have been concentrated). The largest platelet, at the bottom, has a vacuolated hyalomere and a chromomere with dispersed azurophilic granules. Red cell morphology is not evaluable in this very thin area of the film. (C) Giant platelet with an enormous central chromomere, so dense it may be called a pseudonucleus. The distinct boundary between the chromomere and the basophilic hyalomere, which itself has indistinct, delicate peripheral projections, can raise the question of whether one is dealing with a villous mononuclear cell. There are three platelets of normal appearance adherent to the surface of the giant platelet. (D) A hypogranular neutrophil with a hyposegmented peanut-shaped nucleus, and three platelets, one of which appears particularly large and hyperchromic.

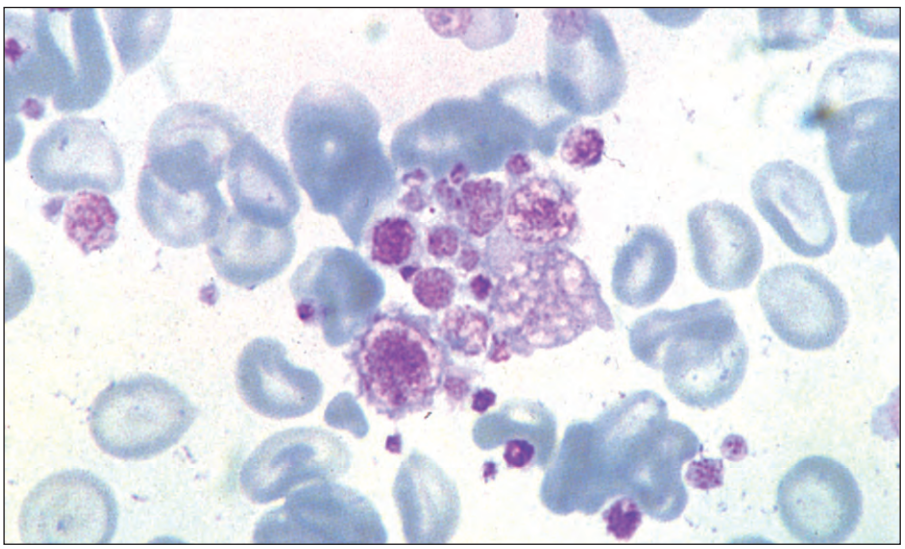
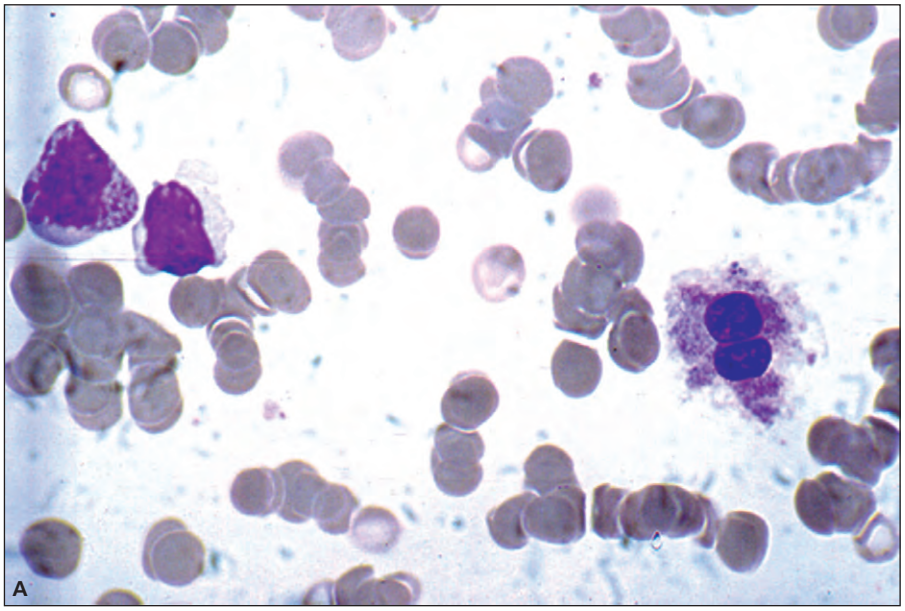
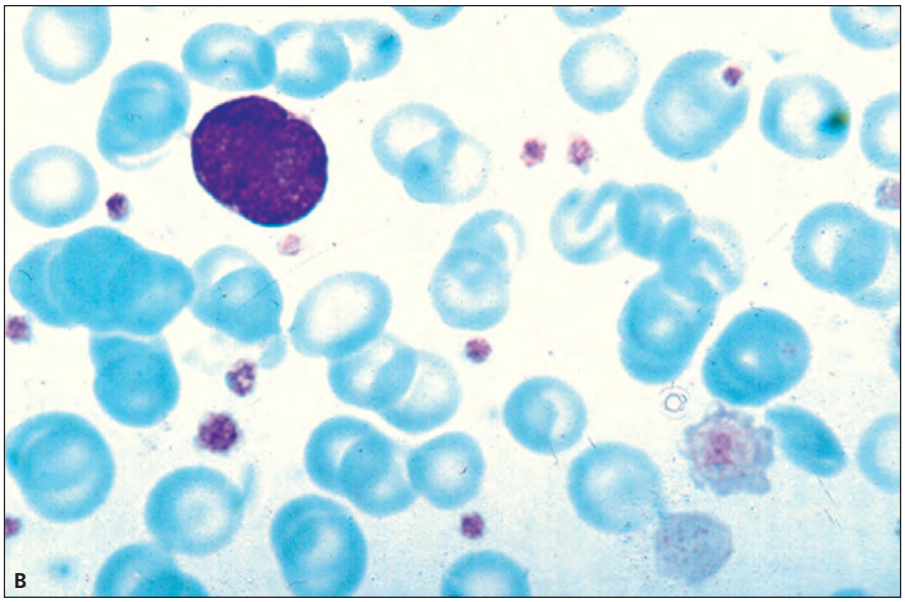


**Fig. 3.24** Platelet dysplasia in a peripheral blood film. A large mass formed of numerous platelets, heterogeneous in size, shape and morphology, clearly distinguishable from each other but nevertheless cohesive: these are not activated platelets that have aggregated but, as one often observes in dysplasia with prominent megakaryocyte involvement, represent a strand of megakaryocyte cytoplasm prematurely liberated into the circulation by a dysplastic megakaryocyte.



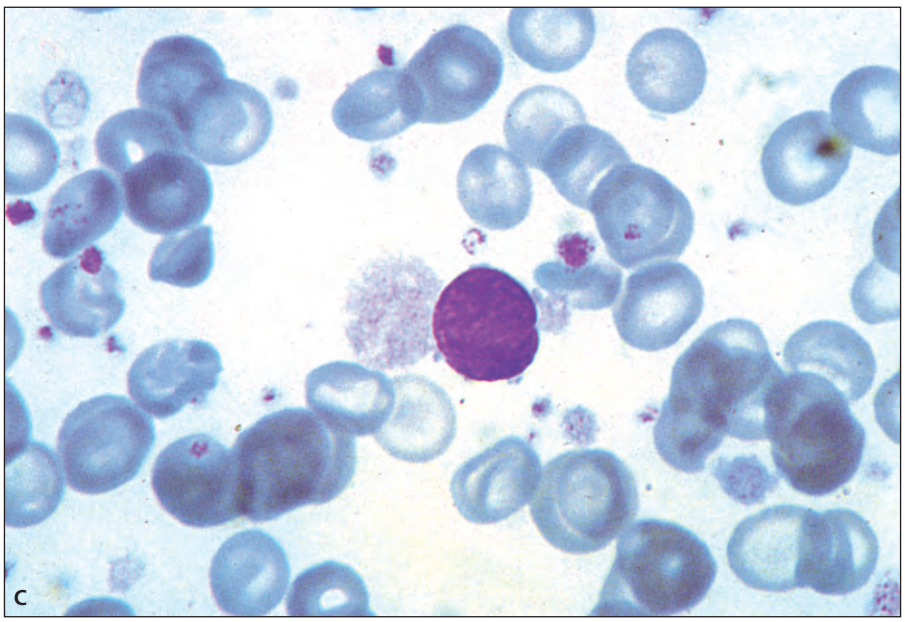




**Fig. 3.25** Micromegakaryocytes in peripheral blood films of patients with various types of MDS. (A) Adjacent to the right-hand edge of the image, a typical micromegakaryocyte, with a nucleus formed of two closely related lobes with very dense chromatin, but still having a structure, represented by small pale spaces. The cytoplasm is very granular and is forming projections with indistinct limits, in an attempt at platelet production that is probably ineffective. On the left, a large lymphocyte and an immature granulocyte with a disorderly distribution of granules. There is rouleaux formation and weakly basophilic background staining, as in patients with a paraproteinemia. (B) Top left, a micromegakaryocyte has a hyperchromatic bilobed nucleus which appears to still be surrounded by a very thin rim of cytoplasm, more easily seen within the two symmetrical notches in the nucleus. There are numerous normally sized hyperchromic platelets and, in the bottom right corner, two pale giant platelets, one with a small chromomere; the other could be called a grey platelet if it were not for the six or seven azurophilic granules scattered through the hyalomere. (C) Another typical micromegakaryocyte, with a round nucleus similar to that illustrated in (B), condensed chromatin, cleaved by a deep horizontal incision that is difficult to visualise but produces two lobes. The cytoplasm is very scanty, greyish with a few fine azurophilic granules.

**Table 3.5** Bone marrow morphology in haemopoietic dysplasia

**Bone marrow aspirate (cytology)**
- Cellularity:
  - Increased (50–60%)
  - Normal (30–40%)
  - Reduced (10–15%)
- Non-specific bone marrow alterations:
  - Increased macrophages (haemosiderin, detritus, nuclei, granules)
  - Haemophagocytosis
  - Lymphocytes, plasma cells, mast cells
  - Fibrin, necrosis
- Morphological alterations specific for dysplasia:
  - Erythroid series
  - Granulocytic (and monocytic) series
  - Megakaryocyte series
  - Blasts
  - Iron deposits
- Very variable myeloid:erythroid ratio:
  - Erythroid hyperplasia
  - Erythroid hypoplasia
  - Normal relationship
- Number of megakaryocytes:
  - Normal or increased
  - Reduced

**Bone marrow biopsy (histology)**
- Evaluation of cellularity
- Disturbance of bone marrow architecture:
  - Myeloblasts in the intertrabecular spaces and abnormal localisation of immature precursors (ALIP)
  - Erythroblasts and megakaryocytes in a paratrabecular position
  - Monomorphic erythroblast islands
- Evaluation of fibrosis

**Erythroid dysplasia**
- Asynchronous nucleocytoplasmic maturation
- Nuclei:
  - Binuclearity or multinuclearity
  - Nuclear lobulation or budding
  - Nuclear hyperlobation
  - Karyorrhexis (fragmentation)
  - Karyoschisis (fissuring)
  - Gigantism
  - Early pyknosis
- Mitosis:
  - Anomalous mitotic figures
  - Detachment of chromosomes from the mitotic spindle (Howell-Jolly bodies)
  - Cytoplasmic bridges
  - Internuclear bridges
- Cytoplasm:
  - Irregular staining, irregular distribution of haemoglobin
  - Vacuoles
  - Basophilic stippling
- Megaloblastoid appearance, asynchronous maturation
- Monomorphic erythroid islands
- Positivity of erythroblasts for PAS, either diffuse or granular
- Perls stain:
  - Increased storage iron (not always)
  - Increased sideroblasts:
    Type I sideroblasts (<5 granules)
    Type II sideroblasts (≥5 granules)
    Type III sideroblasts, ring sideroblasts
    (≥5 granules; >⅓ nuclear circumference surrounded)
    (≥15% type III: RARS)

**Granulocyte dysplasia**
- Nucleus:
  - Lobularity of mature neutrophils:
    Hyposegmentation (pseudo-Pelger)
    Irregular hypersegmentation
  - Chromatin density:
    Increased pyknosis
    Reduced – spongy
  - Howell-Jolly-like bodies
  - Irregular nuclear appendices
- Cytoplasm:
  - Reduced or increased cell size
  - Reduced or absent secondary granules (mature neutrophils)
  - Reduced or absent azurophilic granules (immature granulocytes)
  - Disorderly distribution of granules
  - Coarse, confluent (pseudo-Chédiak-Higashi)
  - Very strongly staining granules
  - Vacuoles
  - Irregularity or absence of the Golgi zone
  - Persistence of peripheral basophilia
  - Deficiency of peroxidase (or other enzymes)
  - Auer rods
- Asynchronous maturation:
  - Myelocytes with nucleoli
  - Cytoplasm appears swollen
- Clasmatosis (cytoplasmic protrusions)
- Dysplasia of eosinophils and basophils

**Megakaryocyte dysplasia**
- Micromegakaryocyte:
  - Small nucleus with dense chromatin, mature or pyknotic
  - Scanty cytoplasm, variable maturity
  - Anomalous platelet formation
- Multiple separate nuclei (multinucleated):
  - Abundant cytoplasm, usually mature
  - Normal or abnormal platelet formation
- Hypolobated or non-lobated nucleus, single, rounded:
  - Eccentric
  - Mature cytoplasm, well differentiated chromatin, not pyknotic
  - Platelet formation usually normal
- Hypogranular cytoplasm, weakly basophilic
- Dysmorphism of various types:
  - Bizarre nuclei, hyperlobulated
  - Bare nuclei
  - Hypertrophic Golgi
  - Atypical platelet formation
  - Dwarfism and/or gigantism
  - Tendency to cluster

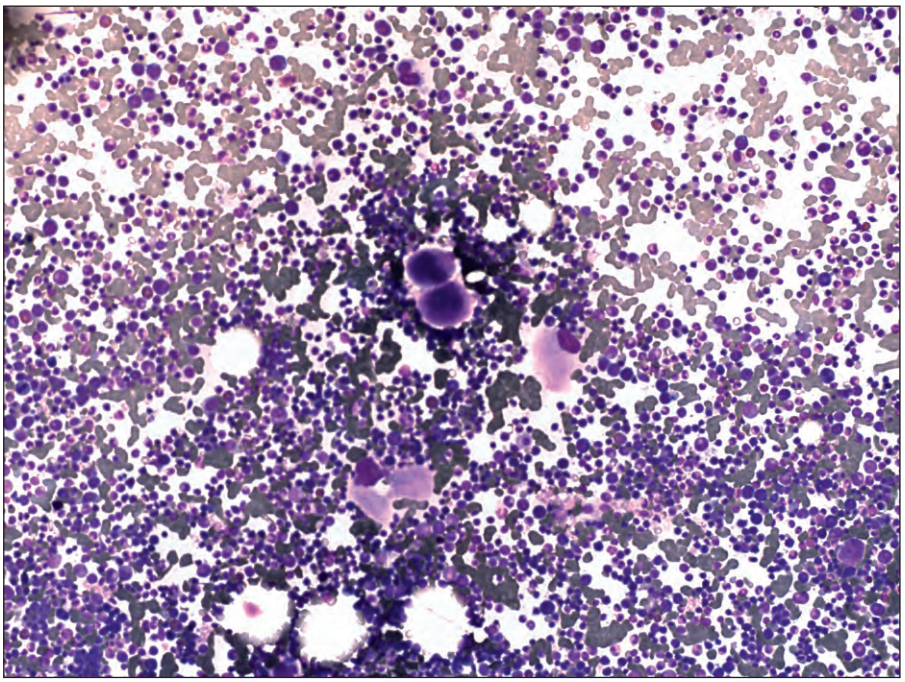


**Fig. 3.26** Bone marrow aspirate, low power, from a case of RCMD. The cellularity appears increased, but the composition is variable with retention of small optically empty circles of fat: this is thus a very different picture from that seen in cases of acute leukaemia in which there is hypercellularity attributable to a monomorphic infiltrate that has completely invaded and replaced the bone marrow. The crowding together of cells is greater in the lower part of the image, which represents the first part of the trail left at the edge of a bone marrow fragment during spreading. The density then reduces in semicircles moving upwards and in this area it is easier to recognise that there are cells belonging to various lineages: e.g. the largest, dark blue cells are immature erythroblasts, while the smaller cells with band-shaped or lobulated nuclei and pink cytoplasm belong to the neutrophil lineage. At this magnification naturally it is not possible to recognise dysplastic features in this lineage. Instead, one should concentrate on the megakaryocytes (or on any other large cells that are present), assessing both their number and their morphology. Here one can observe, around the centre, a small group of megakaryocytes that do not show any dysplastic features: the two less mature ones are adhering to each other and have cytoplasm that is partly obscuring the nuclei and is still basophilic, but is developing an acidophilic tinge towards the periphery; the two more mature cells, at the bottom, have nuclei that can be clearly seen and appear lobulated, in an eccentric position within plentiful, pink, completely differentiated cytoplasm. A smaller megakaryocyte with scanty pink cytoplasm is seen in proximity to the upper edge, close to the centre, while an immature basophilic megakaryocyte precursor, probably a promegakaryocyte, is seen just above the bottom right corner.

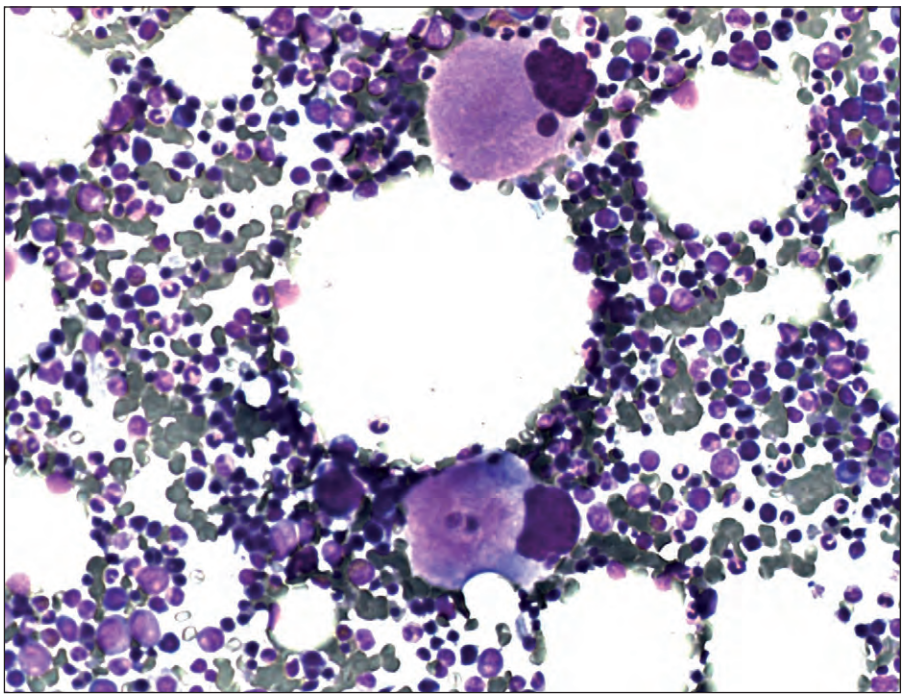


**Fig. 3.27** Bone marrow aspirate from a patient with RAEB-1. At an intermediate power the cellularity appears quantitatively normal. There are numerous fat spaces and two differentiated megakaryocytes with hyperdiploid nuclei and totally mature cytoplasm without any signs of dysplasia. The megakaryocyte at the top has all its nuclear lobes fused or close together, with the exception of one that appears almost detached, like a falling drop; the other, beneath the large clear circle left by an adipocyte, has less homogeneous cytoplasm with large areas of peripheral basophilia and shows emperipolesis of a neutrophil with a bilobed nucleus. At this magnification one can start to appreciate morphological details which permit neutrophil precursors to be distinguished, e.g. in the bottom left corner, where cells with large nuclei and a pale paranuclear Golgi zone can be distinguished. Close to the right edge, a plasma cell with an eccentric nucleus, blue cytoplasm and a large pale paranuclear Golgi zone.

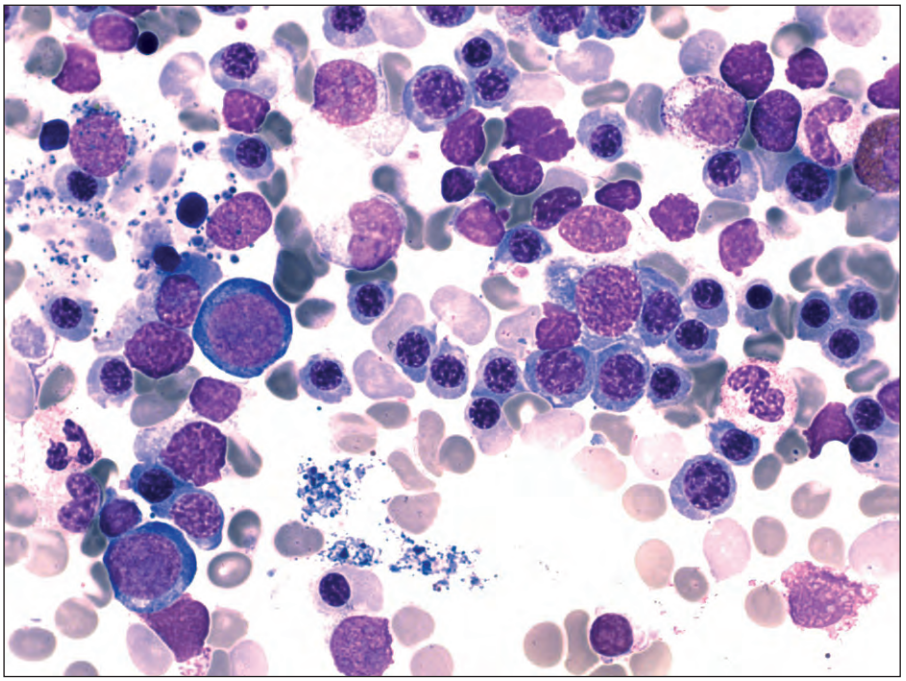


**Fig. 3.28** Erythroid hyperplasia with signs of asynchronous maturation; bone marrow aspirate from a patient with refractory anaemia. The marrow appears hypercellular with a clear predominance of proerythroblasts and erythroblasts at all stages of maturation. There is a generalised moderate megaloblastoid tendency, evident in the finely stippled chromatin pattern of the two large proerythroblasts on the left, recognisable not only from their size but also from the roundness of their nuclei and the intense blue colour of their cytoplasm with paler arcoplasm. Furthermore, the nuclei of all the basophilic, polychromatic and orthochromatic erythroblasts appear to show slight retardation of maturation in relation to the degree of basophilia and haemoglobinisation of the cytoplasm. This nucleocytoplasmic (N:C) asynchrony is evident, e.g. in the small isolated erythroblast at the bottom towards the centre, between a proerythroblast on the left and a lymphocyte on the right: in this cell the nucleus still shows the presence of pale spaces between the chromatin clumps, but the cytoplasm is orthochromatic. In the upper part of the image towards the left corner, one can recognise the round and oval nuclei with a spongy chromatin network of three macrophages, with traces of granular blue-grey cytoplasmic debris, which are adhering to a number of erythroblasts and free pyknotic nuclei.

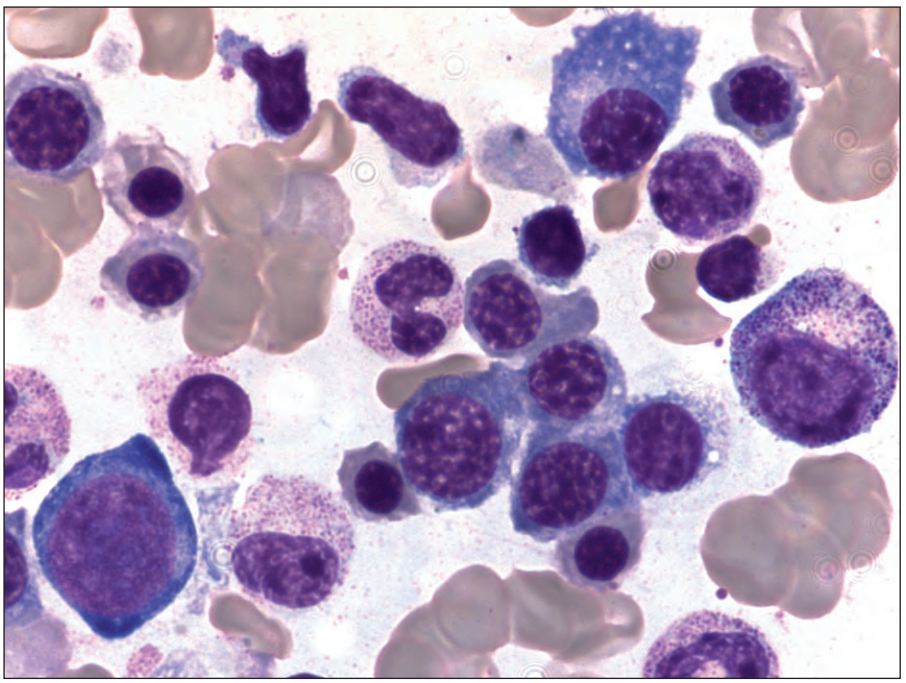


**Fig. 3.29** Erythroid maturation without marked dyserythropoiesis in a case of MDS; bone marrow aspirate. In the bottom left corner, a proerythroblast with a nucleus that has a delicate chromatin pattern and contains a number of ill-defined, pale blue nucleoli. The small group of basophilic and polychromatic erythroblasts in the centre show some delay in maturation; the two orthochromic erythroblasts at the bottom of this cluster are joined by a long thin cytoplasmic bridge, so taut that it is deforming and stretching the cytoplasmic membrane. In the top left corner, an orthochromatic erythroblast shows clear linear defects in the staining, representing defective haemoglobinisation. Along the upper border, two lymphocytes and a mature plasma cell. In the centre of the right edge, a promyelocyte shows non-dysplastic maturation; it has an eccentric nucleus and a large, very pale paranuclear Golgi zone from which granules spread out in an orderly manner, the general appearance resembling a rising sun which illuminates the 'night' of the surrounding basophilia. Adjacent to an erythroblast, on the opposite side, a myelocyte with a small nucleus and a residual weak background basophilia (retarded maturation of the cytoplasm, a phenomenon that is the inverse of megaloblastoid maturation); another myelocyte immediately above has an apoptotic liquefied nucleus.

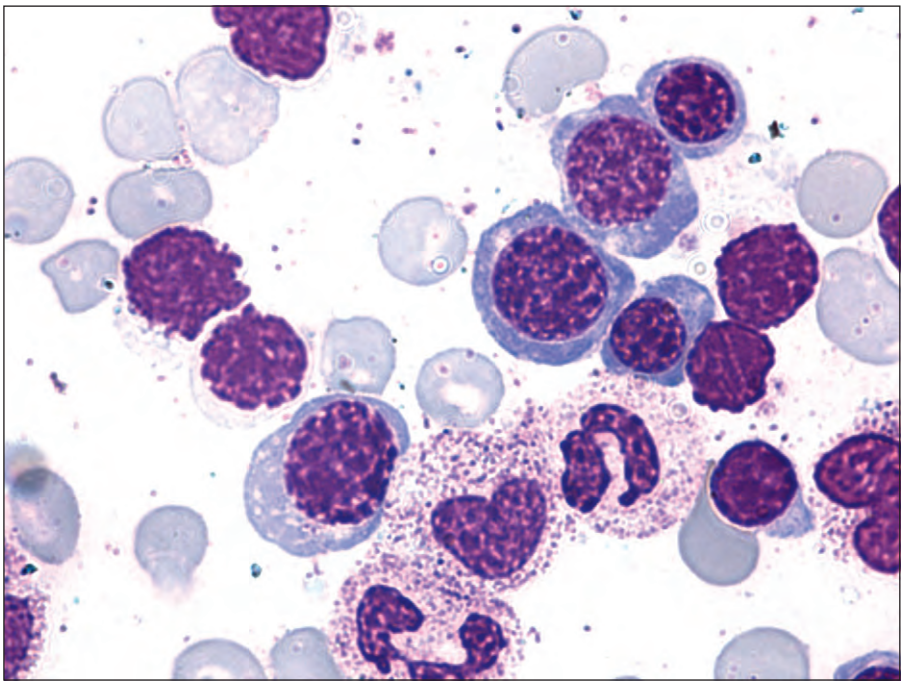


**Fig. 3.30** Asynchronous maturation in refractory anaemia; bone marrow aspirate. The nuclei of the erythroblasts are round with a fairly high N:C ratio; the chromatin is condensed into blocks, characteristic of the basophilic erythroblast stage, while the cytoplasm already shows the variation in staining attributable to the synthesis of haemoglobin.

cytoplasm stains irregularly, in blocks separated by white spaces, fissures or arcs, resulting from a defect in haemoglobinisation or from the persistence of non-staining residues of degenerating structures, such as membranes of the endoplasmic reticulum; basophilic stippling, representing residues of RNA, is often observed, as are Howell-Jolly bodies. In some cases, although not often, erythropoiesis is frankly megaloblastic, with marked asynchrony and notable nuclear immaturity, the chromatin being much finer and more delicate than normal. In regard to megaloblastic erythropoiesis, one cannot overestimate the importance of a correct differential diagnosis between MDS and megaloblastic anaemia resulting from vitamin $B_{12}$ and/or folic acid deficiency; this is not always easy since both may have severe trilineage dysplasia. The presence of macrocytes and hypersegmented neutrophils in the peripheral blood are important features directing one's attention towards investigation of a suspected deficiency state.

In bone marrow aspirates of patients with MDS one often observes islets of erythroblasts (Figs 3.51 through to 3.54), in which the cells have all reached the same stage of maturation, as a result of dissociation of the processes of proliferation and maturation. Typically, the erythroblasts of MDS are PAS positive, with diffuse or finely granular positivity. Diagnosis of MDS requires a Prussian blue stain for iron, which permits quantification of iron deposits in the stromal cells and in erythroblasts (type I or type II sideroblasts, depending on the number of granules) and/or in siderocytes. Ring sideroblasts, in which iron is deposited within mitochondria, must be carefully identified and enumerated in order to assign a case to the appropriate subtype of MDS. From the strictly morphological point of view, they must be considered ring forms (type III) when there are five or more siderotic granules that surround at least a third of the nuclear circumference.[65–67]

Patients with MDS have an increased number of apoptotic cells in comparison with healthy subjects: a reduced frequency of apoptotic cells, associated with a reduction in erythroid hyperplasia, correlates with a favourable response to treatment with growth factors and erythropoietin.[68]

## The granulocytic series

Dysplasia in granulocyte precursors can be absent or very subtle in comparison with dyserythropoiesis, particularly in refractory anaemia (RA), and even more so in RARS. In other cases, conversely, abnormalities in granulopoiesis are the dominant morphological abnormality, with abnormalities of the nuclei or cytoplasmic granules or marked asynchrony of maturation (Figs 3.55 through to 3.68). In obvious cases, the myelocytes appear strikingly hypogranular because of the absence of secondary granules, while an excess of ribosomes conveys an unusual peripheral basophilia to the cytoplasm. Some promyelocytes may lack primary granules, while in others the granules

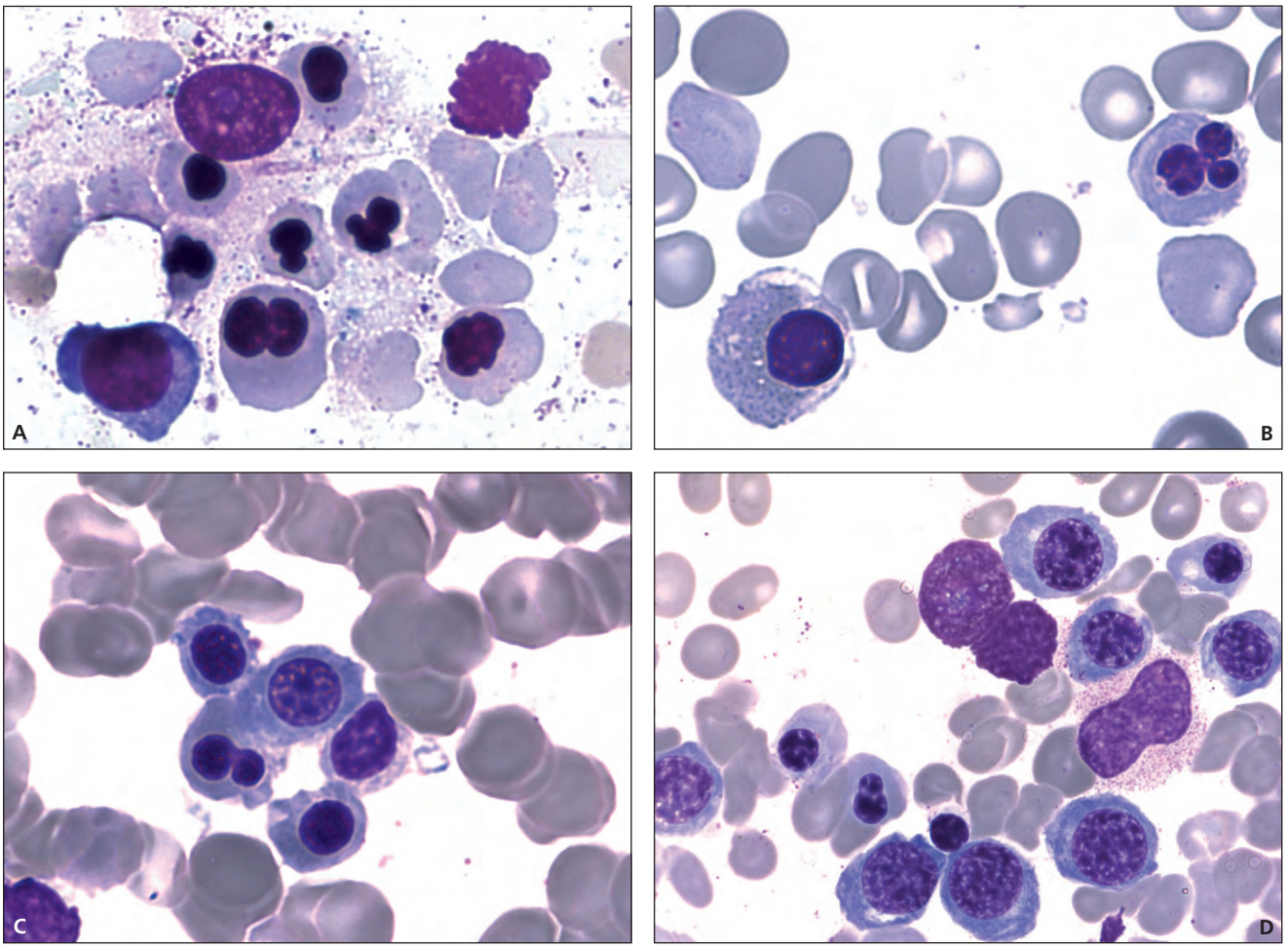



**Fig. 3.31** Marked dyserythropoiesis in bone marrow aspirates of patients with MDS showing, in particular, lobulation of the nuclei of orthochromatic and polychromatic erythroblasts. (A) The nuclei of these mature erythroblasts are multilobated with very dense chromatin, which only in the largest cells still retains some paler spaces. The cytoplasm has a polychromatic tinge, which can be appreciated by comparing these cells with the mature erythrocytes that are partially visible in the bottom right corner. The N:C ratio is very variable, giving the impression of markedly disordered maturation. The largest cell, bottom left, could suggest a plasma cell: however, it is in fact a deformed basophilic erythroblast as is indicated particularly by the shape and position of the Golgi zone, elongated and paranuclear, a type of arcoplasm. At the top, a macrophage with an oval spongy nucleus with prominent dark violet nucleoli; its cytoplasm is spreading over the background, insinuating itself in the form of greyish granular tentacles between the red cells along the left-hand edge and between the deformed erythroblasts towards the centre and lower down, suggesting interaction of the type known as rhopheocytosis. (B) To the right, a polychromatic erythroblast with a trilobed nucleus, of a shape that resembles Mickey Mouse but with asymmetrical ears. Its cytoplasm is not homogeneous but has pale linear structures that tend to interrupt the continuity of the cytoplasmic staining resulting from haemoglobinisation. Bottom left, beside a stomatocyte, another polychromatic erythroblast with a well-formed nucleus and plentiful cytoplasm showing fine basophilic stippling, some microvacuoles and an extensive non-haemoglobinised area along the internal surface of the cytoplasmic membrane. (C) A small group of polychromatic and orthochromatic erythroblasts which show a range of degrees of chromatin condensation: from the presence of distinct masses, like lumps of coal, surrounded by a thick round nuclear membrane in the largest cell, to the almost total liquefaction of the chromatin structure with massive condensation, as in the orthochromatic cell lower down and the bilobed cell more to the left. The cell to the right, similar to a lymphocyte with regard to the shape and the violet colour of the chromatin, is actually a deformed preapoptotic erythroblast. (D) This focus of erythroblasts shows more orderly progression of the morphological changes that occur with erythroid maturation, but in a general context of asynchrony due to more rapid maturation of the cytoplasm. At the bottom, three basophilic erythroblasts with round nuclei, regularly clumped chromatin and clearly visible arcoplasm within cytoplasm that is starting to change colour. The two erythroblasts to the right are joined by a thin internuclear bridge. Upper right, above a degenerating metamyelocyte, are erythroblasts – three polychromatic and one orthochromatic with a round nucleus. Towards the left, other orthochromatic erythroblasts, one of which has a lobulated nucleus, and a pyknotic nucleus, extruded from a newly formed erythrocyte and retaining only a thin rim of cytoplasm.

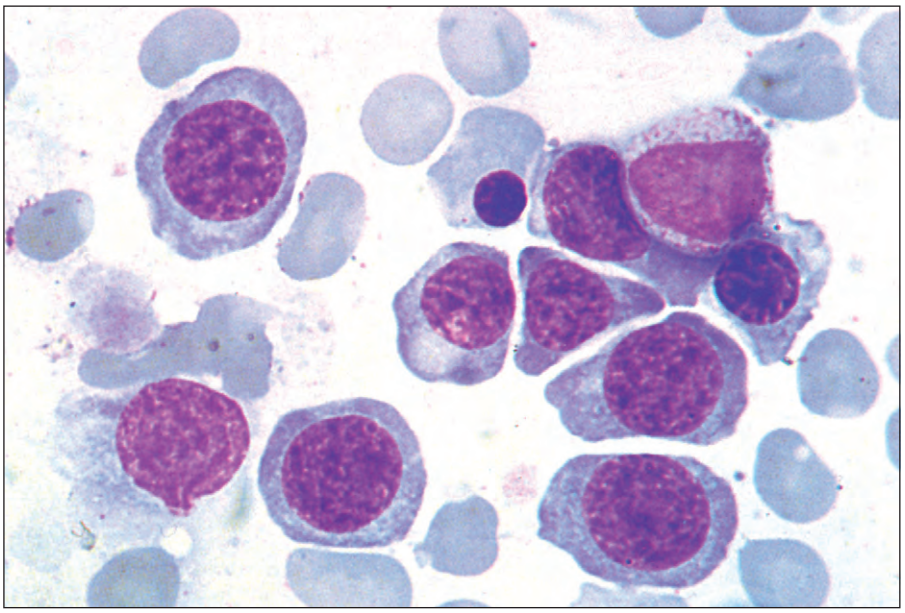


**Fig. 3.32** Dyserythropoiesis: asynchronous maturation; bone marrow aspirate. The seven larger erythroblasts have cytoplasm of intermediate maturity, which allows them to be defined as polychromatic; it is greyish as haemoglobin synthesis has already started. In some cells one can still see the pale paranuclear Golgi zone. The nuclear morphology, however, is more similar to that of the preceding stage of the basophilic erythroblast, with a round nucleus that is generally central and with foci of condensed chromatin now grouped into fairly large clumps, alternating with pale areas, creating a fairly heterogeneous overall picture. The proximity of a number of identical erythroblasts, apparently blocked at the same stage of maturation, is a frequent and important sign of dyserythropoiesis. An eighth polychromatic erythroblast seen further to the right is different from the others since it has a more mature nucleus with chromatin showing more condensation, better synchronised with the cytoplasm. Above it, a small hypogranular neutrophil metamyelocyte and, high up in the centre, an erythroblast with almost orthochromatic cytoplasm with a small nucleus that is about to be extruded, but which still retains some structural heterogeneity (pyknosis is incomplete).

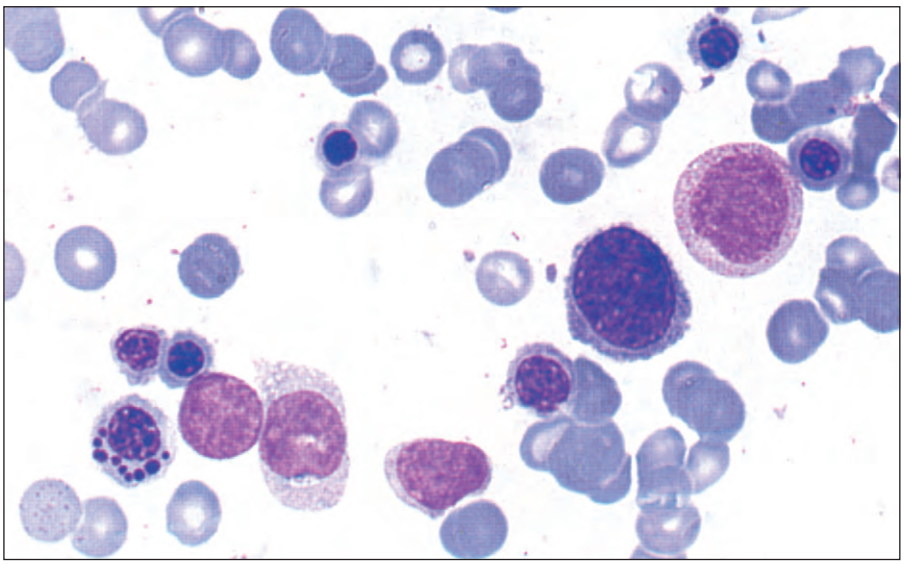


**Fig. 3.33** Dyserythropoiesis: nuclear anomalies; bone marrow aspirate. There are eight erythroblasts, the cytoplasm of which is almost identical to that of erythrocytes (orthochromatic), while the nuclei are more heterogeneous with atypia of various types. In some the nucleus is round with structured chromatin with paler areas that separate dark clumps – thus, not yet pyknotic and homogeneously dark as they should be in orthochromatic erythroblasts; in one of these asynchronous erythroblasts (bottom left corner) the lower half of the nuclear outline is surrounded by a necklace of chromatin masses that originated in a dysplastic mitosis: this phenomenon is known as karyorrhexis. In the small isolated erythroblast in the upper area, the chromatin, conversely, is pyknotic. Lower down, to the left of a neutrophil promyelocyte with a large nucleus and scanty, finely granulated cytoplasm, is a large erythroblast that shows in a single cell all the most typical features of severe dyserythropoiesis: the nucleus is gigantic, hyperdiploid, with chromatin irregularly condensed, enclosed within a distinct, regularly oval nuclear membrane, with cytoplasm that is almost orthochromatic.

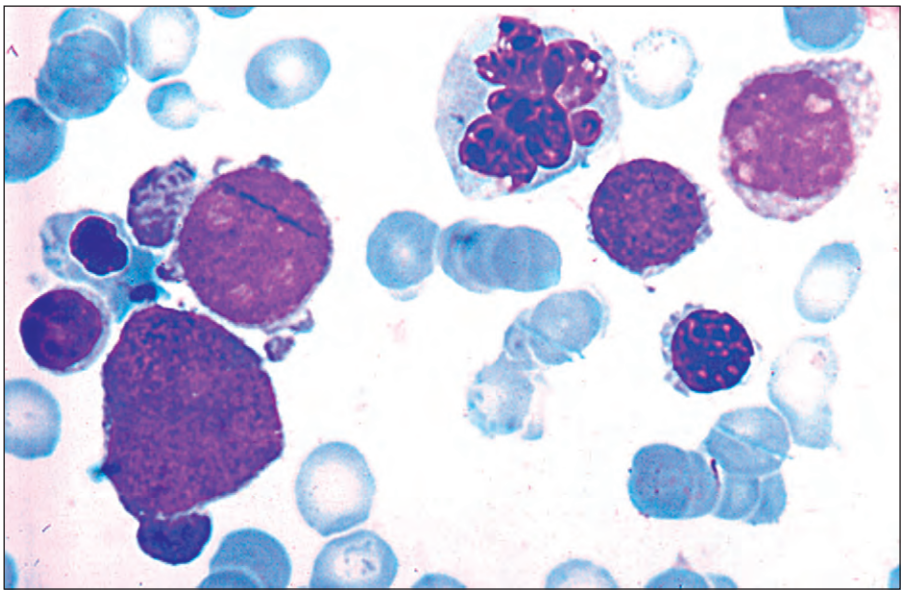


**Fig. 3.34** Severe dyserythropoiesis in a case of RAEB-2; bone marrow aspirate. Starting from the top right corner, a granular blast cell of intermediate size, a small agranular blast with fairly condensed chromatin and almost no cytoplasm, immediately beneath it a polychromatic erythroblast and, in the centre under the upper border of the image, a large erythroblast with polychromatic cytoplasm, with a strange nucleus with ten or so lobes of dense shiny chromatin, which remain joined to each other but project outwards likes the petals of a monstrous flower. Towards the left, from the top downwards, a blast cell that is difficult to assign to a lineage – erythroid or megakaryocytic – with chromatin that is almost liquefied, which is losing almost all of its cytoplasm in large blebs; an orthochromatic erythroblast with a pyknotic nucleus of irregular shape, rather like a coffee bean; a lymphocyte; a giant erythroblast with basophilic cytoplasm, almost all of which is in the form of a large protrusion at the bottom of the cell, and with a compact hyperdiploid triangular nucleus, formed of a single block of granular cytoplasm.

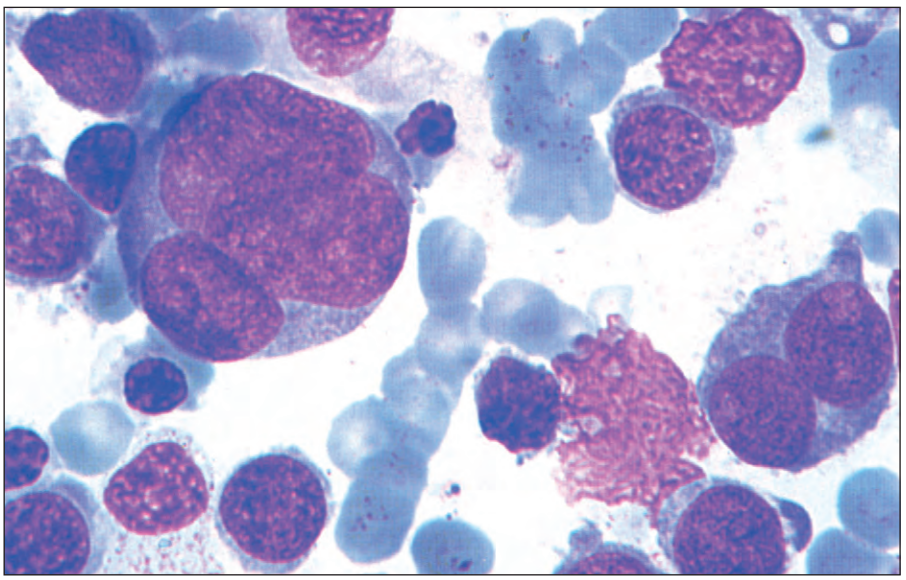


**Fig. 3.35** Marked dyserythropoiesis and erythroid multinuclearity in a bone marrow aspirate. The cells of erythroid lineage are characterised by considerable immaturity, with a prevalence of proerythroblasts and basophilic erythroblasts. There are two giant, multinucleated proerythroblasts, one of which has two symmetrical more regular nuclei; the other is more bizarre, even monstrous, with three hyperdiploid asymmetrical nuclei superimposed on each other. The chromatin of these nuclei is very delicate, quite similar to that of a megaloblast, so that in the area of superimposition it is possible to see the structure of the underlying nucleus; there are also a number of visible, but not prominent, nucleoli. The cytoplasm is abundant and strongly basophilic with extensive pale paranuclear areas and peripheral enhancement of the basophilia. There are also a number of basophilic erythroblasts with fairly scanty cytoplasm and nuclei with a perfectly round outline.

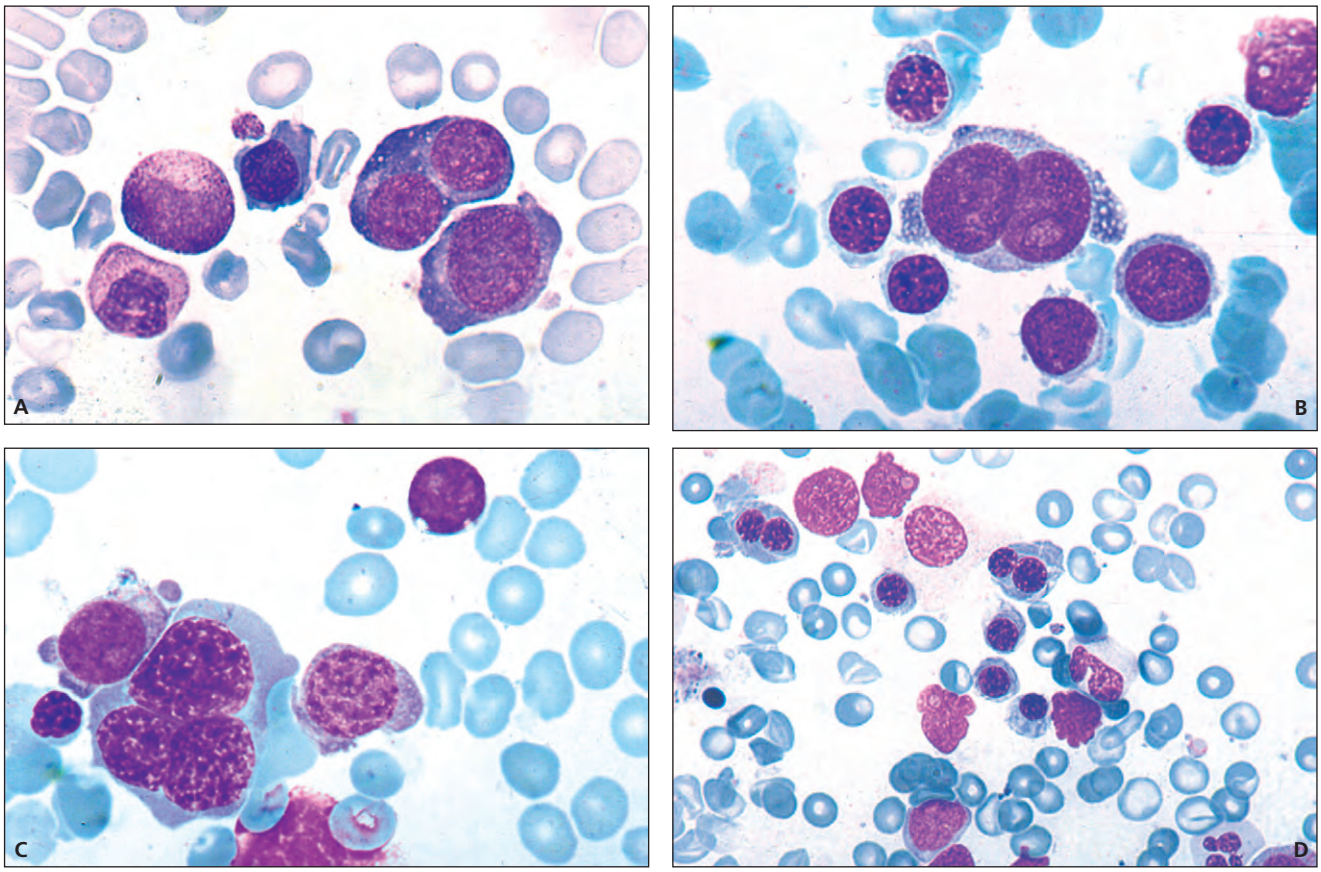



**Fig. 3.36** Erythroblast multinuclearity as a feature of dyserythropoiesis in four different cases of refractory anaemia; bone marrow aspirate. (A) On the left, two granulocyte precursors (a neutrophil band form with a long folded nucleus and a promyelocyte with an immature nucleus and an irregular distribution of granules), a polychromatic erythroblast with a nucleus that has chromatin that is still not well condensed, having a structure of small alternating blocks of dark and pale chromatin and, immediately above, a hyperchromatic platelet. On the right, a binucleated erythroblast, which appears to be in the final stage of telophase in which the round, regular nuclei with granular chromatin, which characterise this first morphologically recognisable phase of erythropoiesis, are completely separated and reformed, while the division of the cytoplasm has not yet started; one notes the presence of pale paranuclear arcoplasm and of pseudo-vacuoles scattered through the blue cytoplasm, corresponding to hypertrophic non-staining mitochondria. The other proerythroblast, on the right, has only one nucleus and is otherwise very similar, but it has undergone endomitosis: the nucleus is in fact double normal size and in terms of DNA content, is tetraploid. (B) A giant proerythroblast in the centre of this small group of cells has two very large nuclei, oval in shape and lined up parallel to the major axis of the cell, with granular chromatin and visible pale nucleoli (particularly prominent in the nucleus on the right); each of these two nuclei, as shown by their size, has double the DNA content of normal cells. The cytoplasm of the binucleate proerythroblast is basophilic and extends into three projections or blebs, a small one at the top and two large ones at the lateral poles, in which the colour is darker and emphasises the presence of numerous small vacuoles. The six cells that surround this large dysplastic precursor are (anticlockwise from the upper right angle): four polychromatic erythroblasts with scanty cytoplasm, a lymphocyte and a basophilic erythroblast. (C) Giant trinucleated erythroblast with polychromatic cytoplasm and nuclei of different sizes (the upper one appears to be double the size of the other two), with shape and chromatin condensation typical of the basophilic erythroblast stage of maturation. To the left, a small orthochromatic erythroblast with an irregular nucleus, and a blast cell with fine granules in its basophilic cytoplasm, formation of surface blebs and an oval nucleus with homogeneous delicate chromatin. (D) Two binucleated late polychromatic erythroblasts are seen towards the top. On the left, a pyknotic nucleus liberated from an orthochromatic erythroblast; the central erythroblasts show a defect in haemoglobinisation. In the centre of the lower edge, an agranular blast cell. Two neutrophils, one of which is only partly included in the bottom right corner, appear to be lacking in granules.

are abnormal – very large, hyperchromatic and sometimes confluent. Nuclear anomalies are most evident in mature neutrophils, in which changes in segmentation and nuclear morphology are very obvious. Metamyelocyte and mature neutrophils can show all the dysplastic features already described in the peripheral blood. The peroxidase activity can vary from cell to cell in an individual patient, depending on the severity and type of dysplasia in the neoplastic clone: it is not rare to see cases with an acquired partial or total peroxidase deficiency. There can be an increase in basophils, often dysplastic, with poor staining of granules. Toluidine blue staining permits the identification of these cells with dysplastic granules.

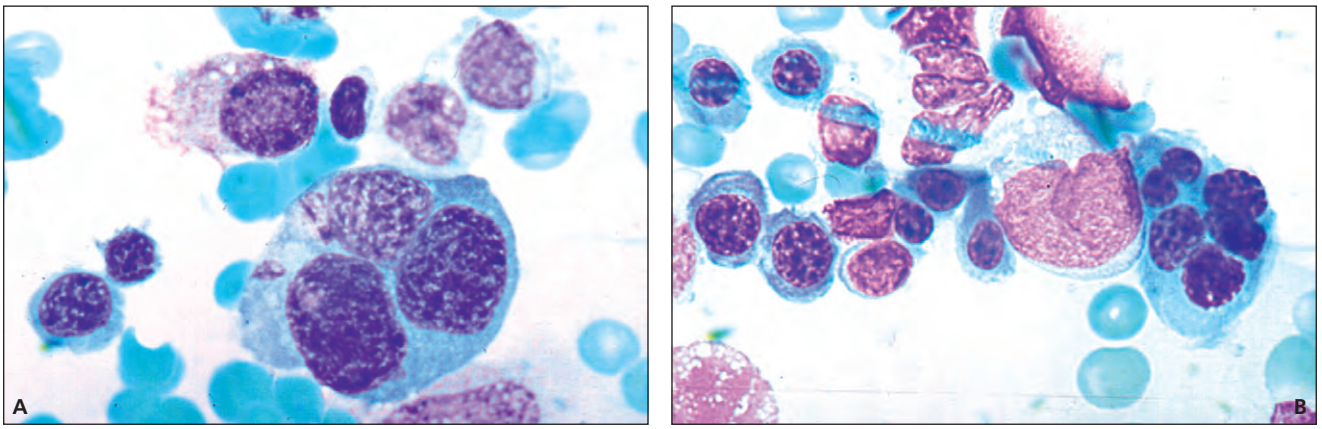



**Fig. 3.37** Dyserythropoiesis in RAEB-1: multinucleated erythroblasts in a bone marrow aspirate. (A) A giant erythroblast with plentiful polychromatic cytoplasm contains three enormous hyperdiploid nuclei: the top one is fragmenting with detachment of nuclear material, while the others retain a typically erythroid structure with alternation of chromatin clumps and paler linear spaces, even if the nuclear outline is not the perfect circle of a normal erythroblast. On the left, a polychromatic erythroblast shows a similar nuclear conformation, although on a much reduced scale; close by is a round nucleus with only a thin rim of cytoplasm of a cell that is about to undergo apoptosis. Top left, a neutrophil myelocyte that can be regarded as dysplastic (because of the persistence of cytoplasmic basophilia, indicating that in comparison with the nucleus, maturation is delayed), a small flattened lymphocyte, a monocyte and an agranular blast cell. (B) Top left, in this field which includes numerous damaged nuclei there are two polychromatic erythroblasts with a lymphocyte to their right. Lower down, across the middle of the image, two erythroblasts, a lymphocyte and three small polychromatic erythroblasts. The large cell with plentiful vacuolated basophilic cytoplasm and an enormous nucleus with pale loose chromatin should be recognised in the context of MDS as an agranular blast cell; its nucleus is folded and has a deep cleft dividing it almost completely into two lobes. On its right, a giant erythroblast, in which the polychromatic cytoplasm contains six, perhaps partly fused, nuclei with mature chromatin; the nuclei are similar to each other with regard to size and chromatin condensation.

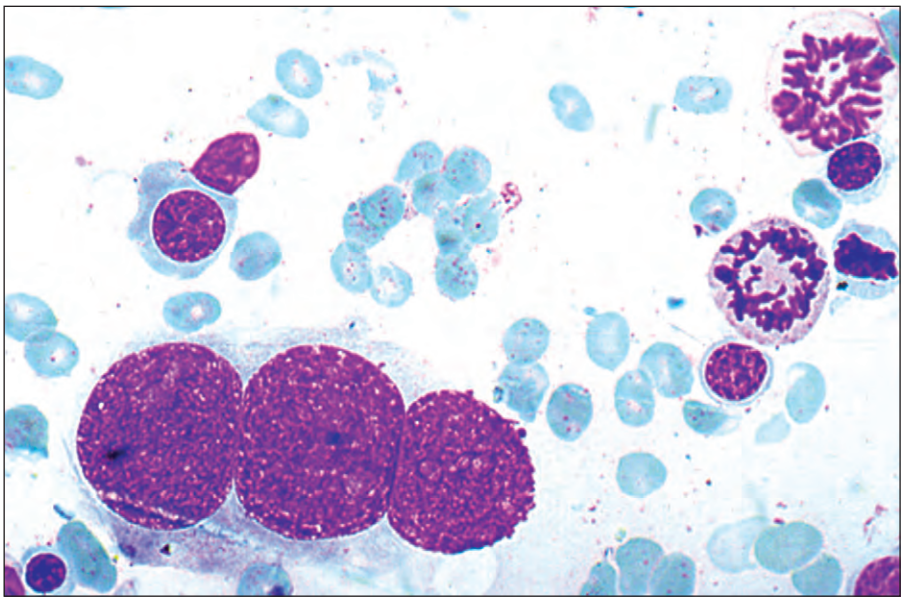


**Fig. 3.38** Extreme dyserythropoiesis; bone marrow aspirate. The giant cell is a proerythroblast with three very large nuclei and is certainly hyperdiploid; the nuclei are rounded but flattened at the points of contact; they have granular chromatin in which, with difficulty, one can see the suggestion of nucleoli. The cytoplasm, which has been damaged during the spreading of the film, is pale blue, weakly basophilic and with margins that are not well defined. The other four erythroblasts present have more normal morphology with the nuclei having small chromatin clumps that are regularly distributed. In the top right corner, three cells in mitosis: the smallest is an orthochromatic erythroblast in which the nuclear morphology has not yet been restored to normal; it has the prepyknotic appearance of karyorrhexis; the other two have polychromatophilic cytoplasm and short chromosomes arranged in a circle: this appearance is common when there is marked dyserythropoiesis and corresponds to late metaphase, the orderly migration of chromosomes to the two poles that characterises the successive anaphase being delayed or permanently blocked.

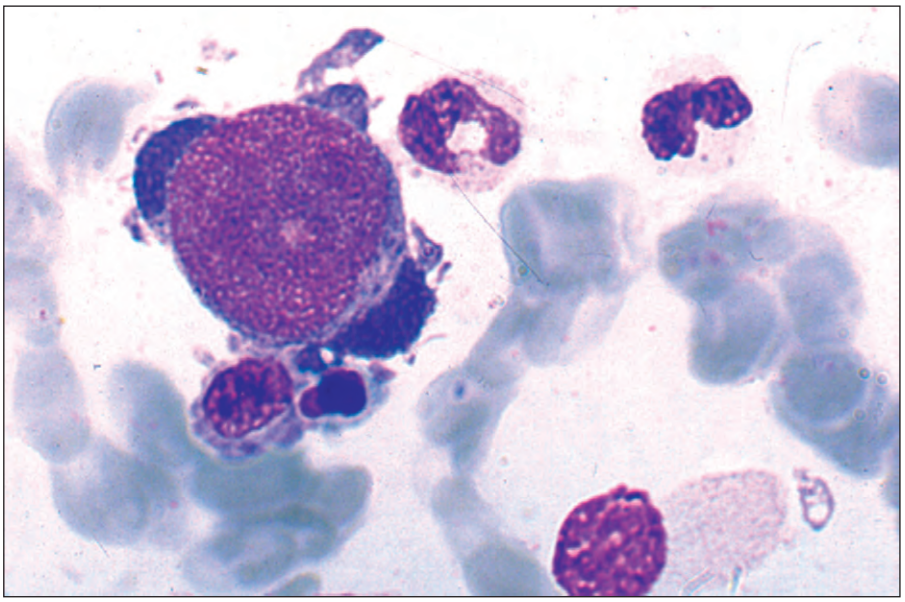


**Fig. 3.39** Dyserythropoiesis in RAEB-1; bone marrow aspirate. A giant proerythroblast, with very homogeneous granular, pearly chromatin that is typically megaloblastoid. In the centre, a paler area corresponding to a poorly defined nucleolus. The nuclear outline is round and regular with a slight flattening on the right corresponding to the pale arcoplasm. The cytoplasm is pale blue in the circle immediately around the nucleus, with basophilia that is quite markedly accentuated in the immense protrusions that characterise the cell outline. All around are fragments and vesicles that seem to have originated from this cell by zeiosis. At the bottom, two more mature erythroblasts, one polychromatic and one orthochromatic, both with a deformed nuclear outline. Top right, two neutrophils with non-segmented nuclei: the one close to the right edge appears clearly dysplastic because of the pyknotic chromatin structure, the irregularity of the nuclear shape and, particularly, the hypogranular cytoplasm.

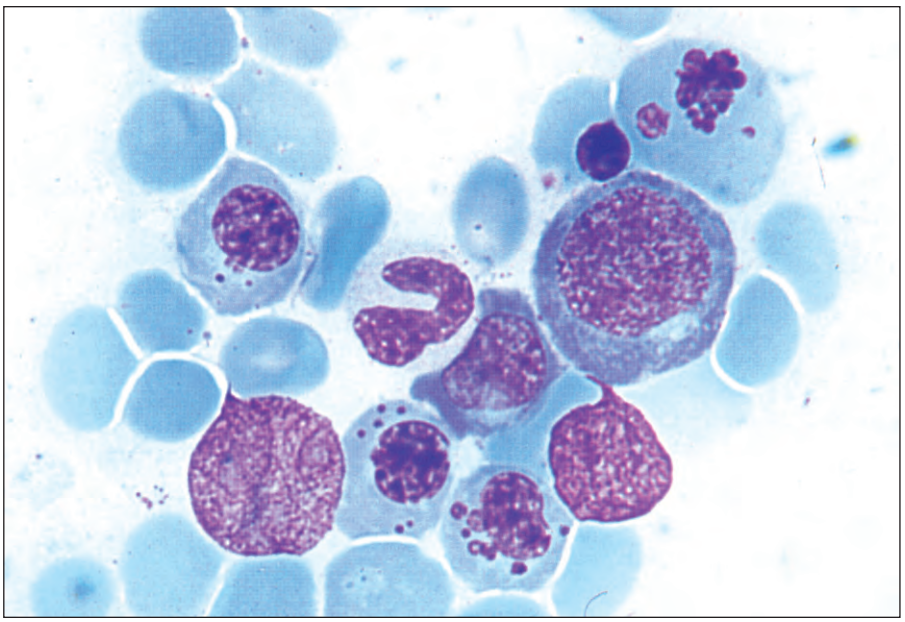


**Fig. 3.40** Karyorrhexis and fragmentation of mature and intermediate erythroblast nuclei in dyserythropoiesis of refractory anaemia; bone marrow aspirate. The majority of the cells are polychromatic erythroblasts characterised by the presence in the cytoplasm of rounded blackish-violet nuclear fragments that represent chromosomes lost in the course of a preceding mitosis (karyorrhexis). The size and number of these Howell-Jolly bodies is variable, but the morphology is consistent – a shiny sphere with a distinct outline. In the lowest cell, some of the larger inclusions appear incompletely stained, as if they are empty or composed of parachromatin, and others seem to have maintained a connection with the nucleus, confirming their nature. The erythroblast top right has a typical appearance of severe myelodysplasia: after cellular division the nucleus has not regained its round shape and the pyknosis that precedes the formation of a mature red cell has altered it into a deformed multilobated structure, like an irreversibly frozen flower. Fragments of nucleus, one larger and one smaller, have become detached. On the left of this cell, however, a small orthochromatic erythroblast is pursuing the normal course of maturation; its small round nucleus with completely condensed chromatin is about to be extruded. A large proerythroblast appears morphologically normal with granular chromatin and worm-like chromatin clumps within a regular round nucleus that appears slightly compressed by a short length of arcoplasm that interrupts the homogeneous basophilia of the cytoplasm.

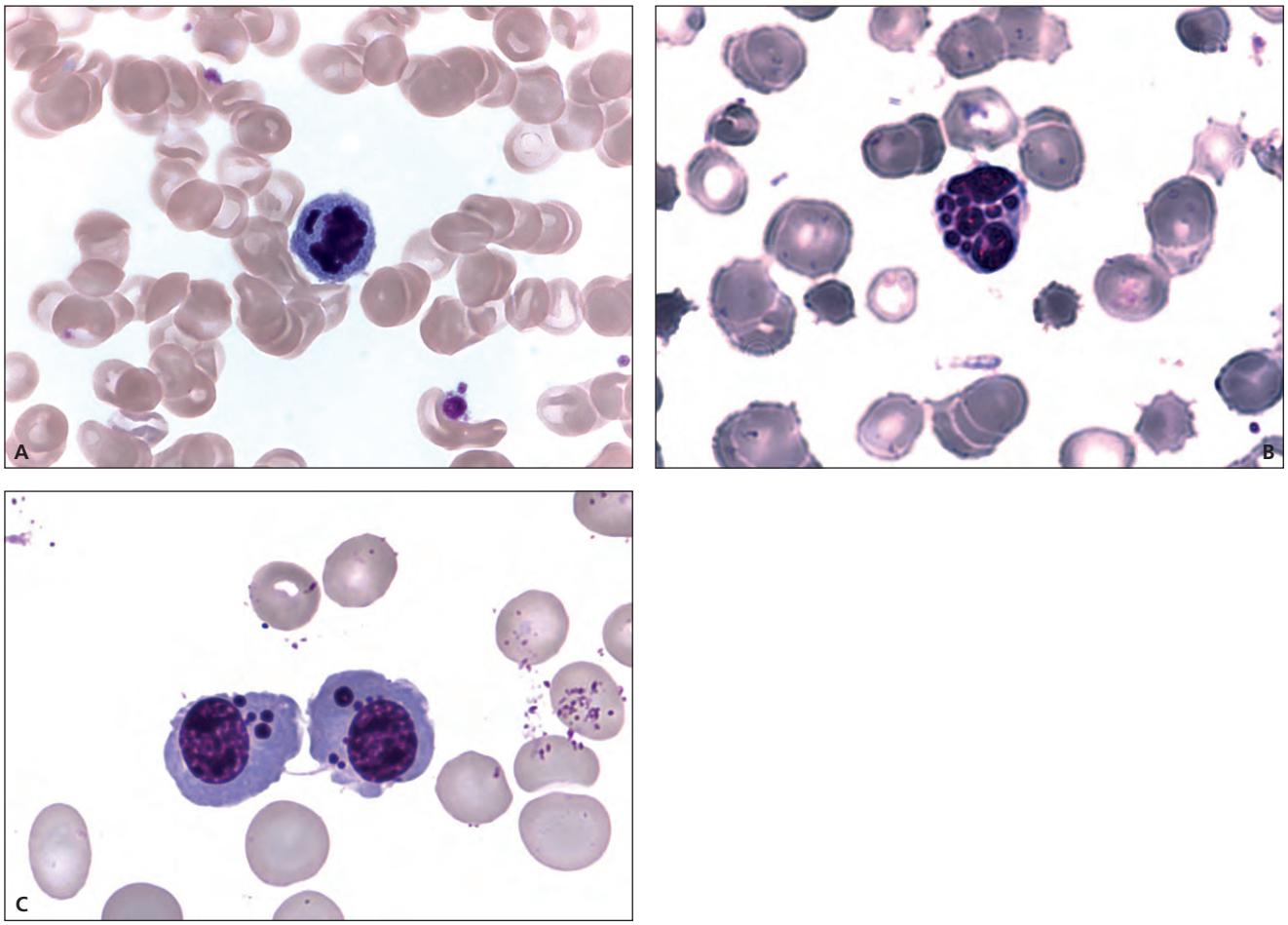



**Fig. 3.41** Dyserythropoiesis in polychromatic erythroblasts in MDS as the morphological equivalent of ineffective erythropoiesis: nuclear fragmentation and formation of Howell-Jolly bodies. The cells shown here will never reach the final stage of erythroblast maturation as they will die prematurely. (A) Peripheral blood film. This polychromatic erythroblast has apparently completed mitosis, having separated from the other erythroblast produced by cytoplasmic division. However, two features show that the process of karyokinesis has not reached a good end: one chromosome, probably complete, has been lost in the cytoplasm and certainly will not be reincorporated into the nuclear structure (see Fig. 3.20); the erythroid nucleus has not reconstituted into its usual rounded shape, but has aborted into a knobbly mass of pyknotic chromosomes. (B) Peripheral blood film. The nucleus of this polychromatic erythroblast appears to have fragmented into three small incomplete pseudonuclei and seven rounded masses of dark condensed chromatin. This is what is classically called fragmentation, but it is very likely due to the failure of reconstitution of the nuclear structure at the end of karyokinesis as a result of degradation of the tubules of the mitotic spindle. There are also some irregularly contracted cells and some echinocytes. (C) Bone marrow film. Cellular division is almost complete, but the reconstitution of the nucleus is incomplete. The oval well-structured nuclei, with continuous and well-defined nuclear membranes, have lost a conspicuous quantity of chromosomal material which has become detached and now forms large Howell-Jolly bodies; some of these remain in continuity with the nucleus being attached by a peduncle (on the left) or adhering to the nuclear membrane (on the right). One also notes that the lower part of the two cells remains attached by a long thin, greyish cytoplasmic bridge that seems to contain a pink filament.

## Blast cells

The identification and quantification of blast cells is one of the most important aspects in the diagnosis of myeloid neoplasms. To make a diagnosis of MDS the percentage of blast cells in the marrow must be less than 20%; otherwise, regardless of the presence or absence of dysplasia, the picture is that of AML. The complete myelogram, indispensable for a quantitative evaluation, must be performed on a minimum of 500 cells, using at least two different slides; to ensure that the count is reasonably precise, at least 100 non-erythroid cells (NEC) must be counted.[66] In MDS, the percentage of blasts, together with the number of cytopenias, is a key element also for distinguishing between the various categories. The 2008 WHO classification indicates clearly that the blast count needed for diagnostic purposes is the morphological count on a cytological preparation, which cannot be replaced by a blast percentage obtained by a different technique, such as flow cytometric immunophenotyping, histology or immunohistochemistry on a trephine biopsy

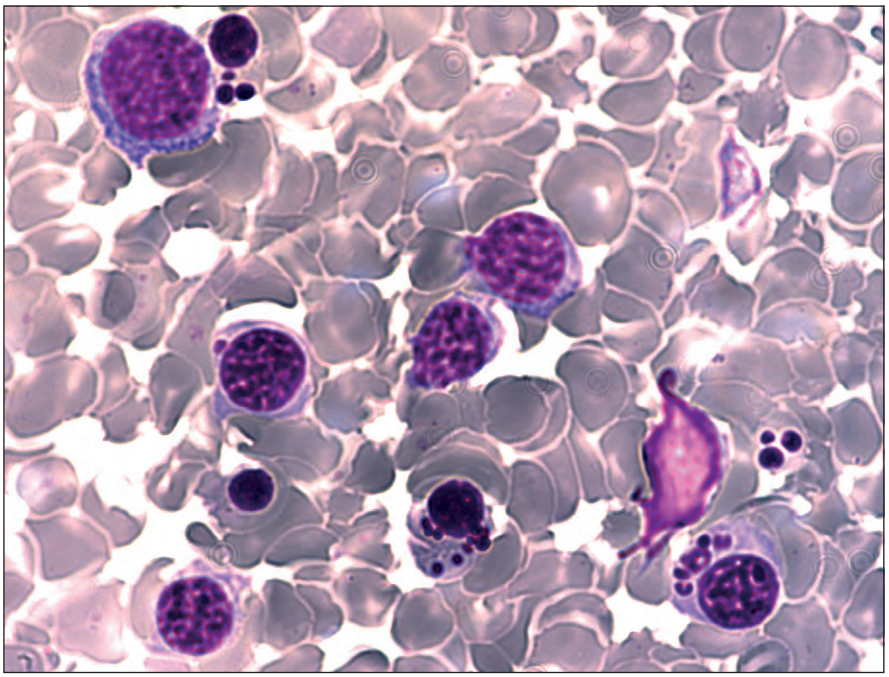


**Fig. 3.42** Bone marrow aspirate from the same patient with refractory anaemia as Fig. 3.41B. The fragmentation (or failure of reconstitution) of the nucleus is a phenomenon that is accentuated in the course of dysplastic erythroid maturation. It is manifest particularly in orthochromatic and polychromatic erythroblasts. Top left, a basophilic erythroblast, morphologically fairly normal and with small pale cytoplasmic vacuoles, is adjacent to an orthochromatic erythroblast with a nucleus fragmented into a main nuclear mass and three small pyknotic spheres. Analogous morphological features, an expression of failed completion of the process of division (which will not even start in this already well-haemoglobinised cell), is recognised in the two erythroblasts at the bottom (one orthochromatic and one polychromatic), and in the free fragment of cytoplasm of another cell, which appears on the right of a red cell containing three Howell-Jolly bodies of various sizes, but sufficiently large that they occupy the entire cytoplasm.

section. Flow cytometric immunophenotyping is useful particularly for defining the lineage to which blast cells belong,[69] for which cytochemistry also retains a place. All these techniques can be useful in situations when the aspirate cytology is diagnostically inadequate because of bone marrow hypocellularity or fibrosis.

It is fundamental that there are precise morphological criteria for the definition of both blast cells and the other immature cells with which they can be confused, such as dysplastic promyelocytes, in order that the latter are not inadvertently included in the blast count (Figs 3.69 through to 3.81). The FAB group proposed the subclassification of blasts cells into type I for morphologically undifferentiated myeloblasts and type 2 for granular blast cells; the approach was confirmed in the 2008 WHO classification, which furthermore suggested the adoption of the terms agranular blast cell and granular blast cell for these two types (Table 3.6). According to the definitions of the IWGM-MDS,[66] accepted by the WHO, the agranular blast is an undifferentiated myeloblast with a high N:C ratio. Its nucleus tends to be central and of variable shape, with chromatin that is usually, but not invariably, delicate with easily visible nucleoli. The cytoplasm shows variable basophilia; a visible Golgi zone, granules and Auer rods are absent. The granular blast cell has analogous nuclear characteristics, but generally more plentiful cytoplasm, again without a visible Golgi zone, with primary, azurophilic granules varying in quantity from scanty to numerous. This definition includes both type II blasts and those with more numerous granules, which were called type III by some members of the FAB group.[70] The lack of even a minimal pale Golgi zone was for these experts an indispensable criterion for the cytological recognition of a blast cell. They did, however, recognise one exception: a small distinct Golgi zone, sometimes with an associated Auer rod, can be present in the blast cells of AML associated with t(8;21). It cannot be excluded, *a priori*, that there are other exceptions, in individual cases, that can and should be recognised in practice to ensure a blast count that is valid from the clinical and therapeutic points of view.

A crucial point for morphological diagnosis is the correct identification of promyelocytes, which is easy when they are normal but sometimes quite difficult when they are dysplastic. Both cytologically normal and cytologically abnormal promyelocytes must be included in the count of differentiating granulocytes, not in the blast cell count: an incorrect cytomorphological diagnosis carries the risk of misclassification with therapeutic and prognostic significance, relevant both when distinguishing

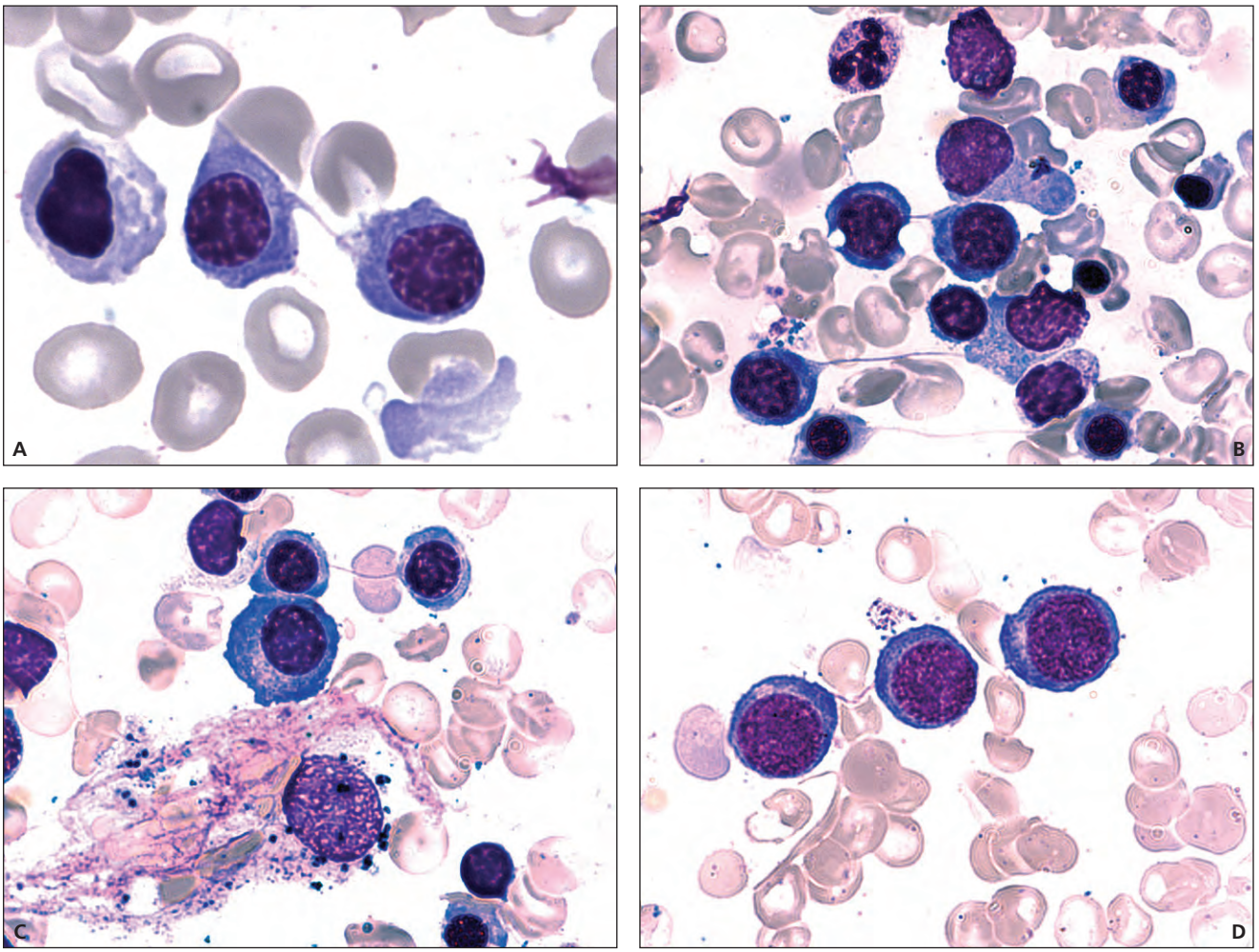



**Fig. 3.43** Dyserythropoiesis in a bone marrow aspirate: formation of internuclear bridges. This phenomenon is not exclusive to, or specific for, MDS, being observed also in various conditions with erythroid hyperplasia and accelerated and disorderly proliferation of erythroid precursors (e.g. in severe acute haemolytic anaemia and in severe thalassaemias). However, in MDS the formation of internuclear bridges can be very abnormal, both in terms of their frequency and their morphology. (A) Two small basophilic erythroblasts, with typical erythroid nuclei with clumps of chromatin separated by thin pale spaces, appear to be joined by a cytoplasmic bridge within which there is a purple-red filament of nuclear material: this can be followed from its origin on the margin of the nucleus on the right, exiting through a cone of cytoplasm and penetrating the cell on the left. On the left, a fairly mature polychromatic erythroblast with a nucleus that shows maximum chromatin condensation, with an irregular bossed outline and pale areas of defective haemoglobinisation in the blue-grey cytoplasm. (B) Unusually long internuclear bridges joining two polychromatic erythroblasts along the lower edge and two basophilic erythroblasts (one of which has been damaged during spreading) a little higher up. The small cell in contact with the damaged erythroblast is a lymphocyte, as is indicated by its chromatin structure with a few very dark and shiny, almost polished, chromatin clumps; there is a small notch at the top of the nucleus. On the right, a small orthochromatic erythroblast with pale areas of incomplete haemoglobinisation and a completely pyknotic nucleus (as is also seen in the cell even further to the right, on the edge of the image, in which the cytoplasm still has a basophilic tinge). Higher up, a chromatin bridge between nuclei of basophilic erythroblasts; above one of these is an agranular myeloid blast cell, with a nucleus that has reticular chromatin and a prominent nucleolus with a perinucleolar accentuation of dark chromatin. (C) This image is traversed, in its lower part, by a comet-shaped macrophage, with a large, round, pale nucleus, which appears spongy because of its pale granularity and the alternating hetero- and para-chromatin. The cytoplasm is frayed and stretched as a result of the spreading of the film (which has created this illusion of a moving comet): this cell contains in its 'tail' granular debris and at least four phagocytosed erythrocytes that have not yet been lysed. Above is a pyriform plasma cell; it is basophilic with reddish areas relating particularly to the paranuclear Golgi zone: the chromatin clumps in the round eccentric nucleus tend to adhere to the inner surface of the nuclear membrane: this appearance, in addition to the shape and the cobalt blue colour of the cytoplasm, confirms the cytological identification of this cell as a plasma cell rather than a basophilic erythroblast. Higher up are two polychromatic erythroblasts with parts of their cytoplasm showing haemoglobinisation; they are joined by a distinct, thick internuclear chromatin bridge. (D) Three well-formed early basophilic erythroblasts, with round nuclei and small chromatin clumps, without visible nucleoli, in two of which a short pale paranuclear Golgi zone is seen within the ultramarine cytoplasm. The two cells to the left are joined by an internuclear filament of the same colour as chromatin, which can be followed to the right across the blue cytoplasm to the nucleus.

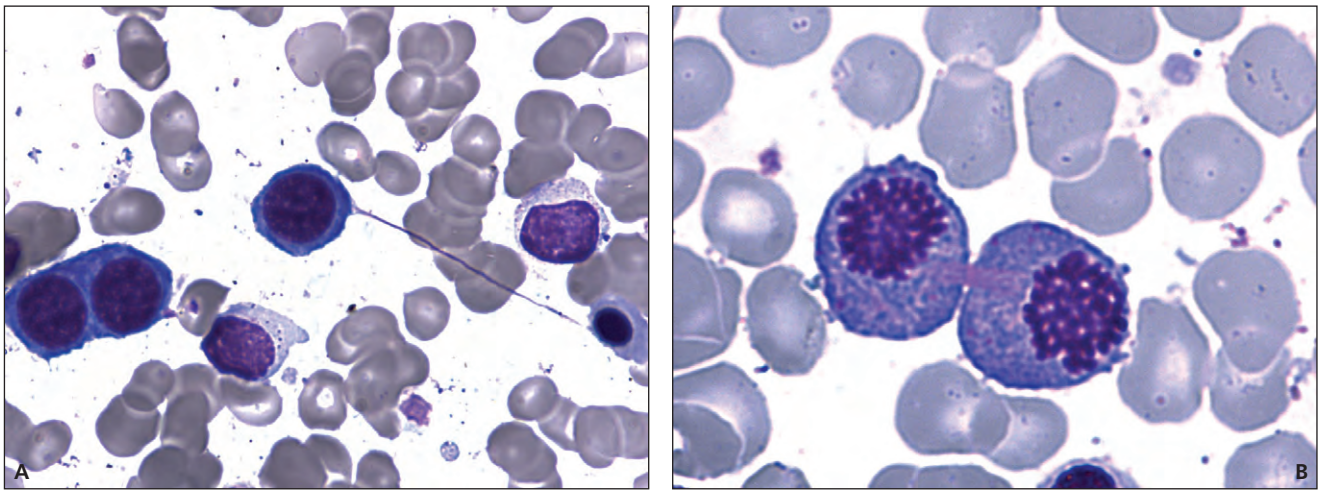



**Fig. 3.44** Dyserythropoiesis in the form of intercellular bridges with aberrant morphology; bone marrow aspirate. (A) Very long internuclear filament with markedly asynchronous maturation of the two erythroblasts that are joined together. The cell on the left remains at the stage of a basophilic erythroblast, while that on the right is an orthochromatic erythroblast, with mature cytoplasm and a completely pyknotic homogeneous and compact nucleus. Adjacent to the left edge, a binucleated basophilic erythroblast, a large granular lymphocyte that has five or six brick-coloured granules clearly visible against the blue-grey background of the plentiful cytoplasm and, top right, a dysplastic hypogranular myelocyte with cytoplasm that is still basophilic. (B) These two basophilic erythroblasts have completed their cytoplasmic division, but the nuclei have not reconstituted their round membrane and they appear as masses of chromosomes tightly bundled together. These abortive nuclei are joined by a short straight band of pale red internuclear filaments; other filaments and azurophilic granules are visible, particularly in the cytoplasm of the erythroblast on the left. This image confirms, with strong visual evidence, that the internuclear bridges in dyserythropoiesis derive from residual filaments of the mitotic spindle that have not been broken down.

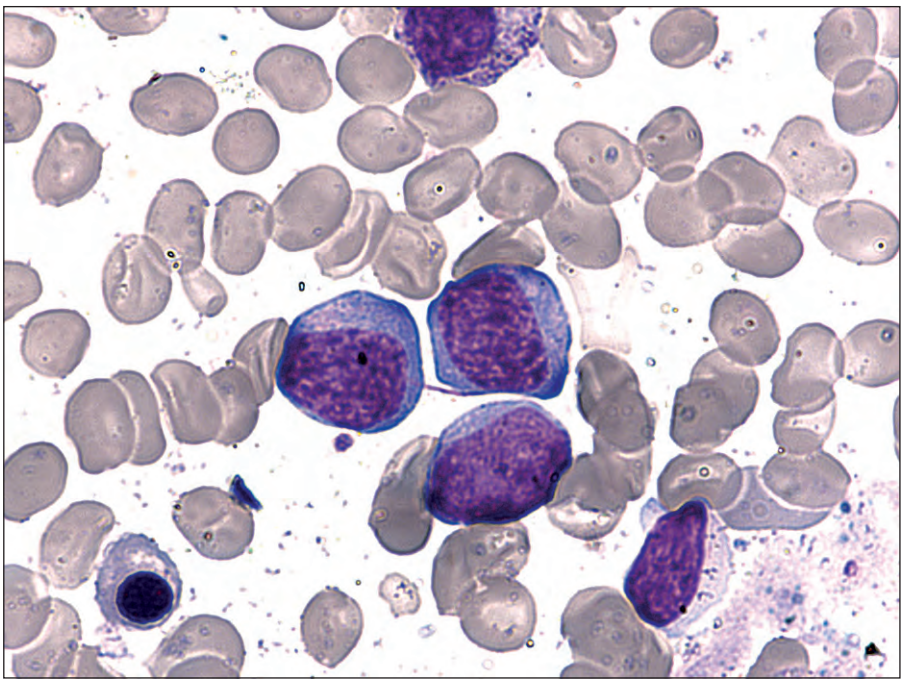


**Fig. 3.45** Dysmyelopoiesis with some interpretative difficulties in MDS; bone marrow aspirate from a patient with RAEB-2, with a bone marrow erythroid component of 54% and with blast cells being 16% of NEC. In the bottom left corner, a polychromatic erythroblast with a nucleus that is now completely mature and pale and with non-haemoglobinised areas in the cytoplasm. In the bottom right corner, a large granular lymphocyte, a feature that is not infrequent in the aspirates of patients with MDS. The isolated cell in the centre at the bottom is an agranular blast cell, easily recognisable from its nuclear characteristics: immature, homogeneous, pale chromatin with small foci that are less condensed and a pale space corresponding to a non-prominent nucleolus, with an angular outline, adherent over a fair extent to the inner surface of the cytoplasmic membrane. Up to this point there is no difficulty in interpretation. However, higher up there are another two almost identical cells, joined by a thick solid internuclear filament of the same wine-red colour as the chromatin. The shape of the nuclei is not the typical round or oval shape of erythroblast nuclei: it is tending to polygonal with angles and straight areas, very reminiscent of the morphology of myeloblasts, as seen in the cell immediately below. However, the structure of the chromatin with large dark clumps that are not in contact with the nuclear membrane (as happens in plasma cells) and thinner pale fissures that separate them (very different from the pale and homogeneous chromatin of the myeloblast beneath), the intense ultramarine colour of the cytoplasm and the presence of the internuclear bridge do not exclude the possibility that these are actually basophilic erythroblasts with deformed nuclei.

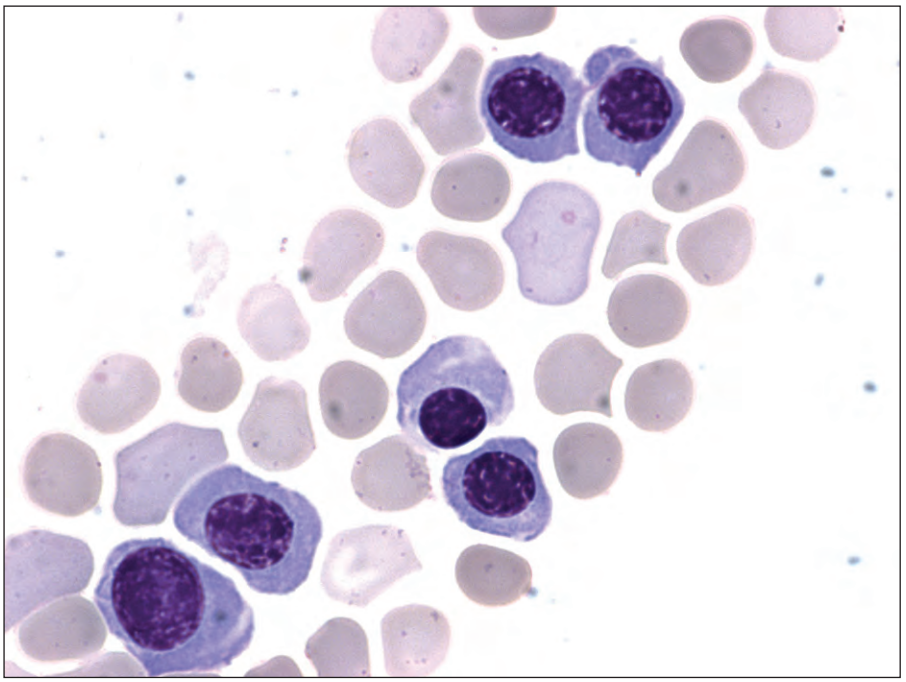


**Fig. 3.46** Dyserythropoiesis and defective haemoglobinisation in a bone marrow aspirate: the abnormalities stand out because of their general uniformity. These cells are all polychromatic erythroblasts, fairly similar to each other if one excepts the presence of linear unstained areas in the cell in the centre with the smallest nucleus and in the cell bottom left with the largest nucleus; it is thus clear that they are synchronised. The nuclei, however, show small but significant differences: the cell in the centre has an almost pyknotic nucleus with very few residual areas of pale parachromatin, while the cell lower down maintains the alternation of pale and dark areas typical of this intermediate stage of erythroid maturation, and the nuclei of the two cells at the top with slightly more basophilic cytoplasm are joined by an internuclear bridge.

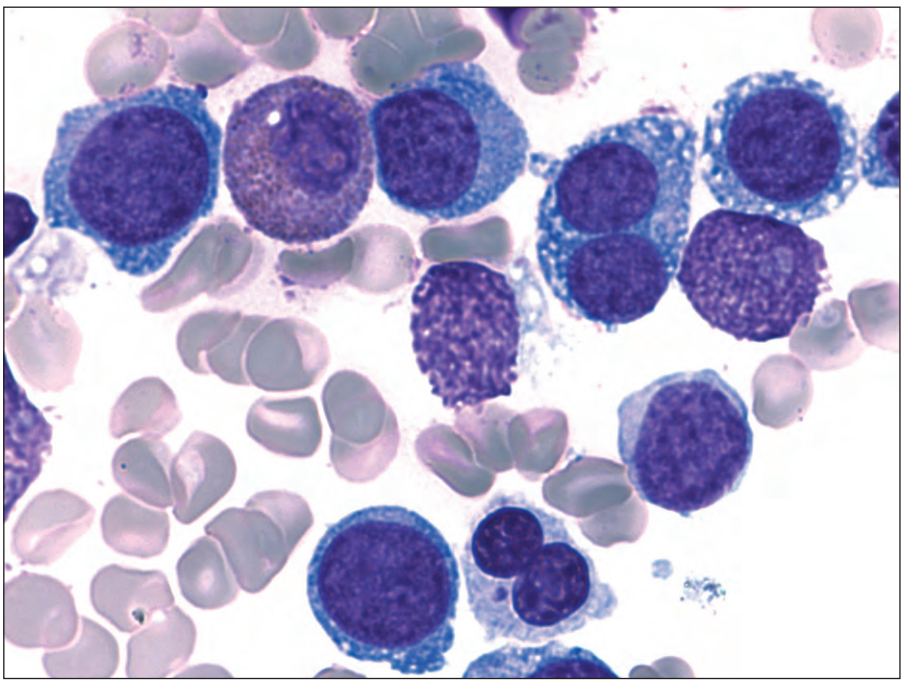


**Fig. 3.47** The erythroblasts of this patient with RCMD show, as a distinguishing characteristic, the presence of numerous delicate cytoplasmic vacuoles. The cell furthest to the left and the left cell of the two cells at the bottom are proerythroblasts with megaloblastoid features, demonstrated particularly by the delicate compact and homogeneous chromatin. The blue cytoplasm is perforated by small vacuoles and extends into protrusions that are particularly accentuated near the lower edge of the image. The other three cells with lacy blue cytoplasm (one of which is binucleated) are globally similar to those already described, but their nuclei have chromatin that has started to condense into dark clumps and they are thus identified as basophilic erythroblasts. At the bottom there is also a binucleated polychromatic erythroblast. The cytological picture is completed by an eosinophil myelocyte at the top, which has weakly basophilic cytoplasm visible between the orange granules and, towards the right, an agranular blast cell with fairly dense chromatin beneath two unrecognisable nuclei that have been torn during the spreading of the film.

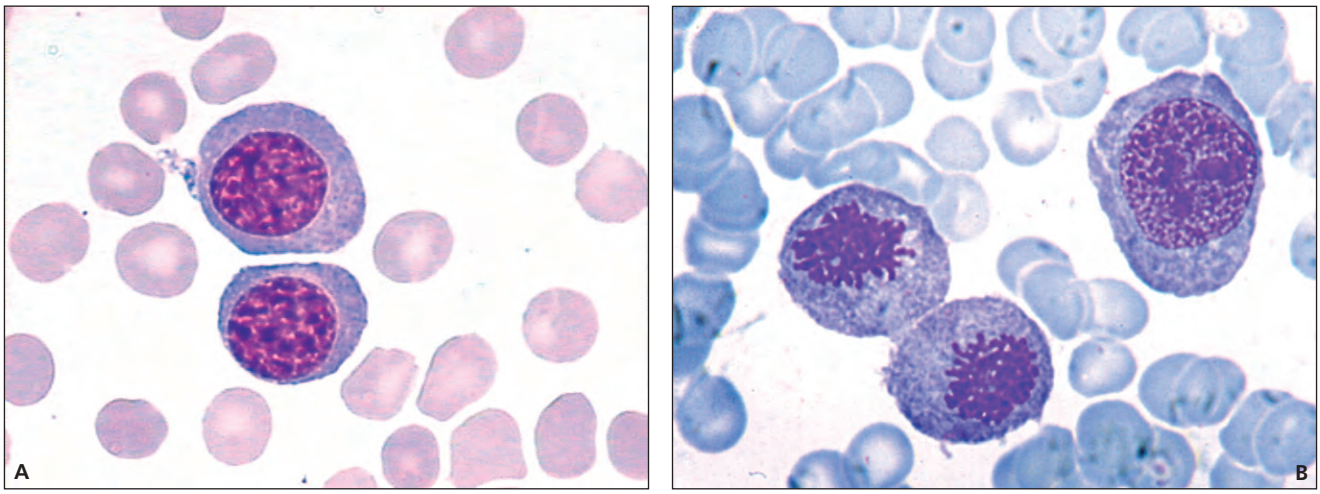



**Fig. 3.48** Dyserythropoiesis with mainly megaloblastoid maturation in MDS. (A) Markedly asynchronous maturation in a case of refractory anaemia: two erythroblasts with plentiful polychromatic cytoplasm have large nuclei with chromatin condensed into small clumps, typical of the basophilic erythroblast stage. (B) On the right, a promegaloblast with a nucleus moving towards the initiation of prophase in which is almost recognisable the condensation into spiral chromosomes separated by pale fissures, surrounding some internal clumps of still compact chromatin. The nuclear membrane is still continuous and clearly seen. On the left, a cell with very similar cytoplasm appears to be in telophase, with polar grouping of two intertwined clusters of chromosomes which are forming new nuclei. The cytoplasmic membranes do not yet seem to be fully separated, appearing to be joined by persisting transparent filamentous structures.

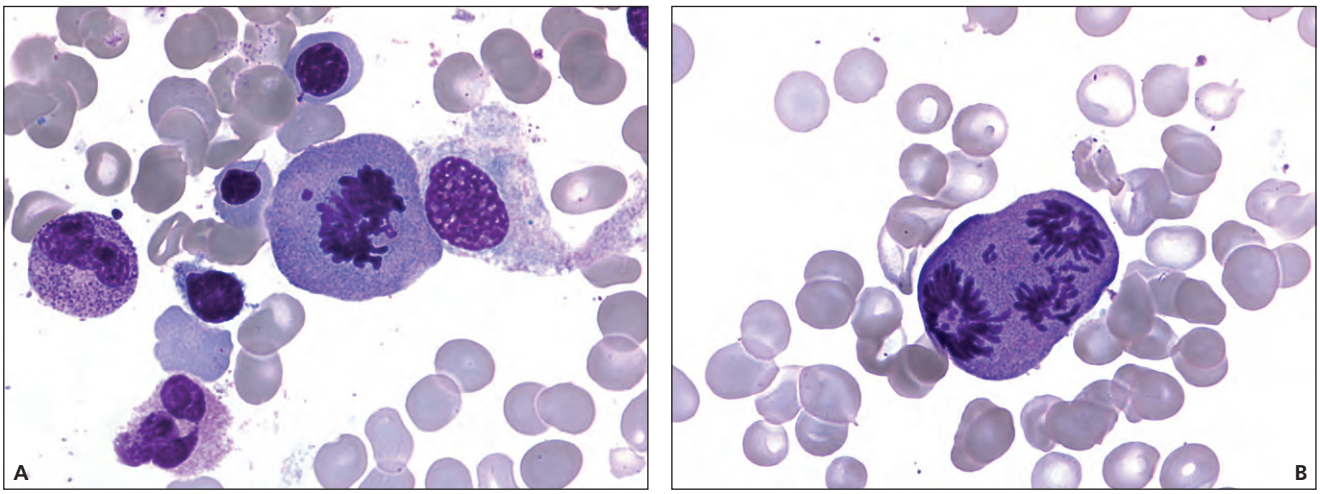



**Fig. 3.49** Dysmorphic mitotic figures in bone marrow aspirates with dysplastic erythropoiesis. (A) On the left, a cell of neutrophil lineage that cannot be categorised using the usual criteria: the cytoplasm is hypergranular with a violet background of residual basophilia and a pale agranular paranuclear area; the nucleus appears lobated like that of a mature neutrophil, but its chromatin appears swollen with many empty spaces and some foci of condensation. At the bottom, a partly damaged neutrophil with hypogranular cytoplasm, and above it a large polychromatic erythrocyte with the outline showing small circles (possibly an effect of flattening of the cell) and an asymmetrical pair of polychromatic erythroblasts, in which the nuclei, one hyperchromatic with an irregular outline and the other better formed and structured, appear to be joined by a thin bridge of material that resembles chromatin, which in the middle appears to lose its continuity as it has been broken or frayed. The centre of the image is dominated by a large erythroid cell in mitosis, in anaphase, with the chromatids already gathered into two groups ready to move towards the poles, with the exception of a chromosomal fragment which, if the cell had survived, would have given rise to a Howell-Jolly body. The enormous amount of cytoplasm shows a tenuous pink mottling, superimposed on the cytoplasmic basophilia. On the right, a macrophage with a spongy, irregularly oval nucleus and plentiful dusty, ragged cytoplasm containing coarse debris derived from phagocytosed material. On the left, a small lymphocyte. (B) This second erythroid mitosis corresponds morphologically to the occurrence, in severe dyserythropoiesis, of erythroid precursors with two or more asymmetrical nuclei, differing in their ploidy and in their degree of chromatin condensation. This giant erythroblast with an immense amount of basophilic cytoplasm has the same rosy granularity as the cell in (A) and appears arrested in a very asymmetric anaphase; the nuclear membrane has not been reconstituted and the re-formed chromosomes have already migrated under the influence of a distorted mitotic spindle. These chromosomes are now forming three groups that are asymmetrical with regard to number, morphology and position with, in addition, a few lost and isolated chromatid fragments. Some chromosomes appear to be still formed of a pair of chromatids joined at the centromere.

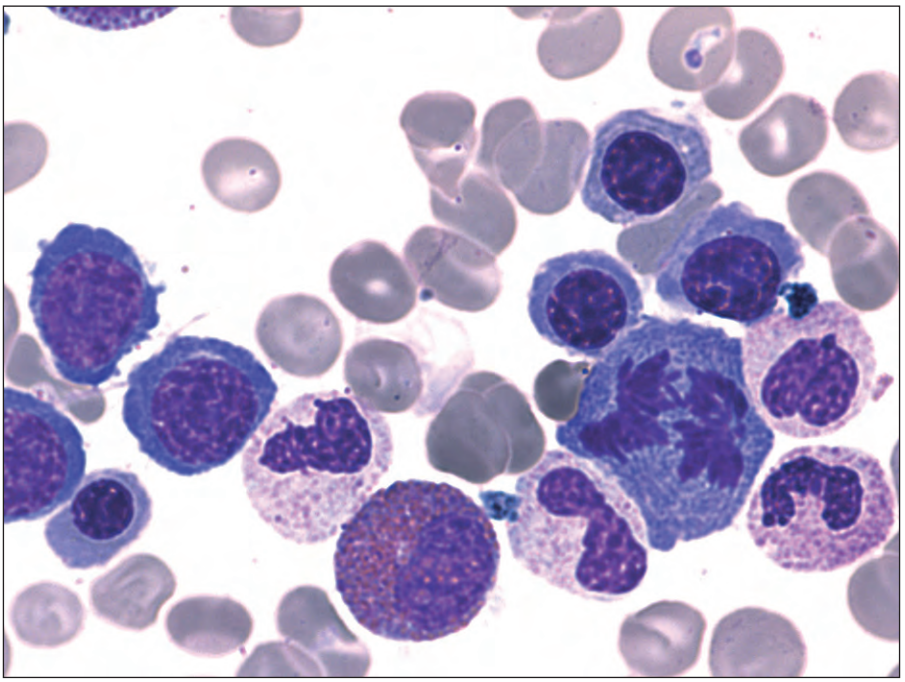


**Fig. 3.50** Dyserythropoiesis and moderate dysgranulopoiesis in a case of RCMD; bone marrow aspirate. From the left, towards the right, one observes: a small polychromatic erythroblast in an advanced stage of maturation; two basophilic erythroblasts that are well formed but joined by a thin internuclear filament; a hypogranular band neutrophil with nuclear appendages of variable thickness; an early eosinophil myelocyte that has large mature orange granules on the background of intensely blue, partly seen, cytoplasm and an eccentric peanut-shaped nucleus with well-condensed chromatin, but still with a nucleolus visible towards the lower pole; another non-segmented hypogranular neutrophil; an erythroid mitosis in which the group of chromatids have gathered in the act of migrating to the poles (one notes that in the basophilic cytoplasm, particularly in the more plentiful lower part, it is possible to see fibres of the mitotic spindle as pale parallel stripes); three polychromatic erythroblasts with nuclei that are not very mature; two neutrophils, the upper one bilobed and hypogranular and the lower one a band form.

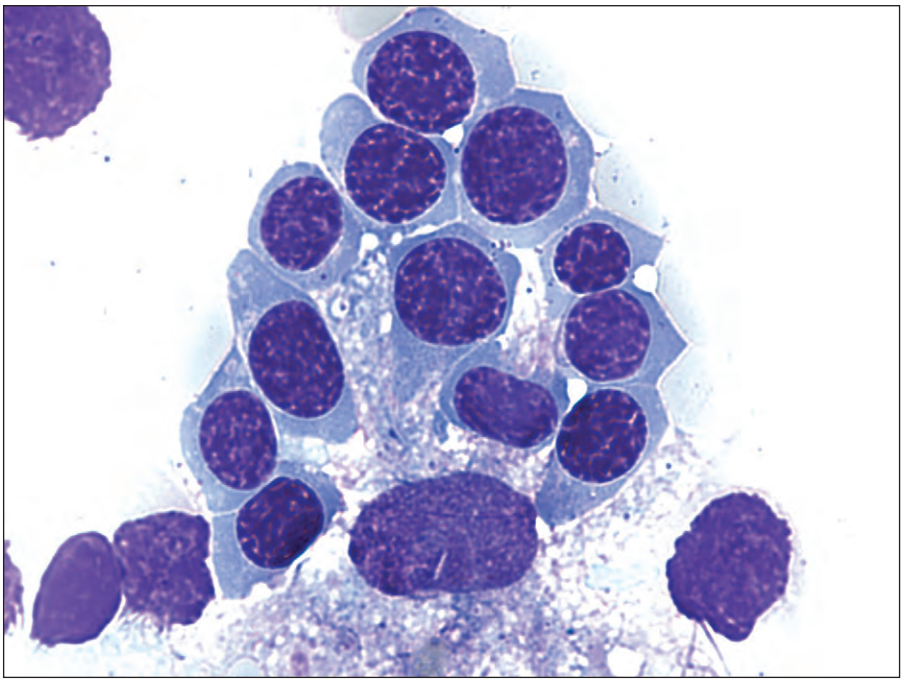


**Fig. 3.51** Erythroblastic synchronisation, a specific feature of dyserythropoiesis in bone marrow aspirates. In normal conditions erythroblasts form fairly numerous erythroid islands in which all the various phases of maturation are represented, often with involvement of a macrophage that has a nutritional function (rhopheocytosis) or a scavenging function (phagocytosis). In dyserythropoiesis, the erythroid islands tend to be monomorphic, i.e. composed of synchronised erythroblasts all at the same stage of maturation. In this image there is a monomorphic carpet of 12 polychromatic erythroblasts, all characterised by retarded nuclear maturation (chromatin condensed into numerous clumps separated by pale fissures as in basophilic erythroblasts). Near the lower edge of the image is the large elliptical nucleus of a macrophage, with pale violet granulated cytoplasm, which appears quite different from the compact mass of erythroblasts. There is a sky-blue nucleolus with well-defined margins. It is interesting to note that the plentiful dusty cytoplasm of this cell is expanding upwards into elongated projections with indistinct margins that are insinuated into the spaces between the erythroblasts; there are suggestions of rhopheocytosis.

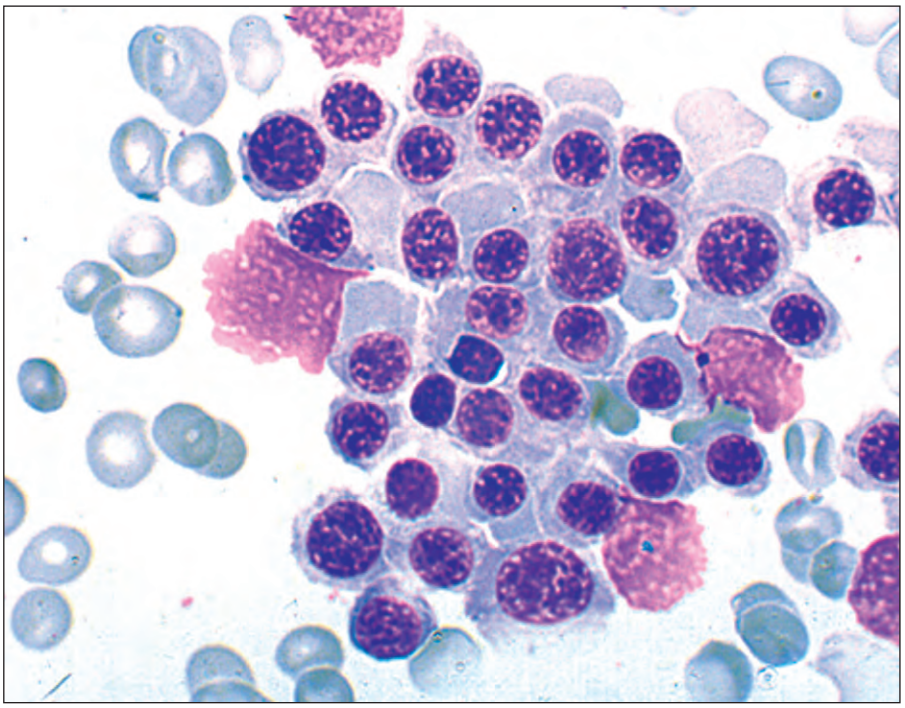


**Fig. 3.52** Synchronised maturation in dyserythropoiesis; bone marrow aspirate. There is a monomorphic carpet of polychromatic erythroblasts, which have identical cytoplasm and perfectly rounded nuclei, the chromatin structure of which, however, shows increasing degrees of condensation. Images of this type have been called 'hot spots' to indicate the vigorous proliferative activity.

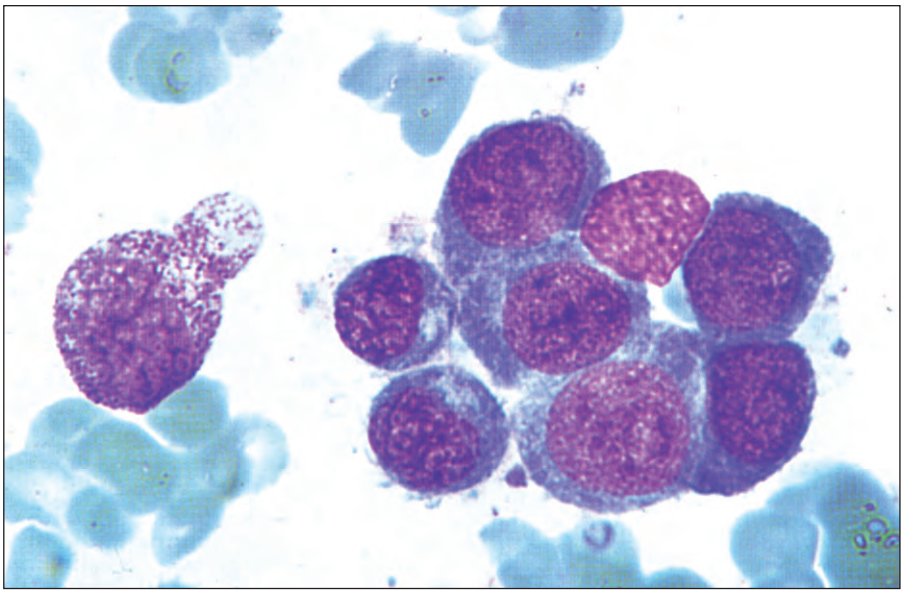


**Fig. 3.53** Synchronised maturation in a bone marrow aspirate from a patient with RAEB-1. This 'hot spot' is formed of five proerythroblasts, so close together that sometimes it is difficult to identify the margins between adjoining cells and the clump thus resembles a syncytium; they have intensely basophilic cytoplasm and nuclei with a granular chromatin structure. On the left, the two basophilic erythroblasts are smaller and have partially condensed chromatin with a vermicular appearance, and visible pale arcoplasm in the ultramarine cytoplasm. In the lower proerythroblast, which is more spread out, a large pale nucleolus is well seen because of the surrounding circle of more condensed chromatin. At the top of the clump of cells, a bare nucleus, testimony to the manner in which a macrophage, the cytoplasm of which has been lost during the spreading of the film and is no longer visible, adheres to the erythroid cells. On the left, an isolated dysplastic early myelocyte (or a late promyelocyte), the numerous granules of which are scattered in a disorderly manner in the sky-blue cytoplasm; the latter has a projection (initiation of clasmacytosis), a frequent feature in dysplastic granulopoiesis.

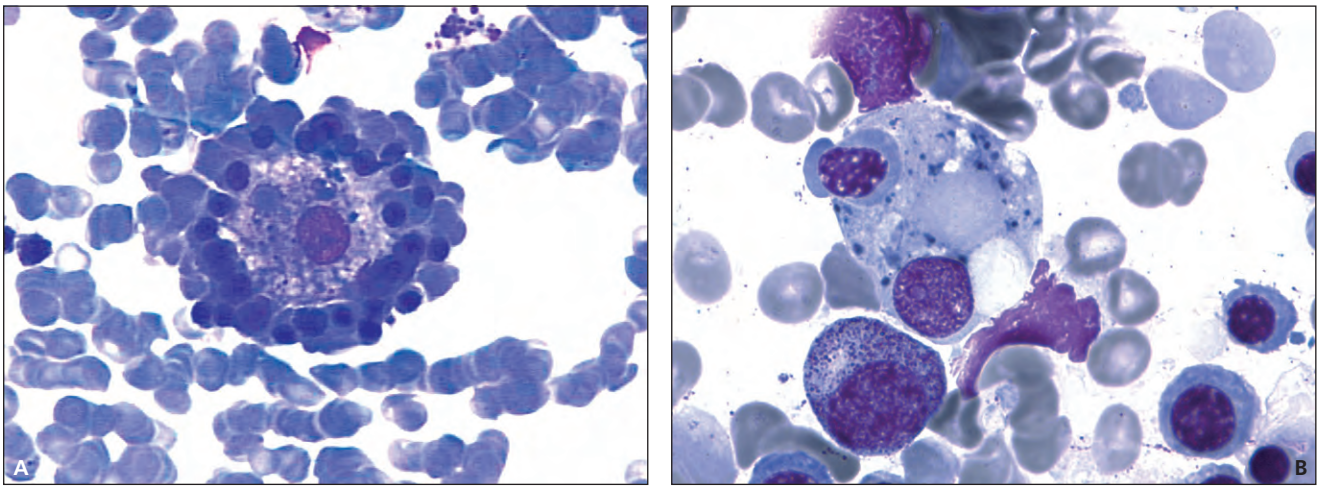



**Fig. 3.54** Interactions between macrophages and erythroid precursors: rhopheocytosis and phagocytosis in bone marrow aspirates of patients with MDS. (A) In the centre of this rhopheocytotic island, a large macrophage with a spongy nucleus in which a round dark blue nucleolus stands out. The greyish cytoplasm is extending in all directions: it contains dark granules, cellular debris, vacuoles and an intact phagocytosed red cell. The external circle is a monomorphic crown of closely adherent erythroblasts, mainly orthochromatic. (B) The central macrophage has a nucleus with spongy reticular chromatin, formed by closely alternating prune-coloured granular chromatin and small hypochromic circles: the nucleolus is prominent, pale and reinforced by a circle of condensed chromatin. The greyish cytoplasm contains dark bluish granules, a large vacuole on the right of the nucleus and other smaller vacuoles, and a still intact erythrocyte. Top right, a polychromatic erythroblast is superimposed on the macrophage cytoplasm, either ready to be phagocytosed or taking up ferritin molecules. The cell at the bottom is a neutrophil promyelocyte, in which the Golgi zone is clearly evident. Bottom right, two erythroblasts which can both be regarded as polychromatic, even if their size and their nuclear density differ.

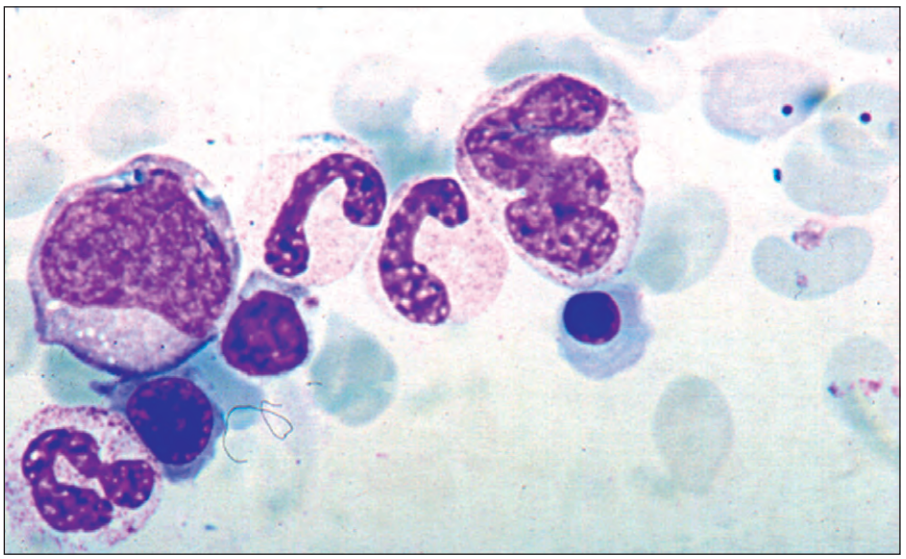


**Fig. 3.55** Dysgranulopoiesis in a bone marrow aspirate. In the centre, two neutrophil band forms with normal chromatin structure and pink cytoplasm with very fine, scattered granules: these cells are considered to be dysplastic because of the hypogranularity of the cytoplasm. On the right, more easily identified as dysplastic, a large mature neutrophil, with a convoluted hyperdiploid nucleus that is not completely segmented, the chromatin of which is normally condensed into violaceous clumps separated by pale spaces: cytoplasmic granules are reduced and in some areas the background, bottom right, appears weakly basophilic. Equally dysplastic is the large myelocyte on the opposite side of the image, which has a large nucleus with preserved chromatin structure and weakly basophilic, cyclamen-coloured cytoplasm that has flecks resembling pink sand, corresponding to very small granules (therefore hypogranular). The band neutrophil in the bottom left corner has a normal complement of cytoplasmic granules and can thus be used to evaluate the hypogranularity of the other neutrophils. On the left, a polychromatic erythroblast, a lymphocyte and an orthochromatic erythroblast.

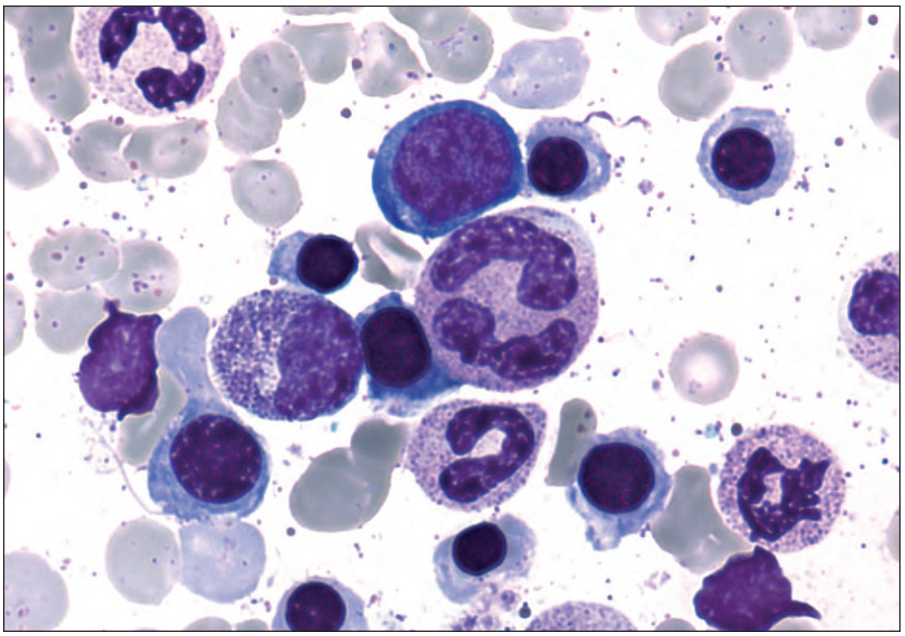


**Fig. 3.56** Moderate bone marrow dysgranulopoiesis in a case of refractory neutropenia. The morphology of the erythroid series, quantitatively well represented, is moderately dyserythropoietic. Some cells show the remains of torn internuclear bridges. There is a proerythroblast high in the centre; an erythroblast, low on the left, can still be considered a basophilic erythroblast because of its nuclear morphology, even if the blue colour of the cytoplasm is fairly attenuated; a number of polychromatic erythroblasts, with chromatin condensation varying from moderate to marked and cytoplasm sometimes showing poor haemoglobinisation. The neutrophil series shows, from top left towards the right: a normally segmented hypogranular neutrophil; a promyelocyte with an eccentric nucleus, a clearly visible Golgi zone and orderly distribution of numerous granules, thus a normal cell; a giant dysplastic neutrophil with two band nuclei, which does not, however, have any anomalies of chromatin condensation or granularity (with the exception of a hypogranular basophilic area at the top, similar to a Döhle body); immediately below, a normal neutrophil band form; a neutrophil, in the bottom right corner, which appears dysplastic because of the very dense chromatin structure and the numerous non-pedunculated nuclear appendages which have strikingly altered the nuclear outline.

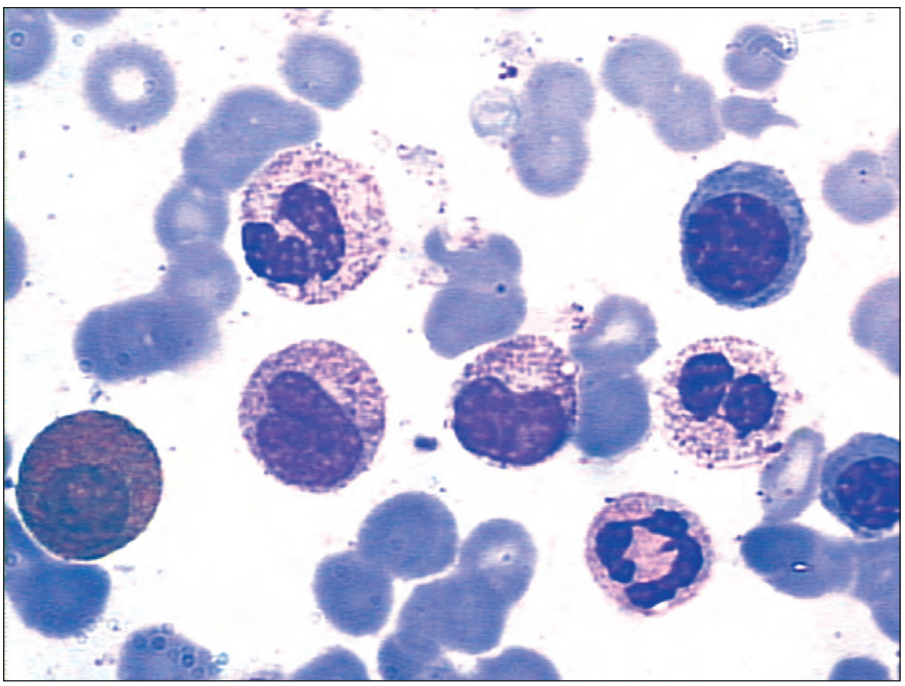


**Fig. 3.57** Dysgranulopoiesis and dyserythropoiesis; bone marrow aspirate. Top right, an erythroblast with N:C asynchrony. Immediately below is a dysplastic neutrophil with a bilobed pelgeroid nucleus and hyperchromatic granules. The neutrophil that is seen lower down, although having a segmented nucleus, is more anomalous, both because of the uniform prepyknotic density of the nucleus, which also has a large protrusion, and because of the pink cytoplasm in which no granules can be identified, but rather multiple Döhle bodies are seen in the form of small agranular basophilic areas adjacent to the cytoplasmic membrane. There are another three neutrophils and, on the left, an eosinophil myelocyte.

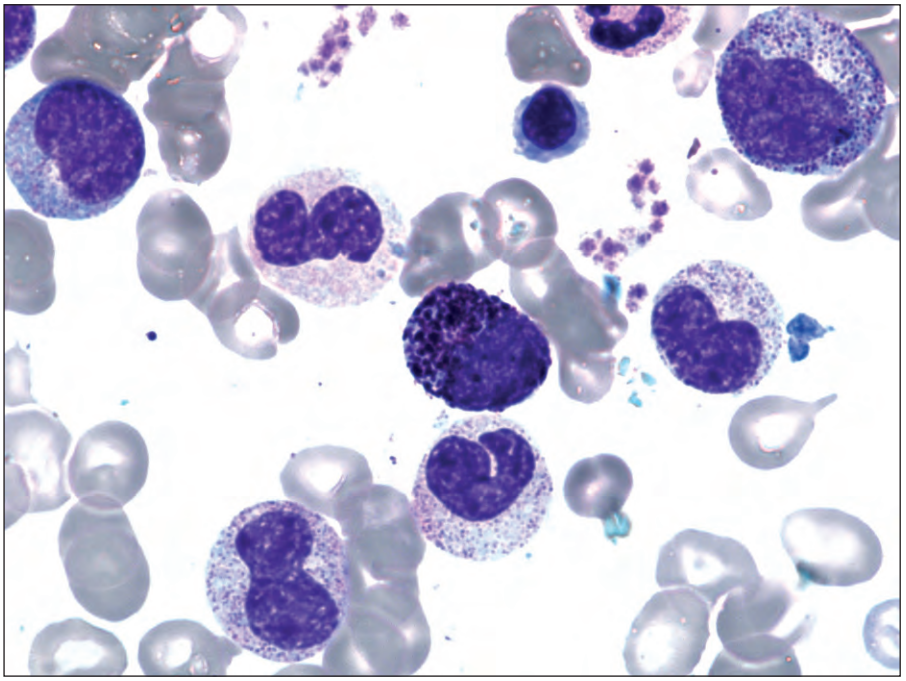


**Fig. 3.58** Dysgranulopoiesis; bone marrow aspirate. This image shows six cells of granulocyte lineage, a small polychromatic erythroblast and some elongated masses of platelets with intensely stained chromomeres, easily individually identifiable although adherent to each other, showing a normal non-activated configuration. In the centre, a small basophil myelocyte stands out, with a non-lobated nucleus with dense chromatin, clearly seen since it is not covered by granules (in contrast to what is observed in mature basophils). The granules are numerous, large and blackish, massed together in the half of the cell that is not occupied by the nucleus; only a few are superimposed on the nucleus at its other pole. The cytoplasmic background, still visible in small areas that lack granules, is basophilic. In comparison with a mast cell, this basophil myelocyte has fewer but larger granules, enabling the distinction to be made. With regard to the cells of granulocyte lineage,, one can consider that at least two of these, the more mature ones, are dysplastic. The early promyelocyte in the top left corner is normal, having an indented eccentric nucleus, moderately clumped chromatin without apparent nucleoli and a small number of granules distributed in an orderly manner in the basophilic cytoplasm, starting from the clearly outlined Golgi zone in the nuclear indentation; similarly normal is the early myelocyte (or late promyelocyte) in the top right corner, the basophilic cytoplasm of which is filled with orderly granules surrounding a pale paranuclear Golgi zone. The neutrophil above and to the left of the basophil myelocyte is certainly dysplastic, being hyposegmented and hypogranular and having areas of residual cytoplasmic basophilia adjacent to the cytoplasmic membrane; the band neutrophil beneath the basophil myelocyte is also dysplastic, because of its hypogranularity and the residual cytoplasmic basophilia. There are difficulties in applying the criteria for dysplasia to the two metamyelocytes at the bottom and to the right. This image clearly illustrates concepts applied in recognising granulocytic dysplasia in the bone marrow: (1) aberrations are seen more clearly in the more mature cells, particularly in segmented neutrophils and band forms; (2) it is common to see myelocytes and metamyelocytes that are more granular than the mature neutrophils; (3) the orderly arrangement of granules within the cell is a fundamental criterion for distinguishing between normal and dysplastic cells; (4) all this, however, must be taken with a pinch of salt, since granulopoiesis is a continuous process, in which alterations can be subtle and when there is dysplasia it can be difficult to assign cells showing N:C asynchrony to a specific stage of maturation.

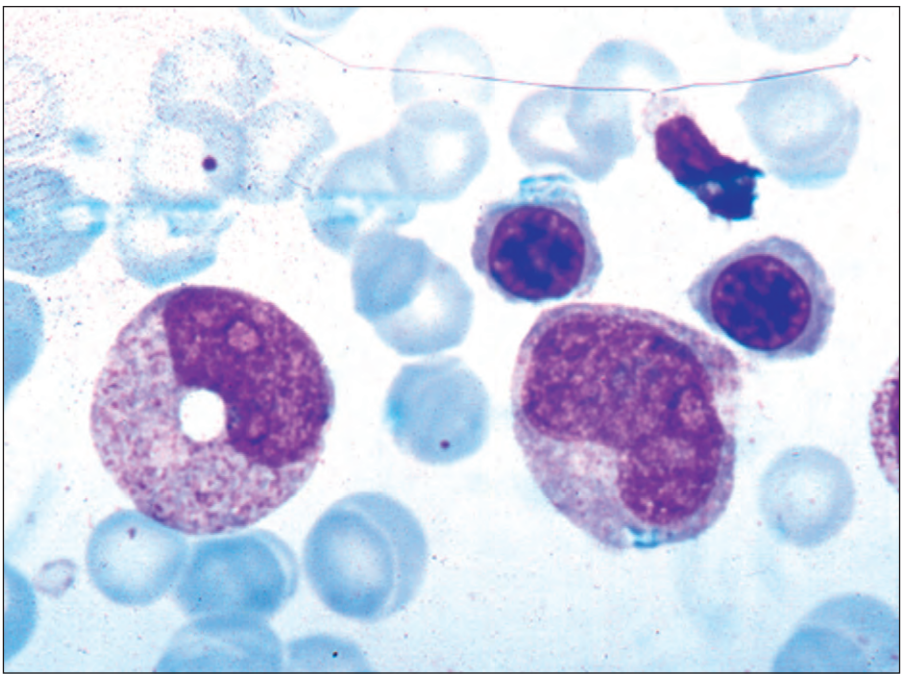


**Fig. 3.59** Dysgranulopoiesis, asynchronous maturation with bizarre features; bone marrow aspirate. On the left, a neutrophil granulocyte precursor with a doughnut appearance resulting from a large vacuole located right in the centre of the cell, strongly suggesting degeneration of the Golgi apparatus; the major anomaly of this cell, however, is the presence of two prominent nucleoli with perinucleolar chromatin condensation in a nucleus with fairly condensed chromatin and cytoplasm appropriate for a myelocyte, rich in mature, brick-red neutrophil granules. In the centre, another large neutrophil precursor with analogous nuclear characteristics in which, however, the cytoplasm contains more delicate granules. Both these cells can be considered dysplastic myelocytes. The cell on the right, however, is a small, normal appearing myelocyte, even though slight cytoplasmic basophilia persists.

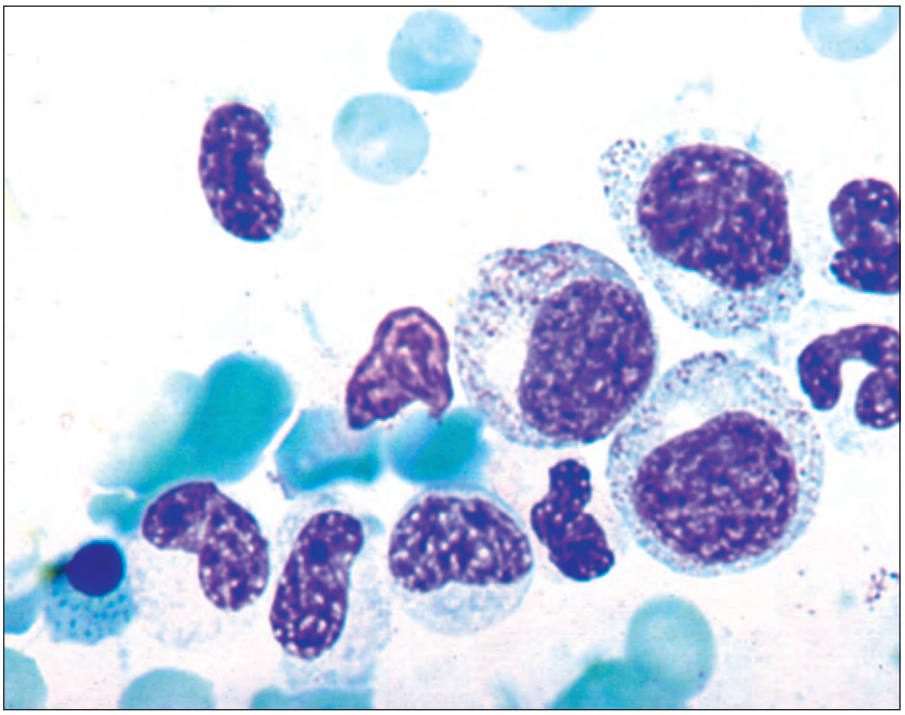


**Fig. 3.60** Marked dysgranulopoiesis in a case of RAEB-1; bone marrow aspirate. All the granulocytes present in this field are recognised as morphologically dysplastic, both because of hypogranularity and because of the abnormal condensation of the chromatin, which appears violet and shiny, with particular prominence of the pale spaces. The three largest cells are myelocytes, classified as such because of the fairly marked condensation of the nuclear chromatin while the cytoplasm is basophilic and contains large but not very numerous granules that are distributed in a fairly disorderly manner. The general appearance of these cells means that dysplasia can be recognised even though there is a pale Golgi zone evident in all three cells. The hypogranularity becomes total in the three small metamyelocytes on the left, close to the lower edge, and in the non-segmented neutrophils on the right. Bottom left, an orthochromatic erythroblast with basophilic stippling.

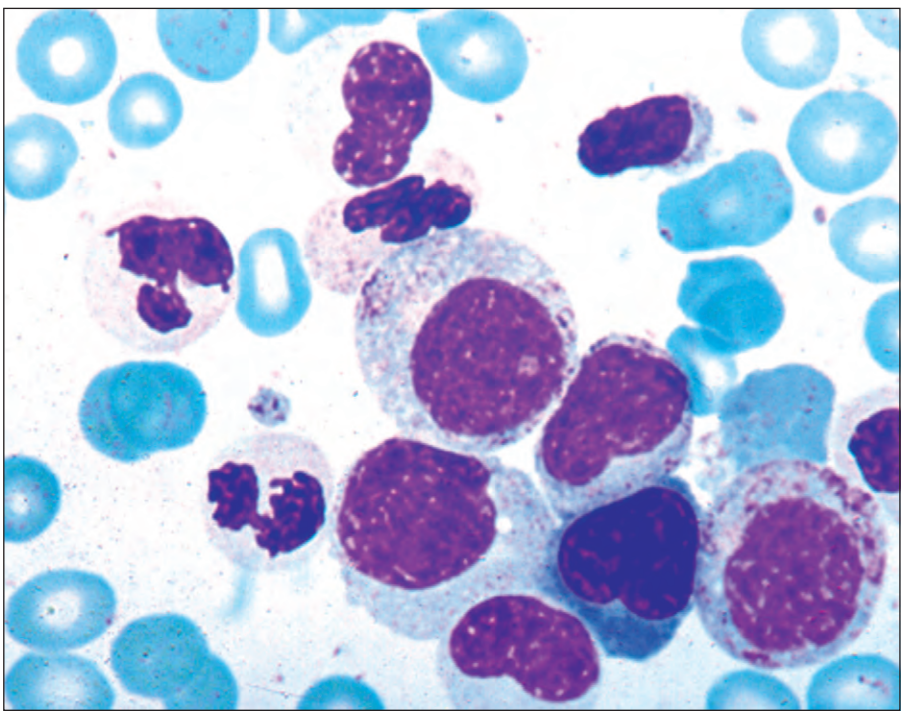


**Fig. 3.61** Marked dysgranulopoiesis with prominent hypogranularity from another patient with RAEB-1; bone marrow aspirate. Also in this case, almost all the cells of the granulocytic series are recognisably dysplastic. In particular, three mature neutrophils (two on the left and one on the right) appear to be totally lacking in granules, with basophilic cytoplasm and pyknotic non-segmented nuclei; the two on the left also have numerous nuclear appendages. The larger cell can be considered a hypogranular promyelocyte, with a prominent nucleolus, while the other three, with nuclei showing a variable degree of notching, can be considered dysplastic, agranular or hypogranular myelocytes. There is also a polychromatic erythroblast with a large bilobed nucleus in the shape of an upside down heart. At the top, a lymphocyte.

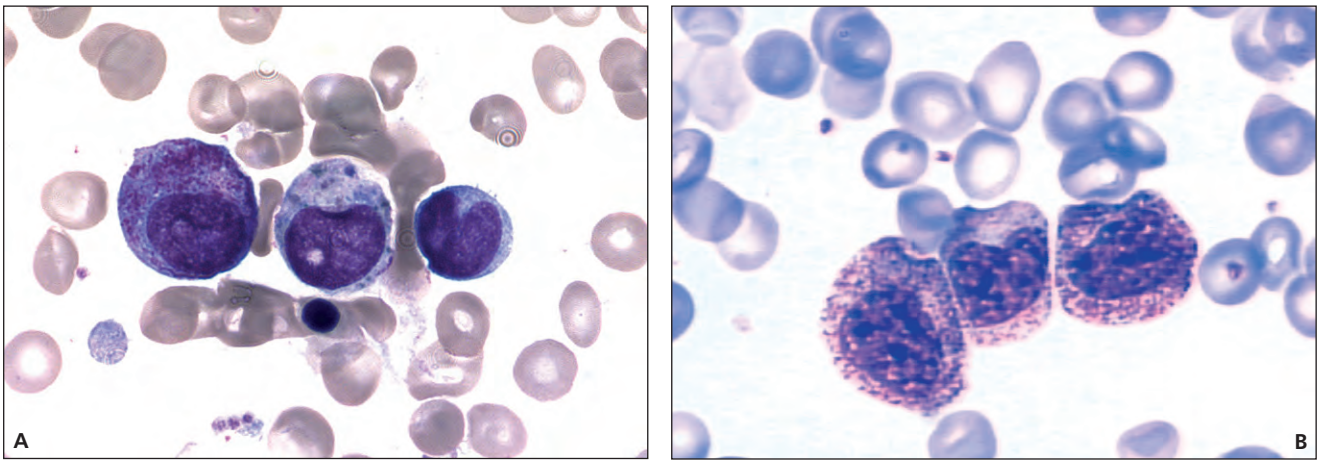



**Fig. 3.62** Anomalies of granulation in dysgranulopoiesis; two examples of bone marrow films. (A) On the left, a dysplastic promyelocyte with an eccentric nucleus and homogeneous chromatin showing little condensation and a prominent nucleolus; the intensely basophilic cytoplasm has a pale area that is slightly ectopic in relation to the nuclear indentation and has numerous very large, intensely azurophilic, cherry red or crimson granules, which in part are coalescent. Cells of this type confirm that the presence of a pale area corresponding to the Golgi apparatus does not exclude a cytomorphological diagnosis of dysplasia if the context is that of general disorganisation of the cellular structure. The cell in the centre is also a dysplastic promyelocyte, similar to the previous one, with features of necrobiosis evident in the coalescence and basophilic degeneration of the granules. There is also an orthochromatic erythroblast and various platelets, some of which are still adherent to each other. The spherical body seen bottom left may be a large grey-blue platelet without a chromomere or, possibly, a fragment of hypogranular cytoplasm of an immature granulocyte. (B) Two hypergranular myelocytes, on either side of a metamyelocyte (the latter recognisable as such because of the deep indentation in the nucleus). The heavy granulation of this cell is sufficient to define it as dysplastic in the context of myelodysplasia. A feature worth noting is the presence, in the top left corner of the cytoplasm of the myelocyte on the right, of two small elongated parallel rod-shaped formations, similar to Auer rods but not definable as such because of the context in which they are found (in a cell of intermediate maturity): this observation, in itself non-specific, can represent a line of superimposed granules, but it suggests a diligent search of the film for blast cells containing Auer rods would be worthwhile.

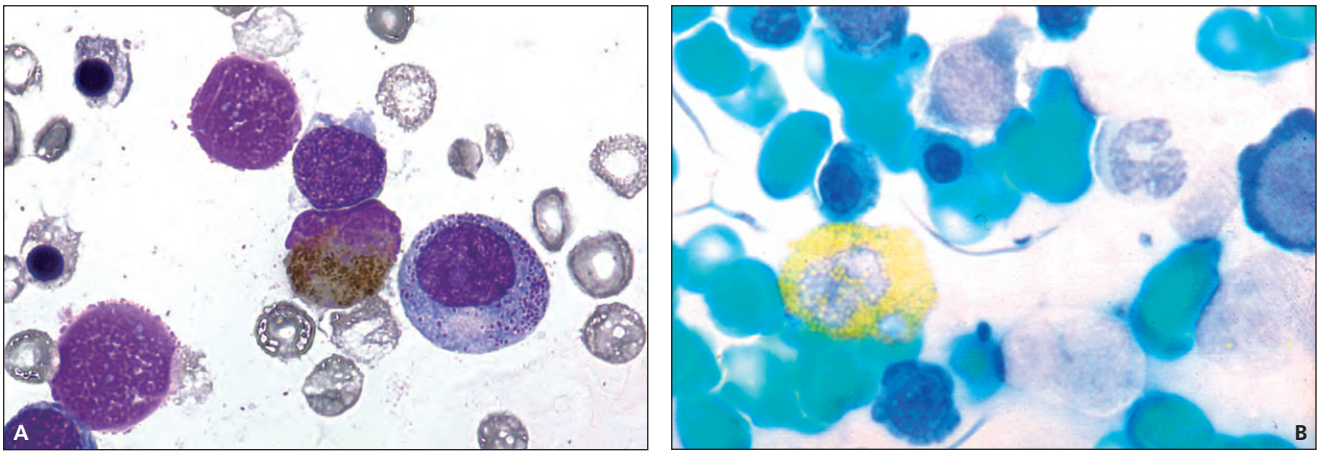



**Fig. 3.63** Dysgranulopoiesis, MPO stain: the total or partial deficiency of peroxidase in a proportion of neutrophils is not a frequent observation, but does suggest myelodysplasia. (A) On the left, two dysplastic orthochromatic erythroblasts, two torn nuclei, an agranular blast cell with scanty ragged cytoplasm, a granulocyte precursor with cytoplasm that is intensely positive for MPO and a neutrophil promyelocyte with a compact nucleus and swollen cytoplasm, which is negative for peroxidase. (B) Adjacent to several erythroblasts there are an eosinophil with very large granules that are intensely positive for peroxidase and a completely negative neutrophil band form.

the different categories of MDS and also in the differential diagnosis with AML. Normal promyelocytes can show subtle cytological differences, depending on a greater or lesser degree of differentiation. They have a round, oval or indented nucleus, centrally placed or eccentric; chromatin is fine or intermediate in density, with a nucleolus that is usually easily visible and prominent. The cytoplasm is generally basophilic (with possible heterogeneity and reduction in basophilia with the progression of differentiation) and they contain azurophilic granules dispersed around the pale Golgi zone in a regular orderly manner.

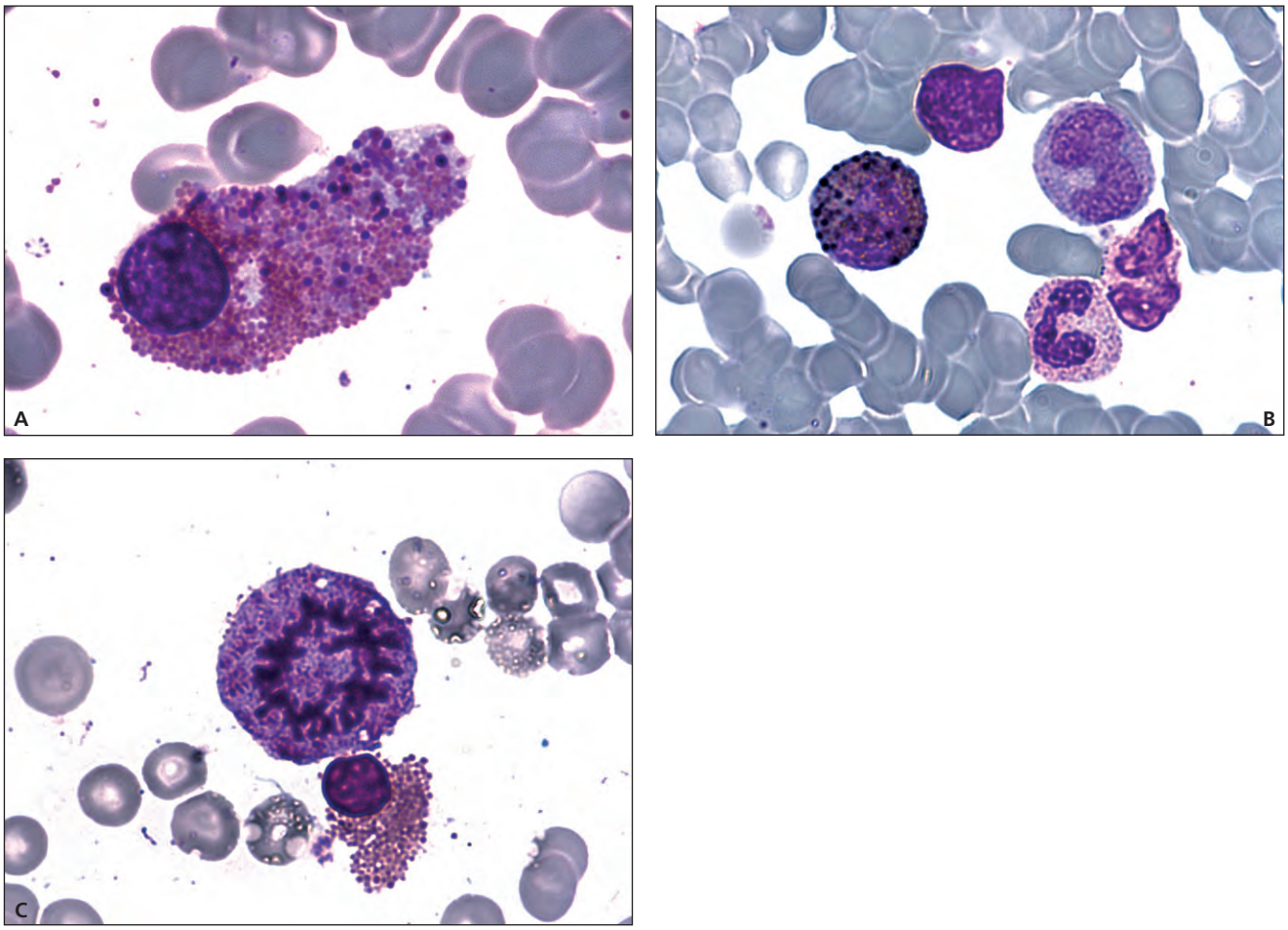



**Fig. 3.64** Dysgranulopoiesis in eosinophil precursors. The criteria for the identification of dysplasia in cells of this lineage are fairly non-specific, since they can also be seen in reactive, idiopathic or clonal eosinophilia. It is thus more appropriate to refer to dysmorphism than to dysplasia in the strict sense. (A) Eosinophil myelocyte with plentiful elongated cytoplasm. The nucleus is round and eccentric with partially condensed chromatin, particularly in relation to the nuclear membrane. The cytoplasm contains very numerous mature orange granules packed around the paranuclear area, in which the agranular pale Golgi zone is clearly seen. In the right side of the cytoplasm there are larger, immature, proeosinophilic granules, pinkish-grey or dark violet, which in fact have been formed first and pushed to the periphery by the newly formed mature granules. (B) Eosinophil myelocyte containing very large newly formed immature blackish granules, which stand out from the background, and some specific eosinophil granules in the background. On the right, a dysplastic monocyte (dysplastic because of the lack of chromatin condensation) and two neutrophils with abnormal nuclear segmentation. (C) A mitotic figure in a cell of neutrophil lineage and a pseudo-Pelger eosinophil.

The most typical characteristic is that which our predecessors described with the metaphor of a 'rising sun', in which the pale Golgi zone appears to rise from the indentation in the nucleus, dispersing the surrounding 'darkness' of the granules and basophilic cytoplasm.

Dysplastic promyelocytes, according to the new definition, have nuclear and cytoplasmic characteristics that are basically analogous to those of normal promyelocytes, with delicate or somewhat condensed chromatin, visible nucleoli and a perceptible Golgi zone, even if sometimes the Golgi zone is seen only with difficulty. However, they are distinguished, and herein lies the dysplasia, by the presence of features such as:

- Nucleocytoplasmic asynchrony;
- Major abnormalities of granules, which can be increased, reduced, irregularly distributed or aggregated;
- Poor development and irregularity of the Golgi zone;
- Reduced or absent cytoplasmic basophilia.

A cytochemical stain for myeloperoxidase (MPO) is useful to confirm the myeloid nature of blast cells. The 2008 WHO classification retains and emphasises the FAB concept of generally expressing the blast cells in the myelogram as a percentage of all nucleated cells (blasts as a percentage of all nucleated cells [ANC]) or, in certain circumstances, as a percentage only of the myeloid compartment, excluding erythroid cells, lymphocytes and plasma

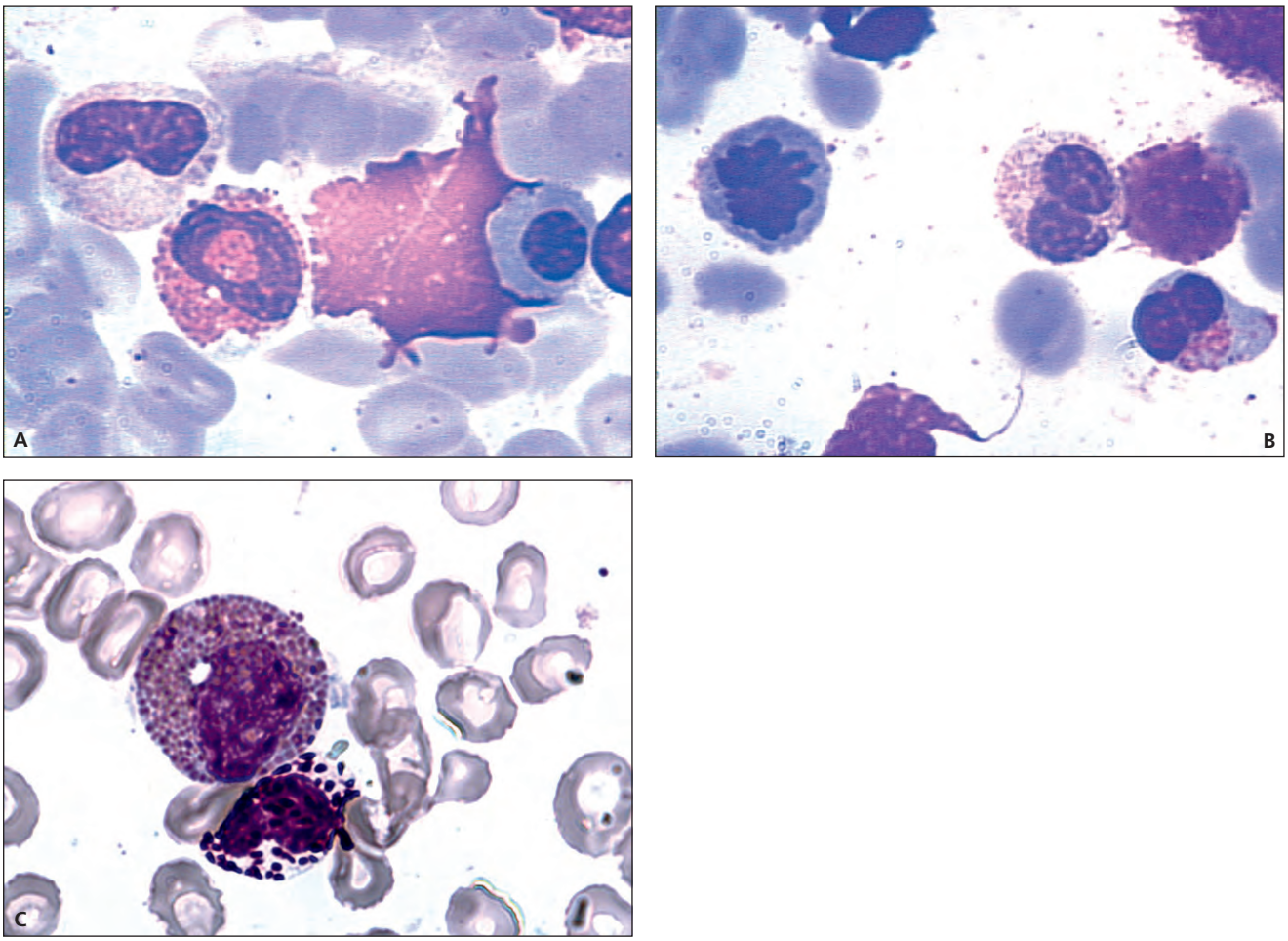



**Fig. 3.65** Dysmorphism and dysplasia of eosinophils in dysgranulopoiesis; three different bone marrow aspirates. (A) Adjacent to a hypogranular myelocyte is a mature eosinophil with cytoplasm that is rich in specific granules and a nucleus that is folded into a sort of ring (which appears at least partially interrupted on the right). This appearance is not specific for dysplasia. (B) On the right, a small very dysmorphic eosinophil, sufficiently abnormal to merit the epithet of dysplastic: the nucleus is peanut shaped, the lobes not having managed to separate, and in fact it is formed from very condensed prepyknotic chromatin; the cytoplasm contains only a few specific granules clustered together near the nucleus, with the rest of the cytoplasm being agranular and greyish, similar to that of the polychromatic erythroblast on the left, the nucleus of which has a cauliflower shape as a result of dyserythropoietic karyokinesis. The bilobed granulocyte between these two cells is difficult to identify because of the small greyish granules that are blending with the pink cytoplasmic background: it should, however, be counted as a dysplastic cell. (C) An eosinophil myelocyte with specific granules, a few dark proeosinophilic granules and a Golgi zone reduced to a vacuole; it almost overlaps a hypogranular basophil. These are both dysmorphic cells, neither of which can be specifically regarded as dysplastic.

cells (blasts as a percentage of NEC).[71,72] Blast cells as a percentage of NEC must be used only for the differential diagnosis between MDS, acute leukaemia with dysplasia, pure erythroid leukaemias and acute myeloid/erythroid leukaemia. Once the diagnosis of MDS has been established, the WHO expert group did not reach a consensus as to whether the blast cells should be counted as a percentage of ANC or as a percentage of NEC, but the majority view was that blasts counted as a percentage of ANC was preferred. Table 4.2 shows the diagnostic algorithm that should be used, following the majority view, to define the blast percentage in a bone marrow aspirate in those patients who at diagnosis have 50% or more erythroid cells in the myelogram.

## The megakaryocytic series

The morphological assessment of megakaryocytes is important when MDS is suspected, inasmuch as these cells show cytological features of dysplasia that are very specific and characteristic, the recognition of which can be determining when there is diagnostic difficulty.

The number of megakaryocytes can be increased or reduced. The morphological features of dysplasia in this lineage are fundamentally of three types (Figs 3.82 through to 3.102), which are:

1. Micromegakaryocytes, which are small megakaryocytes (with a nucleus similar in size to that of a promyelocyte or

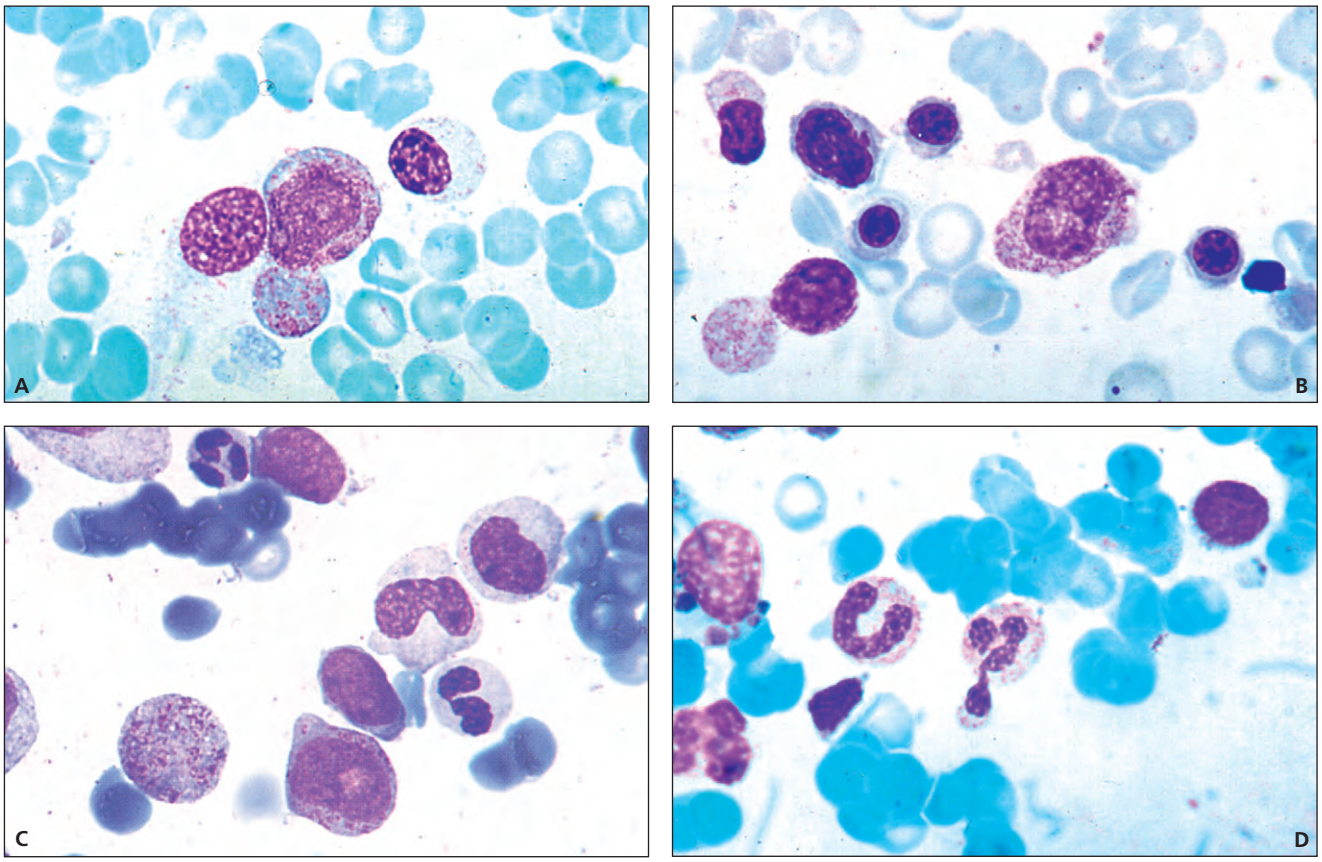



**Fig. 3.66** Clasmacytosis: a frequent observation, even though non-specific, in bone marrow aspirates characterised by dysgranulopoiesis. (A) A large dysplastic promyelocyte with a nucleus that has fairly dense chromatin and contains two prominent nucleoli. The basophilic cytoplasm is rich in azurophilic granules. At the bottom of the cell the cytoplasm is forming a large vesicular protrusion, which also contains granules; this appearance represents the first phase of clasmacytosis, the detaching of a portion of the cytoplasm. On the right, a mature neutrophil with a non-lobed pseudo-Pelger nucleus and blue, almost agranular cytoplasm. The nucleus on the left, with spongy chromatin, and the attached cytoplasm with ill-defined margins belong to a macrophage. (B) The cell bottom left is a neutrophil myelocyte, which is losing most of its cytoplasm (containing azurophilic granules); the cytoplasm looks like a large saddlebag joined to the rest of the cell only by a short peduncle. The nuclear chromatin is very clumped because of the preapoptotic state that is also responsible for the clasmacytosis. Starting from top left, a large granular lymphocyte, various polychromatic erythroblasts, one of which has a dysplastic polyploid and irregular nucleus, and another myelocyte rich in brick-red azurophilic granules. (C) In a setting of severe granulocytic dysplasia, bottom left, there is a large sphere formed of granular basophilic cytoplasm, which has detached from a granulocyte precursor by the process of clasmacytosis. Rather more important from the prognostic point of view is the recognition of three blast cells: the one at the bottom is granular with delicate homogeneous chromatin, an enormous nucleolus and large red-purple azurophilic granules scattered through the cytoplasm without there being an apparent Golgi zone; the other two, one immediately above and the other at the top close to the edge of the image, are smaller and lack granules. The diagnosis of myelodysplasia is confirmed by the presence of two metamyelocytes and two mature neutrophils (with pyknotic chromatin) with completely agranular blue cytoplasm. (D) In the centre, a hypogranular non-segmented neutrophil and at the top another with a four-lobed nucleus; one of the lobes is within a vesicular portion of cytoplasm that is close to being detached by clasmacytosis.

even smaller), often with a fairly high N:C ratio, scanty cytoplasm that is partially mature and a small, round nucleus that is non-lobed or bilobed, with dense mature chromatin[73]; their size can be similar to that of a lymphocyte, with a diameter between 7 and 10 μm.[74] Thus, one is not dealing just with small megakaryocytes, but with anomalous forms with a characteristic morphology, which must also be distinguished from more immature undifferentiated precursors, in which the nuclear chromatin has not yet become dense; these forms represent the most reliable evidence of megakaryocytic dysplasia.

2. Atypical megakaryocytes with multiple separate nuclei, usually with abundant cytoplasm.
3. Megakaryocytes with hypolobated nuclei, rounded in shape, small and eccentric, with normal chromatin density, and relatively ample cytoplasm that is usually well differentiated.

One can also observe hypogranular megakaryocytes, which are frequent if less specific, with cytoplasmic basophilia and marked atypia of platelet formation; or megakaryocytes with nuclei of bizarre shapes, and bare nuclei at the end of platelet production. Emperipolesis is the presence of extraneous cells

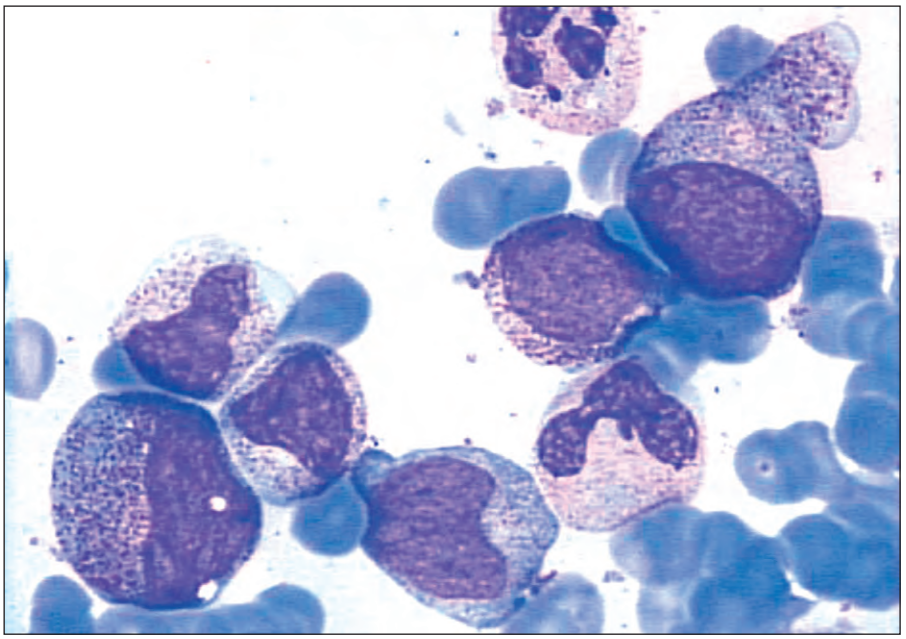


**Fig. 3.67** Severe dysgranulopoiesis seen at all stages of maturation in a case of RAEB-2; blasts and promyelocytes in the bone marrow aspirate of a male patient. In the centre at the bottom, a granular blast cell: this cell must be included in the blast count even though the pale outline of a Golgi zone is seen in a paranuclear position; it is regarded as a blast cell more because of the delicate reticular homogeneous structure of the chromatin than because of the presence of a double very prominent nucleolus and the fairly intense basophilia of the cytoplasm (which contains very delicate azurophilic granules distributed in a disorderly manner). The large cell in the bottom left corner, however, should be considered a dysplastic promyelocyte, since the granules, although they have created a 'rising sun' appearance, are distributed in a haphazard manner in the cytoplasm. The cell in the top right corner can also be classified as a dysplastic promyelocyte because of the disorderly appearance of the blue cytoplasm, in which the Golgi zone appears as a pale area detached from the nucleus and the numerous granules are scattered in an irregular manner throughout the cytoplasm, including within the large pseudopodial structure that represents clasmacytosis. The neutrophil at the top is interesting from the point of view of criteria for dysplasia since it has a hypersegmented nucleus with extremely condensed chromatin and numerous sessile or pedunculated nuclear appendages, one of which, arising from the lowest lobe, has the form of a pseudo-Barr body. In the well-granulated cytoplasm there are some basophilic paranuclear areas.

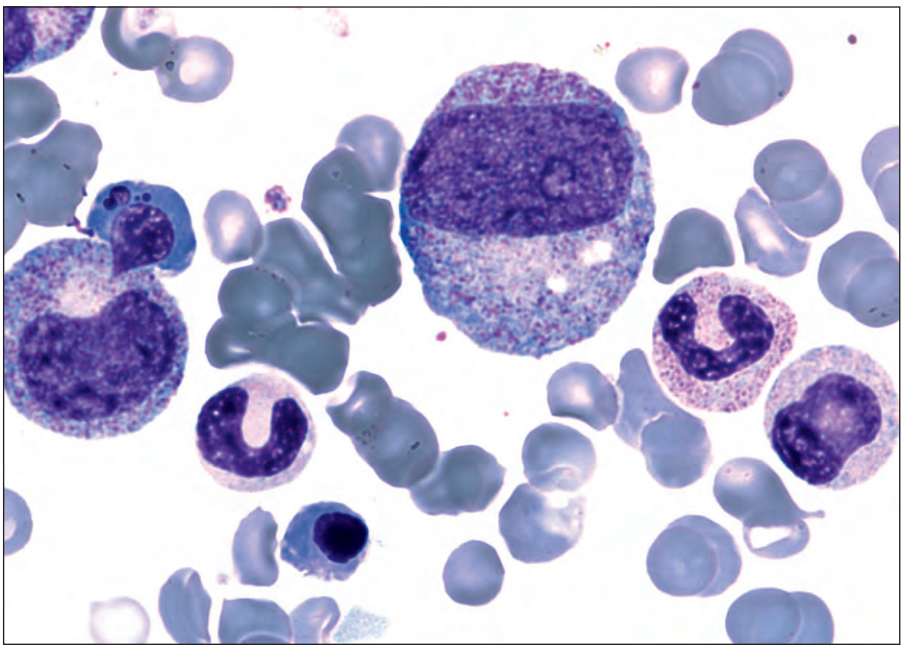


**Fig. 3.68** Granulocytic and erythroid dysplasia; bone marrow aspirate. The large cell in the centre is a promyelocyte with an eccentric rectangular nucleus with granular chromatin and a visible nucleolus, which must be considered dysplastic because of the quite irregular distribution of the granules, which are more numerous in the region distant from the paranuclear Golgi zone: the latter, however, appears too large and ill-defined for a normal promyelocyte. Among the other cells, the neutrophil precursor on the left has the nucleus of a myelocyte, but the cytoplasm of a promyelocyte (with a well-formed Golgi zone): because of this asynchrony it is regarded as dysplastic. Partially superimposed on it is a polychromatic erythroblast with numerous large Howell-Jolly bodies and a teardrop-shaped nucleus, resulting from stretching by an internuclear bridge that is now ruptured. Immediately to the right, a neutrophil with a horseshoe-shaped nucleus with almost liquefied chromatin and degenerated cytoplasm. On the right, a dysplastic myelocyte and another neutrophil band form with more normal morphology.

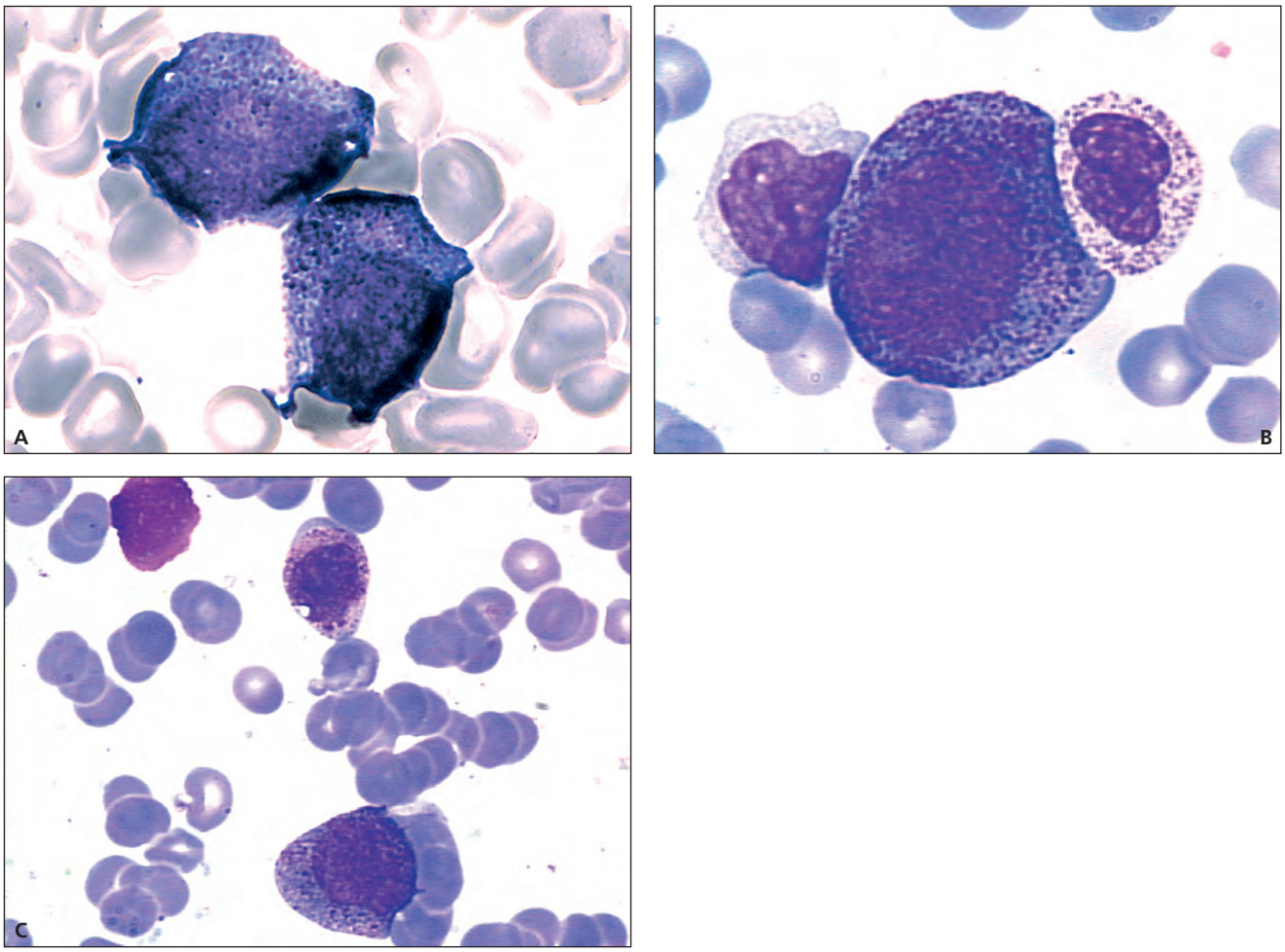



**Fig. 3.69** Dysplastic promyelocytes in myelodysplasia; bone marrow film. (A) Two immature granulocytes that have all the characteristics of dysplastic promyelocytes: chromatin density intermediate between that of blasts and that of promyelocytes; presence of nucleoli (one is visible in the upper cell); marked atypia of the cytoplasmic morphology with background basophilia, Golgi zone absent or difficult to discern and chaotic distribution of azurophilic granules, which also vary in size with some features of coalescence. (B) The giant cell in the centre, the nuclear dimensions of which suggest that it is tetraploid, otherwise has characteristics identical to those described in (A). It is thus not included in the blast count when making a cytomorphological diagnosis of myelodysplasia. On the left, a monocyte with vacuolated cytoplasm; on the right, a morphologically dysplastic metamyelocyte. (C) At the top, a myelocyte with nuclear appendages and at the bottom a promyelocyte, both dysplastic.

with well-conserved morphology within megakaryocyte cytoplasm, seen particularly when megakaryocytes are large and numerous. Occasionally, dysplastic megakaryocytes are present in small groups or clusters, which must be carefully sought in relation to fragments. Even rarer is a total absence of megakaryocytes.

Following the 2008 WHO criteria for assessing megakaryocyte dysplasia, it is important to examine at least 30 megakaryocytes; the presence of only one or two dysplastic megakaryocytes was reported in nearly 40% of bone marrow aspirates performed on 50 healthy subjects and hence this is not sufficient to make a diagnosis of dysplasia in this lineage.[30] Some morphological aspects of megakaryocyte dysplasia, when they are frequent, correlate with specific cytogenetic abnormalities: micromegakaryocytes, in particular, are associated with monosomy 7; hypo- and non-lobated megakaryocytes correlate with deletion of part of the long arm of chromosome 5.

With the aim of defining morphological criteria for megakaryocytic dysplasia, bone marrow aspirates from patients affected with MDS were analysed using the APAAP technique and monoclonal antibodies directed at glycoprotein IIb/IIIa, proteins specific for the megakaryocyte/platelet lineage; the aim was to confirm morphological observations and identify any atypical megakaryocytes with cytological features similar to lymphocytes. Five types of megakaryocyte were identified: types 1, 2 and 3 were defined as atypical megakaryocytes, type 4 as mature megakaryocytes and type 5 as megakaryoblasts. Type 1 is a micromegakaryocyte, the diameter of which is less than

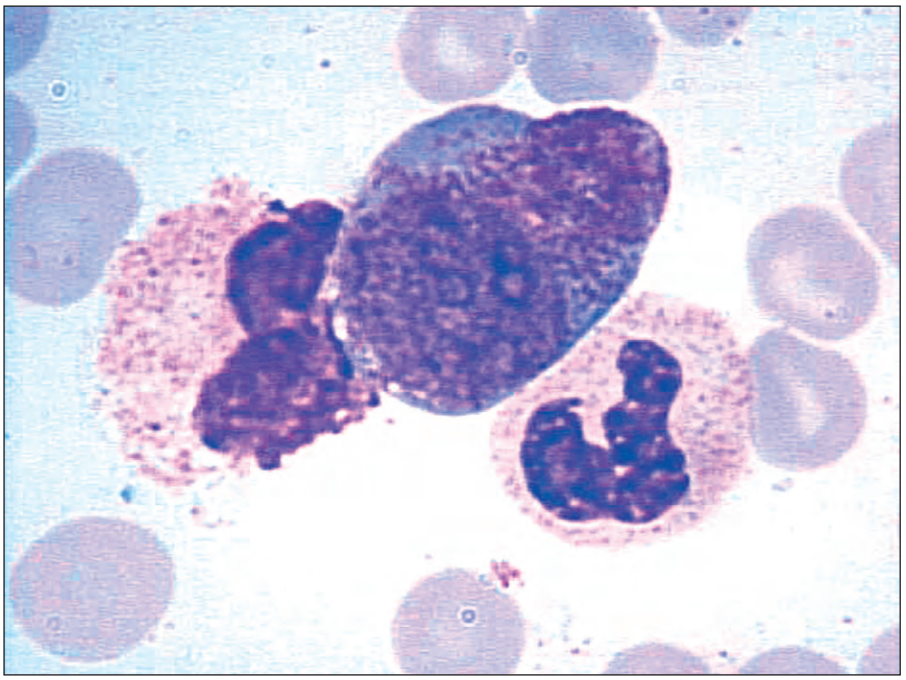


**Fig. 3.70** Promyelocytic and neutrophil dysplasia in a case of refractory anaemia with multilineage dysplasia; bone marrow aspirate. In the centre, a dysplastic promyelocyte: the most important features indicating that this cell is not a blast are both nuclear, such as condensation of the chromatin into compact heterogeneously distributed clumps, and cytoplasmic, such as the crowding of numerous granules into a limited part of the cytoplasm, which is forming an extrusion. However, some features of this cell, such as the presence of multiple nucleoli, the intense cytoplasmic basophilia and the particular abundance of granules give it the resemblance of an abnormal promyelocyte in acute promyelocytic leukaemia: this diagnosis can, nevertheless, be excluded not only on the basis of morphology, but also by considering the context, which in myelodysplasia is that of heterogeneity of lineages involved and of the maturity of the affected cells rather than the monomorphism of acute promyelocytic leukaemia. The two mature neutrophils have clear signs of nuclear dysplasia; the cell on the left has a pedunculated appendage at the top and a sessile nodule at the bottom; the cell on the right has a filiform appendage on the left.

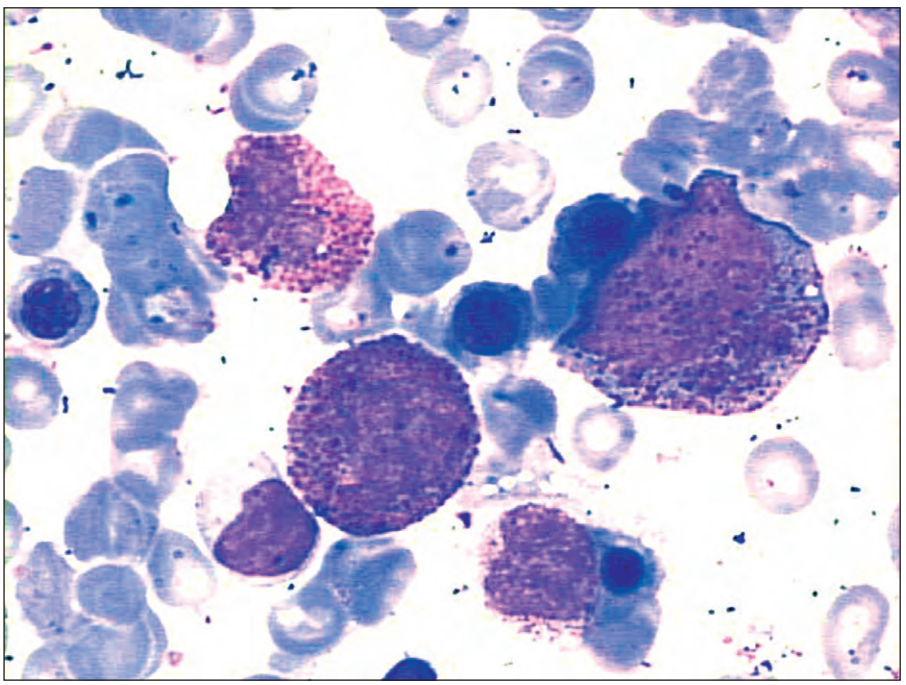


**Fig. 3.71** Differential diagnosis between blast cells, promyelocytes and dysplastic promyelocytes in myelodysplasia; bone marrow aspirate. The cell below and to the left of centre, between an eosinophil, some erythroblasts and a large granular lymphocyte, is a dysplastic promyelocyte, characterised by an abundance of large dark granules, which crowd the cytoplasm leaving only a part of the nucleus exposed, which shows clearly its clumped chromatin. The classification of the large hypergranular cell on the right is more difficult. It is intensely basophilic, packed with fairly large dark granules and lacks a visible Golgi zone; the nucleus is eccentric and angular, with fairly fine and homogeneous chromatin. Because of the very immature nucleus it is appropriate that this cell is included in the count of granular blasts.

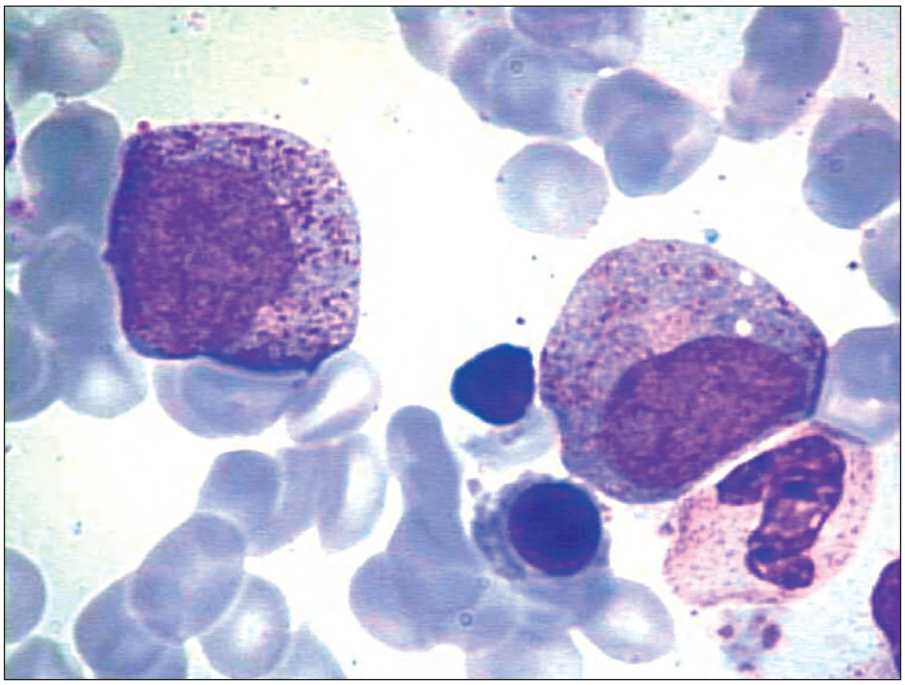


**Fig. 3.72** Differential diagnosis between blast cells, promyelocytes and dysplastic promyelocytes in myelodysplasia; bone marrow aspirate. Based on the criteria that have been explained, the large cell on the right, between a non-segmented neutrophil and two small erythroblasts, is a dysplastic promyelocyte. The nucleus is anomalous: it is eccentric with chromatin that is compact but not very condensed, a large nucleolus and an indentation in the nucleus which corresponds to the clearly visible Golgi zone. The cytoplasm is intensely basophilic, slate blue, and appears to meet the criteria for dysplasia: the granules are distributed in a fairly disorderly manner, and are heterogeneous with regard to size and staining characteristics, the larger and darker ones suggesting that coalescence has occurred. The neutrophil promyelocyte on the left is dysplastic, repeating the same nuclear characteristics but with more advanced and more abnormal cytoplasmic maturation (an abundance of large and heterogeneous granules with the Golgi zone poorly seen).

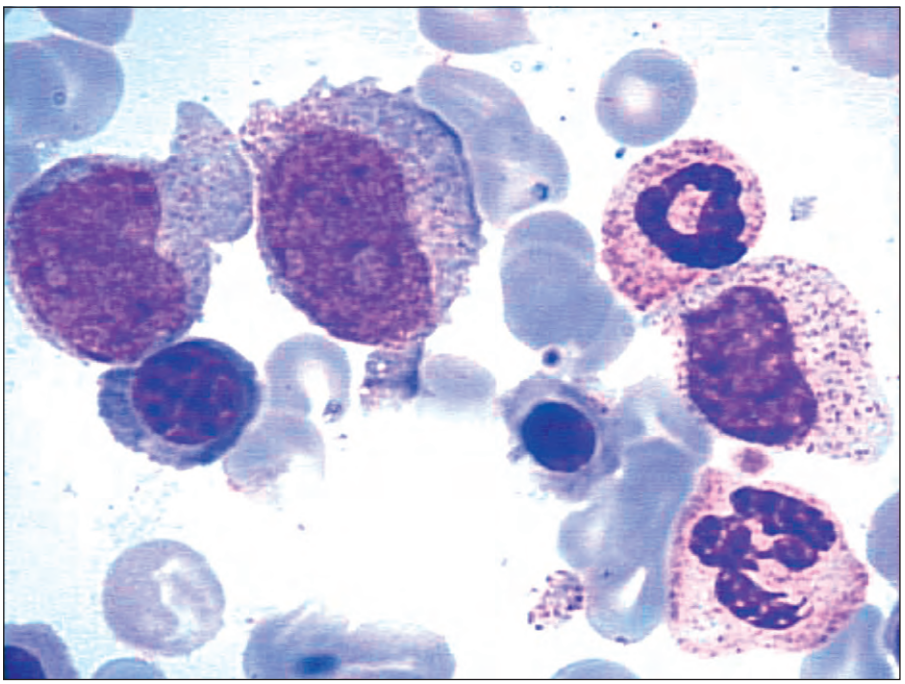


**Fig. 3.73** Differential diagnosis between blasts and dysplastic promyelocytes in myelodysplasia; bone marrow aspirate. On the right, three maturing granulocytes: the cell in the middle is a myelocyte, while the other two are dysplastic neutrophils with abnormal nuclei. Two erythroblasts, one orthochromatic and one polychromatic, appear cytologically normal. The two larger cells, top left, are not identical. The one closer to the centre is a promyelocyte, but it can be considered dysplastic because of the bizarre distribution of the granules, which are mainly crowded together at the upper pole, a long way from the indistinct Golgi zone that can nevertheless be recognised in the nuclear indentation; there are two nucleoli, one quite large, which are pale and show peripheral reinforcement. The other cell, on the left, has plentiful, more weakly basophilic, grey-blue cytoplasm and is deformed by a large extrusion that may indicate clasmacytosis. The eccentric nucleus has delicate homogeneous chromatin; there are two nucleoli; the outline is angulated and shows a clear notch beneath a small pale area. There are a few fine azurophilic granules that are separated from each other and distributed without any apparent pattern. This cell can be included in the blast count.

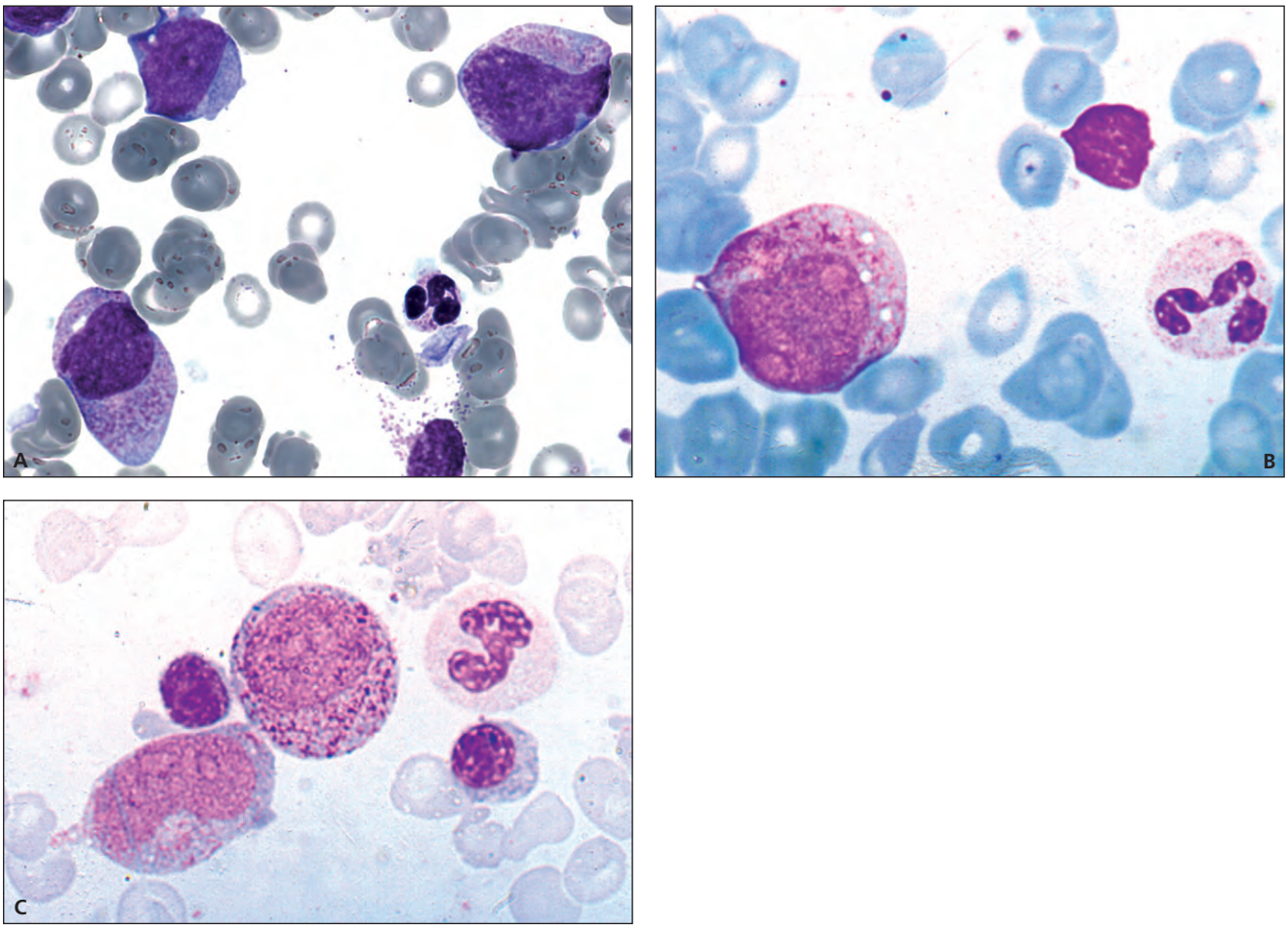



**Fig. 3.74** Differential diagnosis between blasts and dysplastic promyelocytes in myelodysplasia; bone marrow aspirate. (A) A very small dysplastic neutrophil, similar in size to the red cells, with a nucleus that has three lobes composed of very condensed chromatin. The three immature cells in the corners of the image present problems of interpretation. These are not major for the cell seen top left, an agranular blast cell, as shown by its uniformly basophilic cytoplasm without granules or pale spaces, its nuclear outline, which on the side bordering the cytoplasm appears straight, without any incision and the fine homogeneous structure of the chromatin, which has small foci of condensation and ill-defined nucleoli. The cell top right is larger with a nuclear outline that is more irregular, but similarly shows straight areas and is angulated with a fold that resembles a notch; the chromatin is denser without evident nucleoli. There are no traces of a Golgi zone, but the basophilic cytoplasm contains many azurophilic granules, which are uniformly small and stain crimson. Their distribution appears random. This is the cell that is most difficult to classify, but rigorously applying the criteria proposed by the International Group for Morphology of MDS (IGM-MDS),[66] it appears necessary to define it as a granular blast cell, particularly because of the characteristics of the cytoplasm, including the lack of a visible Golgi zone. The oval cell seen bottom left, although similar overall, is better classified as a dysplastic promyelocyte, because of the greater regularity of the nucleus and cytoplasm with evidence at the bottom of a pale Golgi zone (not to be confused with a transparent vacuole at the top), because of the less basophilic cytoplasm and, particularly, because of the orderly distribution of the granules, which are larger, heterogeneous and a less vivid red. (B) This large immature granulocyte precursor shows very irregular distribution of the numerous crimson-red granules, which are partly massed together and coalescent, contained within plentiful, weakly basophilic, vacuolated cytoplasm. The nuclear outline shows, in its upper part, a depression corresponding to a pale area in the cytoplasm where the granules are more crowded: it is the existence of this indistinct Golgi zone that leads to the cell being classified as a dysplastic promyelocyte. The nuclear chromatin is granular and homogeneous, with two enormous nucleoli, one of which shows peripheral reinforcement by condensed chromatin. Adjacent, a dysplastic neutrophil. (C) In the centre, a dysplastic promyelocyte with the same characteristics as described in (B). Bottom left, a granular blast cell with chromatin that could be described as fine, loose, granular and open; there are a number of nucleoli, some with peripheral reinforcement. The difference, in terms of chromatin density and the quantity and characteristics of the granules, between a dysplastic promyelocyte and a granular blast cell is evident here. The blast has a visible Golgi zone in the nuclear hollow from which a few fine azurophilic granules spread out. The visibility of the Golgi zone is shown here to be a less absolute criterion than has recently been suggested. The other cells are a lymphocyte, on the left, a hypogranular neutrophil and a polychromatic erythroblast.

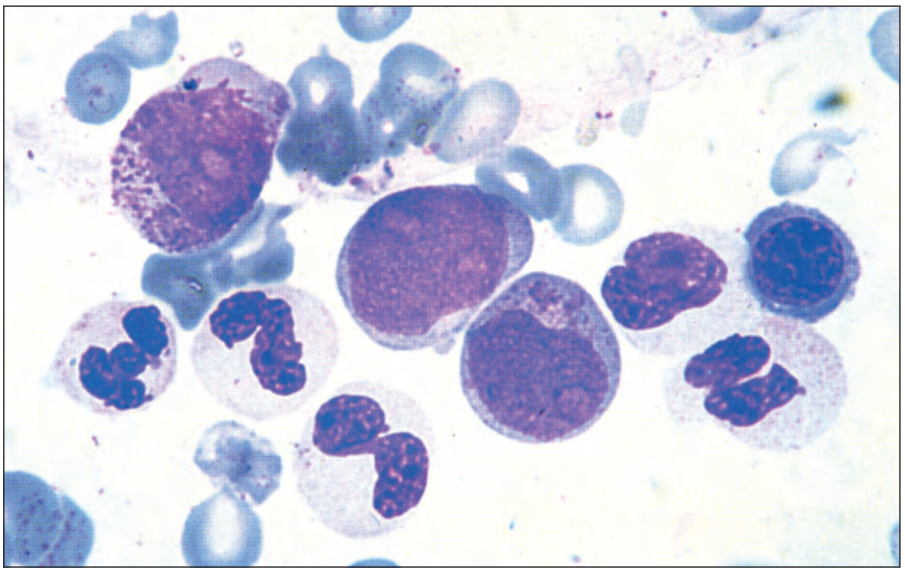


**Fig. 3.75** Differential diagnosis between blasts and dysplastic promyelocytes in myelodysplasia; bone marrow aspirate. Five dysplastic hypogranular neutrophils, an erythroblast with asynchronous maturation and three immature granulocyte precursors. In the past these cells would have been classified as blasts. The WHO classification has modified the criteria for blast cells from those proposed in the FAB classification and has simultaneously lowered the diagnostic threshold for acute leukaemia from 30% to 20% blasts. Only one of these three cells, that in the centre, satisfies the WHO criteria for a blast cell (non-granular): the nucleus has reticular granular chromatin with two visible, although poorly defined, nucleoli; the cytoplasm is basophilic and has a vague pale paranuclear area without there being any apparent granules. The cell on the right, using a strict definition, is a non-dysplastic promyelocyte: it has an immature nucleus with a prominent nucleolus, basophilic cytoplasm with a pale Golgi zone corresponding to an indentation in the nucleus and orderly arrangement of the granules around the Golgi zone. The cell on the left is classified as a dysplastic promyelocyte because its granules are large and dysmorphic, and crowded into one part of the cytoplasm without any apparent relationship to the Golgi zone. The WHO and IWGM-MDS criteria, which are being described and utilised for making a distinction between granular and agranular blast cells, on the one hand, and promyelocytes showing a greater or lesser degree of dysplasia, on the other, provide a useful and necessary basis for making a judgement. However, at the same time, it is also important to recognise that the criteria may sometimes be inadequate both because of the subjectivity of interpretation and because of the fact that in many cases the blast cells can undergo differentiation, so that the cytological context is that of a continuum in which it is sometimes impossible to draw a clear distinction between granular blast cells and dysplastic promyelocytes.

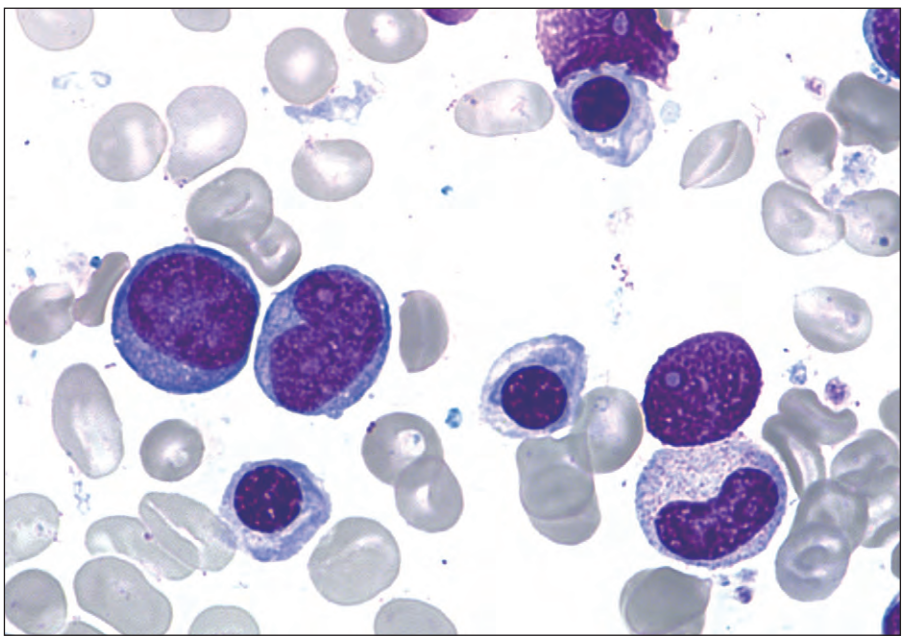


**Fig. 3.76** Blasts and myelodysplasia; bone marrow aspirate. Three polychromatic erythroblasts with cytoplasm that contains large, pale, non-haemoglobinised spaces; the cell at the top has a nucleus that is already homogeneously pyknotic, while the others have pale areas of parachromatin. Bottom right, a neutrophil metamyelocyte, with plentiful grey-blue cytoplasm and some granules. The other two cells on the left are two agranular blast cells: their nuclear outlines differ, that on the left being oval while that on the right shows a deep notch, but they do not differ in the structure of their chromatin, which is fairly compact and homogeneous but with some areas of greater condensation. Nucleoli are present in both the nuclei, but are more distinct in the nucleus of the blast on the right, there being one nucleolus in each lobe. The cytoplasm is moderately abundant and strongly basophilic, ultramarine.

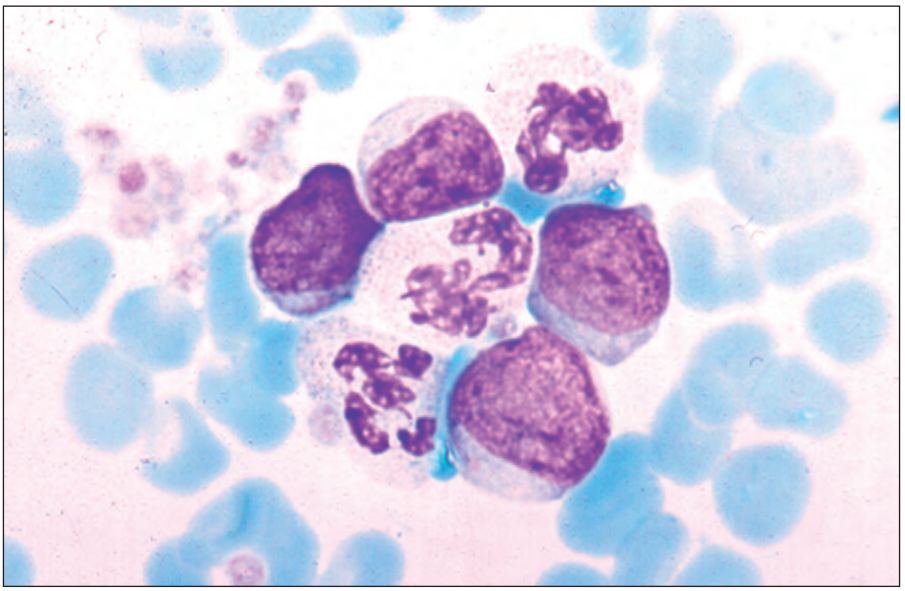


**Fig. 3.77** Blasts and neutrophil dysplasia in dysgranulopoiesis. A small group of cells adhering to each other from a patient with RAEB-2; bone marrow aspirate. There are three neutrophils showing a tendency to nuclear hypersegmentation: this is associated, as often happens, with the presence of a number of nuclear appendages of various shapes, sessile and pedunculated, particularly prominent in the central cell. Top, a small hypogranular neutrophil myelocyte. The other three cells are blasts with non-indented nuclei and chromatin composed of a loose, coarsely woven reticulum with some foci of condensation. The cytoplasm is homogeneously basophilic without a Golgi zone, but with some fine azurophilic granules, the presence of which was confirmed, at least for two of them, by altering the fine focus of the microscope.

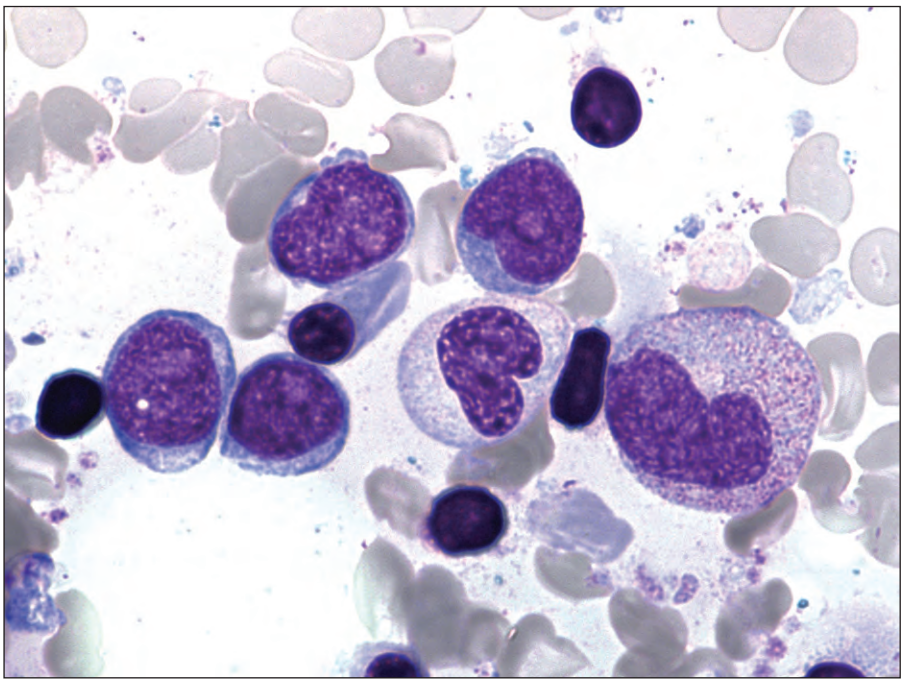


**Fig. 3.78** Blasts and neutrophil dysplasia in dysgranulopoiesis; bone marrow film from the same patient in Fig. 3.76. The morphology of the four agranular blast cells is characterised, and does not leave any cytodiagnostic doubts, by: an irregularly rounded outline, sometimes with notches; chromatin formed of a dark homogeneous reticulum, thicker in some cells and looser in others, evidence of a nucleolus in the three cells to the right and with two or possibly three pale indistinct nucleoli in the cell further to the left; the blue cytoplasm with some pale areas, sometimes paranuclear (without this feature, however, alternative diagnostic possibilities would be raised). There are pyknotic nuclei, certainly of erythroid origin, and an orthochromatic erythroblast with linear non-haemoglobinised areas (to be counted among the dysplastic cells when evaluating the dysplasia in each lineage), and two cells of neutrophil lineage, which, although they both fit the definition of a dysplastic metamyelocyte, show substantial morphological differences. That in the centre has almost completely agranular cytoplasm, and a folded nucleus with prune-coloured chromatin that is condensed into clumps separated by pale lines and circles (this cell could also be regarded as a dysplastic neutrophil band form). The cell on the right has fewer signs of cytological maturation: the nucleus, indented and almost horseshoe shaped, has chromatin that is paler and more open, which although very different could be compared with the homogeneous chromatin (albeit more compact and dark) of the blast cell. On the right, some morphologically normal platelets (at the bottom) and others that are grey-blue, without a chromomere; the swollen spherical particle above the myelocyte on the right could be a cytoplasmic fragment detached by clasmacytosis.

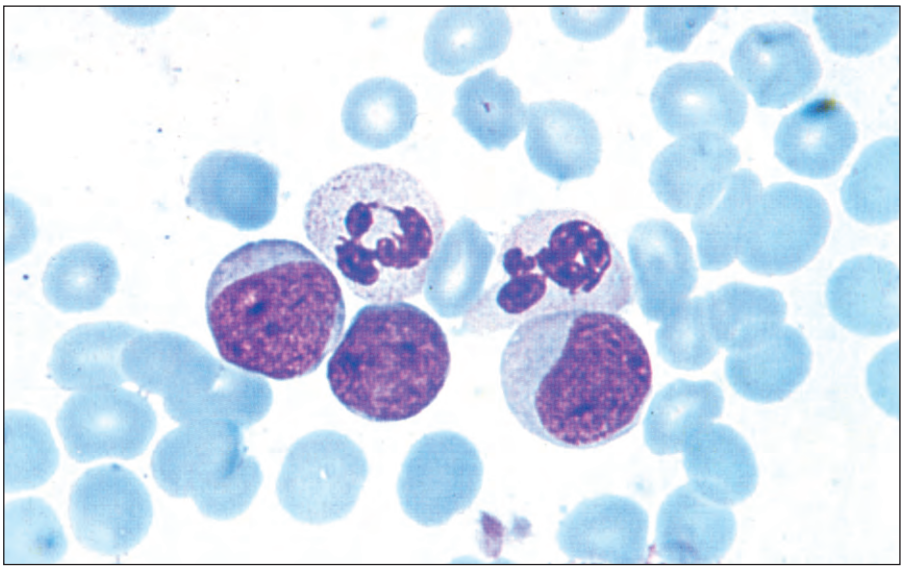


**Fig. 3.79** Granular blasts and neutrophils in myelodysplasia; bone marrow aspirate from a case of RAEB-2. There are two neutrophils with normally segmented nuclei with some filiform appendages and, in the case of the cell on the right, agranular cytoplasm. The other three cells are agranular blasts (which would have been type I blasts in the FAB classification): the one in the centre has only a thin half-moon of cytoplasm above the nucleus, while the other two have eccentric nuclei within moderately more abundant cytoplasm which shows a variable degree of basophilia. In the blast on the left, there may be some fine azurophilic granules. One notes that, in the absence of any recognisable Golgi zone, the chromatin structure of this blast cell appears relatively more condensed than that in the blast cells of many myeloid leukaemias.

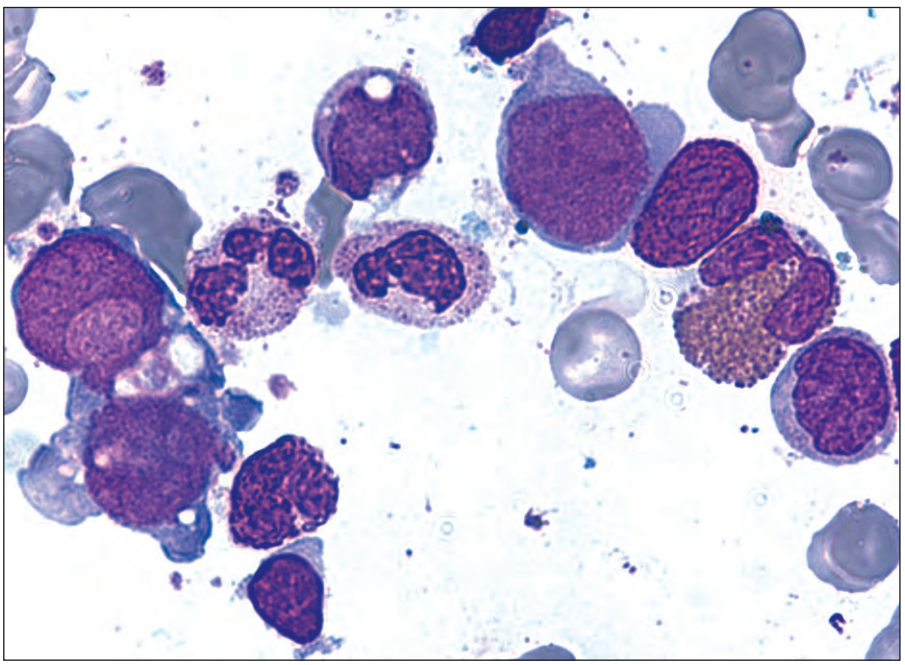


**Fig. 3.80** Blasts with bizarre morphology in the bone marrow aspirate of a patient with RAEB-2. In the centre at the top, a large blast cell with plentiful agranular basophilic cytoplasm which is forming blebs; the chromatin of the enormous nucleus, certainly tetraploid, is finely granular: there is a large nucleolus with a pale delicate chromatin network, adjacent to which the chromatin in condensed into small clumps. Bottom left, another large blast cell in which the characteristics described above are exaggerated: in fact, it contains two large reticular nuclei, joined by an intracytoplasmic chromatin bridge, with giant vesicular nucleoli and striking cytoplasmic protrusions. The morphology of these abnormal cells resembles that of megakaryoblasts, the nature of which can be verified by flow cytometric immunophenotyping or immunohistochemistry. In this field, in addition to the blasts, it is curious that there are present all five types of mature leucocyte found in the peripheral blood: from bottom left, a lymphocyte, a basophil, two neutrophils and, further to the right, an eosinophil and a monocyte.

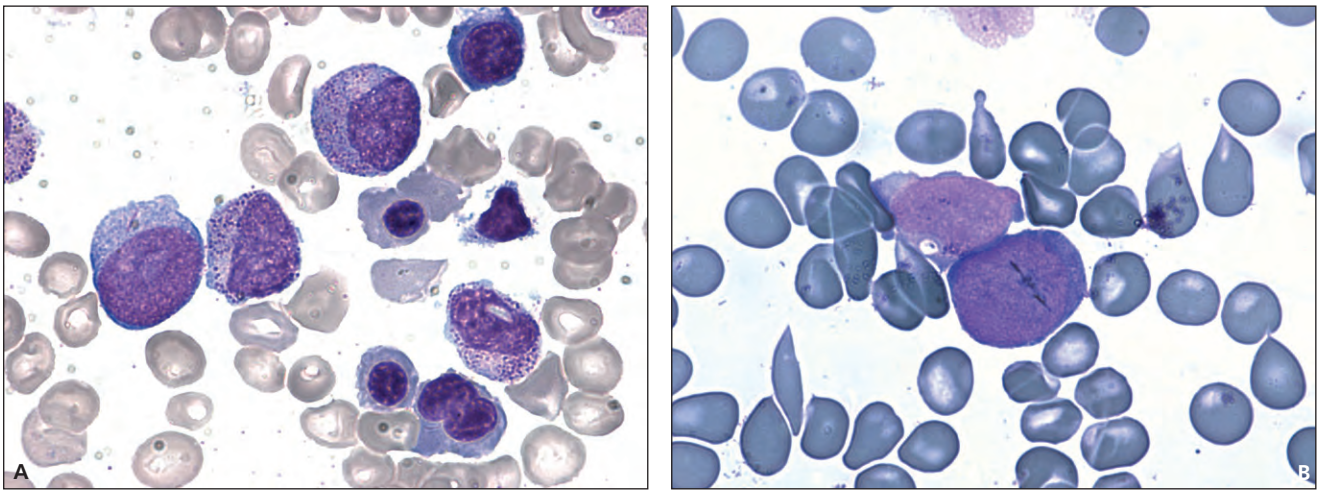



**Fig. 3.81** Blasts with Auer rods in MDS: the presence of these inclusions leads to the inclusion of a case in the RAEB-2 category; bone marrow aspirates. (A) On the left, a blast cell apparently without granules, with basophilic, fairly abundant cytoplasm with projections; the cytoplasm is not homogeneous and contains a single, thin, pointed Auer rod; the nucleus is oval, dark violet-red, with homogeneous, open, reticular, loose chromatin and a large pale lilac-grey nucleolus. On its right, two dysplastic myelocytes (recognised as such particularly because of the cytoplasmic immaturity), a dysplastic neutrophil with a ring nucleus, a lymphocyte and four erythroblasts of which that at the bottom appears frankly dyserythropoietic because of the presence of a double or bilobed nucleus. (B) Same patient as (A). A MPO reaction shows very fine paranuclear granular positivity in the upper blast cell, and the presence of another two needle-like Auer rods, which are lined up in tandem, in the absence of any other positively-staining granules.

**Table 3.6** Characteristic morphology of blast cells and promyelocytes in the myelodysplastic syndromes

**Agranular blast cells (FAB type I)**
- Central nucleus, oval or angulated
- Fine chromatin, one or more nucleoli
- Basophilic cytoplasm
- Absence of granules, absence of Auer rods
- Absence of a clear Golgi zone

**Granular blast cells (FAB type II plus type III)**
- Central nucleus, fine chromatin, nucleoli
- More ample cytoplasm, variably basophilic
- Presence of granules (from a few to numerous)
- Possible presence of Auer rods
- Absence of a clear Golgi zone

**Normal promyelocytes**
- Nucleus central or eccentric
- Nuclear outline oval or indented
- Chromatin finely dispersed or showing an intermediate grade of maturation
- Nucleoli: usually one, visible and prominent
- N:C ratio lower than in blast cells
- Cytoplasm more abundant than in blast cells with variable basophilia
- Paranuclear Golgi zone clearly visible
- Uniform and orderly distribution of granules

**Dysplastic promyelocytes**
- Nucleus central or eccentric
- Nuclear outline oval or indented
- Chromatin finely dispersed, or moderately and irregularly more dense
- Nucleoli: usually one, visible and prominent
- N:C ratio variable
- Cytoplasm more abundant than in blasts cells and less basophilic
- Reduced basophilia or poorly or irregularly developed Golgi zone
- Hypo- or hyper-granularity, irregular distribution of granules with possibly some dense clumps

double the diameter of a normal neutrophil: it is differentiated from a megakaryoblast by the degree of nuclear maturation and the higher N:C ratio. Type 2 is a megakaryocyte with a single round nucleus, while type 3 is multinucleated. The presence of type 1 or type 3 megakaryocytes would be, for these authors, indicative of dysplasia.[75]

## Histology

In the diagnosis of MDS, histology permits a precise evaluation of cellularity, and also of the distribution and localisation of various cellular components and of the degree of fibrosis[63,76]: the various diagnostic methods should be integrated with the aim of excluding reactive and secondary myelodysplasia. Histological examination is particularly important when there is a hypocellular aspirate or a 'dry tap'. In histological sections, medullary architecture may be found to be disturbed, with anomalous localisation of granulocyte precursors in the intertrabecular area, while the erythroid precursors and megakaryocytes are abnormally sited in the paratrabecular region. The presence of granulocyte precursors grouped together in an inappropriate position (ALIP) is particularly common in high risk MDS, such as RAEB. Small accumulations of blast cells can thus be found in the central zone of haemopoietic tissue, distant from vascular structures and from the endosteal surface of the bony trabeculae. Megakaryocytes are sometimes found in clusters. An assessment of erythroid islands permits the recognition of erythroblasts that are all at the same stage of maturation, an expression of dyserythropoiesis. Further useful information that may be

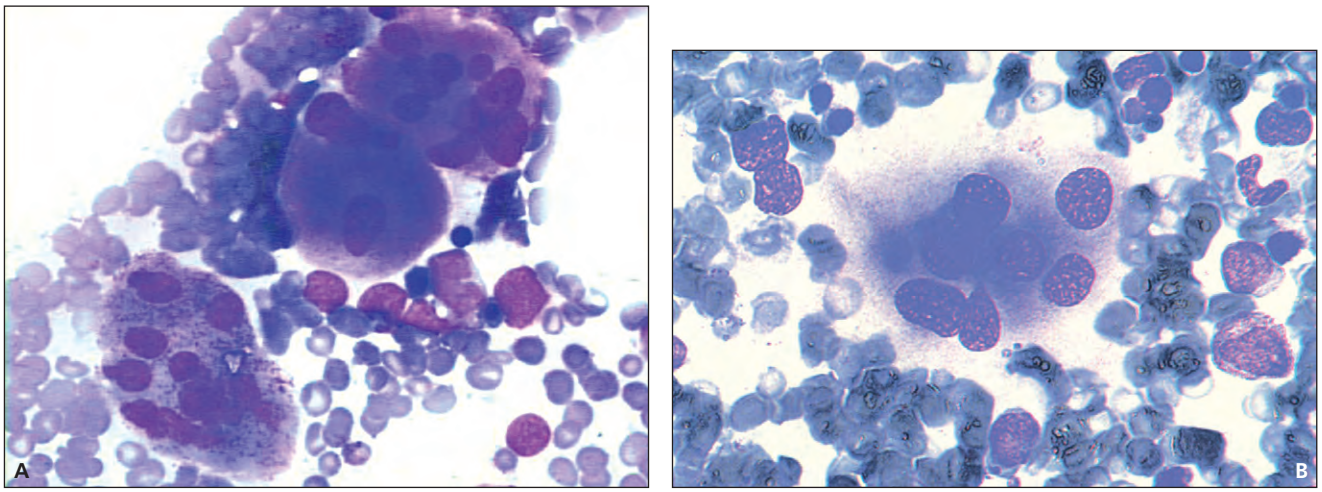



**Fig. 3.82** Megakaryocytes in a bone marrow aspirate from two cases of MDS. (A) In addition to some blast cells and damaged nuclei, there are three gigantic multinucleated cells. The upper one is a hyperdiploid megakaryocyte, with numerous nuclear lobes of variable size, some of which could have lost continuity with the main nuclear mass. That in the middle is a megakaryocyte with two widely separated nuclear lobes, which are probably not joined, within cytoplasm that still retains intense basophilia in the central parts of the cell. The lowest cell is not a megakaryocyte but an osteoclast: the nuclei are oval, monomorphic, spongy, superimposed on each other and located towards the periphery of the cell; the cytoplasm is greyish and full of large granules that characterise cells of macrophage lineage. (B) A large megakaryocyte with intermediate cytoplasmic maturity, with residual central basophilia, has a nucleus composed of seven lobes, some of which (on the right) are separated from the others. As is observed in dysplasia, separated nuclear lobes tend to become rounded, while those that remain joined by a filament are deformed into a teardrop shape. The chromatin structure, as far as can be seen, appears condensed in an abnormal manner into masses separated by pale spaces. Top left, a bare bilobed megakaryocyte nucleus.

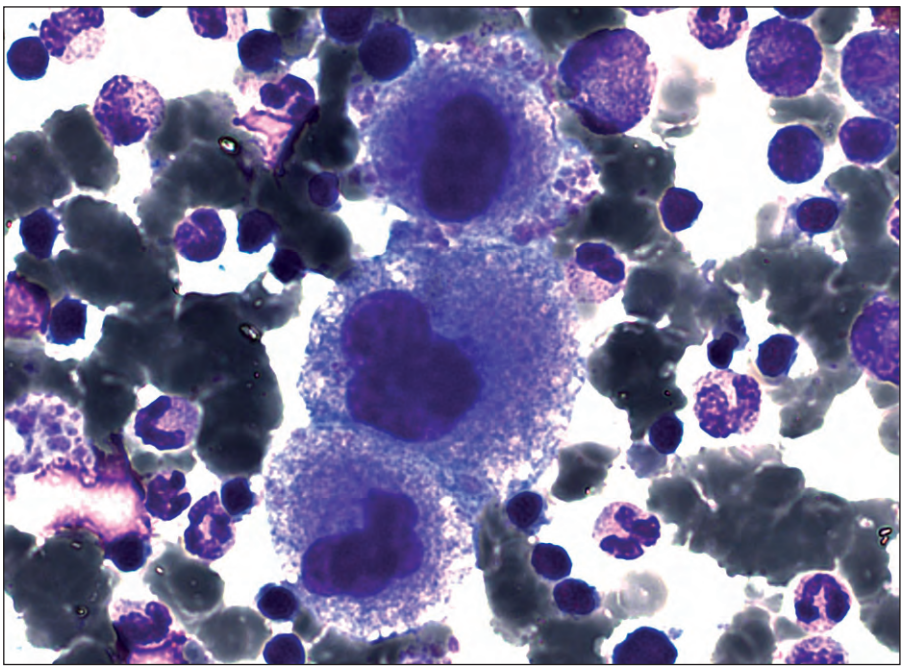


**Fig. 3.83** Bone marrow aspirate from a patient with RCMD. In the context of a cellular marrow with granulocytic and erythroid precursors, there is a small group of three megakaryocytes that vary in size, but are otherwise similar to each other. Their nuclei are hyperdiploid and their outlines include segments of circles; chromatin is condensed and dark violet. The cytoplasm forms a strongly basophilic background, but appears lacking in homogeneity as a result of abnormal and ineffective platelet formation. The periphery of the cell, where in normal megakaryocytes the microtubules of the platelet demarcation system are found, appears disorganised by numerous optically empty vacuoles. Ill-defined images of platelet formation can be discerned in the context of basophilia, e.g. on the right of the central megakaryocyte and at the upper pole of the lower megakaryocyte, in the form of aggregates of bluish masses surrounded by a pink halo. The periphery of the top megakaryocyte, however, is surrounded by masses of newly formed platelets, certainly derived from pseudopodial formations that are functionally more efficient. This dysmorphism, while indicating a disturbance in the mechanism of platelet formation, cannot be considered specific for dysmegakaryopoiesis.

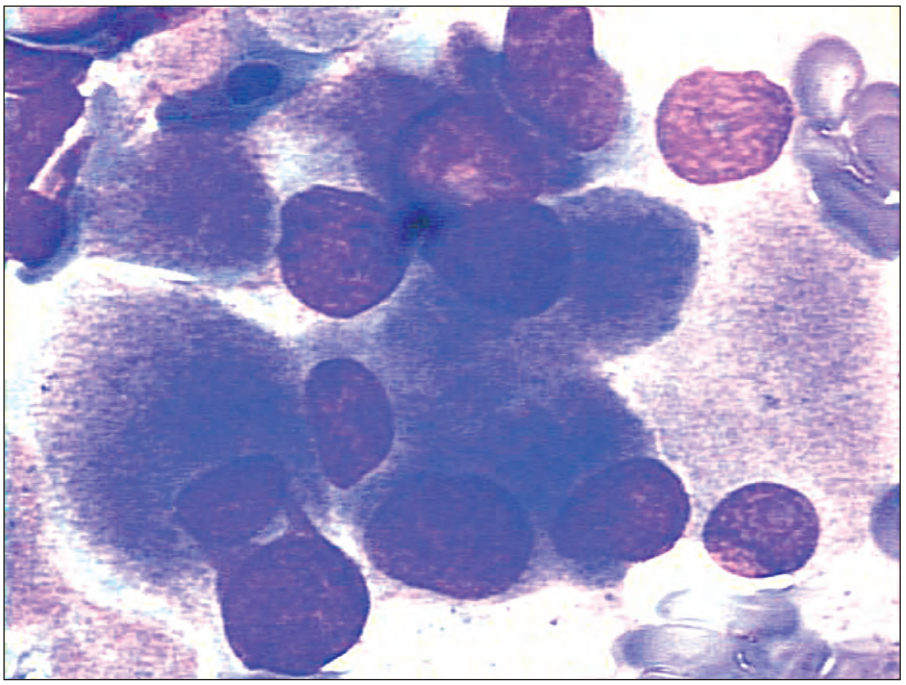


**Fig. 3.84** A tight cluster of small megakaryocytes with nuclear anomalies in a case of RAEB-1; bone marrow aspirate. In this cluster of superimposed megakaryocytes it is not possible to recognise with certainty the limits of the individual cells. The general characteristic of these cells is that the nuclear masses tend to be oval, maintaining a connection between lobes, as on the left, or forming two detached nuclei, as in the cell in the centre. The mononuclear cell on the right is similar to the non-lobated megakaryocytes seen in the 5q- syndrome, in this instance rather flattened.

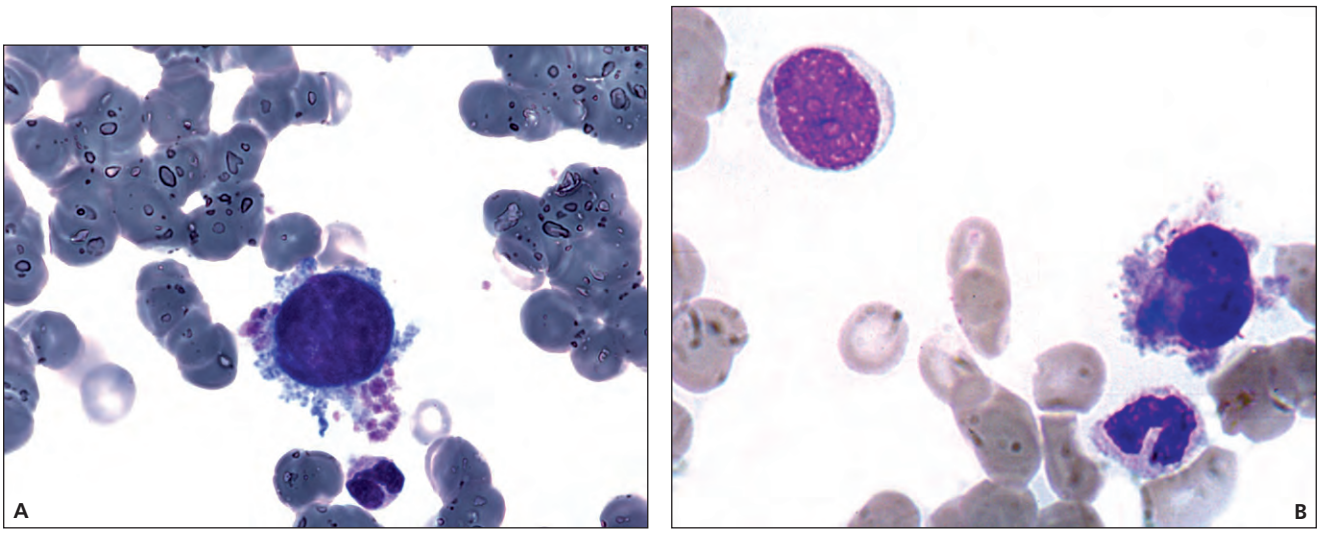



**Fig. 3.85** Micromegakaryocytes: examples of morphological variation; bone marrow aspirates from different patients. (A) Micromegakaryocyte with a large round nucleus. The nuclear outline is an almost perfect circle; the chromatin is condensed but retains traces of structure with alternating dark and pale areas. The N:C ratio is very high, the cytoplasm being reduced to ragged bluish pseudopods which in two areas have formed filaments of proplatelets, not yet detached but already well formed as individual structures. At the bottom, a small dysplastic neutrophil band form. (B) Micromegakaryocyte with a pyknotic reniform nucleus with a notch that is tending to divide the nucleus into two lobes. Also in this example, the N:C ratio is very high and the ragged cytoplasm indicates the beginning of platelet formation. This cell is very small for a megakaryocyte, similar in size to the agranular blast cell that is seen top left.

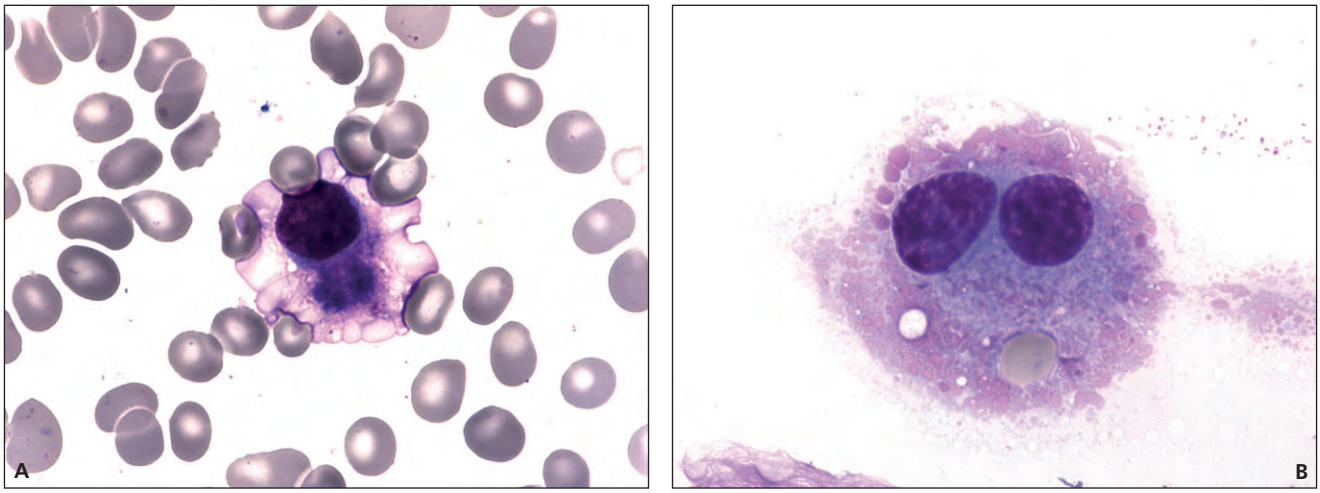



**Fig. 3.86** Micromegakaryocytes: examples of morphological variation ; bone marrow aspirates from different patients. (A) Micromegakaryocyte with a pyknotic non-lobated nucleus and cytoplasm that has degenerated peripherally into a halo of large bubbles of hyaline material. Only the paranuclear zone retains an area of diffuse basophilia with indistinct margins, in which one can see small dark masses that may correspond to an abortive attempt at platelet formation. The very dense nucleus has a diameter about double that of a red cell. There is a polychromatic erythrocyte with an irregular outline in the bottom left corner. (B) In this binucleated micromegakaryocyte, the two nuclei differ in shape, triangular in one instance and round in the other. The chromatin structure is compact with ill-defined pale areas. The central part of the cytoplasm in which the nuclei are immersed has tinctorial features identifying this as an intermediate megakaryocyte, with background basophilia and scattered and coalescent azurophilic granules. The peripheral zone, however, around the entire circumference, has been transformed into pink hyaline material, which is forming globules and tending to become detached, in a parody of normal platelet production. Close to the lower pole, a red cell appears to have been included, by emperipolesis, between the two concentric zones of the cytoplasm.

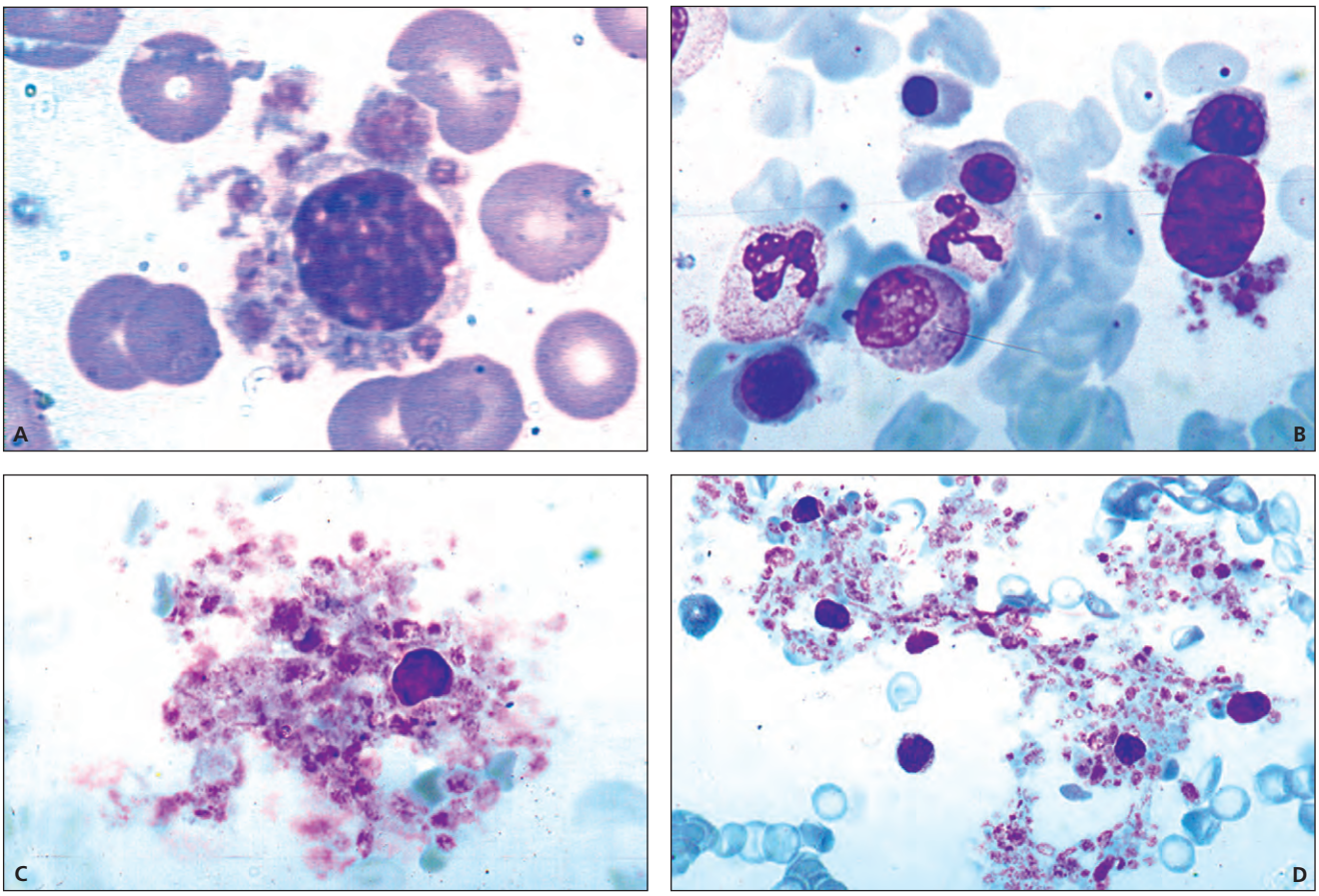



**Fig. 3.87** Micromegakaryocytes: examples of morphological variation; bone marrow aspirates from different patients. (A) Small micromegakaryocyte with a diameter that is double that of an erythrocyte, composed of a compact round nucleus with a small notch, surrounded by a halo of cytoplasm that has been in large part transformed into platelets that vary in size, but are well formed with regard to the relationship between the chromomere and the hyalomere. (B) The micromegakaryocyte on the right has quite anomalous morphology that is typical of severe dysplasia and quite often seen in this context, and is sometimes observable also in the peripheral blood. The appearance is of a nucleus with compact chromatin and a nuclear notch in which the presence of a thin rim of cytoplasm is more readily appreciated; elsewhere, the cytoplasm is forming small fronds of proplatelets, which give the false impression of arising directly from the nucleus. (C) Another morphological form of micromegakaryocytic dysplasia is the presence of small compact and irregular nuclei, with the surrounding cytoplasm totally transformed into proplatelets, which remain joined and interwoven with each other; they can be detached as masses or chains of non-activated platelets. (D) Micromegakaryocytes and pyknotic nuclei with cytoplasm completely differentiated into proplatelets are sometimes seen as numerous masses, which can show analogies with the picture that can be observed in essential thrombocythaemia or primary myelofibrosis.

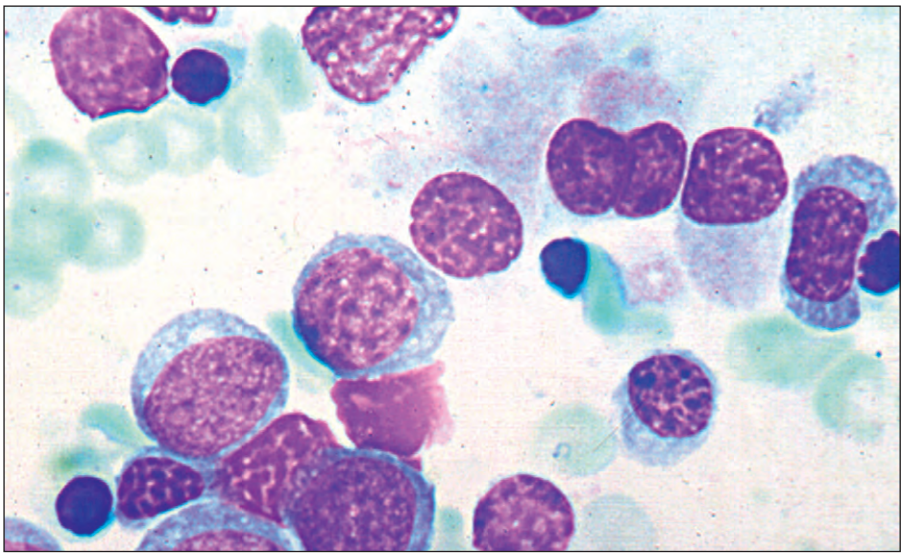


**Fig. 3.88** Bone marrow aspirate from a patient with RAEB-1. There are a number of basophilic, polychromatic and orthochromatic erythroblasts. The large cell on the left with an eccentric oval nucleus, reticular coarsely woven chromatin with small foci of condensation, and basophilic cytoplasm with a pale paranuclear area is an agranular blast cell. Towards the right and to the right of a spongy macrophage nucleus, two micromegakaryocytes with typical chromatin density and basophilic cytoplasm that is more granular in the cell on the left; this cell has a bilobed nucleus and appears in a more advanced stage of dysplastic maturation than the cell on the right.

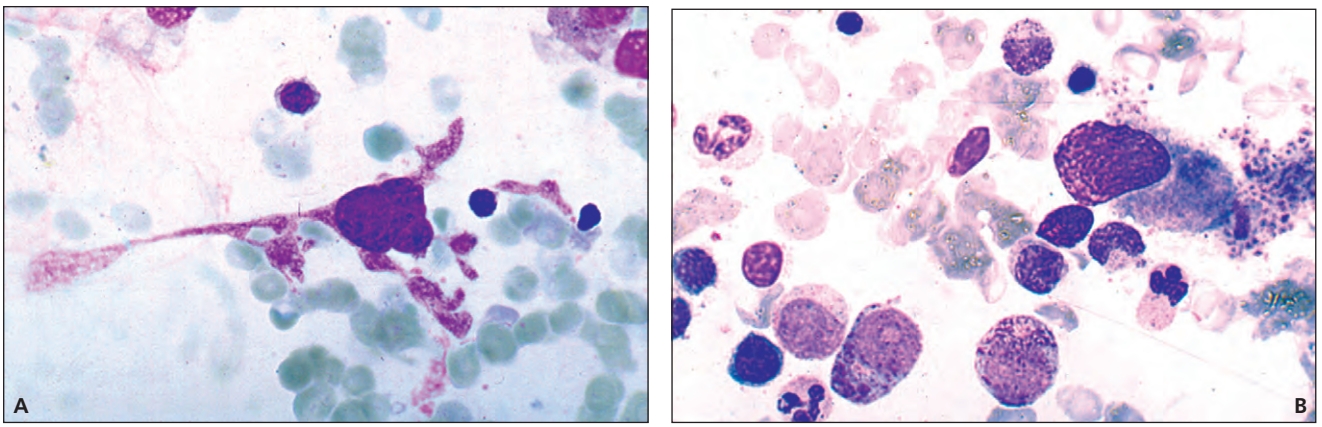



**Fig. 3.89** Dysmorphism of megakaryocytes in MDS: not all small megakaryocytes are micromegakaryocytes; bone marrow aspirate. (A) This image shows a megakaryocyte with a very high N:C ratio, in which the nucleus is composed of three asymmetric lobes with quite compact chromatin and a size that is much greater than that of the erythroblast above it. The cytoplasm is mature, intensely granular and stretched into three radiating projections from which arise tentacle-like pseudopodial extensions that represent ineffective attempts at platelet formation. (B) Top right, a megakaryocyte with a nucleus that has vermicular, not fully condensed chromatin and cytoplasm with asymmetrical areas of paranuclear basophilia and peripheral platelet formation. Neither the nuclear morphology nor the dimensions of the cell, which is much larger than the erythroid and granulocytic cells present, permits the recognition of this cell as a micromegakaryocyte. The two hypergranular precursors at the lower edge are dysplastic promyelocytes.

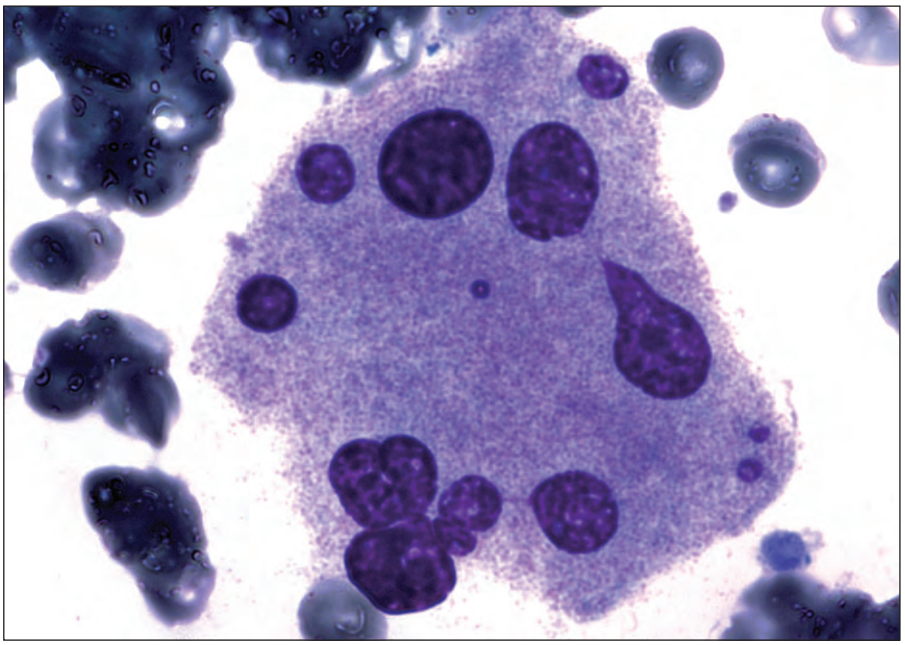


**Fig. 3.90** Megakaryocytes with separate nuclei; the second specific type of megakaryocyte dysplasia; bone marrow aspirate. This giant megakaryocyte has almost mature cytoplasm with fine homogeneously distributed granules and a residual tinge of basophilia. The numerous nuclear components are in part isolated and rounded, in part joined by chromatin filaments (as in the two on the right), in part gathered in multilobated formations and in part reduced to fragments. The compact structure of the chromatin ('salami slice appearance') with a clear nuclear outline is very typical of the megakaryocyte lineage.

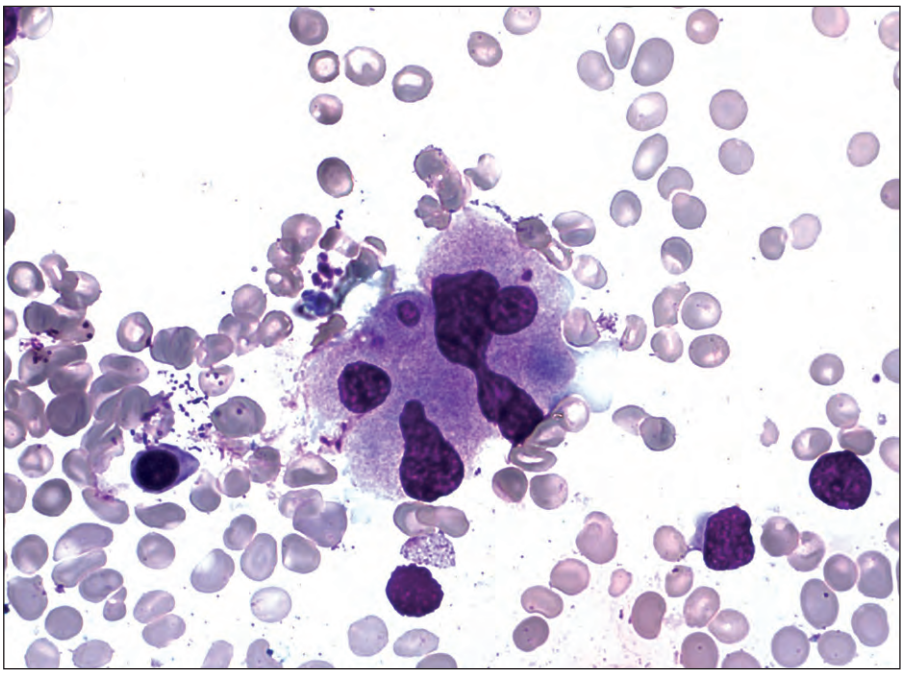


**Fig. 3.91** Megakaryocytes with separate nuclei; bone marrow aspirate. Between a dyserythropoietic erythroblast and some bare nuclei is a medium-sized megakaryocyte with mature cytoplasm, heterogeneous distribution of its numerous granules and basophilic cytoplasm with a ragged periphery. The hyperdiploid nucleus is fragmented into formations that vary in size and shape, but have the same chromatin structure that is typical of the megakaryocyte lineage.

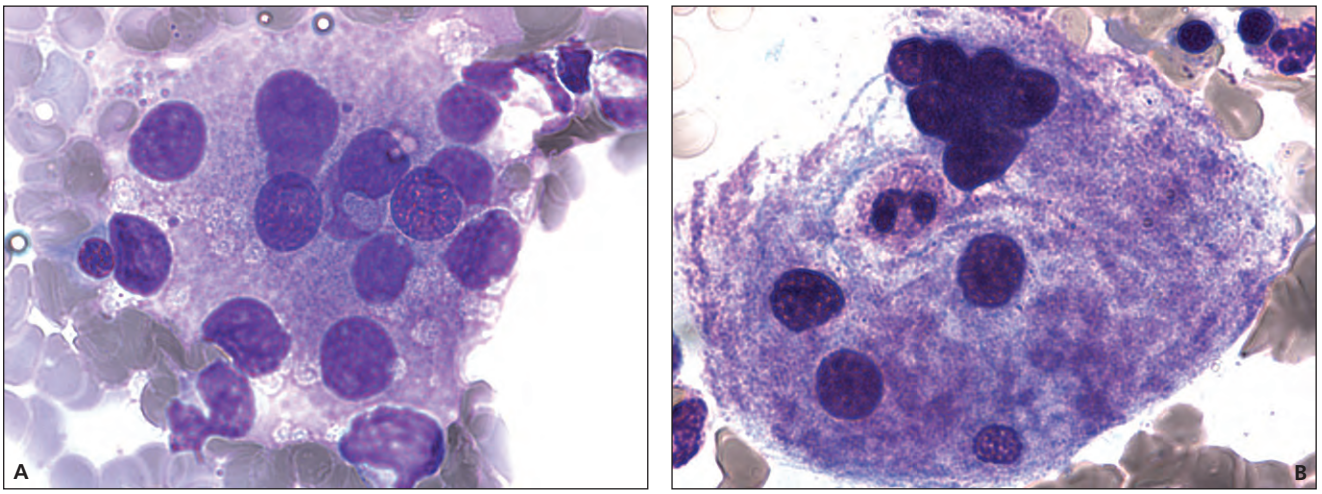



**Fig. 3.92** Differential cytodiagnosis of dysmegakaryopoiesis; two different bone marrow aspirates. (A and B) Giant multinucleated cells of different types. The structure and position of the nuclei, the cytoplasmic features and the relationship with other cells are the elements that permit a correct recognition of lineage. (A) A large osteoclast. The cytological features that permit its identification are the spongy structure of the nuclei, which have open pale violet chromatin, their uniform morphology, their regular oval shape and the superimposition of nuclei that are sufficiently transparent that the underlying nucleus is visible. (B) Giant dysplastic megakaryocyte. That this cell is a megakaryocyte is confirmed by the presence of a hyperdiploid multilobated nucleus in the upper part of the cell, by the characteristics of the condensed chromatin with its pale spaces; by the differentiation of the cytoplasm, which is very granular with the granules tending to condense within demarcated areas of cytoplasm, and by the emperipolesis of a neutrophil.

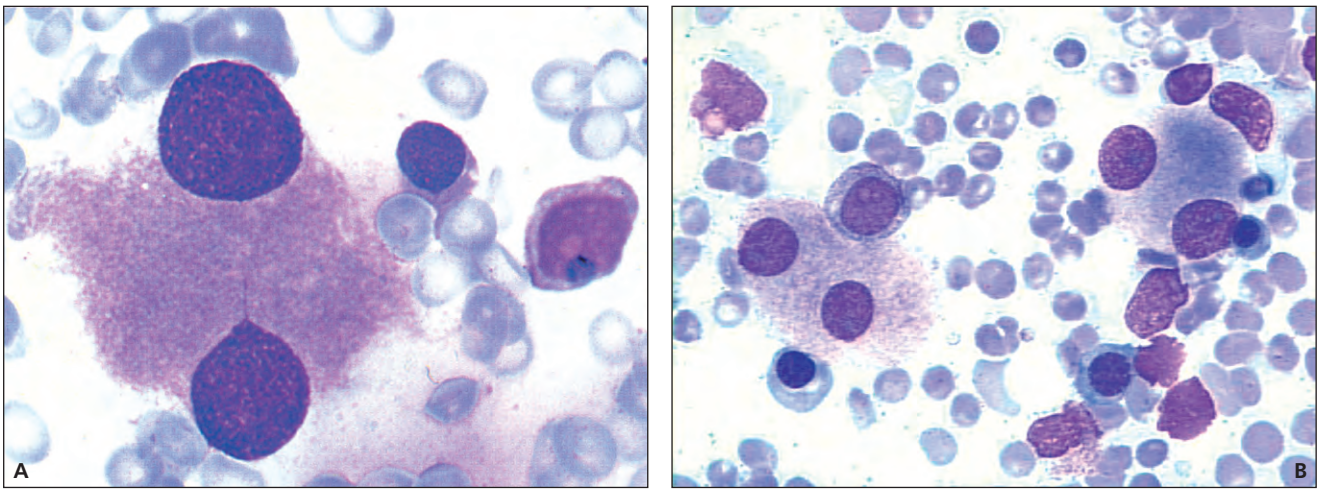



**Fig. 3.93** Dysplasia of megakaryocytes in RCMD; bone marrow aspirates of different patients. (A) Medium-sized megakaryocyte, the mature cytoplasm is full of fine granules distributed in an orderly manner; the two large rounded nuclei have spongy chromatin, relatively less mature than the cytoplasm, and appear to be joined by a very fine filament with a similar appearance to the internuclear bridges of dysplastic erythroblasts. (B) The two large cells are small binucleated dysplastic megakaryocytes, in a general context of myelodysplasia as apparent from the features of dyserythropoiesis (bottom left, an orthochromatic erythroblast with incomplete haemoglobinisation) and of dysgranulopoiesis (a dysplastic promyelocyte above the binucleated megakaryocyte on the left).

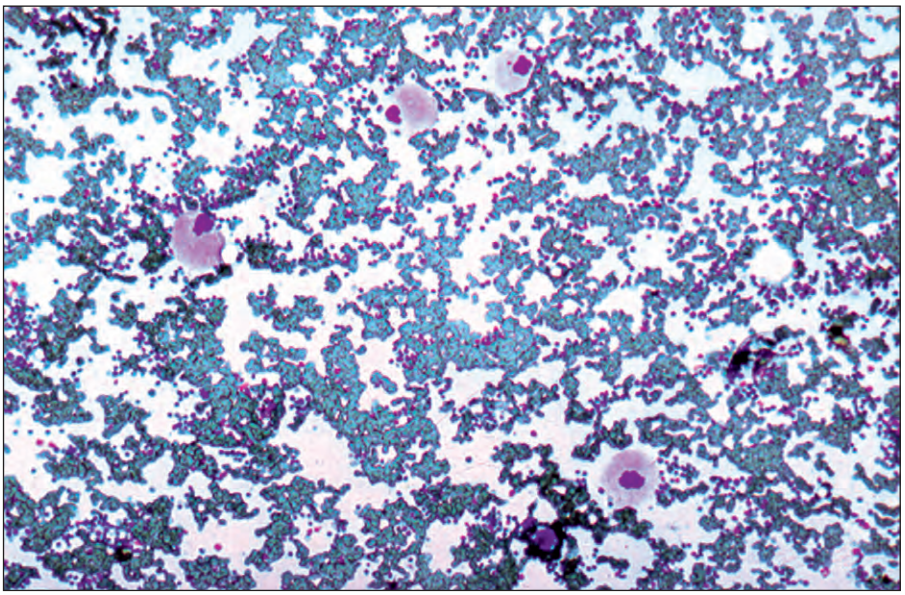


**Fig. 3.94** Normal megakaryocyte morphology; bone marrow aspirate from a patient with refractory anaemia. In this low power field, the marrow appears cellular and there are four mature megakaryocytes, with eccentric hyperdiploid multisegmented nuclei within plentiful pink cytoplasm.

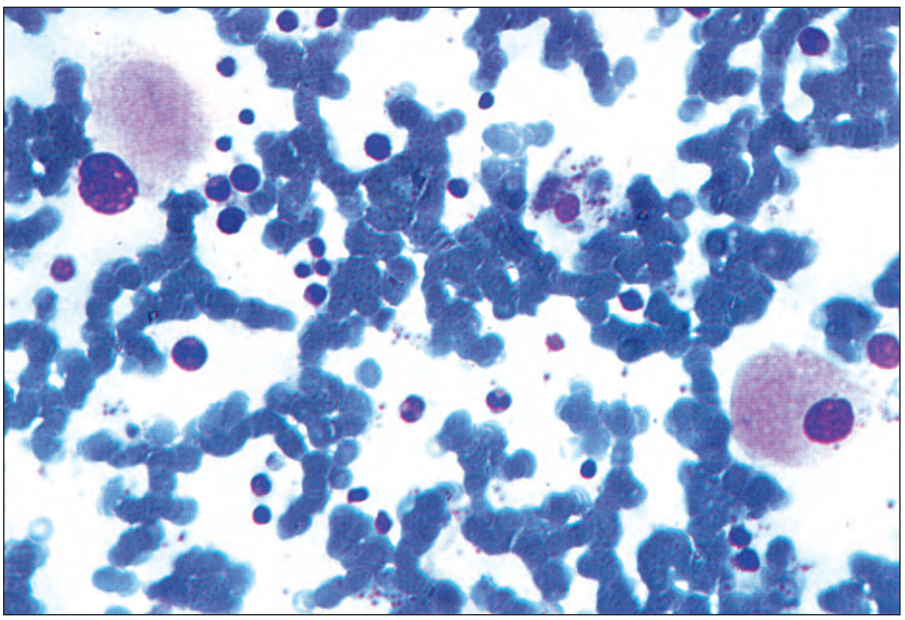


**Fig. 3.95** Dysmegakaryopoiesis in the 5q- syndrome; bone marrow aspirate. The two medium-sized megakaryocytes have eccentric non-lobed nuclei with well-condensed chromatin and a regular ovoid nuclear outline, within plentiful pink cytoplasm that appears mature and capable of producing platelets.

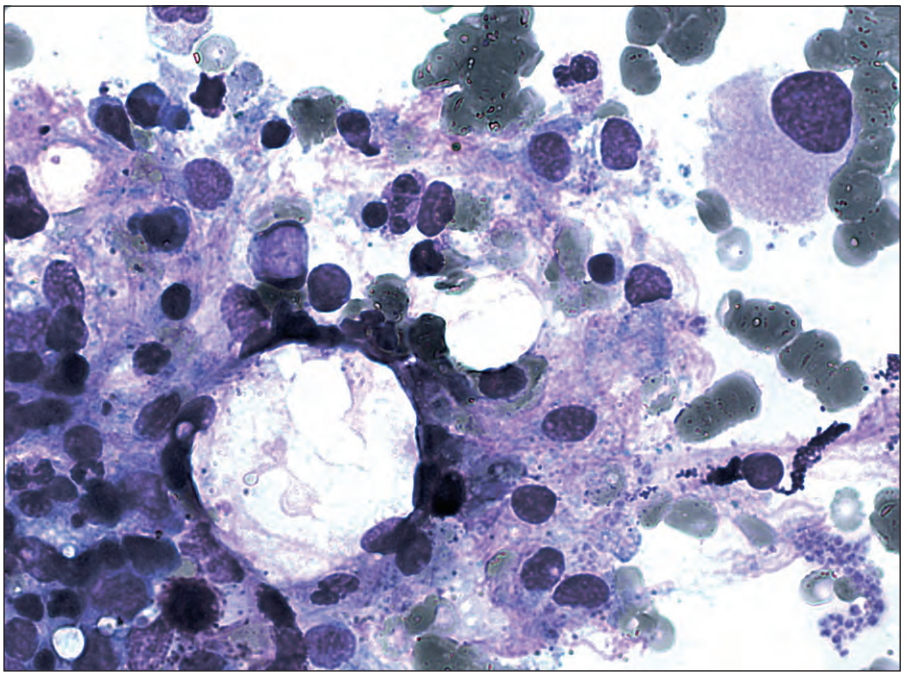


**Fig. 3.96** Megakaryocyte with a non-lobed nucleus in a patient with the 5q- syndrome, macrocytic anaemia (MCV 106 fl) and a platelet count of $385 \times 10^9$/l; bone marrow aspirate. The image was captured close to a bone marrow fragment that was rich in fibrinous material; one can identify a blast cell above a larger fat cell, various macrophage nuclei and two mast cells, one rounded, on the left near the lower edge, and one fusiform with two elongated projections loaded with black granules, on the right immediately above a festoon of platelets with well-preserved morphology. The megakaryocyte with a non-lobed nucleus in the top right corner shows the typical morphological characteristics of a medium-sized cell with granular cytoplasm destined to be transformed into platelets, and a nucleus with a chromatin structure that is also typical.

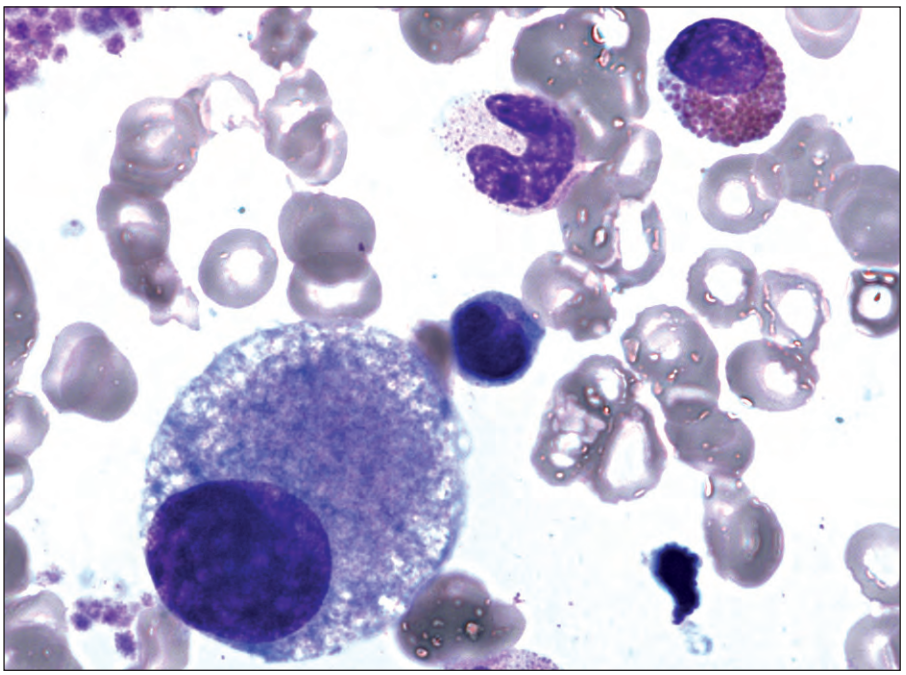


**Fig. 3.97** Megakaryocyte with a non-lobed nucleus in another patient with the 5q- syndrome; bone marrow aspirate. This megakaryocyte, although maintaining the main morphological characteristics of this type of MDS (eccentric hypolobated nucleus, very compact chromatin condensed into rounded clumps that are indefinite rather than clear), has a defect in cytoplasmic maturation with the area that is destined for the final phase of platelet formation being occupied around its full circumference by pseudovacuolar formations; this indicates a functional or structural defect in the formation of microtubules and platelet membranes. The central paranuclear area of the cytoplasm has better preserved morphology with alternation of basophilic and pink cytoplasm, seeming to suggest a tendency to form platelet demarcation fields. Top right, a moderately dysmorphic eosinophil with a non-segmented nucleus and well-differentiated cytoplasm with plentiful granules that leave free only a small area of weakly basophilic cytoplasm.

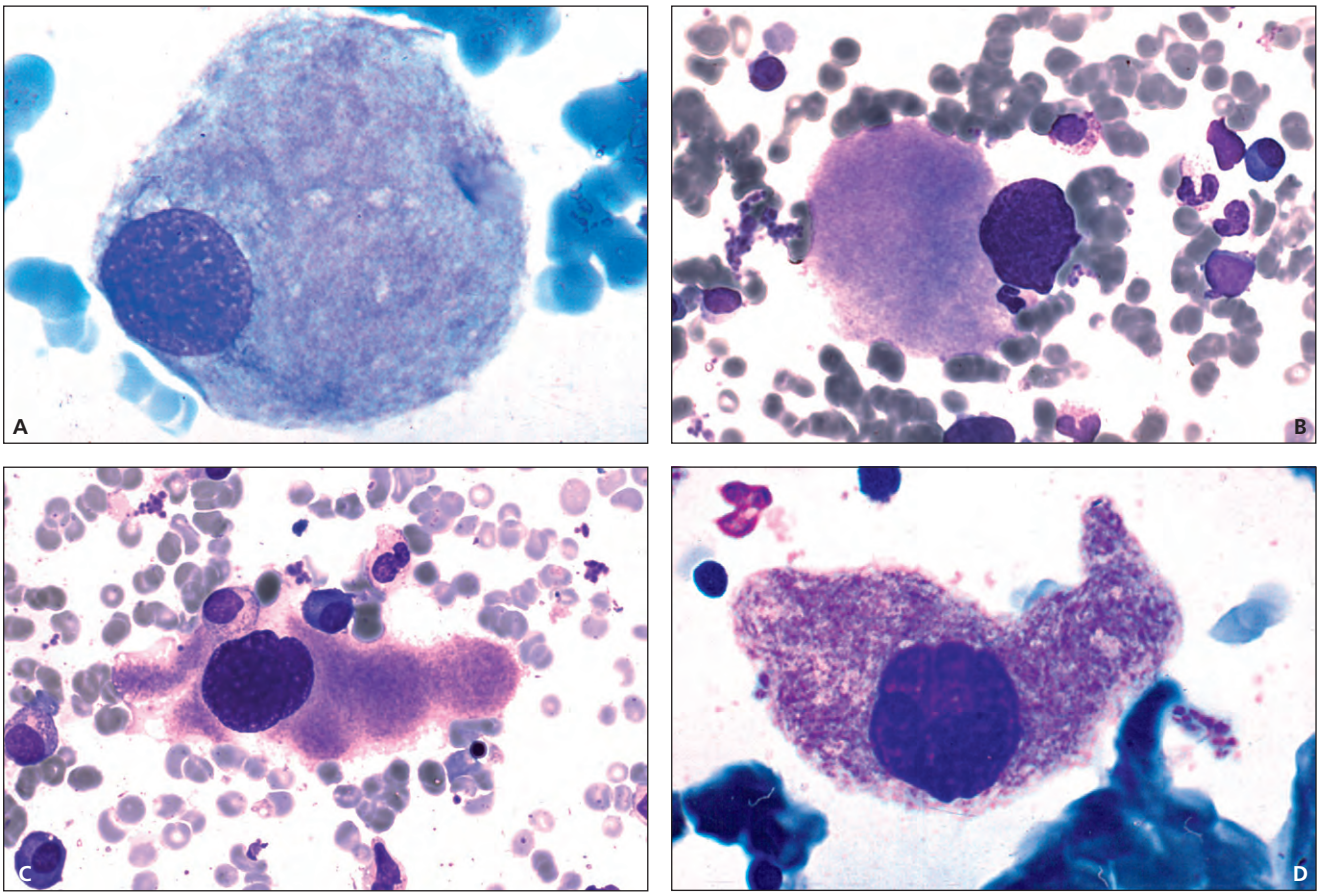



**Fig. 3.98** Consistency and specificity of the third criterion of dysmegakaryopoiesis: the megakaryocyte with an eccentric non-lobated or hypolobated nucleus; from bone marrow aspirates of patients with deletion of 5q. This appearance, associated with specific genetic lesions, is fairly consistent and therefore not difficult to recognise. Some variation can be seen, particularly with regard to different degrees of maturation (even though the morphological defect is most typical in the most mature megakaryocytes), the morphological features of platelet production and the presence of superimposed dysplastic features. (A) Dysplastic megakaryocyte with an eccentric, round, mature nucleus and plentiful pear-shaped, very basophilic cytoplasm which is showing delayed maturation; there is a network of pale spaces and many fine azurophilic granules with some platelet demarcation fields. (B) Large megakaryocyte (if compared with the adjacent cells) with a hyperdiploid, but hyposegmented, nucleus with an irregular outline. The cell has reached full maturity and has voluminous cytoplasm that is almost fully differentiated, with numerous fine platelet-type granules, a basophilic area adjacent to the nucleus and a pinker area on the opposite side; it is detaching from the nucleus. (C) Large megakaryocyte, similar to the previous one, with a hyperdiploid nucleus which is notched but not lobated, within elongated cytoplasm that is already mature, in which one can recognise, particularly on the right, platelet demarcation fields corresponding to future platelets. (D) In this megakaryocyte the large nucleus is round and not lobated. The plentiful cytoplasm has retained its integrity, although platelet demarcation fields are well developed; these have masses of large wine-red granules separated by short linear spaces.

revealed by histological examination is the presence of lymphoid nodules, which requires an assessment of whether there is a co-existing lymphoproliferative disorder.[77,78]

For a more accurate prognostic stratification, a pathological accumulation of blast cells can be confirmed by immunohistochemistry, using an anti-CD34 antibody.[79,80] It is necessary to remember, however, that in MDS not all blast cells are CD34 positive and that, furthermore, positivity for this marker is also a feature of endothelial cells: analysis also of CD117 can be useful for a final assessment. Flow cytometric assessment of the same markers can give further useful information, but it is not rare to find discordance between flow cytometry and immunohistochemical morphology, which may be attributable to the presence of CD34-negative blast cells or analysis of a haemodilute bone marrow aspirate. However, according to the 2008 WHO classification, the reference method for the diagnosis of MDS remains the blast percentage obtained by morphological assessment of a bone marrow aspirate.[69,81] Flow cytometry can be used to study the characteristics of maturation of precursors of the various haemopoietic series, looking for anomalous expression of immunophenotypic markers as possible indicators of dysplasia of a particular lineage.

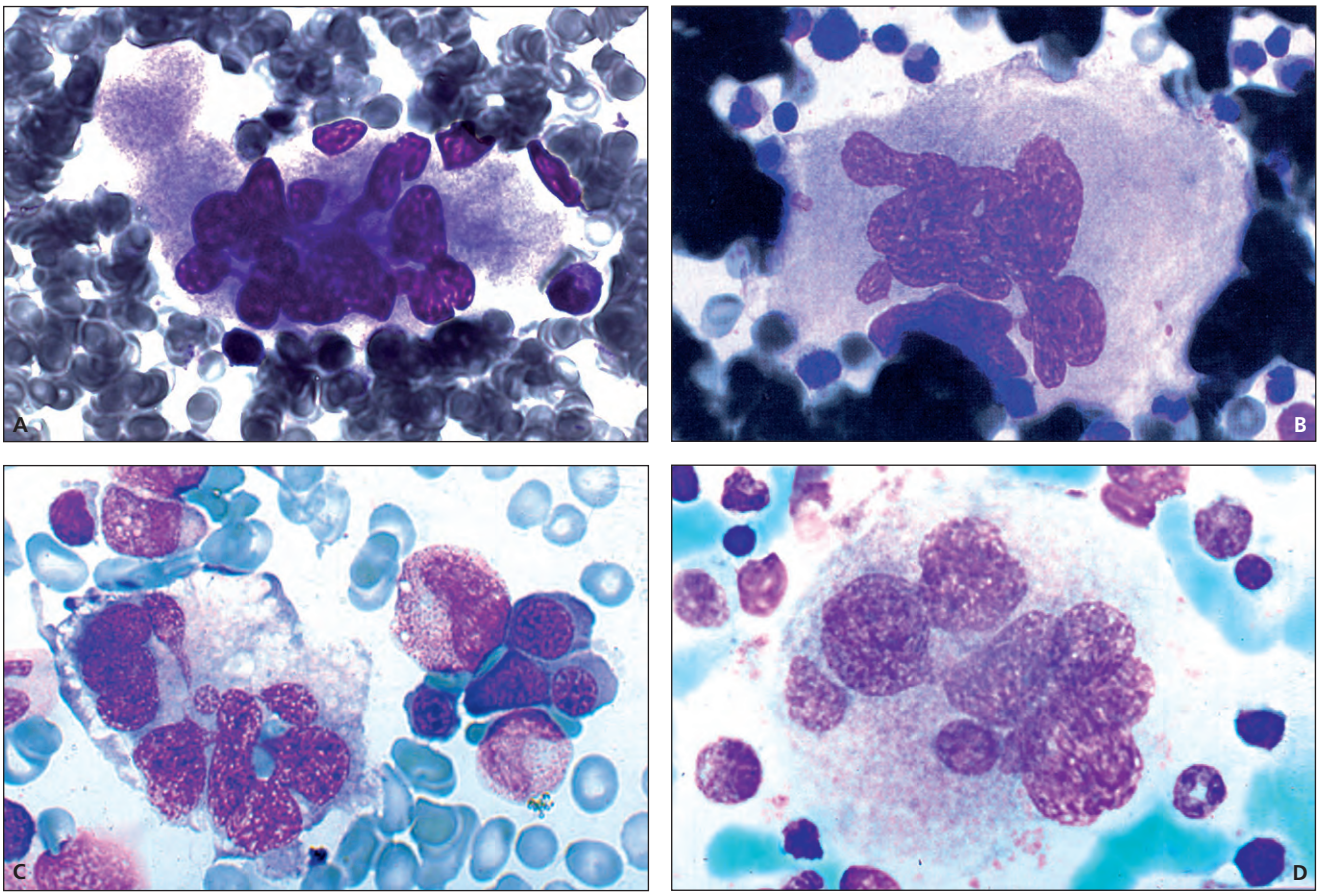



**Fig. 3.99** Dysmorphism of megakaryocytes with hypogranular cytoplasm and bizarre cytoplasmic and nuclear anomalies: another, less specific, feature of dysmegakaryopoiesis; bone marrow aspirates of different patients with MDS. Analogous morphological abnormalities can be seen in MPN, such as essential thrombocythaemia and primary myelofibrosis but, in the context of suspected MDS, cells with these features should be classified as dysplastic (when assessing 30 megakaryocytes to determine whether or not this lineage is dysplastic). (A) Giant megakaryocyte with an extremely hyperdiploid nucleus with more than a dozen lobes, some of which appear to be detached, while the majority are still joined by thick interlobar chromatin bridges. This nuclear form, together with other key features of the chromatin structure – compact, smooth, not spongy, of a dark violet-red or prune colour – permits this cell to be distinguished from other giant multinucleated cells that can be found in bone marrow aspirates. The cytoplasm has a foamy structure with a colour that varies from dark pink to lilac-grey. (B) Large megakaryocyte with weakly basophilic, hypogranular cytoplasm. The nucleus, irregularly folded on itself, has chromatin that is partly condensed into elongated clumps with plentiful pale spaces (a vermicular or worm-like appearance). No platelet demarcation fields are seen. (C) Marked abnormalities of morphology and maturation characterise this degenerating megakaryocyte, which has a hypersegmented nucleus with lobes of bizarre shapes, abnormal condensation of the chromatin which contains numerous non-staining microvacuoles, and scanty basophilic cytoplasm that is heavily vacuolated, particularly in its outer part, beneath the cytoplasmic membrane. (D) Large megakaryocyte with a giant nucleus with many asymmetrical and irregular lobes, indicating extreme hyperdiploidy. The incomplete chromatin condensation has produced a confused mixture of darker areas and pale areas of parachromatin. The cytoplasm shows a background weak basophilia with pink azurophilic granules and some platelets, possibly newly formed, in some parts of its circumference.

## Genetic alterations

Investigation of cytogenetic and molecular genetic abnormalities has become a cornerstone in the diagnosis and prognostic and therapeutic evaluation of MDS.[82] These investigations can confirm clonality and also reveal the presence of anomalies that correlate with specific clinical and morphological features. As already mentioned, the karyotype at diagnosis is one of the fundamental biological characteristics that is used for the prognostic stratification of patient with *de novo* MDS (see Table 3.2).

Chromosomal anomalies and molecular abnormalities, often multiple, are found in 50% of patients affected by *de novo* MDS, with variation in different series of cases from 30% to 80%, this in part being dependent on the sensitivity of the technique employed. Most of these are not specific for a particular subtype of MDS; some, however, are associated with specific morphological anomalies affecting the megakaryocyte and erythroid series:

- Inversions and translocations that involve chromosome 3 are found in AML and MDS with an increase of abnormal megakaryocytes;

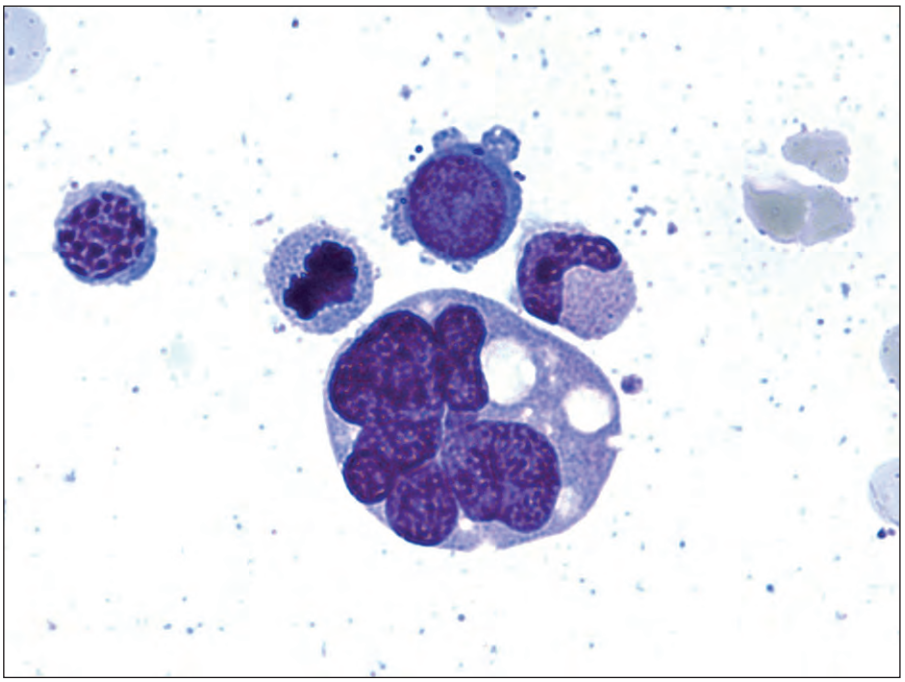


**Fig. 3.100** Dysmegakaryopoiesis with hypogranularity in a patient with RAEB-1. The megakaryocyte, at the bottom in the centre, is very large and has a hyperlobated nucleus with marked asymmetry of the various nuclear segments. The chromatin is violet and it appears compact with a smooth, shiny surface. In contrast with the large prominent nucleus, which appears to be the result of vigorous endomitotic activity, the cytoplasm is relatively sparse, grey-blue, with very few fine scattered granules; it is degenerating, as shown by the presence of various large vacuoles. Top left, a polychromatic erythroblast with chromatin clumps in a mosaic formation; adjacent is another erythroblast, containing a cauliflower-shaped mitotic figure. Next is a blast cell with a high N:C ratio, a perfectly round nucleus with a large central nucleolus, homogeneously condensed chromatin and scanty, intensely basophilic cytoplasm with numerous blebs: this morphology, in particular the structure of the chromatin, is suggestive of a megakaryoblast rather than a proerythroblast. There is also a metamyelocyte without any particular anomalies.

- An isolated deletion of 5q is found particularly in women with hyposegmented and non-segmented megakaryocytes, refractory anaemia with an increased MCV, normal or increased platelet count, and a good prognosis with long survival: the WHO classification recognises this complex of anomalies as a specific type of MDS, which has a marked sensitivity to lenalidomide, in terms of both erythroid and cytogenetic response;
- Monosomy 7 is associated with micromegakaryocytes and has a negative prognostic significance because of rapid clinical evolution leading to death;
- Deletion of 11q is associated with increased iron deposition;
- Deletion of 17p is generally associated with small neutrophils with pseudo-Pelger nuclei and vacuolated cytoplasm, in the context of MDS or AML, often therapy-related and with a very poor prognosis; there is deletion of one allele of *TP53* and usually also a mutation of the other allele;
- Isolated deletion of 20q is associated with marked dyserythropoiesis and dysmorphic megakaryocytes[83,84];
- Recently, deletion of 20q as an isolated cytogenetic abnormality has been associated with a new category of MDS identified as 'refractory thrombocytopenia'.[85]

Following the 2008 WHO classification, if one or more specified cytogenetic alterations is found in a patient with persistent cytopenia without an evident cause, it is possible to make a diagnosis of MDS even in the absence of morphological dysplasia. The presence of complex anomalies (more than three in a single case) is generally associated with an unfavourable disease evolution.

Recently, genetic analysis of molecular anomalies has been extended by the use of new very sensitive methods, such as the analysis of single nucleotide polymorphisms, telomere dynamics or epigenetic alterations (such as DNA hypermethylation and histone modification) plus new techniques for DNA sequencing and gene expression profiling[8]: these techniques will certainly lead to the identification of new and specific lesions critical in the development of MDS.

## The 2008 WHO classification of the myelodysplastic syndromes

Table 3.7 lists the seven subtypes of MDS currently recognised, together with the minimum criteria necessary for their diagnosis. These are based on:

- The presence of one or more peripheral blood cytopenias;
- Quantification of blast cells in the peripheral blood and the bone marrow;

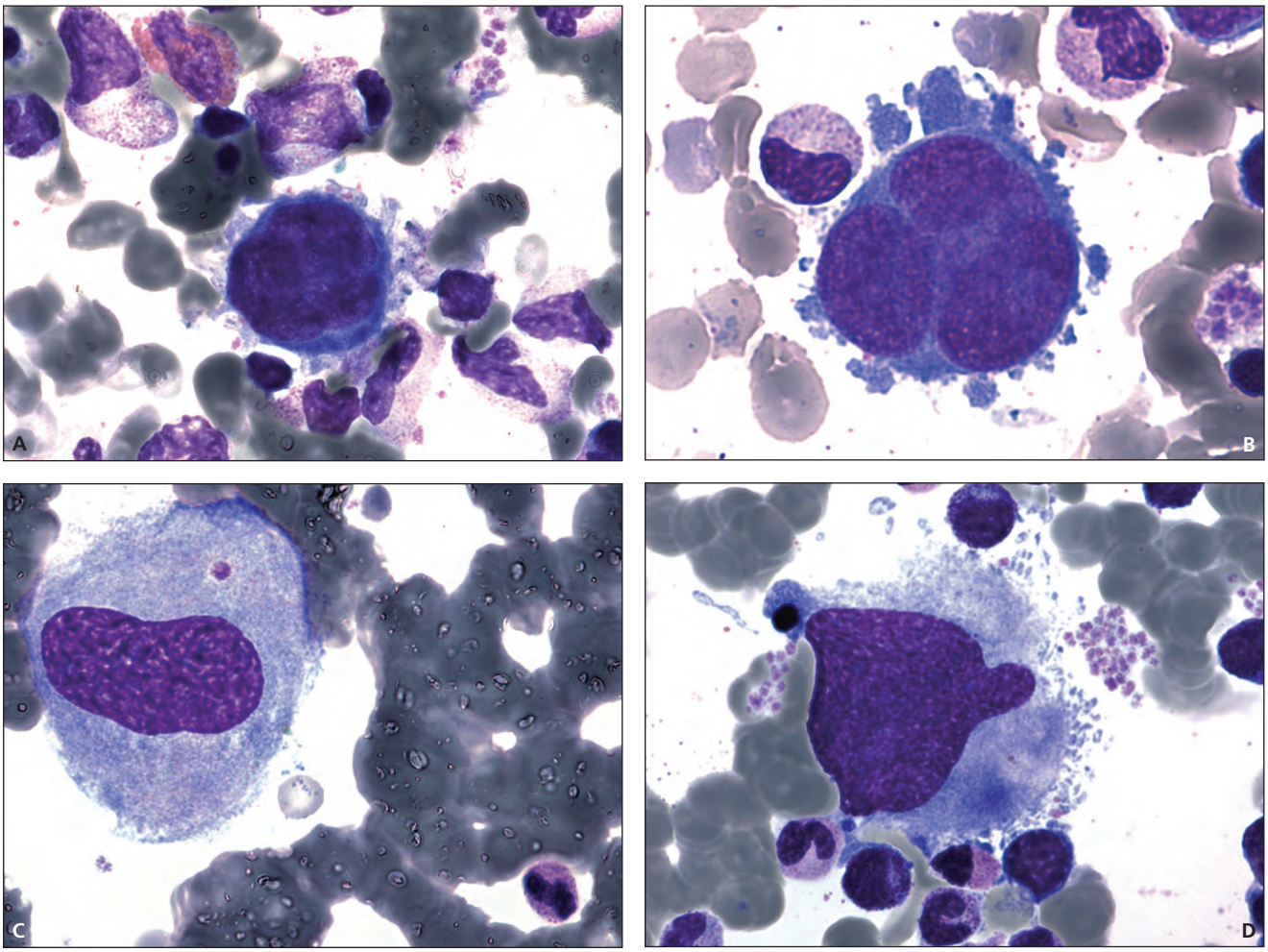



**Fig. 3.101** Immature megakaryocytes, asynchrony and dysmegakaryopoiesis in MDS; bone marrow films. (A) The megakaryocyte in the centre shows markedly asynchronous maturation. The nucleus is in fact formed of numerous lobes, packed together, and certainly numerous endoreduplications of chromosomes have occurred; the chromatin is dark and condensed, as happens in mature megakaryocytes. The cytoplasm, however, is very scanty and intensely basophilic without any clear granules or platelet formation, thus similar to the cytoplasm of an immature megakaryocyte precursor. The outer surface of the cytoplasmic membrane is forming numerous pseudopodia, resembling thin grey-blue veils. The few platelets that are seen as small red bodies on the surface have adhered to the membrane rather than having been produced by this megakaryocyte, which can be classified, particularly because of its scanty cytoplasm and relatively small size, as a promegakaryocyte with N:C asynchrony. (B) Multinucleated promegakaryocyte. The nucleus is divided into three lobes; the chromatin has a granular structure; the nuclear outline is regular and linear; the cytoplasm is intensely basophilic, without granules, with numerous cytoplasmic blebs. Following a rigorous interpretation of the WHO criteria, the anomalies illustrated in (A) and (B) are not part of the canonical typology of megakaryocyte dysplasia, and these cells are therefore not counted as dysplastic for a diagnosis of MDS. (C) A large dysplastic megakaryocyte at an intermediate stage of maturation, with a hyperdiploid but non-lobated nucleus, with fairly condensed, prune-coloured chromatin with small blue circles that suggest the presence of nucleoli. The cytoplasm is weakly basophilic and finely granular; these small azurophilic dots correspond to the granules of future platelets. The mature platelet that appears to be within the cytoplasm is actually extrinsic, an adherent platelet superimposed on the megakaryocyte. (D) This megakaryocyte is also immature with asynchronous maturation and meets the WHO criteria for dysplasia because of the defect in nuclear segmentation. The cytoplasm is basophilic and agranular, with numerous small pseudopods, resembling achromic platelets lacking a granulomere, that are detaching from the surface membrane. It does not seem plausible that the clump of well-structured platelets to the right could have been produced by this megakaryocyte.

- The identification of the type and degree of dysplasia in individual lineages;
- Karyotypic analysis.

To these seven categories one can add a provisional category of MDS, designated 'refractory cytopenia of children'. Patients with persistent cytopenia, in the absence of dysplasia and specific cytogenetic alterations, are classified as 'idiopathic cytopenia of undetermined significance (ICUS)' and must be kept under close observation.

One of the new features of the 2008 WHO classification is that it establishes specific quantitative and qualitative criteria for the definition of dysplasia: the erythroid and granulocytic lineages

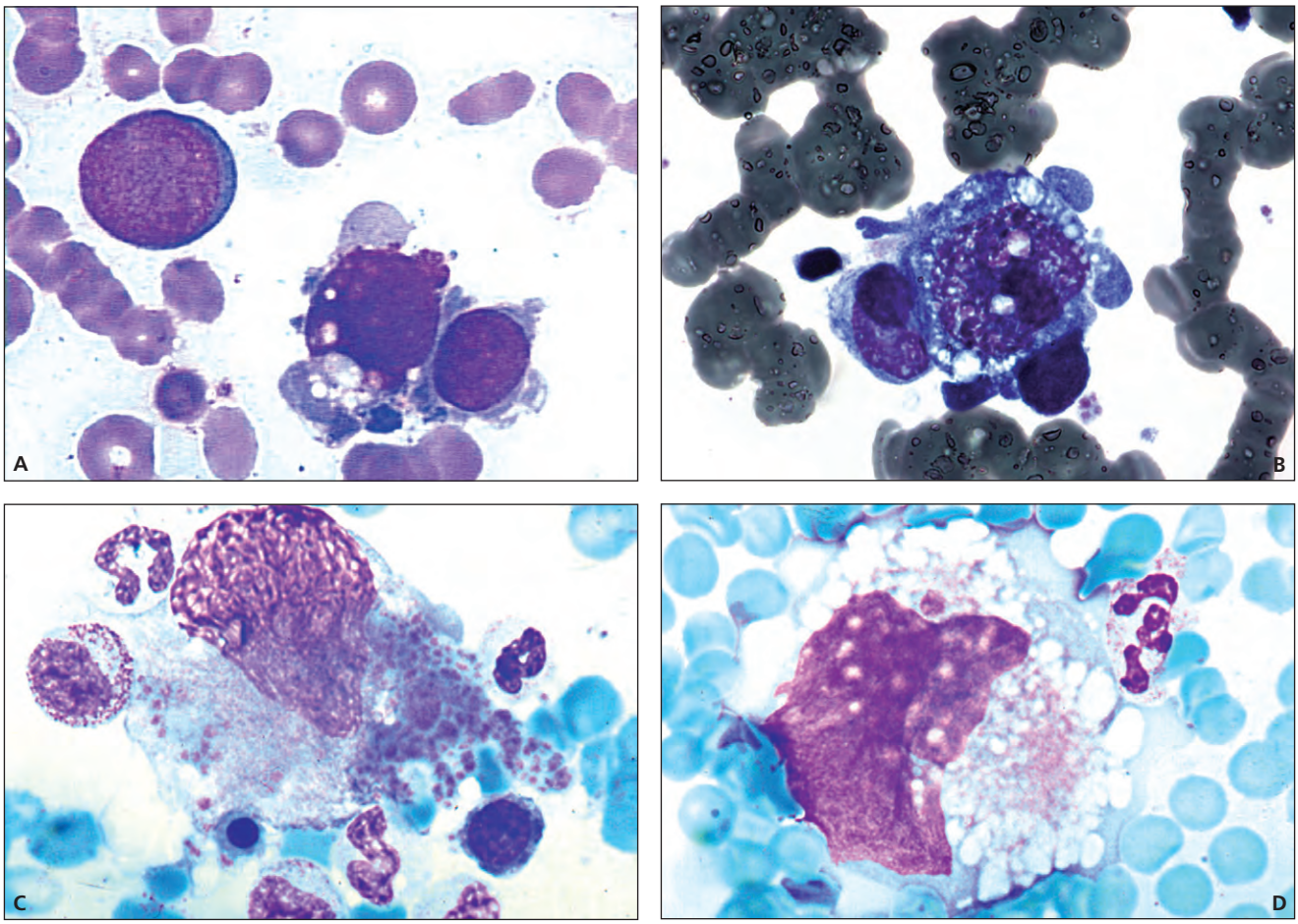



**Fig. 3.102** Bizarre morphology of dysmegakaryopoiesis; bone marrow aspirates of different patients with MDS. All these cells are included in the count of dysplastic megakaryocytes. (A) Top left, a megakaryoblast with finely granular chromatin and a large prominent nucleolus. The two cells adhering to each other, lower down, differ in size but have similar morphological aberrations, of a type that suggests that they are micromegakaryocytes: the N:C ratio is very high and the nucleus is oval and non-lobated with very compact and homogeneous chromatin; the scanty cytoplasm is greyish and agranular, without any platelet demarcation fields and is projecting as pseudopods that at some points show staining that verges on intense basophilia. The larger cell contains a number of irregular cytoplasmic and nuclear vacuoles that indicate necrobiosis. (B) Small megakaryocyte containing numerous degenerative vacuoles both in the cytoplasm and the nucleus. The cytoplasm is basophilic with numerous pseudopods. (C) Megakaryocyte with a nucleus that is hyperdiploid but not segmented, with some signs of immaturity (vermicular chromatin condensed into long filamentous structures separated by paler segments and at least three nucleoli). It is large, as can be appreciated by comparing it with the myelocyte, the hypogranular neutrophil and the erythroblasts that are in contact with its surface. The plentiful cytoplasm is heterogeneous with regard to staining characteristics and structure: there are weakly basophilic hypogranular areas, other areas with fine pink granules and yet others where platelet demarcation is occurring and well-formed proplatelets are present; the right part of the cytoplasm is represented by masses of completely differentiated mature platelets, ready to be released. (D) Large megakaryocyte with a hyperdiploid but non-lobated nucleus, which has a quite irregular and angular outline and homogeneous chromatin containing a number of degenerative vacuoles. The cytoplasm is almost completely occupied by vacuoles, which are large and form a continuous crown immediately beneath the cytoplasmic membrane. Otherwise the cytoplasm is weakly basophilic and contains, in a context of general hypogranularity, very fine granules packed together in the paranuclear zone.

are considered to be dysplastic when at least 10% of cells in each series show specific morphological anomalies on a count of 200 cells of each lineage; the megakaryocyte lineage is considered to be dysplastic when at least 10% of megakaryocytes, on a count of a minimum of 30 cells, show typical dysplastic features.

Other relevant points in this updated diagnostic scheme are the possibility of making a diagnosis of MDS in the presence of:

- Unilineage or multilineage dysplasia, with less than 5% blast cells in the bone marrow and 2–4% in the peripheral blood;
- Unilineage cytopenia and dysplasia, with less than 5% blast cells in the marrow and less than 1% in the peripheral blood, in the absence of the cytogenetic abnormalities that are associated with MDS;
- Cytopenia, unequivocal dysplasia in less than 10% of cells in one or more lineages, and recognition of one or more of the cytogenetic alterations associated with MDS.

When making a diagnosis it is necessary to have all relevant clinical information available in order to be able to exclude the

**Table 3.7** 2008 WHO classification of the myelodysplastic syndromes (adapted from Swerdlow 2008,[28] with permission of IARC)

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numerous dysplastic manifestations that can result from cytotoxic chemotherapy, use of growth factors including erythropoietin, exposure to toxic substances and infection. Table 3.8 lists the principal conditions that it is necessary to consider. In particular, the differential diagnosis with vitamin deficiency is fundamental in cases with megaloblastoid dyserythropoiesis. The granulocyte growth factors (G-CSF and GM-CSF) produce prominent alterations in the granulocyte series, such as hypergranularity and reduction or increase of nuclear lobes, with ring nuclei and a left shift of the maturation curve.[86] These alterations can be recognised with automated analysers that measure MPO activity.[87] Transitory and reversible neutrophil dysplasia, not associated with anomalies of other lineages or an excess of blast cells, can be produced by various immunosuppressive, cytotoxic and other drugs, which induce nuclear hypolobulation and hypercondensation of nuclear chromatin of a pseudo-Pelger type.[88–92]

### Refractory cytopenia with unilineage dysplasia

This category includes refractory and persistent unilineage cytopenia with the bone marrow showing unilineage dysplasia. It includes refractory anaemia, refractory neutropenia and refractory thrombocytopenia: together these represent around 20% of cases of MDS, without any difference between men and women and occurring usually after the age of 65 years. The clinical course is usually protracted and these cases fall into the low risk

**Table 3.8** Clonal and non-clonal conditions to be considered in the differential diagnosis of the myelodysplastic syndromes

- Megaloblastic anaemia (deficiency of vitamin $B_{12}$ or folate)
- Copper deficiency
- Exposure to or poisoning by heavy metals (lead, arsenic, zinc)
- Drugs: antifolates, antivirals, mycophenolate mofetil, cytokines and growth factors (G-CSF)
- Antineoplastic irradiation and/or drugs
- Chronic infection
- Parvovirus B19 infection
- Immune deficiency syndromes, particularly HIV infection
- Haemophagocytic lymphohistiocytosis
- Primary myelofibrosis
- Myeloproliferative neoplasms
- Myelodysplastic/myeloproliferative neoplasms
- Paroxysmal nocturnal haemoglobinuria
- Aplastic anaemia
- Selective unilineage aplasia/hypoplasia, congenital or idiopathic
- Acute paucicellular non-lymphoid leukaemia
- Various congenital syndromes:
  - Myelokathexis and WHIM syndrome
  - Pelger-Huët anomaly
  - Thalassaemia
  - Sickle cell anaemia
  - Congenital dyserythropoietic anaemia
  - Congenital sideroblastic anaemia

group, according to the IPSS, with evolution to acute leukaemia in 2% of cases. The commonest of the three is refractory anaemia. The basic diagnostic criteria are:

- Peripheral blood: unilineage cytopenia and blast cells less than 1%;
- Bone marrow: unilineage dysplasia, blast cells less than 5% (without Auer rods) and ring sideroblasts less than 15%.

The peripheral cytopenia is defined in accordance with the criteria of the IPSS,[13] using the following values:

- Haemoglobin concentration less than 100 g/l;
- Neutrophils less than $1.8 \times 10^9$/l;
- Platelets less than $100 \times 10^9$/l.

However, with the new revision of the WHO classification it is possible to make a diagnosis of MDS also above these threshold values if there is dysplasia and/or cytogenetic alterations that are considered specific.[93] However, the WHO proposes that to avoid a potentially unreliable diagnosis in cases without a cytogenetic marker, a diagnosis of MDS should be made only after a 6-month period of observation.

In general, there is a correspondence between the lineage that is dysplastic in the marrow and the cytopenic lineage in the peripheral blood, with uncommon exceptions having been reported.[93] The rare cases that at diagnosis have bicytopenia, but unilineage dysplasia, are also assigned, according to the WHO classification, to the refractory cytopenia with unilineage dysplasia(RCUD) category. Refractory pancytopenia with unilineage dysplasia is assigned to the MDS, unclassifiable (MDS-U) category. If there is neither dysplasia nor a specific cytogenetic alteration, the diagnosis of ICUS is made, this not necessarily indicating an early stage of MDS. If there is bi- or tri-lineage dysplasia, the diagnosis is RCUD. The presence of blast cells in the peripheral blood excludes a diagnosis of RCUD: according to the WHO classification, the patient who at diagnosis has at least 1% but less than 2% circulating blast cells must be categorised as MDS not classifiable (while those with blast cells between 2% and 4% are classified as RAEB-1).

## Refractory anaemia

Refractory anaemia represents a little less than 20% of cases of MDS: the course is variable with a median survival of 6–7 years. Infection (30%) and, less often, leukaemic transformation (5–10%) represent the main causes of death.[94]

The peripheral blood shows anaemia, which can be normocytic and normochromic or macrocytic; microcytosis is much rarer. A double erythrocyte population may be present. There is variable anisopoikilocytosis, which is rarely marked; the RDW is usually increased. Occasional cases show neutrophil dysplasia or dysmorphic platelets. Rarely, there is also neutropenia or thrombocytopenia, usually only of moderate degree. Circulating blasts cells are, by definition, absent or less than 1%.

Bone marrow films are generally normocellular or hypercellular, with a tendency to inversion of the myeloid:erythroid (M:E) ratio. Erythroid dysplasia is recognised when at least 10% of erythroid cells are abnormal (Figs 3.103 through to 3.108). Bone marrow iron stores are usually increased, but are variable according to the clinical features. Ring sideroblasts can be present but, by definition, must be less than 15%. The granulocytic and megakaryocyte series are most often morphologically normal; dysplastic changes are present in 10% or more of erythroid cells. Blasts cells are less than 5% of ANC. Auer rods are absent. Cytogenetic abnormalities are seen in more than 50% of cases, most often del(20q), trisomy 8 or abnormalities of chromosomes 5 and 7. An accurate differential diagnosis, particularly in cases without any cytogenetic abnormality, must obviously include the numerous non-clonal conditions that cause anaemia with erythroid hyperplasia and some degree of dyserythropoiesis, such as autoimmune haemolytic anaemia, megaloblastosis, anaemia of chronic disease, thalassaemias and haemoglobinopathies, and certain haemophagocytic syndromes.[95]

## Refractory neutropenia

Like refractory thrombocytopenia, but differing from refractory anaemia, this is a rare condition, which represents only 1–2% of cases of MDS. The diagnosis requires the exclusion of exposure to certain drugs and other myelotoxic substances, infection, autoimmune pathology and other primary causes of neutropenia. In refractory neutropenia at least 10% of cells of granulocyte lineage are dysplastic (Figs 3.109 and 3.110): neutrophil hypogranularity and hypolobation are the most characteristic dysplastic features and, in contrast to refractory anaemia, dysplasia is often more apparent in the peripheral blood film or a buffy coat film than in a bone marrow film. Recognition of

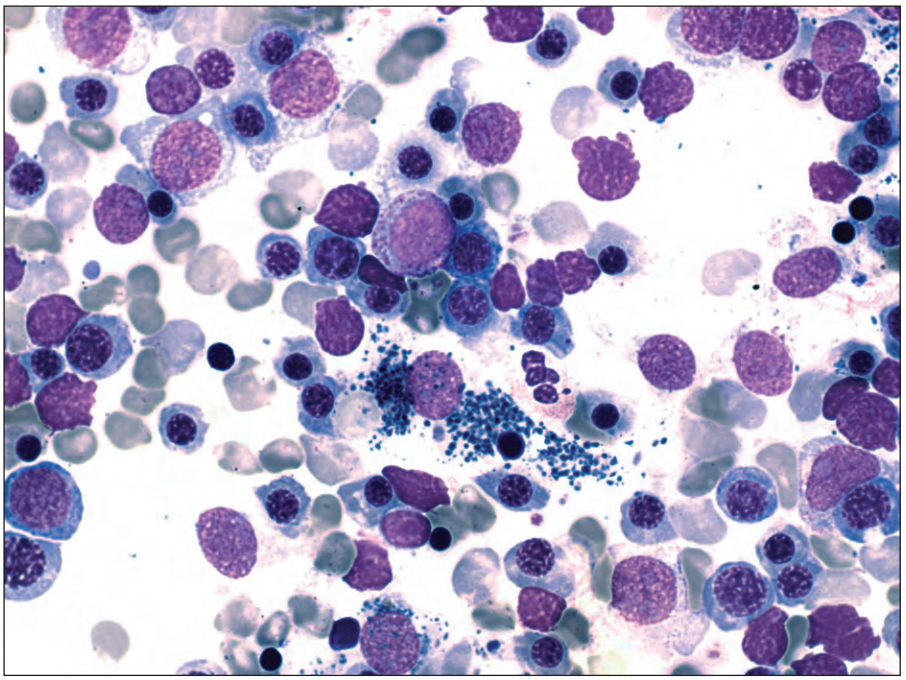


**Fig. 3.103** Refractory cytopenia with unilineage dysplasia (RCUD), specifically refractory anaemia, in a female patient. The bone marrow aspirate shows marked erythroid hyperplasia with preserved maturation (predominance of polychromatic and orthochromatic erythroblasts) and moderate signs of dysplasia, particularly nucleocytoplasmic asynchrony. Top left, two proerythroblasts with a megaloblastoid tendency, suggested by the polychromasia of the cytoplasm and the immaturity of the nucleus, which contains a number of pale nucleoli. In the centre, a non-dysplastic promyelocyte with, on its right, two small basophilic erythroblasts and, immediately beneath, a normally lobulated and granulated neutrophil with a nuclear drumstick (Barr body); adjacent, a macrophage with extensive elongated cytoplasm containing abundant phagocytosed material in the form of blackish granules and, on the left, an entire intact erythrocyte (erythrophagocytosis). On the right, another three oval macrophage nuclei with spongy chromatin, associated with barely visible cytoplasm in the form of tentacle-like strands with indistinct margins.

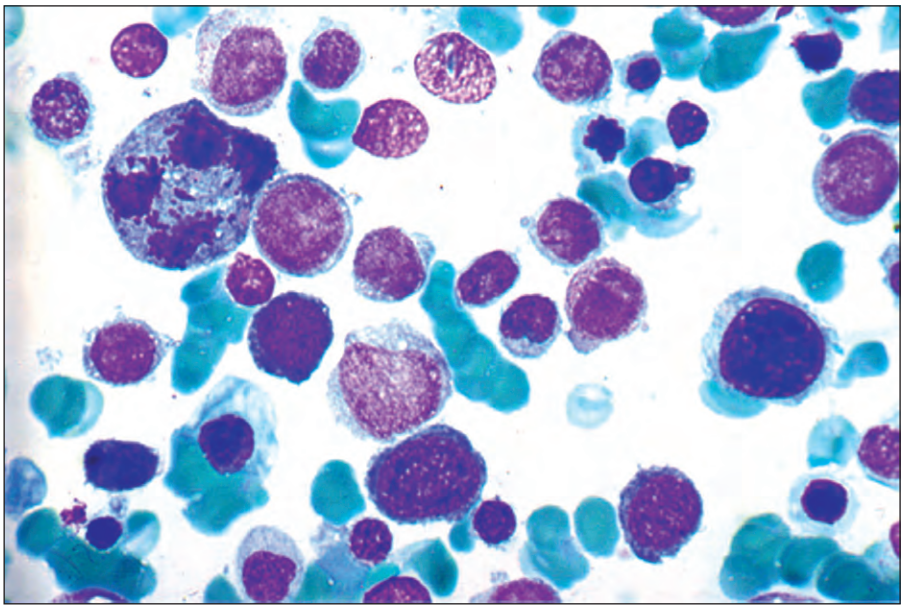


**Fig. 3.104** Refractory anaemia: bone marrow aspirate. Erythroid hyperplasia with markedly dysplastic features. On the left, a giant tetrapolar mitosis with polychromatic cytoplasm containing numerous lost chromatid fragments. On the right and in the centre at the bottom, very dysplastic erythroblasts with large hyperdiploid nuclei, irregular nuclear outlines, irregular chromatin condensation and scanty cytoplasm. There are also poorly haemoglobinised erythroblasts, karyorrhexis, a dysplastic promyelocyte top left and two blast cells, one small and one large, towards he centre.

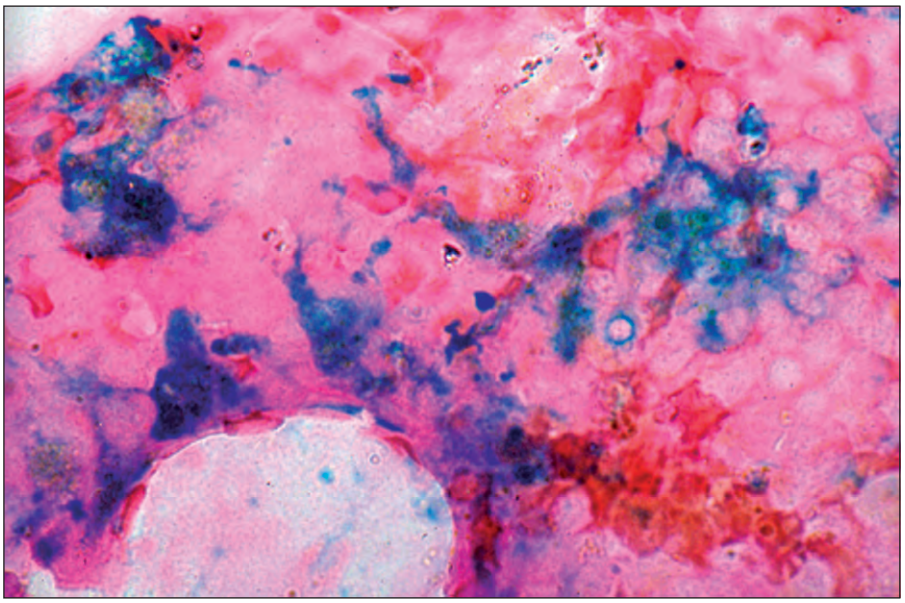


**Fig. 3.105** Refractory anaemia: bone marrow aspirate, Perls stain. Iron deposits are increased in this bone marrow fragment and the blue precipitate appears well localised within the plentiful, irregularly shaped cytoplasm of numerous macrophages. A single small erythroblast shows a distinct circle of diffuse paranuclear staining, a different appearance from that of a ring sideroblast.

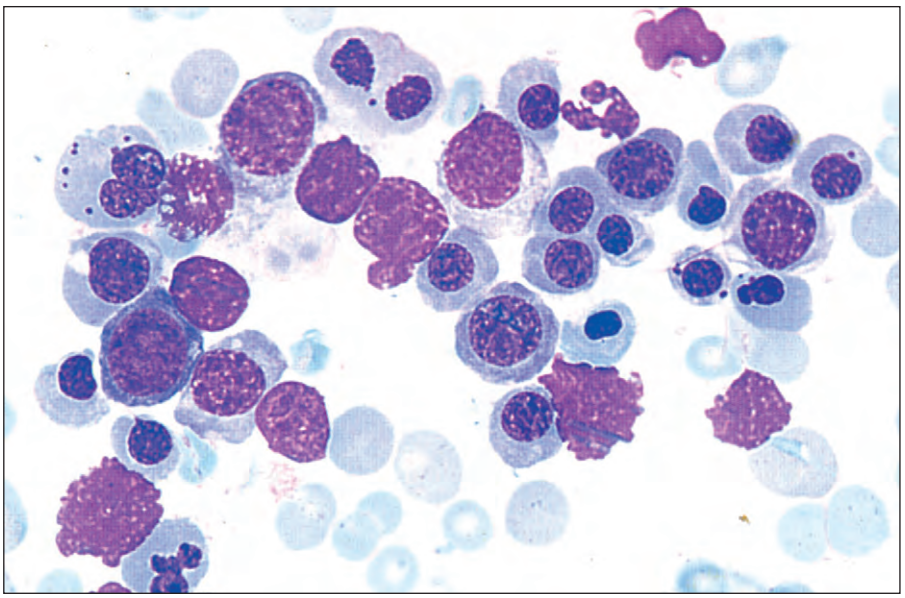


**Fig. 3.106** Refractory anaemia: bone marrow aspirate showing a field with a tendency to synchronisation of erythroblast maturation, with megaloblastoid asynchrony. Only two basophilic erythroblasts are visible on the left, one with the remains of a nucleolus visible within the clumped chromatin and the other, higher up, with torn cytoplasm. The other erythroblasts, all dyserythropoietic, have cytoplasm with very similar staining characteristics, with only minimal variation in the greyish polychromatic tint and with frequent Howell-Jolly bodies. There are some nuclei that still have chromatin condensed into clumps (of the type that is seen in basophilic erythroblasts), thus showing a megaloblastoid tendency. Other nuclei are more compact, others completely pyknotic; some are joined by internuclear bridges and others have an irregular or lobulated nuclear outline. Top left, a polychromatic erythroblast with a multilobated nucleus and a number of Howell-Jolly bodies; adjacent is an erythroblast with an abortive telophase.

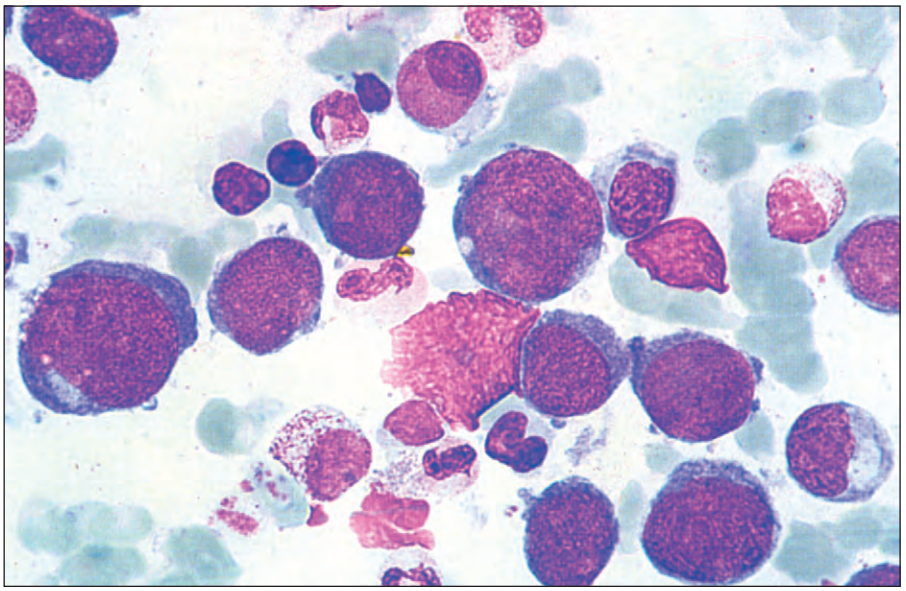


**Fig. 3.107** Refractory anaemia: bone marrow aspirate. In this patient erythroid hyperplasia is associated with marked deviation to the left of the maturation curve, with an excess of proerythroblasts and basophilic erythroblasts that have ultramarine cytoplasm and very immature chromatin, almost megaloblastoid. The large erythroblast in the centre and that on the extreme left are certainly polyploid. Top centre, a small eosinophil myelocyte with a part of the cytoplasm being agranular and sky-blue; the granules are tightly packed into the remaining part of the cytoplasm. At the bottom in the centre and top right, small myelocytes with rounded eccentric nuclei.

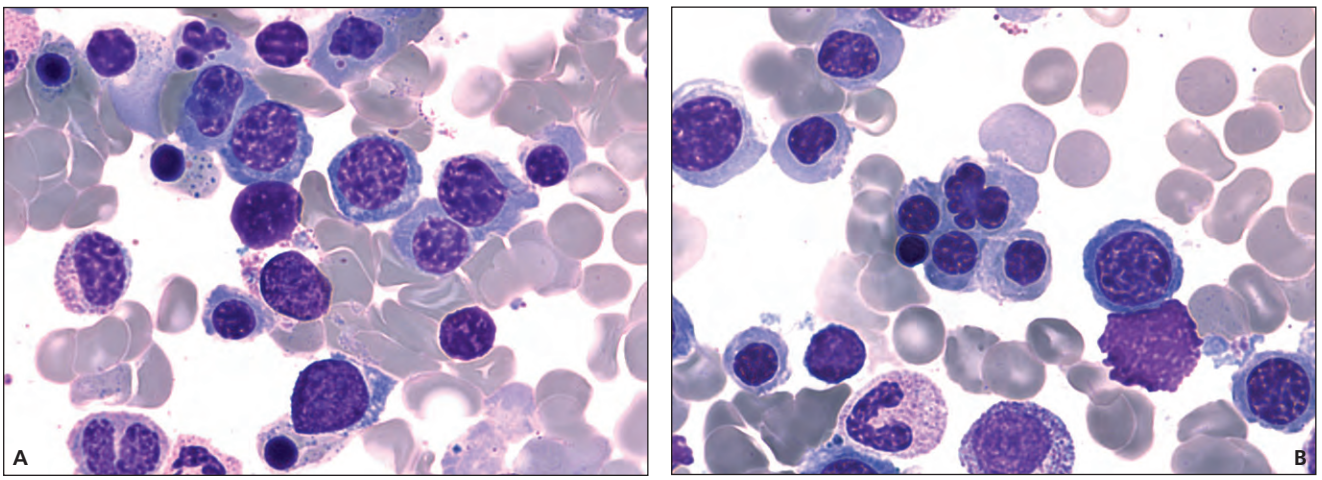



**Fig. 3.108** Refractory anaemia: features of dyserythropoiesis; bone marrow aspirate. (A) Abundance of basophilic, polychromatic and orthochromatic erythroblasts. While the basophilic erythroblasts are fairly normal in appearance, in some polychromatic erythroblasts and in many orthochromatic erythroblasts there are abnormalities such as karyorrhexis, basophilic stippling and defective haemoglobinisation. The nuclei in the centre could belong to megakaryocytes or micromegakaryocytes with the cytoplasm having been lost. On the left, a small neutrophil myelocyte. (B) Nuclear irregularity in polychromatic and orthochromatic erythroblasts. In the centre, a multilobated nucleus with the nuclear lobes being circular; far left, cut off by the border, is a larger cell with a violet nucleus and cytoplasm with circular spaces lacking haemoglobin; adjacent is a polychromatic erythroblast with nodular nuclear protuberances.

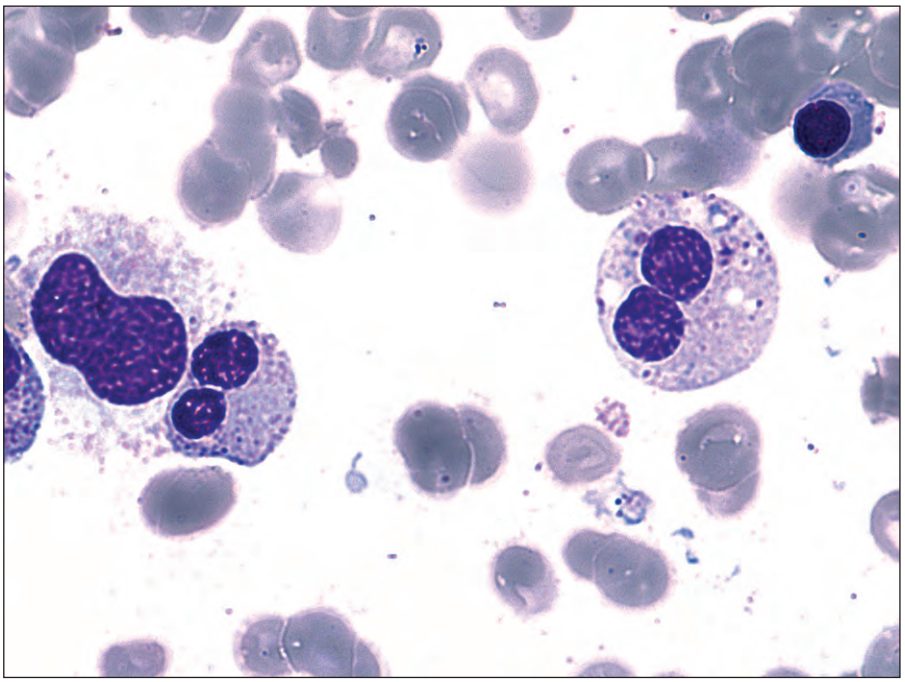


**Fig. 3.109** RCUD, refractory neutropenia; bone marrow aspirate. There are neutrophils showing marked dysplasia, both bilobed nuclei (pseudo-Pelger) and hypogranular cytoplasm; the cytoplasm is pinkish-grey with some vacuoles and a few irregularly distributed bluish granules. Top right, a polychromatic erythroblast.

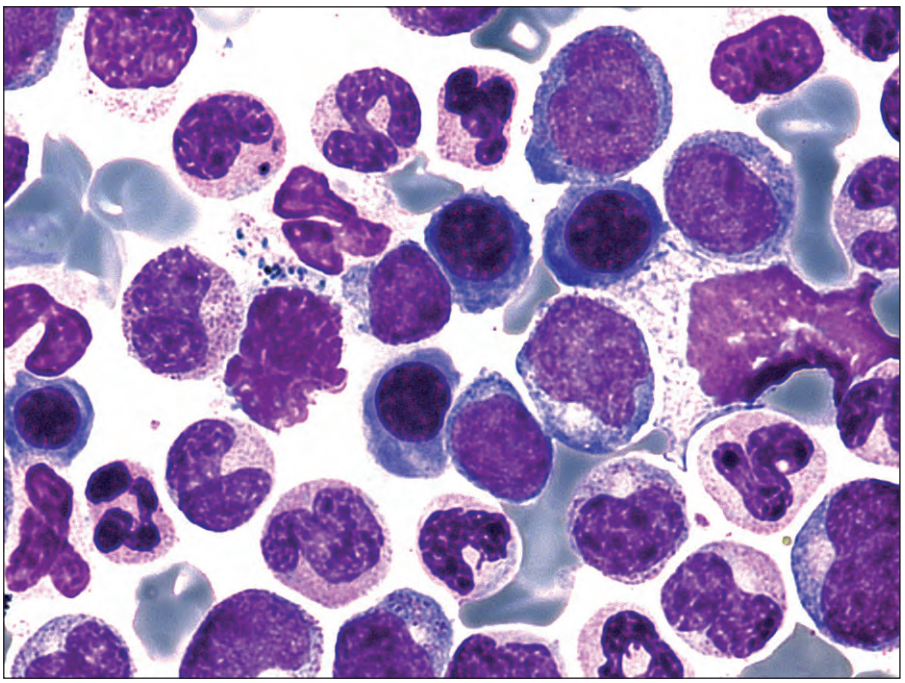


**Fig. 3.110** Bone marrow aspirate in a different case of refractory neutropenia. This microscopic field appears hypercellular and contains, in addition to four erythroblasts and a lymphocyte, numerous granulocyte precursors and three agranular blasts. The most significant feature with regard to dysplasia is the tendency of the nuclei in the mature cells to absent or incomplete segmentation, with very marked chromatin condensation in some cells and the presence in the pink cytoplasm of very fine, almost invisible granules. There are Howell-Jolly body-like inclusions (in a metamyelocyte top left), filiform nuclear appendages and areas of cytoplasmic basophilia (in a band neutrophil in the centre at the bottom).

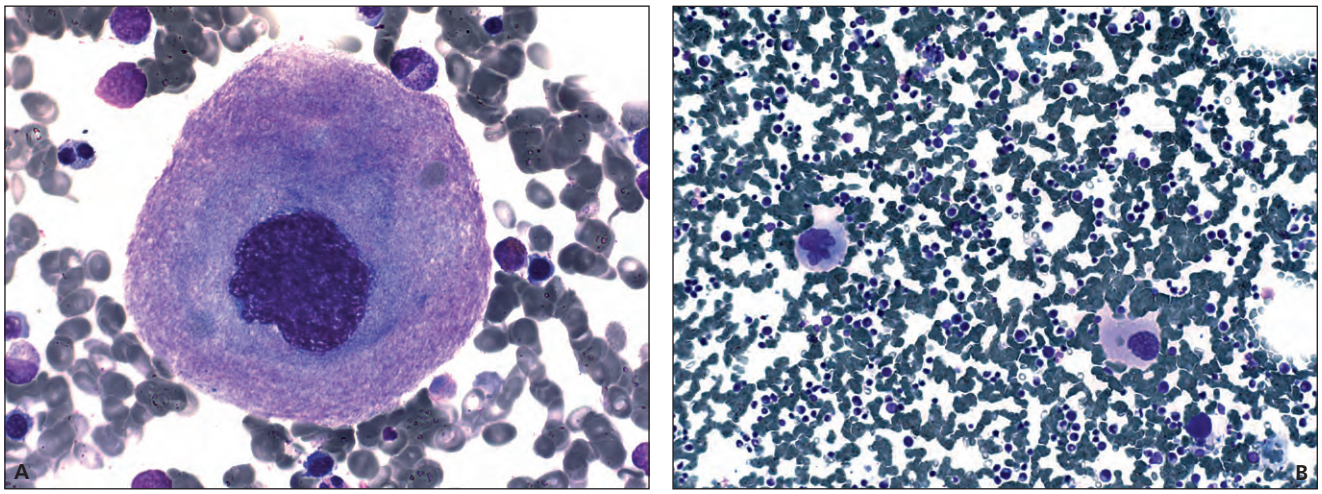


**Fig. 3.111** RCUD, refractory thrombocytopenia. In the bone marrow aspirates of these patients with thrombocytopenia of variable severity there is in general a normal or increased number of megakaryocytes, which rarely have prominent dysplastic features. The differential diagnosis with thrombocytopenia of peripheral origin is often impossible purely on the basis of morphology. (A) A large polyploid megakaryocyte with the nuclear outline including some segments of circles and with normal cytoplasmic differentiation: there are numerous platelet demarcation fields recognised by the concentrations of alpha granules in a large central perinuclear area with more accentuated basophilia. There are, however, no signs of platelets being released. An erythrocyte is included within the cytoplasm, top right. (B) Bone marrow megakaryocytes in a case of refractory thrombocytopenia. The megakaryocyte on the left has an enormous hypersegmented nucleus and cytoplasm that is relatively well developed and basophilic (N:C asynchrony); the megakaryocyte on the right has a cauliflower-shaped non-segmented nucleus and shows emperipolesis. In the bottom right corner is an intensely basophilic promegakaryocyte.

dysplasia of immature granulocytes is important, particularly in making a distinction between dysplastic promyelocytes and granular blast cells: an erroneously high blast count can, in fact, lead to assignment to an inappropriately adverse prognostic group and *vice versa*.

### Refractory thrombocytopenia

In refractory thrombocytopenia the megakaryocytes in general are present in normal or increased numbers: at least 10% of megakaryocytes are dysplastic (Fig. 3.111). In the absence of a cytogenetic abnormality, the differential diagnosis of autoimmune thrombocytopenia must be considered. The presence of isolated del(20q) in patients with refractory thrombocytopenia correlates with a good prognosis.[85]

### Refractory anaemia with ring sideroblasts

RARS, like other MDS, is caused by a clonal defect arising in a haemopoietic stem cell which, in this instance, as well as leading to ineffective erythropoiesis, leads to an alteration of iron metabolism in erythroid cells that is responsible for intramitochondrial accumulation of iron. RARS is distinguished from refractory anaemia by the presence, often in the context of a hypercellular marrow with erythroid hyperplasia and dyserythropoiesis, of at least 15% ring sideroblasts (expressed as a percentage of total erythroid precursors) (Figs 3.112 through to 3.116). The definition has been standardised: they are erythroblasts with at least five siderotic granules, blue on a Perls stain, which surround at least a third of the nuclear circumference.[66] Their recognition thus does not require complete encirclement of the nucleus. These cells are exclusively type III sideroblasts, according to the definition already provided. They correspond to cells with intramitochondrial iron accumulation with deposition of ferritin and haemosiderin, which can be caused by acquired genetic defects of nuclear or mitochondrial DNA. In the more obvious cases, ring sideroblasts may be as high as 40% of erythroblasts. With the passage of time, often the proportion of pathological sideroblasts increases in parallel with increasing iron stores and serum ferritin. A small percentage of patients develop the clinical picture of haemochromatosis.[96] RARS strikes people over the age of 60 years without any gender predilection. The median survival from diagnosis varies from 7 to 9 years, even if, as in refractory anaemia, this condition is often diagnosed incidentally and late in its course, for which reason the true duration is unknown. Acute leukaemic evolution is uncommon (1–2%).

The peripheral blood shows normocytic or, more often, macrocytic anaemia; in contrast to other types of MDS, there is often a dimorphic picture, resulting from the presence of a variable proportion of microcytic erythrocytes, which are generally also hypochromic (see Report 3.1). Automated haematological analysers show an increased proportion of hypochromic red cells, on average more than 40% of cells, which increase notably following treatment with growth factors: it is important to be aware of this in order, during erythropoietin therapy, not to

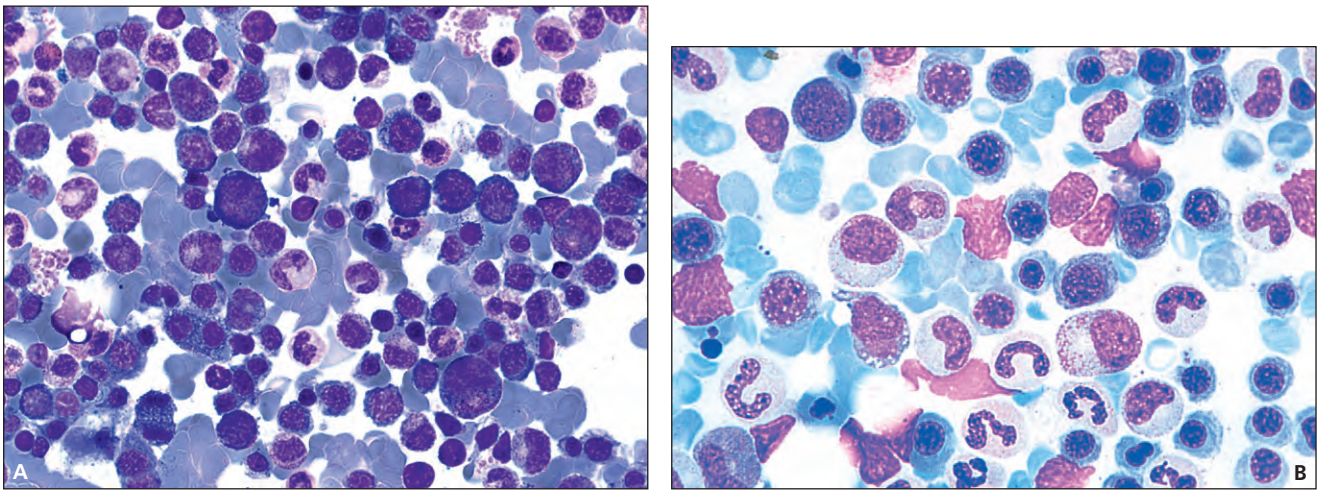



**Fig. 3.112** Refractory anaemia with ring sideroblasts (RARS); bone marrow aspirates of different patients. (A) Marked hypercellularity with erythroid hyperplasia; more than 50% of cells are erythroblasts at all stages of maturation with moderate dysplasia. Bottom left, three mitotic figures and vacuolisation of the cytoplasm; slightly to the left of the centre, a large multinucleated basophilic erythroblast. There are some promyelocytes on the right side of the image. (B) The marrow is cellular and heterogeneous, with inversion of the myeloid:erythroid ratio as a result of moderate erythroid hyperplasia with some features of dyserythropoiesis. The erythroblasts show mildly asynchronous maturation with nuclear dysmorphism that becomes evident only in the most mature cells, in which the nuclei with homogeneous pyknotic chromatin are often bilobed. The granulocytic precursors sometimes have hypogranular cytoplasm, but otherwise show normal maturation.

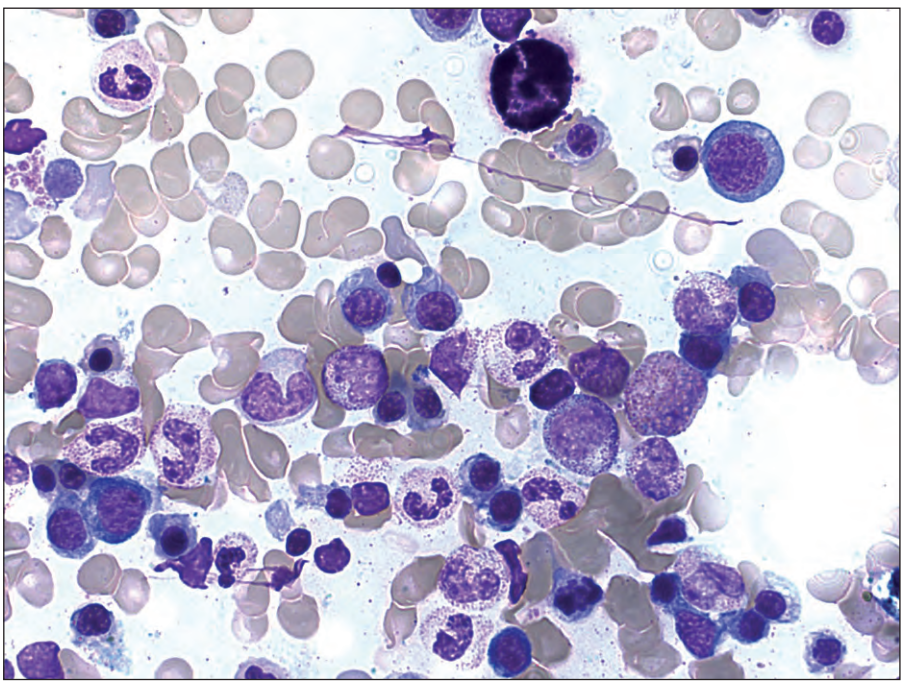


**Fig. 3.113** RARS; bone marrow aspirate. The cellularity is not increased in this preparation, and the erythroid series differs from that shown in Fig. 3.112 in not being hyperplastic. The erythroblasts show fairly normal maturation from proerythroblasts to orthochromatic erythroblasts, but have features of abnormal maturation in the form of defects in haemoglobinisation (e.g. in the pair of basophilic erythroblasts in the centre at the top or in the polychromatic erythroblast in the bottom right corner); at the bottom there is a mitotic figure. The granulocytic series is well represented and does not show dysplastic features. Adjacent to the upper border is a mast cell with a triangular pale violet nucleus and cytoplasm that is packed with black granules. On the left border, a strand of platelets that includes a giant bluish platelet.

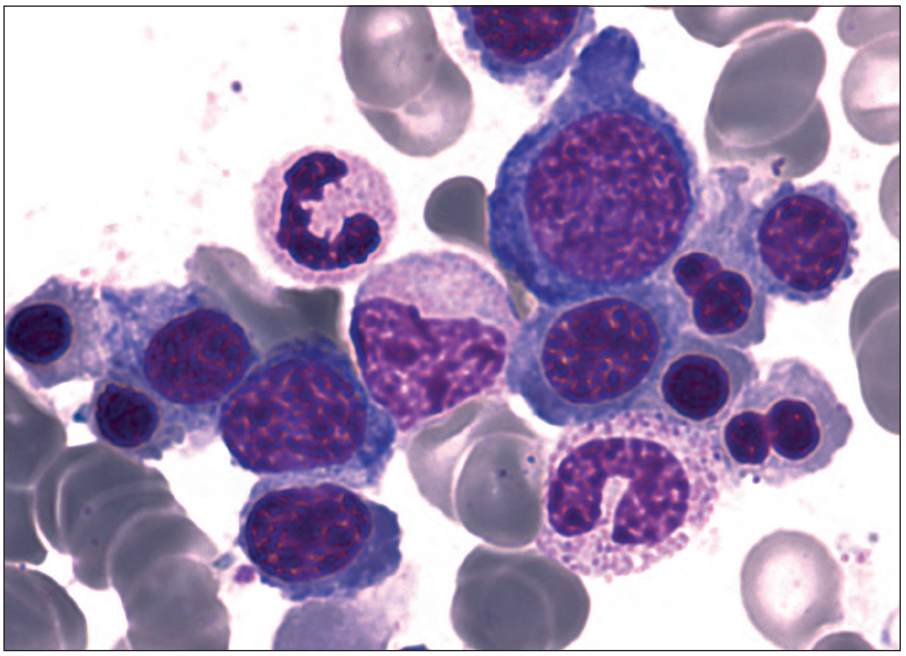


**Fig. 3.114** RARS; bone marrow aspirate. Also in this case microscopic examination of a film stained by a panoptic method (May-Grünwald-Giemsa [MGG]) shows mild erythroid hyperplasia with moderate dysplasia of the more mature cells, as shown by the two orthochromatic erythroblasts with bilobed nuclei visible on the right. In total there are: a well-differentiated proerythroblast, a single basophilic erythroblast, four early polychromatic erythroblasts with almost identical nuclei, but with cytoplasmic staining varying from blue to dark blue-grey, and five orthochromatic erythroblasts. The granulocytic series is represented by a myelocyte, a metamyelocyte and a dysplastic neutrophil (very dense chromatin, various nuclear appendages and few granules).

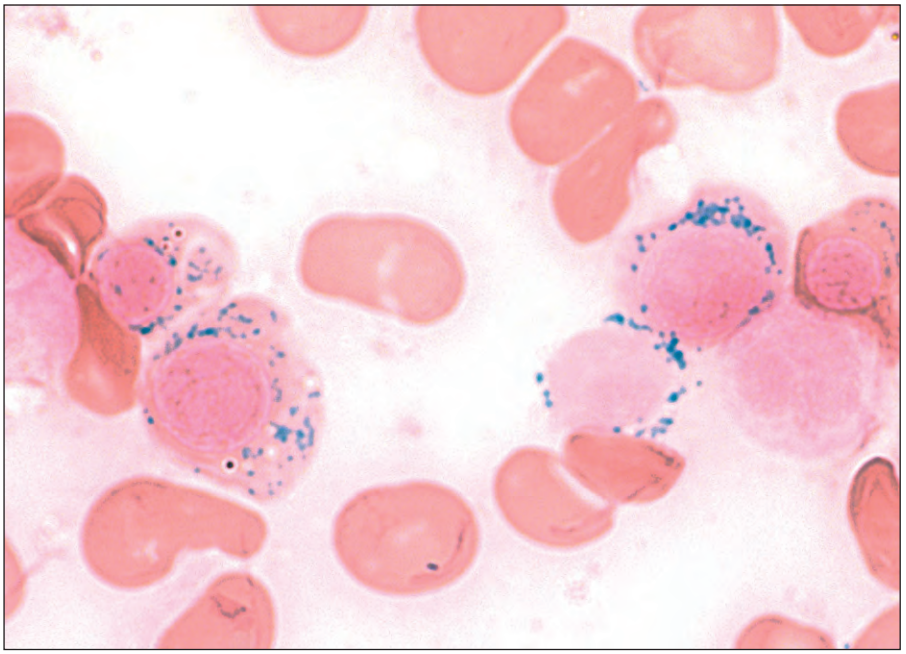


**Fig. 3.115** RARS; bone marrow aspirate, Prussian blue (Perls) stain. Ring sideroblasts of various sizes (which could suggest different degrees of differentiation), with very numerous blue granules that entirely surround the nucleus. On the right, the nucleus of a larger cell, possibly a metamyelocyte, with the cytoplasm being negative for iron.

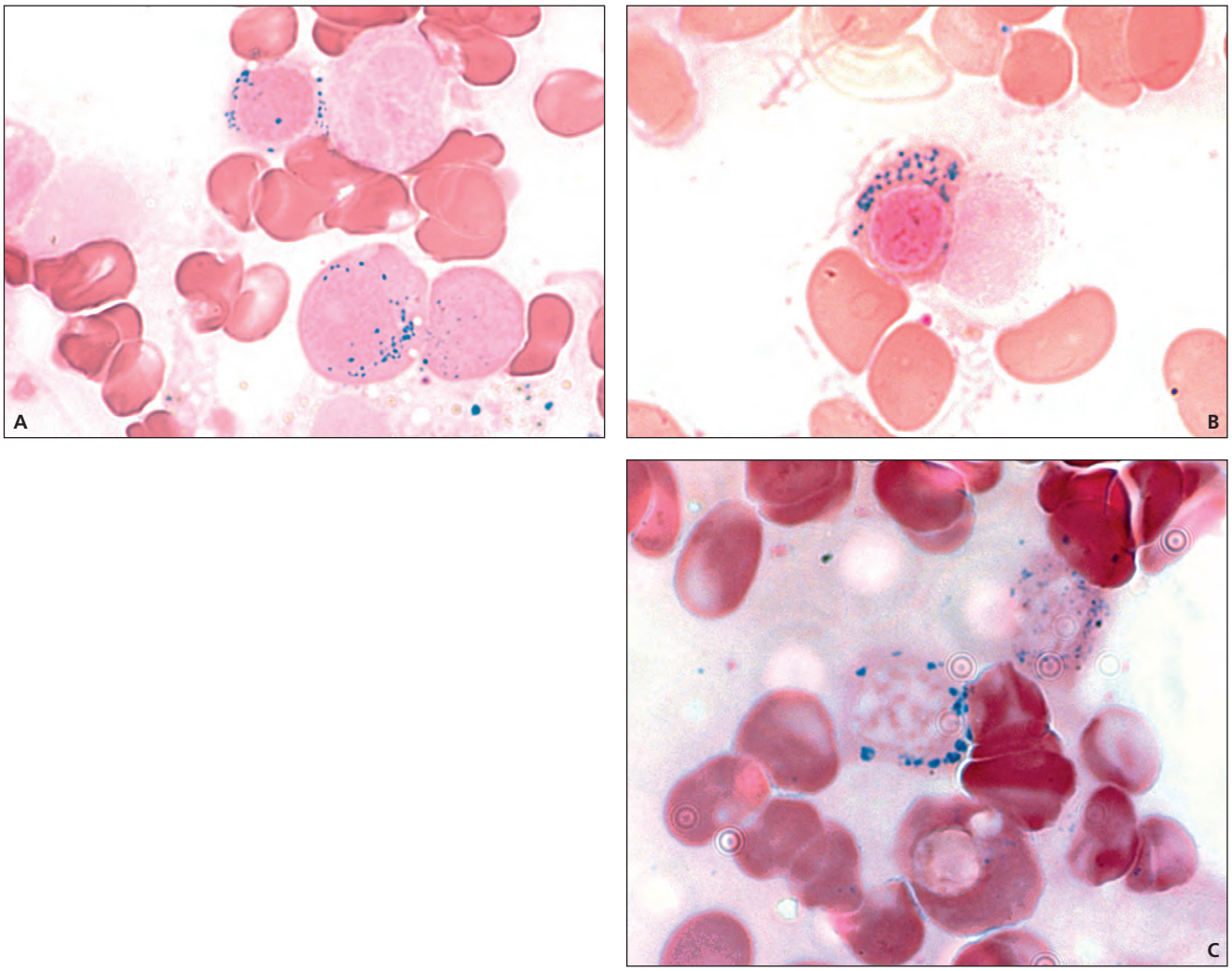



**Fig. 3.116** RARS; bone marrow aspirate, Prussian blue (Perls) stain. Various forms of ring sideroblast. (A) Incomplete rings of blue granules around the nuclei of two sideroblasts at different stages of maturation. (B) In this erythroblast, certainly polychromatic on the basis of the cytological features, the positive granules are numerous and surround more than a third of the nuclear circumference: one is thus dealing with an accredited ring sideroblast. (C) Sideroblast with a mature nucleus with very large positively-staining granules surrounding most of the nucleus. Top right, another cell shows a larger quantity of very delicate granules that surround the nucleus (in a somewhat atypical manner) in three dimensions: this cell also should be included in the count of ring sideroblasts.

suspect functional iron deficiency (and then give inappropriate iron treatment). In these patients the hypochromia, often associated with macrocytosis, is principally due to an intrinsic defect in erythropoiesis.[36] Reticulocytes also can have a lower haemoglobin content than normal.[47] This abnormality causes an alteration in the distribution of signals in the ADVIA system red cell volume/concentration cytogram that is strongly predictive of a diagnosis of RARS: the red cell population appears more dispersed than normal in all directions, occupying eight of the nine sectors of the two-dimensional cytogram.[97]

Moderate anisopoikilocytosis, basophilic stippling and rare erythroblasts can be present in the blood film. Blast cells are less than 5% in the marrow and are absent in the peripheral blood. Leucocytes and platelets are usually normal: rarely, there are moderate quantitative and/or qualitative abnormalities. If the platelet count is at least 450 $\times$ $10^9$/l, the diagnosis is that of RARS with thrombocytosis rather that RARS, and morphological abnormalities in megakaryocytes are present; RARS with thrombocytosis is assigned, as a provisional category, to the MDS/MPN group of disorders described in Chapter 7.

In the bone marrow, there is increased erythropoiesis with dyserythropoiesis of variable severity, but the granulocytic and megakaryocytic series are generally morphologically normal or at least show dysplastic changes in less than 10% of cells. According to the WHO classification, when the presence of at least 15% ring sideroblasts occurs together with a second

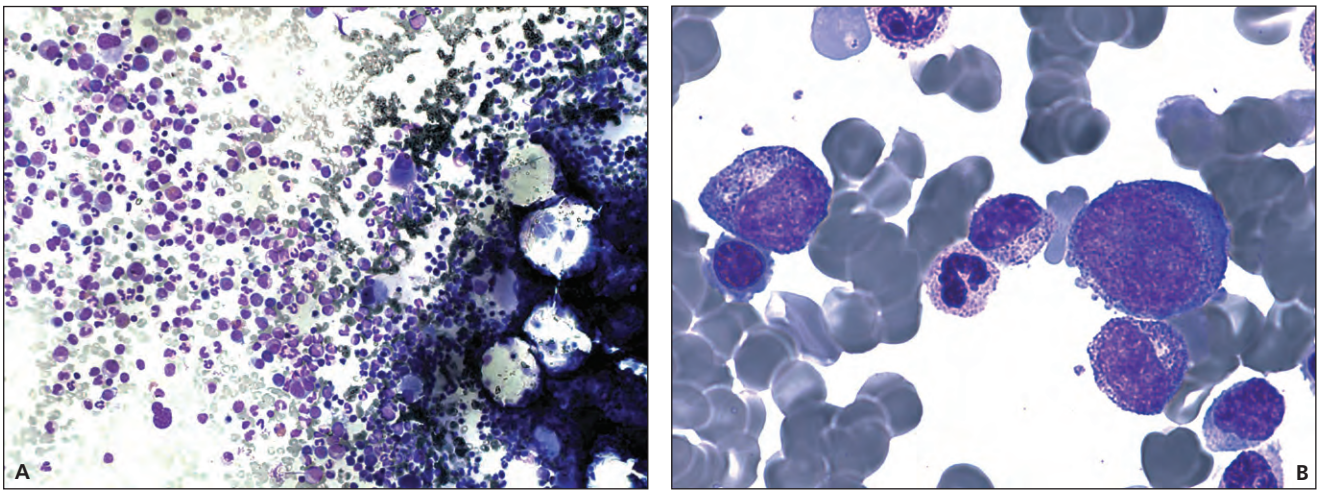



**Fig. 3.117** RCMD; bone marrow aspirate. (A) At low power, in a trail left behind a small fragment there are some fat cells and a number of small to medium-sized megakaryocytes with fairly basophilic cytoplasm; a bare nucleus is seen bottom left. The marrow is fairly cellular in this area with the cells being mainly of granulocytic lineage with fairly normal maturation plus a few erythroblasts. (B) At higher power, in this field there is a granular blast cell with an enormous hyperdiploid nucleus with chromatin condensed into clumps and with intensely blue cytoplasm without a visible Golgi zone, which is forming small pseudopodia, but can be distinguished from a proerythroblast by the presence of dark granules around the nucleus; immediately beneath it, a myelocyte and a monocyte; to the left of the granular blast cell, a dysplastic metamyelocyte, definable as such because of its small size even if the nucleus is bean shaped rather than horseshoe shaped; a dysplastic non-segmented neutrophil, with very dark chromatin and a large sessile appendage; still further to the left, a myelocyte/promyelocyte (see discussion of Fig. 3.58), recognised as dysplastic because of the marked asynchrony between the blue of the cytoplasm, in which however the granules are distributed in a fairly orderly manner around the Golgi zone, and the condensed chromatin of the eccentric nucleus; a polychromatic erythroblast.

cytopenia in addition to the anaemia, the case is classified as refractory cytopenia with multilineage dysplasia (RCMD), and tends to have a worse prognosis than that of RARS. Similarly, if blast cells are present in the blood or are increased in the marrow, the diagnosis becomes RAEB-1 or RAEB-2, even though ring sideroblasts are 15% or more. The presence of a chromosomal abnormality, usually single, is reported in less than 20% of patients.

With regard to the differential diagnosis, ring sideroblasts can be observed in other subtypes of MDS, in non-clonal anaemia (anaemia of chronic disease, megaloblastic anaemia), in copper deficiency and as a feature of toxicity of alcohol, various drugs and zinc. Various inherited defects in haem synthesis must be excluded, specifically congenital sideroblastic anaemia and Pearson syndrome (which affects the pancreas as well as the marrow, is caused by a deletion of mitochondrial DNA and has a haematological picture similar to that of RARS). Finally, in the presence of 15% or more ring sideroblasts and a platelet count of $450 \times 10^9$/L or greater it is indispensable to exclude the *JAK2* V617F mutation (which often occurs in this provisional MDS/MPN category of RARS with thrombocytosis).[43]

## Refractory cytopenia with multilineage dysplasia

This is a new MDS category, introduced in the 2008 WHO classification, which represents around 30% of cases of MDS (see Report 3.2). It includes cases with one or more peripheral cytopenias associated with bilineage or trilineage dysplasia, with blast cells less than 1% in the peripheral blood and less than 5% in the bone marrow (Figs 3.117, 3.118 and 3.119). The bone marrow aspirate is often hypercellular.

Criteria that exclude this diagnosis are monocytosis ($>1 \times 10^9$/l), which indicates a diagnosis of CMML, and the presence of Auer rods, which leads to the automatic inclusion of the case in the RAEB-2 category. The presence of 15% or more ring sideroblasts should be noted, but does not alter the classification since there has not yet been any evidence of this feature indicating a different prognosis (see Report 3.3).The severity of cytopenia and dysplasia are indicative of varying prognosis and correlate with survival. Cytogenetic abnormalities including complex karyotypes are present in about 50% of cases; the prognosis is worse than that of RCUD, with a median survival of about 30 months and a frequency of leukaemic transformation within two years of about 10%.

## Refractory anaemia with excess of blasts

RAEB is the most frequent type of MDS, corresponding to about 40% of newly diagnosed cases, characterised by an increase in blast cells that is insufficient for a diagnosis of AML. To make this diagnosis, the total granular and agranular blast cells must be between 2% and 19% in the peripheral blood and between 5% and 19% in the bone marrow.

The full blood count usually shows normocytic or macrocytic anaemia, with anisopoikilocytosis and an increased RDW. Often there is an associated neutropenia, thrombocytopenia or

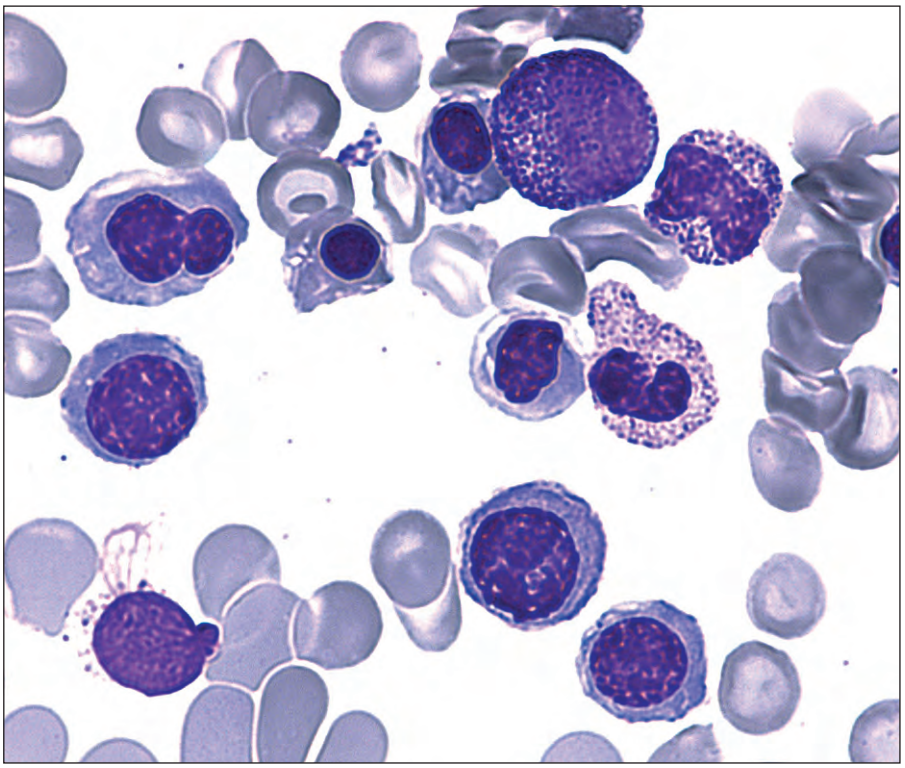


**Fig. 3.118** RCMD; bone marrow aspirate from another patient. There are features of dyserythropoiesis with N:C asynchrony, nuclear irregularity and pale spaces in the cytoplasm. Cells of the granulocytic series are very heavily granulated, but these three cells are not dysplastic.

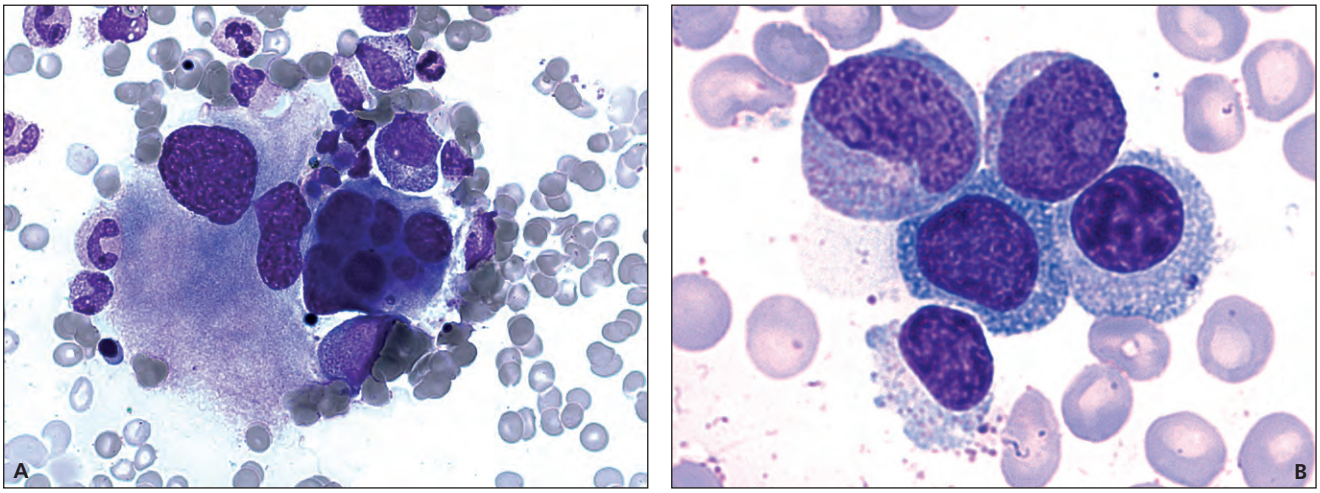



**Fig. 3.119** Two examples of RCMD; bone marrow aspirates. (A) The megakaryocyte on the left has a bilobed nucleus with spongy chromatin and basophilic cytoplasm with granular violet flecks. The megakaryocyte on the right is small and intensely basophilic and has a number of nuclear lobes, partly separated from each other. At the bottom, a dysplastic promyelocyte. The three orthochromatic erythroblasts are also dysmorphic. (B) From the left, clockwise: a granulocyte precursor with the nucleus of a myelocyte but the cytoplasm of a promyelocyte, with disorderly distribution of granules; a granular blast cell; two very dyserythropoietic erythroblasts with vacuolated cytoplasm; a micromegakaryocyte. From the same patient as Fig. 3.47.

both. The circulating neutrophils show reduced granulation and nuclear anomalies in many cases. The platelets also can be dysmorphic, with gigantism and an increase or reduction in the granulomere. Circulating blast cells can be granular, agranular or both.

On bone marrow films, cellularity is usually increased. The dysplasia can be in two or, more often, three lineages and can show all the anomalies of maturation that have already been described. There is almost always present a significant proportion of differentiated granulocytic precursors, which are often

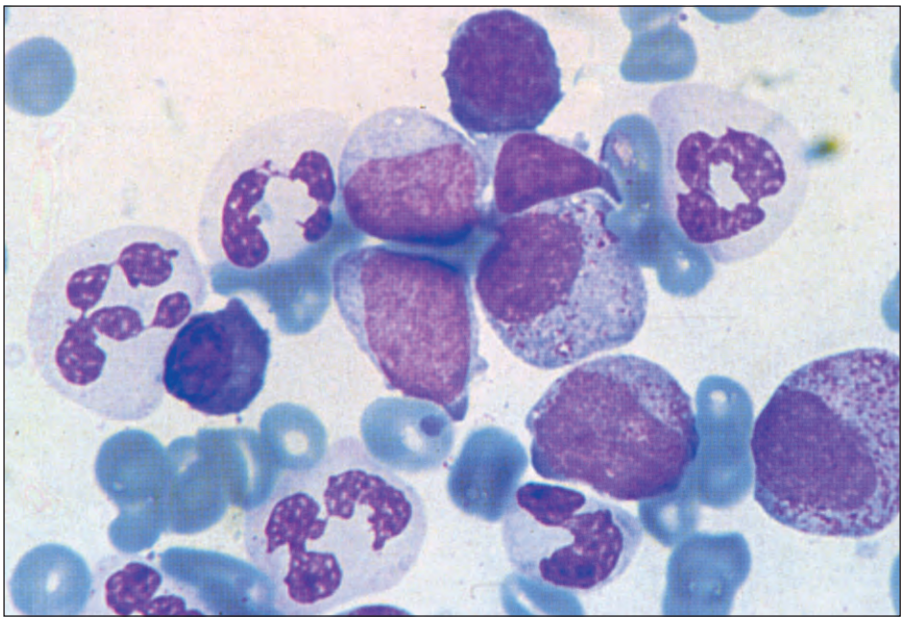


**Fig. 3.120** RAEB-1; bone marrow aspirate. In the centre, two agranular blasts with a high N:C ratio; the nuclei have open, pale, reticular chromatin with a visible nucleolus, particularly in the upper cell; the cytoplasm is basophilic, sky-blue and not uniform. On the right, three immature granulocytes with basophilic hypogranular cytoplasm, disorderly distribution of granules and little evidence of a Golgi zone. On the basis of the nuclear characteristics, the cell on the left is a myelocyte, while the other two are dysplastic promyelocytes. The five mature neutrophils are very dysplastic both because of the total absence of granules and because of the glassy appearance of the chromatin, which is forming irregular and incomplete lobes with appendages of various shapes. At the top, a basophilic erythroblast and on the left a small plasma cell.

morphologically abnormal. Megakaryocytes are usually present in good numbers, but are markedly abnormal and tend, not rarely, to form small groups adjacent to fragments. Blasts may have cytoplasmic granules or Auer rods, or may appear undifferentiated, but in general they are peroxidase positive. Immunophenotyping usually shows positivity for CD34, CD117, CD38, HLA-DR and granulocyte differentiation markers; it is not uncommon to have anomalous expression of other markers, such as CD7 or CD56.

It is in RAEB that abnormal localisation of haemopoietic cells is most readily detected on histological examination, with erythroblasts and megakaryocytes situated close to the paratrabecular area, where normally granulocyte precursors are found. Histological sections are also better for detecting blast cells (using CD34) in the uncommon form of RAEB with reduced cellularity; in cases associated with fibrosis (for which the abbreviation RAEB-F has been suggested), megakaryocytes are often increased, being particularly numerous and dysplastic.

Clonal cytogenetic abnormalities with or without a complex karyotype are seen in more than 50% of patients.

Two subtypes of RAEB are recognised, based on the number of blast cells; these differ in their prognosis and median survival:

- RAEB-1, in which the percentage of blasts is between 2% and 4% in the peripheral blood or between 5% and 9% in the bone marrow; the median survival in these patients is less than two years (Figs 3.120 and 3.121) (see Report 3.4);
- RAEB-2, in which the percentage of blast cells is between 5% and 19% in the peripheral blood or between 10% and 19% in the bone marrow; the median survival is less than a year (Figs 3.122, 3.123, 3.124 and to 3.125) (see Report 3.5).

The presence of blast cells containing Auer rods leads to a diagnosis of RAEB-2, regardless of the number of blasts, and when these are seen, the survival is less than 12 months.[98]

In cases in which the erythroid component is more that 50% of cells, it is necessary to quantify the blasts as a percentage of NEC in order to have an accurate differential diagnosis.

The percentage of bone marrow blasts at diagnosis correlates with prognosis, which is often poor with median survivals of 16 and 9 months respectively in RAEB-1 and RAEB-2. The duration of the illness is less than three months, and thus is not dissimilar to that of AML in patients in whom the blast count in the peripheral blood is 5% or higher.[99] The most frequent causes of death in RAEB are infection and haemorrhage (50% of patients) and evolution to acute leukaemia (30%) (see Report 3.6).

### Myelodysplastic syndrome with isolated del(5q)

Described for the first time in 1974, this subtype represents an unusual form of MDS with an indolent clinical course, distinguished from other forms of MDS by certain quite typical aspects that correlate the morphology with the genotype:

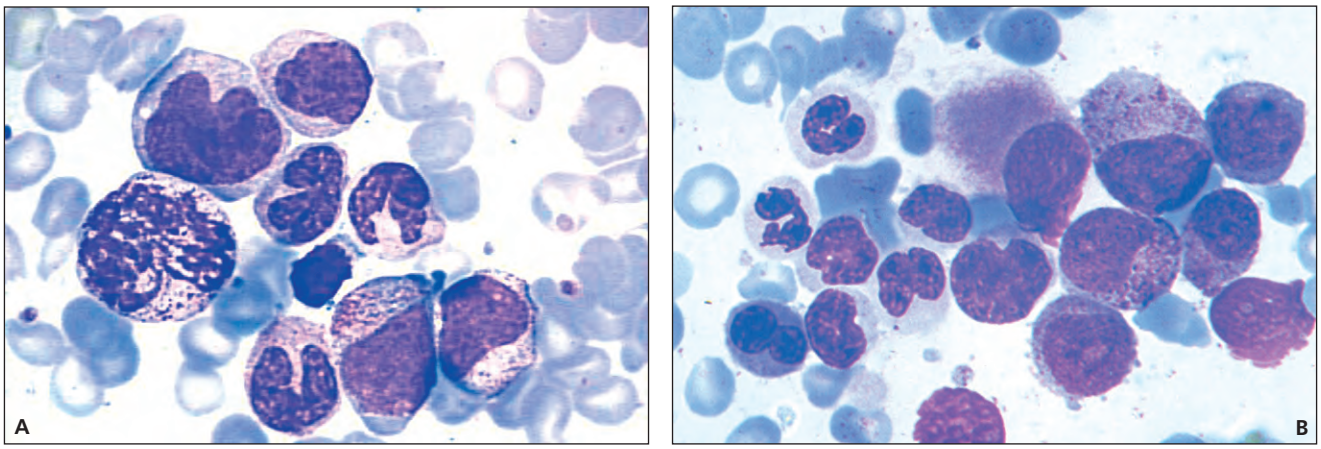



**Fig. 3.121** RAEB-1; bone marrow aspirates from two different patients. (A) At the bottom, a granular blast cell (defined as such because of the nuclear morphology with an angulated outline and delicate homogeneous chromatin), a metamyelocyte and a dysplastic hypogranular myelocyte. On the left, a frozen mitotic figure with highly atypical morphology. The other four cells, above a lymphocyte, are probably granulocyte precursors with very atypical, lobulated and deformed nuclei. One cannot exclude that the three at the top belong to the monocyte lineage: such uncertainty is not uncommon in the presence of dysplastic cells with very atypical nuclei and granules. (B) Clear signs of trilineage dysplasia. Bottom left, a binucleated erythroblast; on the left, numerous agranular neutrophils and metamyelocytes. Centre top, a micromegakaryocyte and, on the right, some immature cells that according to the criteria currently in use should not be included in the count of granular blast cells, but rather should be considered dysplastic promyelocytes.

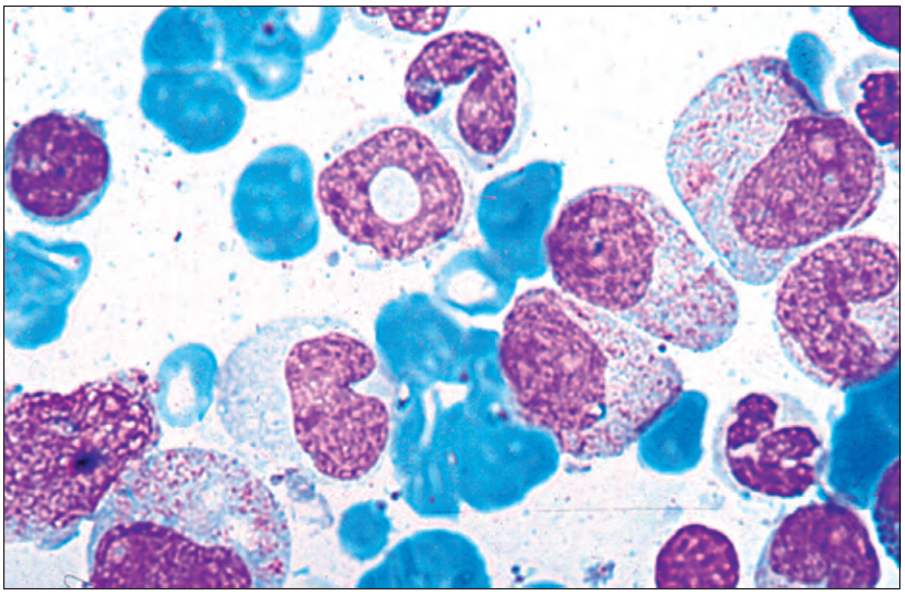


**Fig. 3.122** RAEB-2; bone marrow aspirate. The dysplasia in cells of granulocyte lineage is severe, with hypogranularity, hyposegmentation and abnormal chromatin condensation in the mature cells, one of which has a ring nucleus. The three immature cells have eccentric nuclei with moderately condensed chromatin, nucleoli, abundant basophilic cytoplasm with numerous azurophilic granules scattered in a disorderly manner, and the mere suggestion of a Golgi zone. These morphological features place the cells on the limits between granular blast cells and dysplastic promyelocytes. The decision, difficult and very relevant to determining the prognosis and the therapeutic approach, must be taken case by case, considering also the clinical and haematological context. In this case, the cell that should be included in the blast count is that at top right.

1. Severe macrocytic anaemia;
2. Normal or increased platelet count with increased megakaryocytes, which are small to medium sized with a non-lobated nucleus;
3. A specific cytogenetic anomaly, an interstitial deletion of the long arm of chromosome 5, occurring as an isolated anomaly.[100,101]

The genetic lesion responsible is in a gene that encodes a ribosomal protein in multipotent haemopoietic stem cells. Essential negative diagnostic criteria (to exclude a diagnosis of RAEB) are:

- Peripheral blood blast cells less than 1%;
- Bone marrow blast cells less than 5%;
- Absence of Auer rods.

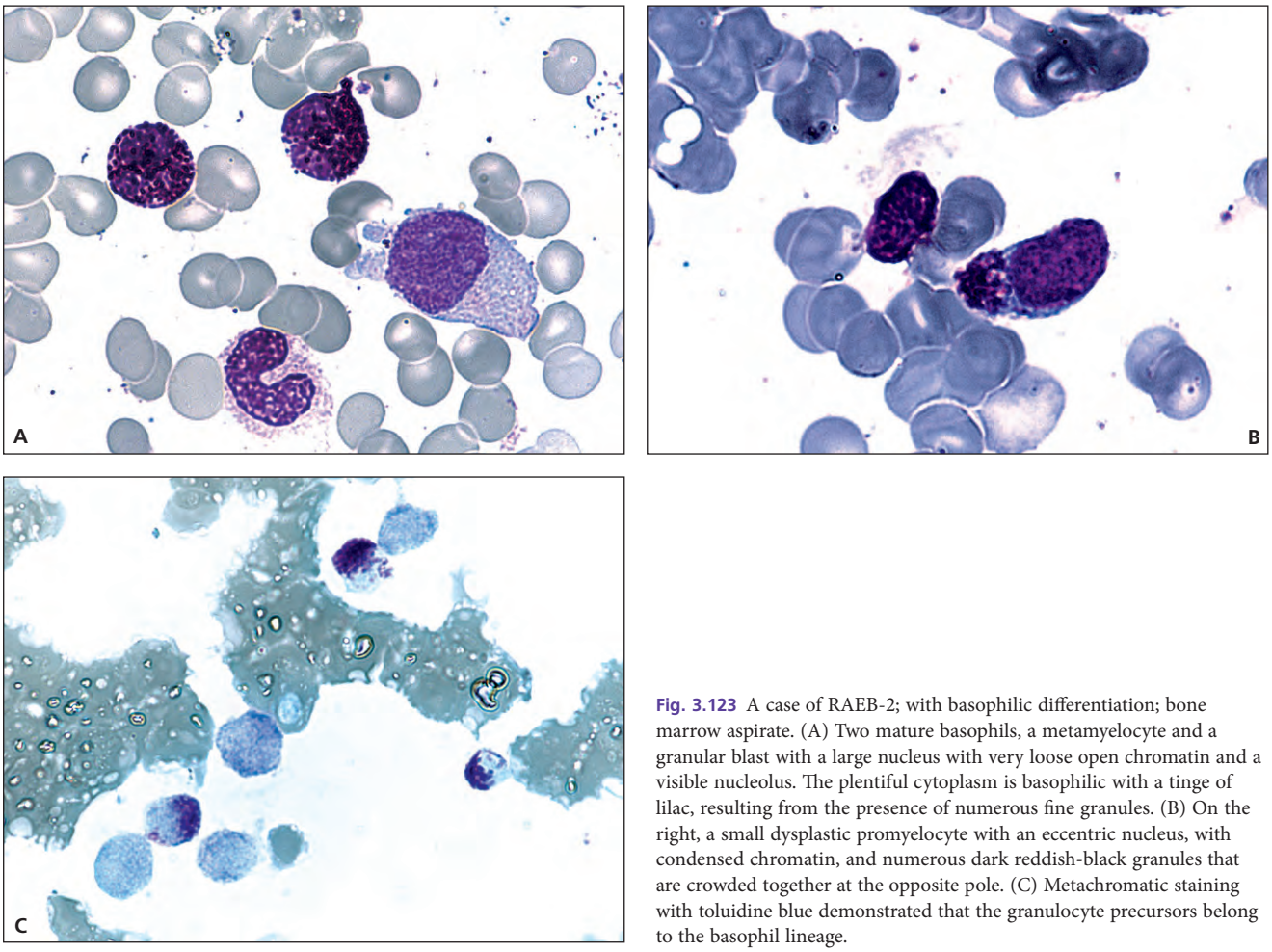



**Fig. 3.123** A case of RAEB-2; with basophilic differentiation; bone marrow aspirate. (A) Two mature basophils, a metamyelocyte and a granular blast with a large nucleus with very loose open chromatin and a visible nucleolus. The plentiful cytoplasm is basophilic with a tinge of lilac, resulting from the presence of numerous fine granules. (B) On the right, a small dysplastic promyelocyte with an eccentric nucleus, with condensed chromatin, and numerous dark reddish-black granules that are crowded together at the opposite pole. (C) Metachromatic staining with toluidine blue demonstrated that the granulocyte precursors belong to the basophil lineage.

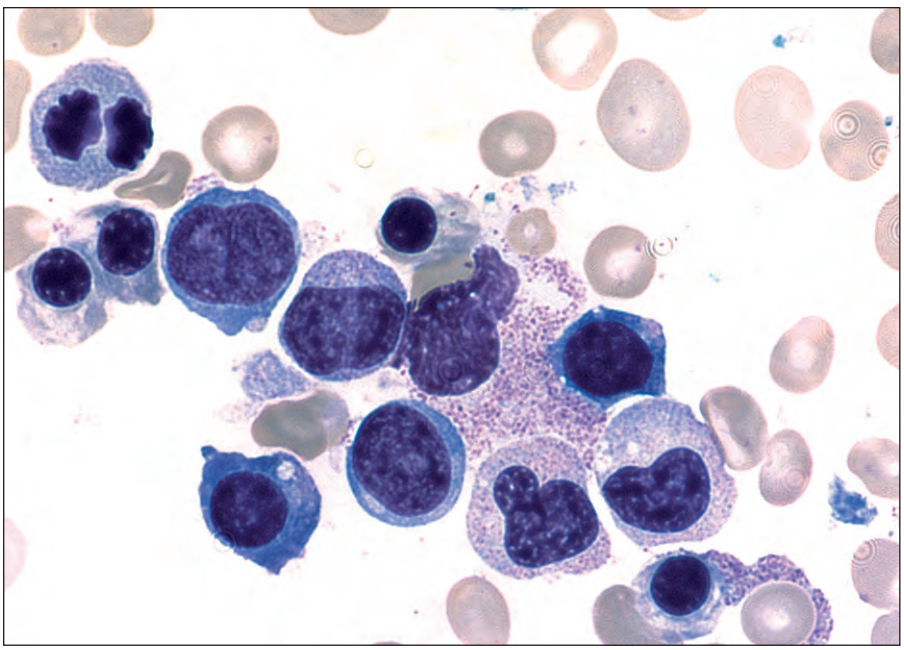


**Fig. 3.124** RAEB-2; bone marrow aspirate. Two agranular blast cells, one of which has a hyperdiploid nucleus separated into two lobes by a deep cleft; two myelocytes (one of which has torn cytoplasm); two hypogranular neutrophil metamyelocytes; a basophilic erythroblast; four polychromatic erythroblasts and a mitotic figure in an erythroid cell, all of these providing evidence of dyserythropoiesis; a plasma cell.

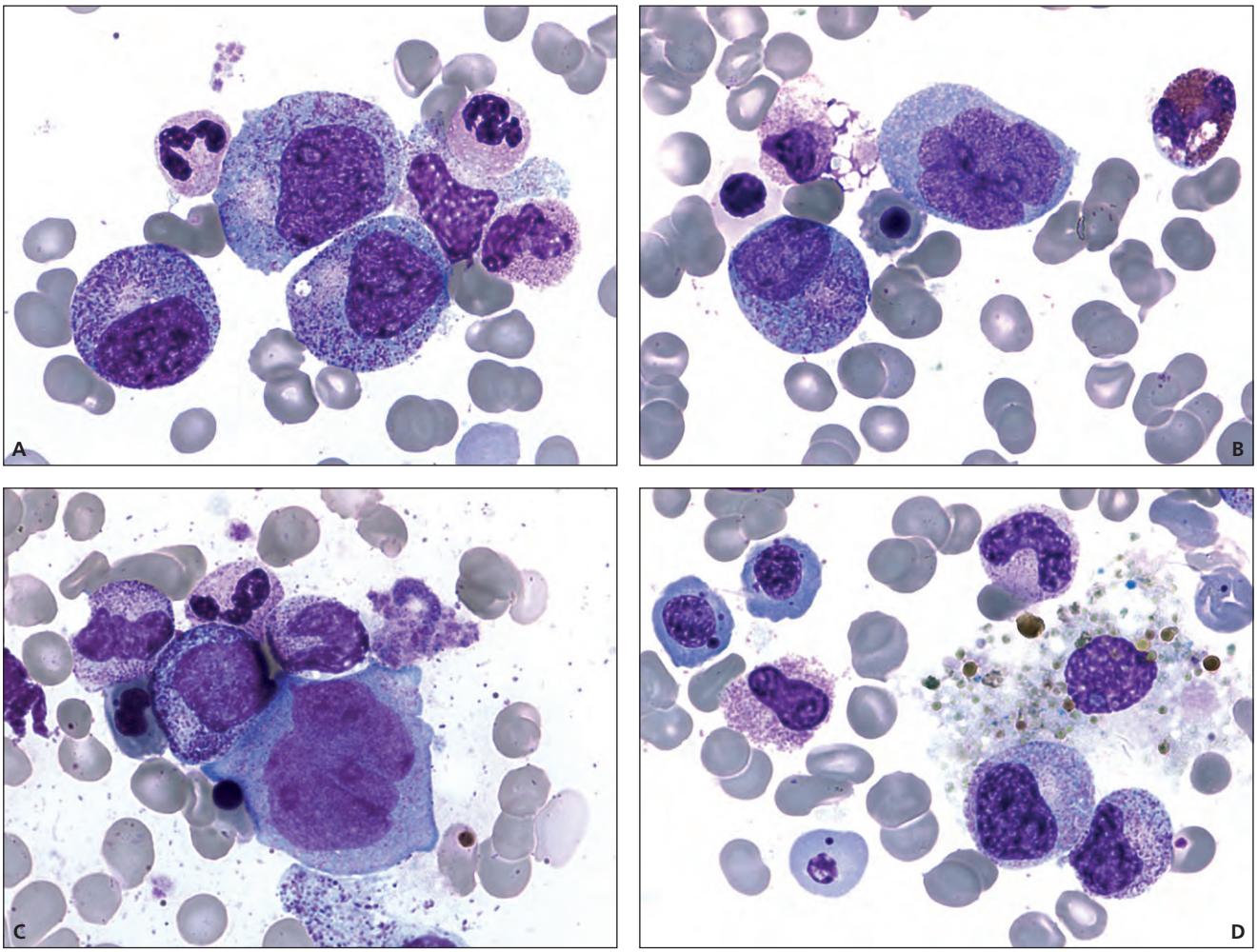



**Fig. 3.125** RAEB-2 in a patient who had previously received cytotoxic chemotherapy for Hodgkin lymphoma (therapy-related MDS; see Chapter 4). (A) Dysgranulopoiesis. In the centre, two giant promyelocytes with prominence of the nucleoli, the Golgi zone and the granules, within azure cytoplasm. Bottom left, a hypergranular myelocyte with still basophilic background cytoplasm. At the top two sides, two non-segmented neutrophils with hypogranular cytoplasm. This absence of maturational harmony (hypergranular precursors, hypogranular neutrophils) is a sign of dysplasia. (B) From the left, another quite granular promyelocyte; an orthochromatic erythroblast; a large blast with some fine granules within the plentiful azure cytoplasm, and a voluminous multilobated, hyperdiploid nucleus with loose, reticular, expanded chromatin; a vacuolated eosinophil. (C) A blast cell similar to (B), but with more granules (the percentage of blast cells in a 500-cell count was 11%). The surrounding cells show dyserythropoietic and dysgranulopoietic features; top right, a strand of megakaryocyte cytoplasm composed of dysmorphic platelets. (D) Dyserythropoiesis. On the left, two polychromatic erythroblasts with N:C asynchrony, Howell-Jolly bodies still partly joined to the nucleus and an internuclear bridge. On the right, a macrophage with heterogeneous yellowish granules.

Typical features that are apparent in the full blood count are: anaemia that is usually macrocytic and fairly marked; the leucocytes do not show any specific anomalies, while the number of platelets is normal in half of cases, with frank thrombocytosis in the other half (see Report 3.7). Rarely, neutropenia or thrombocytopenia is present at diagnosis.

In the bone marrow, cellularity is normal in 40% of cases, increased in 40% and reduced in 20%. The erythroid series is not hyperplastic, tending to be rather less well represented than in refractory anaemia, with less than 20% of nucleated cells being erythroblasts in half of patients. Dyserythropoiesis, defined by the restrictive criteria of the WHO, is observed in only 15%.[102] Granulocytic dysplasia is moderate or totally lacking: its presence, however, correlates with a worse prognosis.[103,104] The characteristic bone marrow feature is the presence of megakaryocytes that are slightly reduced in size with an eccentric nucleus that is hypolobated or non-lobated (Figs 3.95 through to 3.98 and 3.126); megakaryocyte numbers are usually increased.

This form of MDS is more common in women with a median age of 65 years and is associated with a clinical course that is particularly favourable: the median survival is 145 months and the risk of leukaemic transformation is less than 10%. The favourable prognosis, however, is restricted to those patients

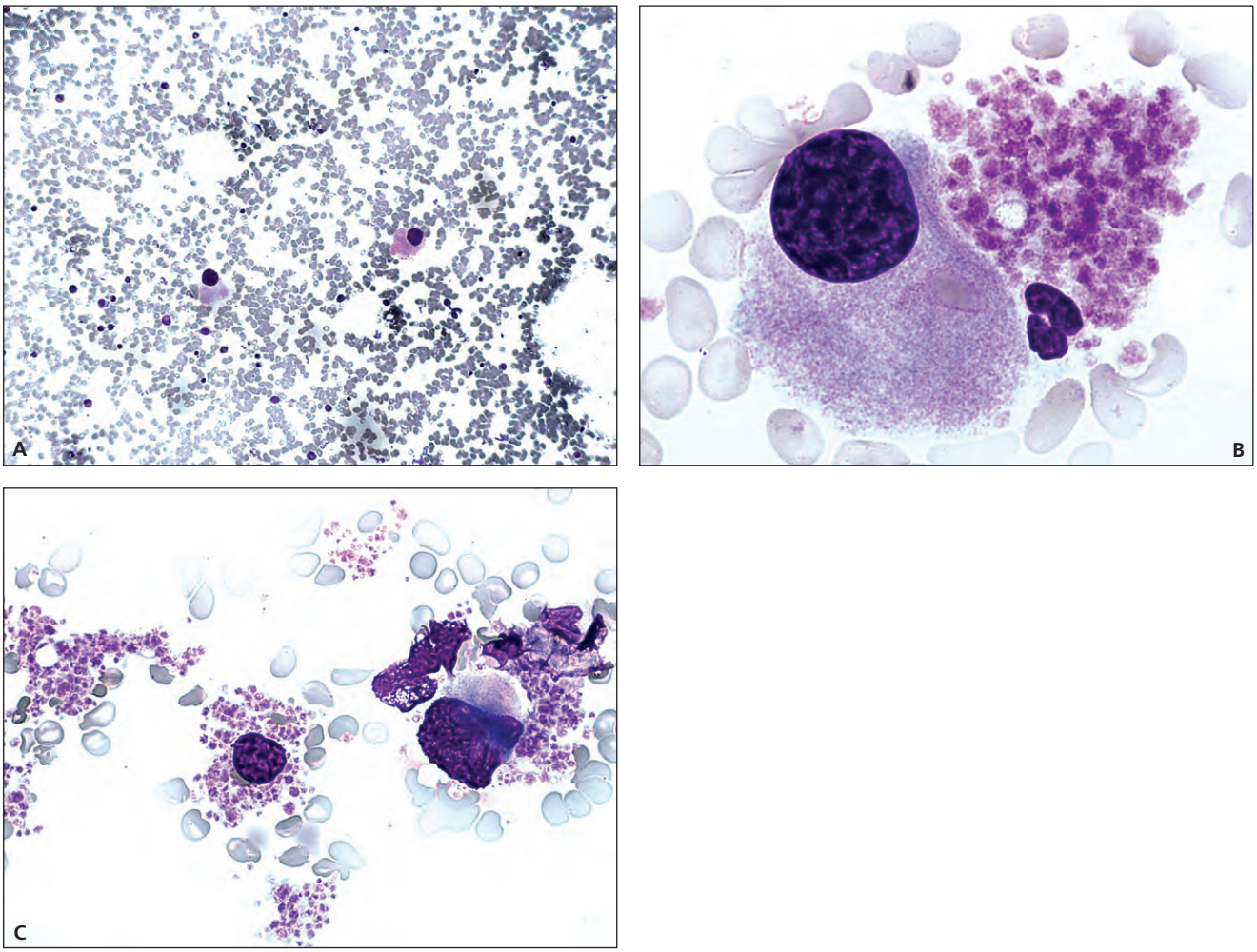



**Fig. 3.126** Morphology of megakaryocytes in MDS with del(5q) as the sole cytogenetic anomaly. (A) At low power, two megakaryocytes of intermediate size, with non-lobated nuclei and mature cytoplasm in a film showing reduced cellularity. (B) Typical non-lobated megakaryocyte, with marked and heterogeneous chromatin condensation; the nucleus is eccentric within mature cytoplasm which contains numerous azurophilic granules that in some areas are more dense. There has also been emperipolesis of an erythrocyte. There is a large mass of hyperchromatic, fairly large platelets. (C) A number of masses of platelets that are still joined. In the centre, the nucleus of a non-lobated megakaryocyte with cytoplasm that has been completely transformed into platelets and that has enveloped an intact erythrocyte. On the right, a non-segmented nucleus of a larger megakaryocyte, with some granular cytoplasm clearly seen.

who meet the strictly defined WHO criteria: the presence of other cytogenetic abnormalities in addition to del(5q) or assignment to the WHO RAEB category, because of the presence of blast cells, indicates a much less indolent clinical course.[104,105]

Another favourable feature of this category of MDS is the notable sensitivity to lenalidomide or thalidomide,[106] which are able to suppress the dysplastic clone and to abolish the transfusion dependence in a third of patients. A problem in differential diagnosis occurs in cases in which there is a concomitant *JAK2* V617F mutation[107]: in the absence of other diagnostic criteria in these patients, they are currently included in this MDS category, so that the possibility of a potentially efficacious specific treatment is available to them.

## Myelodysplastic syndrome, unclassifiable

Cases are assigned to this category when, at diagnosis, there are no quantitative or qualitative morphological characteristics sufficient for inclusion in any of the preceding subtypes of MDS. As a consequence, there are no positive diagnostic morphological criteria.

The patients require close monitoring to establish the clinical course and follow disease evolution in real time.

More specifically, patients are included in this category:

- With criteria otherwise diagnostic of RCUD or RCMD, but when on at least two occasions there has been recognition and confirmation of 1% of blast cells in the peripheral blood;
- With pancytopenia associated with unilineage dysplasia;

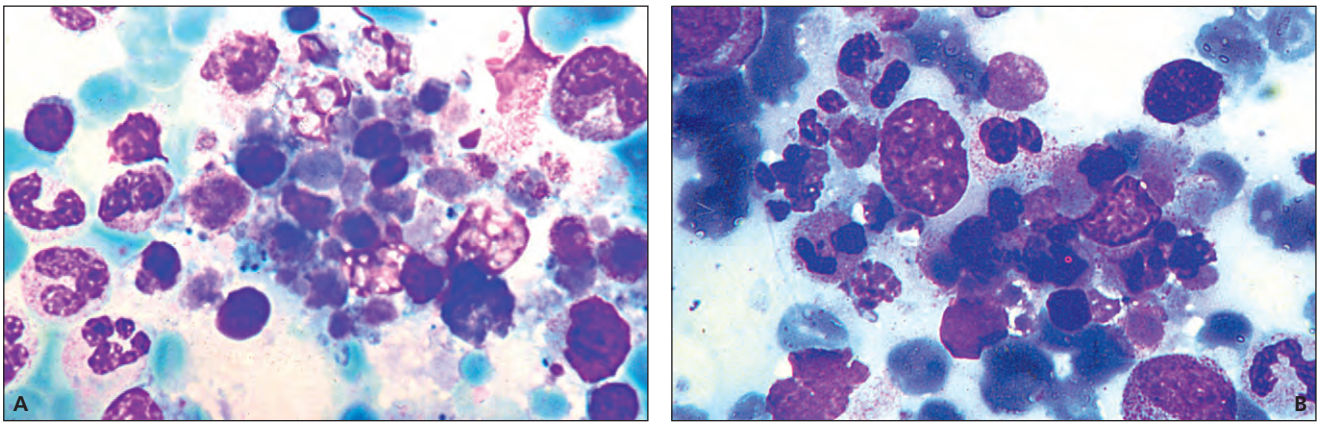



**Fig. 3.127** Necrosis and bone marrow degeneration with haemophagocytosis reflecting ineffective haemopoiesis; bone marrow aspirates from patients with MDS. This phenomenon is part of the stromal reaction to ineffective haemopoiesis, in which there is a high rate of intramedullary death; it is also more frequently seen in MDS associated with myelofibrosis, reduced cellularity or both. (A) From a patient with MDS with fibrosis (MDS-F). In this marrow there were numerous macrophages laden with cellular debris. Here the mass of necrotic material is composed mainly of pyknotic nuclei and erythrocytes. (B) From a patient with refractory anaemia. Numerous degenerating neutrophils, some erythroblast nuclei and probably some platelets have been engulfed; the almost invisible macrophage cytoplasm surrounds the nucleus with its spongy cytoplasm.

- When the quantitative and qualitative criteria for dysplasia are not met, but there are cytogenetic abnormalities that provide presumptive evidence of MDS.[62]

### Refractory cytopenia of childhood

This provisional category was included in the 2008 WHO classification; it represents around 50% of MDS in children, without any gender difference. The diagnostic criteria are:

- Peripheral blood showing persistent cytopenia with circulating blasts less than 2% and sometimes neutrophil dysplasia;
- Bone marrow blasts less than 5%;
- Dysplasia recognised in at least two cell lines.

In the peripheral blood there is macrocytosis with anisopoikilocytosis and anisochromasia. There may be giant platelets and dysplastic neutrophils. The bone marrow aspirate is often hypocellular, making evaluation of dysplasia in the various lineages more difficult; dysplasia is, however, marked. Megakaryocytes are reduced in number; typically, there is an abundance of micromegakaryocytes. Blasts are less than 5%.[108]

The differential diagnosis is with secondary cytopenia (infection, autoimmune disorders, and vitamin deficiency), aplastic anaemia, paroxysmal nocturnal haemoglobinuria (PNH) and Pearson syndrome (due to deletion of mitochondrial DNA). The most frequent cytogenetic abnormality is monosomy 7, which conveys a worse prognosis.[109]

## Primary bone marrow disorders that are not WHO 2008 categories

### Myelodysplastic syndrome with fibrosis

The presence of bone marrow fibrosis at diagnosis of MDS is found in a proportion of patients varying from 10% to 15%. It is manifest as pancytopenia due to bone marrow failure in the absence of organomegaly.[110–114] The identification of fibrosis requires examination of bone marrow trephine biopsy sections, appropriately stained for reticulin by the silver impregnation method of Gomori. With grading of fibrosis from 0 to 3 (absent, mild, moderate, severe), the presence of grade 2 or 3 indicates bone marrow fibrosis.

There is normocytic, normochromic anaemia with the presence of red cell fragments and dacrocytes, associated with reticulocytopenia. Neutropenia and thrombocytopenia are consistently present. There may be some circulating blast cells together with erythroblasts and granulocyte precursors. Attempted bone marrow aspiration usually leads to a 'dry tap' or provides hypocellular material showing necrosis and degeneration (Fig. 3.127). Histological examination documents the presence of:

- Megakaryocytic dysplasia with numerous atypical and dysplastic forms;
- Small clusters of blast cells (ALIP), identifiable with immunohistochemical techniques (CD34, CD61, CD117), found in an intertrabecular position, distant from the endosteum and vascular niches;
- Evident dysplasia in other haemopoietic cells (ring sideroblasts or other dyserythropoiesis, dysgranulopoiesis);
- Intense myelofibrosis with bone remodelling and focal deposits of collagen.

The majority of cases of MDS-F fall into the RAEB category: it is in fact rare to have myelofibrosis in the lower risk categories. The 2008 WHO classification does not have a separate category for MDS-F, but suggests that the presence of fibrosis at diagnosis should be noted because of its negative prognostic significance.

The differential diagnosis includes therapy-related MDS (which is often characterised by bone marrow fibrosis), acute

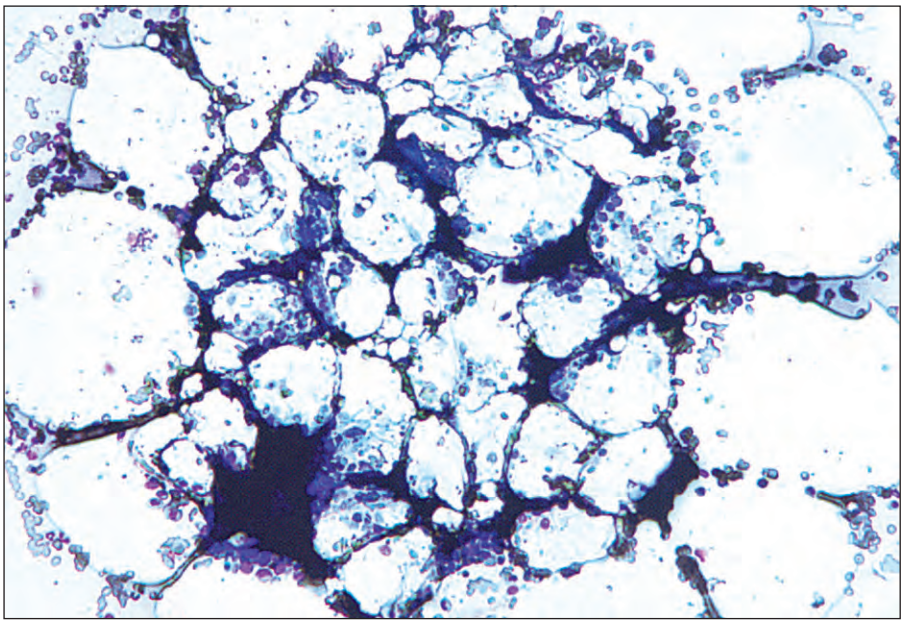


**Fig. 3.128** Hypoplastic myelodysplastic syndrome (MDS-H); bone marrow aspirate. In this patient with pancytopenia, the aspirate has yielded a number of clearly hypocellular fragments. There are small clusters containing a few haemopoietic cells within a structure that is mainly adipose cells and other stromal elements.

panmyelosis with fibrosis, acute megakaryoblastic leukaemia, MPN associated with fibrosis (above all primary myelofibrosis), MDS/MPN associated with fibrosis and non-myeloid neoplasms such as hairy cell leukaemia and bone marrow metastases of solid tumours.[80]

## Hypoplastic myelodysplastic syndromes

The bone marrow is hypocellular in 10% of adult cases of MDS (with a higher frequency in women) and in 70% of childhood cases (Figs 3.128 and 3.129). Histological examination is fundamental in these cases, since the material obtained on aspiration is diluted with peripheral blood and the blast percentage may thus be underestimated. Morphological criteria for hypocellularity are a reduction of cellularity below 30% (in histological sections) in patients under the age of 60 years and a reduction to less than 20% cellularity at a more advanced age. Careful attention must be paid to the differential diagnosis of hypocellular MDS and MDS with fibrosis with aplastic anaemia, hypocellular AML and toxic and autoimmune myelopathy; this is of crucial importance because of the therapeutic and prognostic implications.[115] The differential diagnosis with aplastic anaemia is often difficult and sometimes impossible, because there are no clear features on which a distinction can be based:

1. Some patients with aplastic anaemia have cytogenetic abnormalities of the same types as are seen in MDS;
2. Some patients with aplastic anaemia with a normal karyotype show evolution into MDS;
3. Small PNH clones can be recognised in both conditions.

In general, the presence of ring sideroblasts, ALIP and alterations of chromosomes 5 and 7 orient the diagnosis towards hypoplastic myelodysplastic syndromes (MDS-H). The literature does not ascribe a clear prognostic significance to hypoplasia in MDS, although these patients do show a fairly favourable prognosis.[116] It is, however, very relevant to identify these cases at diagnosis because of the implications for choice of treatment: in fact, some patients respond to immunosuppressive treatment, while certain cytotoxic drugs, particularly azacitidine, may not be indicated.

## Idiopathic cytopenia of undetermined significance

The IWGM-MDS in 2005 grouped together under this designation, patients with cytopenia with no identifiable cause, persisting for more than six months, affecting one or more haemopoietic lineages and with insufficient morphological evidence of dysplasia to establish a diagnosis of MDS.[117,118] The diagnosis of ICUS is not an entity included in the 2008 WHO classification of MDS, since cases do not necessarily represent an early stage of MDS; it is necessary to exclude a large number of possible causes of cytopenia, e.g. excluding by means of the clinical history, the use of various medications and possible exposure to toxic substances (Fig. 3.130). It is also necessary to explore fully, by means of the clinical history and laboratory investigations, the possibility of nutritional deficiency, chronic infection and chronic inflammatory states.

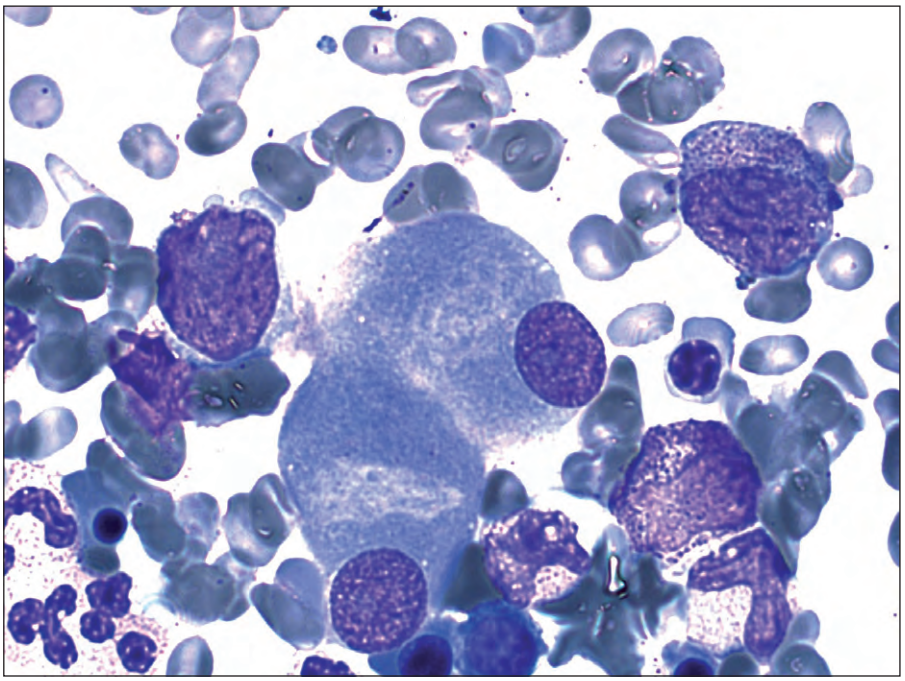


**Fig. 3.129** MDS-H; bone marrow aspirate. In this aspirate from another patient with reduced bone marrow cellularity, the stromal elements of the marrow and bone are present in increased proportions and are readily seen; these are two osteoblasts, characterised by their nuclei with spongy chromatin with nucleoli, similar to the nucleus of a macrophage, and by their plentiful cerulean cytoplasm, which contains a hypertrophied Golgi zone that is distant from the nucleus, a feature that has not occurred in any other cell type yet seen. On the left, the bare nucleus of a non-segmented megakaryocyte; on the right, a promyelocyte at the top and other granulocytes at the bottom, all recognisable as dysplastic.

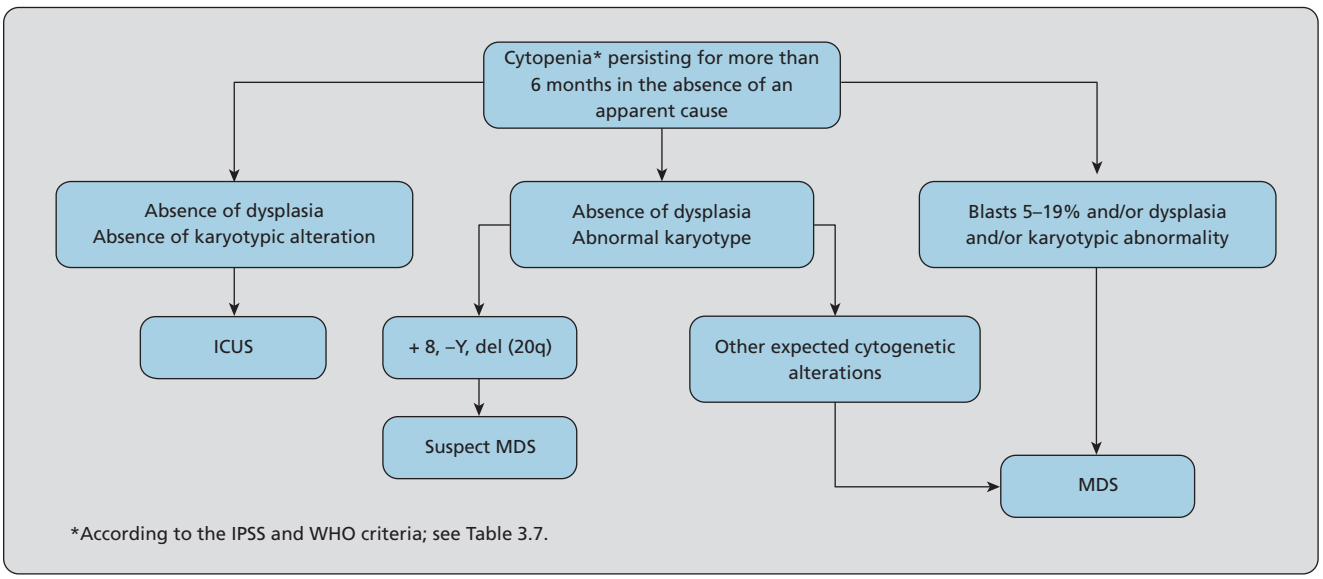



**Fig. 3.130** Diagnostic algorithm for MDS versus idiopathic cytopenia of undetermined significance (ICUS).[117]

Evaluation of the peripheral blood, the bone marrow aspirate and trephine biopsy sections, together with immunohistochemistry, flow cytometric immunophenotyping, cytogenetic analysis and molecular genetic analysis yields results that are not sufficient to establish a diagnosis of MDS.[119] The presence of the cytogenetic abnormalities del(20q), trisomy 8 and –Y in the absence of dysplasia are not sufficient to establish an unequivocal diagnosis of MDS, nor of ICUS: however, these patients must be followed carefully because of a suspicion of MDS. In the literature, ICUS appears to be rare: generally, patients are elderly

with a low serum erythropoietin; sometimes fluorescence *in situ* hybridisation (FISH) reveals the presence of a small clone. Around 60% of cases of ICUS evolve into MDS in between one and ten years.

### Idiopathic dysplasia of undetermined significance

The condition provisionally identified as IDUS is the reverse of ICUS[34]: it is characterised by the presence of morphological dysplasia with or without an abnormal karyotype in the absence of peripheral blood cytopenia. The criteria for a diagnosis of MDS are not met. The diagnosis is difficult and requires the exclusion of all the potential causes of dysplasia of haemopoietic cells. The cases of IDUS reported in the literature are even less frequent than those of ICUS: in general, these are young patients in whom an abnormality, often a pseudo-Pelger type of nuclear alteration or unexplained macrocytosis, is detected during routine blood tests.[117]

## Non-clonal myelodyplasia in HIV infection

Lymphopenia, resulting from the specific loss of CD4-positive lymphocytes, is the most characteristic haematological feature of infection by the human immunodeficiency virus (HIV). The development of cytopenia affecting other lineages is not, however, a rare event during the course of this infection.[120] The pathogenetic mechanisms that are responsible for this occurrence are complex and multiplex, including direct infection by the virus of monocytes-macrophages and bone marrow fibroblasts, alterations in the production of cytokines that have an adverse effect on haemopoiesis, the peripheral destruction of cells by antibodies or immune complexes and the actions of various drugs.[121] Direct infection of CD34-positive stem cells by the virus has been shown to occur, but appears to be rare.[122,123] In addition to the quantitative defects, morphological alterations are also recognised, particularly during the progressive phase of the infection; these are dysplastic features affecting precursor cells of all three haematological lineages as well as the mature cells in the peripheral blood.[124] These alterations are associated with a low blast cell count and do not show evolution to AML.[125] Recognition of bone marrow fibrosis is common.[126]

Nowadays, thanks to early recognition and effective antiretroviral treatment, it is rare in western countries to recognise the characteristic haematological features of the advanced phase of the disease. In many cases it is not possible to make a distinction between the underlying pathological condition, the effects of opportunistic infections and, particularly, the effects of the numerous drugs being used, which are often related to cytotoxic drugs and can cause dysplasia. Zidovudine, in particular, can cause alterations in haemopoiesis, which becomes megaloblastic, with marked macrocytosis.

Anaemia and thrombocytopenia can be associated with anisopoikilocytosis, basophilic stippling and Howell-Jolly bodies, and can be caused by ineffective haemopoiesis resulting from immune disturbances. Leucopenia, the frequency of which increases with disease progression, is the result not only of lymphopenia but also of neutropenia. Neutropenia, observed in advanced cases, is nowadays less frequently observed in developed countries, and can be accompanied by characteristic morphological alterations in circulating neutrophils[127] (Figs 3.131 and 3.132). These neutrophils are larger than normal with

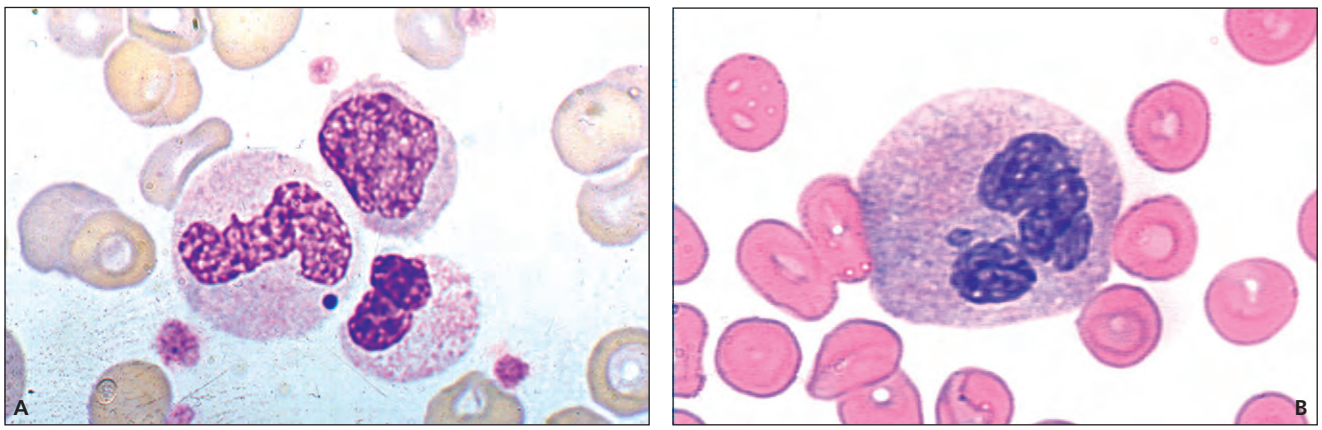



**Fig. 3.131** Non-clonal dysplasia in neutrophils in HIV infection; peripheral blood of two male patients with AIDS. (A) On the left, a giant neutrophil with hypogranular cytoplasm containing a pseudo-Howell-Jolly body; the nucleus is hyposegmented, band shaped, with glassy looking chromatin with numerous pale spaces. Inside the cytoplasmic membrane, at 8 o'clock, is a small basophilic structure corresponding to a Döhle body. The cell higher up has similar features. The cell bottom right is a bilobed eosinophil with scanty fine granules, which appear more pink than orange because of the background cytoplasmic basophilia. The platelets are large and hyperchromatic, without hyalomeres. (B) Giant neutrophil with very dysmorphic nuclear segmentation. From the lower lobe there extends a pedunculated structure of pseudo-drumstick type, the morphology of which illustrates a possible mode of formation of the pseudo-Howell-Jolly bodies. The cytoplasm is very plentiful, alternating pink and blue, with very fine granules.

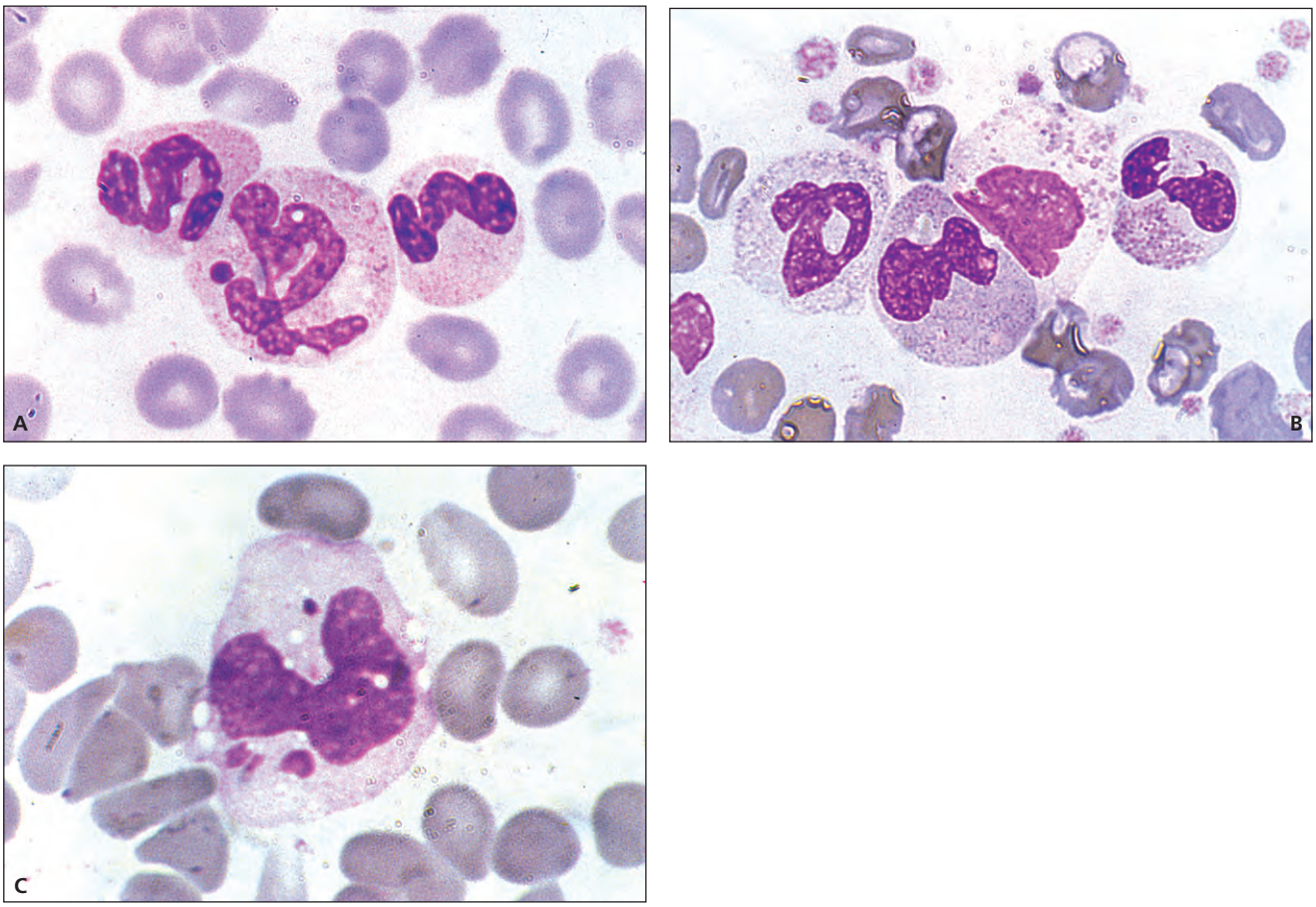


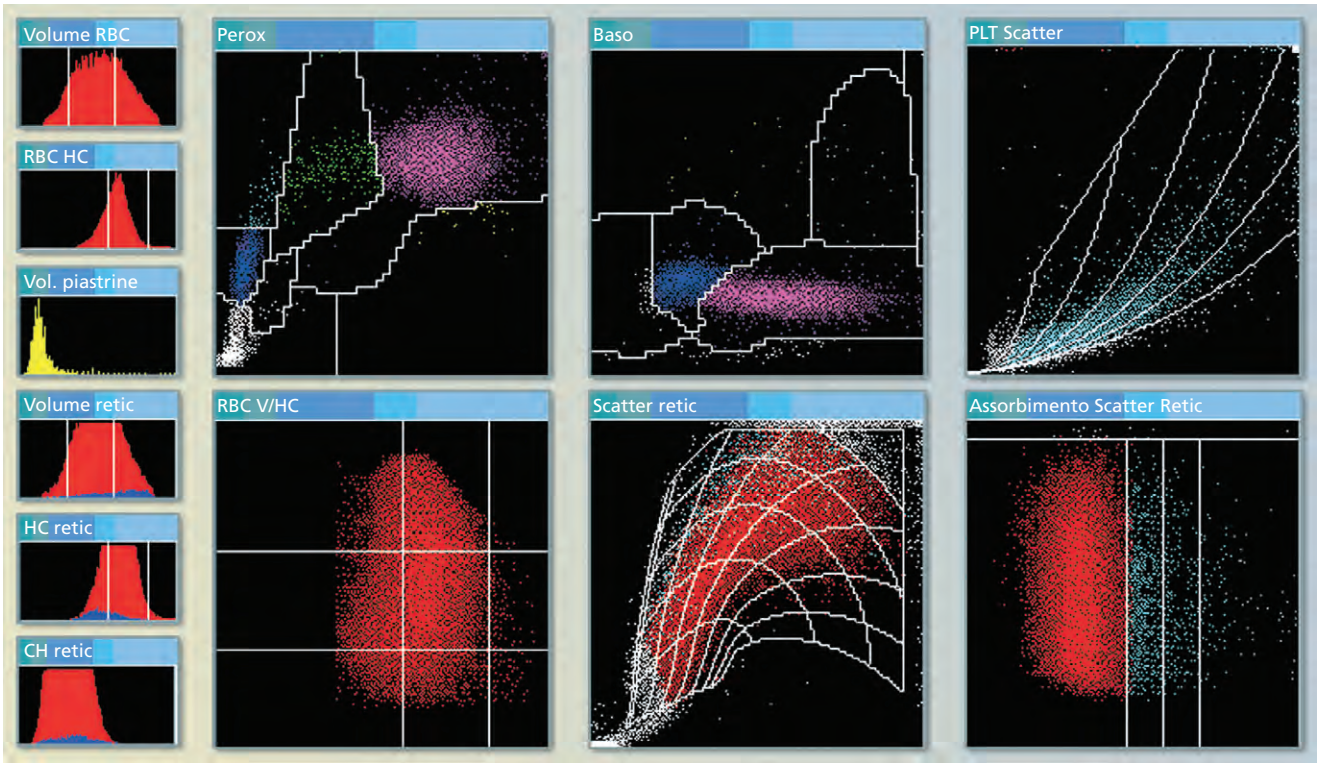

**Fig. 3.132** Non-clonal dysplasia in neutrophils in HIV infection; peripheral blood of three male patients with AIDS. (A) At the two sides, neutrophils of intermediate size with nuclei formed of a long snake of chromatin of normal structure, which is folded on itself without segmentation into lobes. The giant neutrophil in the centre has paler chromatin and vacuolated cytoplasm; bottom left in the cytoplasm one notes round masses of nuclear material, almost detached from the main part of the nucleus. (B) The two cells to the left are neutrophils with plentiful finely vacuolated cytoplasm and striking abnormalities of nuclear segmentation, similar to those seen in (A). The two cells on the right are dysmorphic eosinophils, characterised by a marked reduction in specific granules, leaving large, pale, empty areas of cytoplasm; the nucleus is bilobed in the cell further to the right, even though the conformation of the lobes is irregular with appendages; the other nucleus has been damaged during spreading of the film. There are a number of morphologically normal platelets showing a moderate increase in physiological variation in size. (C) A large dysmorphic monocyte with vacuolated cytoplasm and an irregular nucleus. One notes the presence of nuclear fragments in the cytoplasm, probably endogenous in origin (chromosomes lost during an anomalous mitotic division). The pseudo-Howell-Jolly body at the top may be joined to the nucleus by a thin pyknotic filament (in which case it could be regarded as a pseudo-Barr body).

plentiful cytoplasm and increased or decreased granules and not rarely are vacuolated with increased peroxidase activity; the nuclei show anomalous segmentation with hypolobated forms (with less chromatin condensation than in other dysplastic pseudo-Pelger neutrophils) or hyperlobated, similar to the macropolycytes of megaloblastic anaemia; there may also be, although fairly rarely, binucleated neutrophils and neutrophils with ring-shaped nuclei. In some patients there are myelocytes and metamyelocytes in the circulation. It is particularly frequent to note, in the cytoplasm of neutrophils, the presence of small rounded fragments of isolated nuclear material, detached from the main chromatin mass, similar to the Howell-Jolly bodies that may be seen in red cells[127–129]: like Howell-Jolly bodies, these inclusions, which have been called karyopyknotic bodies, are positive with a Feulgen reaction indicating that they are composed of DNA.[130,131] Even more frequently than in HIV infection, Howell-Jolly-like inclusions[60] have been described in transplant patients and patients being treated with cytotoxic drugs for malignant or autoimmune conditions.[132] Eosinophils, increased in 15–20% of patients with AIDS, can show morphological abnormalities which, however, are different from those seen in neutrophils; these include dysmorphism and numerical reduction of granules, vacuoles and areas of basophilia in the cytoplasm. Monocytes are usually reduced in number but

relatively less so than lymphocytes and neutrophils, such that their percentage is often increased: it is not rare for them to appear hypertrophic and dysmorphic, with plentiful weakly basophilic vacuolated cytoplasm and irregular or convoluted nuclei with pale chromatin.

The reduction in mature cells in the peripheral blood is generally associated with bone marrow cellularity that is normal or increased, even though an increase in the fibrous component often leads to failure of attempted aspiration and requires recourse to a trephine biopsy for histological examination. Dyserythropoiesis is often manifest as asynchronous maturation, karyorrhexis or nuclear fragmentation, binuclearity and basophilic stippling. Another characteristic feature of the bone marrow aspirate in HIV infection is an increase in plasma cells and lymphocyte; immunophenotypically the lymphocytes are B cells, which tend to form nodules that are easily recognised on histological examination. Macrophages are also increased and can be phagocytic, ingesting erythrocytes, platelets and nucleated cells; this may be so prominent that the picture is that of a haemophagocytic syndrome. Sometimes, in the presence of peripheral cytopenias, the bone marrow aspirate shows invasion by B-lineage lymphoma cells: the lymphomas that most often develop in HIV-infected patients are Burkitt lymphoma and diffuse large B-cell lymphoma, which have features that are not readily distinguishable from the forms not associated with HIV infection.

## REPORT 3.1 Refractory anaemia with ring sideroblasts and false reticulocytosis



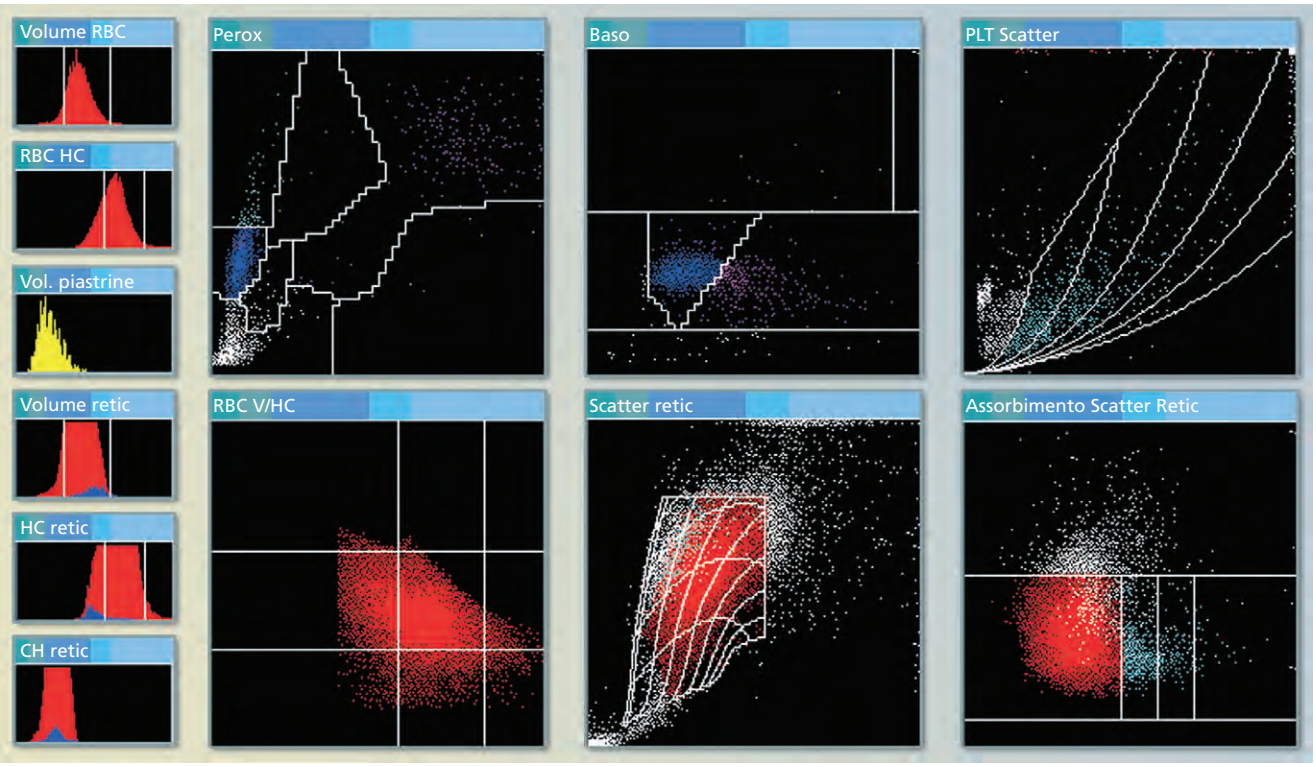

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 95 | g/l | |
| RBC | 3.01 | $\times 10^{12}$/l | |
| HCT | 0.312 | l/l | |
| MCV | 103.8 | fl | |
| MCH | 31.5 | pg | |
| MCHC | 303 | g/l | |
| CHCM | 308 | g/l | |
| RDW | 30.7 | % | Anisocytosis +++ |
| HDW | 4.08 | g/dl | Anisochromasia ++ |
| HYPO | 23.6 | % | Hypochromia +++ |
| HYPER | 0.6 | % | |
| MICRO | 8.7 | % | Microcytosis ++ |
| MACRO | 80.4 | % | Macrocytosis +++ |
| RBC fragments | 0.05 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 3.65 | % | * |
| RET | 109.9 | $\times 10^{9}$/l | * RTCADA, RTCINT |
| CHr | 35.1 | pg | |
| IRF (M+H) | 32.0 | % | |
| PLT | 166 | $\times 10^{9}$/l | |
| MPV | 8.5 | fl | |
| PDW | 63.4 | % | |
| Large platelets | 13 | $\times 10^{9}$/l | LPLT ++ |
| MPC | 25.4 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 8.52 | | |
| WBC Perox | 7.74 | | |
| NEUT | 6.47 | 75.9 | |
| LYMPH | 1.64 | 19.2 | |
| MONO | 0.28 | 3.3 | |
| EOS | 0.03 | 0.3 | |
| BASO | 0.02 | 0.3 | |
| LUC | 0.08 | 0.9 | |
| NRBC | | | |
| LI | 1.95 | | |
| MPXI | 0.8 | | |
| MN | | 28.1 | IG + |
| PMN | | 71.2 | |
| BLASTS | | 0.6 | |
| Perox HPX | | 0.9 | |
| Perox SAT | | 0.4 | |

Moderate macrocytic anaemia with dimorphism, anisocytosis and anisochromasia. The increased values of the two erythrocyte indices of heterogeneity translate into quantitative terms the abnormalities shown in the histograms: particularly the Volume RBC histogram is greatly broadened at the base, as the result of the fusion of at least two peaks. The double erythrocyte population, with a simultaneous increase in microcytic and macrocytic red cells, is best visualised on the Scatter Retic plot and the two-dimensional RBC V/HC cytogram, in which the red cells overflow into at least five of the eight peripheral areas of the distribution. The percentage of reticulocytes shows an unusual increase, as does the immature reticulocyte fraction (IRF), which could suggest an anaemia with regeneration rather than a refractory anaemia. The appearance of the reticulocyte graphics is, however, atypical and shows increased dispersion. The leucocyte analysis does not show any anomaly, either quantitative or qualitative, with the exception of an IG+ flag, which is not explained by the morphology of the populations in either of the two leucocyte analytical channels, which appear normal. The platelet count is normal as is the mean platelet volume: one observes, however, an increased volumetric heterogeneity (increased PDW) and an excess of large platelets.

**Morphology.** Marked anisocytosis, with macrocytic red cells, polychromasia, basophilic stippling and Howell-Jolly bodies (sometimes two or three in a single cell); a small proportion of normocytic hypochromic cells. The differential count is superimposable on the automated count. The IG flag has a curious explanation in the mathematical calculation that has led to its activation. In fact, the ADVIA system gives an IG+ flag when the difference between the sum of neutrophils and eosinophils in the Perox channel and the percentage of PMN in the Baso channel is equal to or greater than 5.0: in this sample these values are 76.2 and 71.2; the difference is exactly 5.0. The increase in reticulocytes is not genuine, but is caused by the presence of numerous erythrocytes containing Howell-Jolly bodies. This interpretation is confirmed by the system flag, RTCADA (reticulocyte absorption distribution abnormal), which indicates an anomalous distribution of the reticulocyte absorption histogram (which is observed, for example, in sickle cell anaemia) and of the flag, RTCINT (reticulocyte platelet interference), which indicates platelet interference because of a poorly positioned threshold.

## REPORT 3.2 Refractory cytopenia with multilineage dysplasia and neutrophils with high peroxidase activity



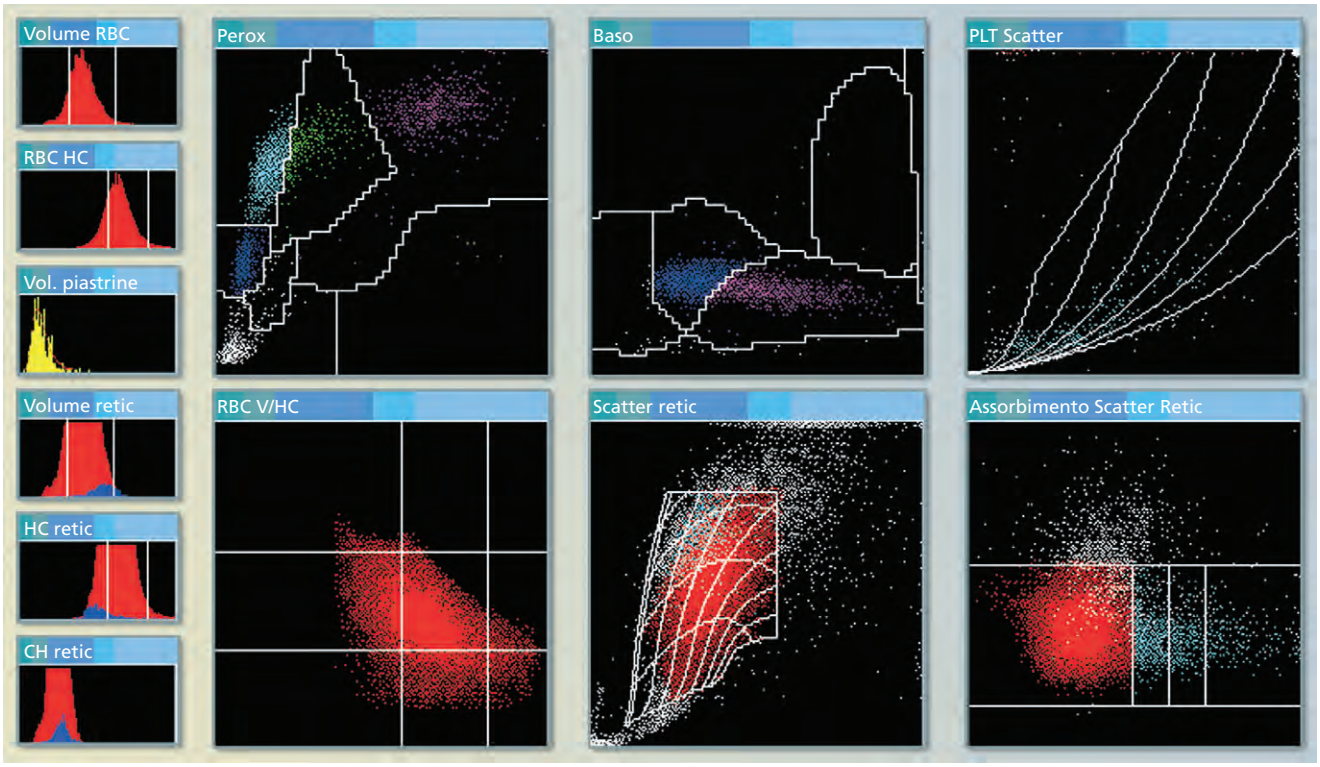

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 99 | g/l | |
| RBC | 3.90 | $\times 10^{12}$/l | |
| HCT | 0.313 | l/l | |
| MCV | 80.3 | fl | |
| MCH | 25.4 | pg | |
| MCHC | 317 | g/l | |
| CHCM | 312 | g/l | |
| RDW | 18.2 | % | Anisocytosis ++ |
| HDW | 4.42 | g/dl | Anisochromasia +++ |
| HYPO | 23.9 | % | |
| HYPER | 1.4 | % | Hypochromia +++ |
| MICRO | 6.5 | % | Microcytosis ++ |
| MACRO | 0.3 | % | |
| RBC fragments | 0.03 | $\times 10^{12}$/l | |
| RBC ghosts | 0.07 | $\times 10^{12}$/l | |
| RET | 1.70 | % | * |
| RET | 66.4 | $\times 10^{9}$/l | * |
| CHr | 25.9 | pg | * |
| IRF (M+H) | 22.7 | % | |
| PLT | 86 | $\times 10^{9}$/l | |
| MPV | 13.0 | fl | |
| PDW | 32.0 | % | |
| Large platelets | 4 | $\times 10^{9}$/l | |
| MPC | 18.2 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 2.35 | | |
| WBC Perox | 2.48 | * PXIFR | |
| NEUT | 0.22 | 9.5 | * |
| LYMPH | 2.02 | 85.9 | * |
| MONO | 0.01 | 0.4 | * |
| EOS | 0.01 | 0.3 | * |
| BASO | 0.02 | 0.7 | |
| LUC | 0.08 | 3.3 | * |
| NRBC | 0.00 | 0.0 | |
| LI | ND | | |
| MPXI | 13.1 | | |
| MN | | 83.5 | |
| PMN | | 15.7 | |
| BLASTS | | 0.7 | |
| Perox HPX | | 1.6 | |
| Perox SAT | | 0.6 | |

Pancytopenia with severe neutropenia; anaemia and thrombocytopenia are moderate. The red cell population shows anisocytosis and marked anisochromasia, with the MCV at the lower limit of normal and an increase in hypochromic erythrocytes. The reticulocyte count is slightly increased. The absolute lymphocyte count is normal. In the Perox cytogram, the few residual neutrophils are positioned more to the right than normal, indicating increased peroxidase activity, confirmed by the increased MPXI. In the Baso cytogram, the PMN tail is replaced by a few dispersed signals (fuchsia). The platelet count is reduced with a marked increase in the MPV and a fairly low level for the MPC. The PXIFR alarm (peroxidase irregular flow rate) indicates irregular flow in the analytical cell of the Perox channel, attributed in this case to the neutropenia, sufficient to interfere with the calculation of the lobularity index (LI).

**Morphology.** The white cell differential count, difficult to do because of the leucopenia, confirms the instrument count. The neutrophil granulocytes appear large, hypogranular and hyperlobated, with a minority of Pelgeroid elements; peroxidase staining demonstrates, in contrast to the morphological hypogranularity, a strong cytoplasmic positivity. The platelets are variable in size, with numerous giant forms and little evidence of a chromomere (correlating with the low MPC). The diagnosis is confirmed by examination of a bone marrow aspirate which shows trilineage dysplasia, increased iron stores and the absence of ring sideroblasts.

## REPORT 3.3 Refractory cytopenia with multilineage dysplasia, partial peroxidase deficiency and ring sideroblasts in the bone marrow



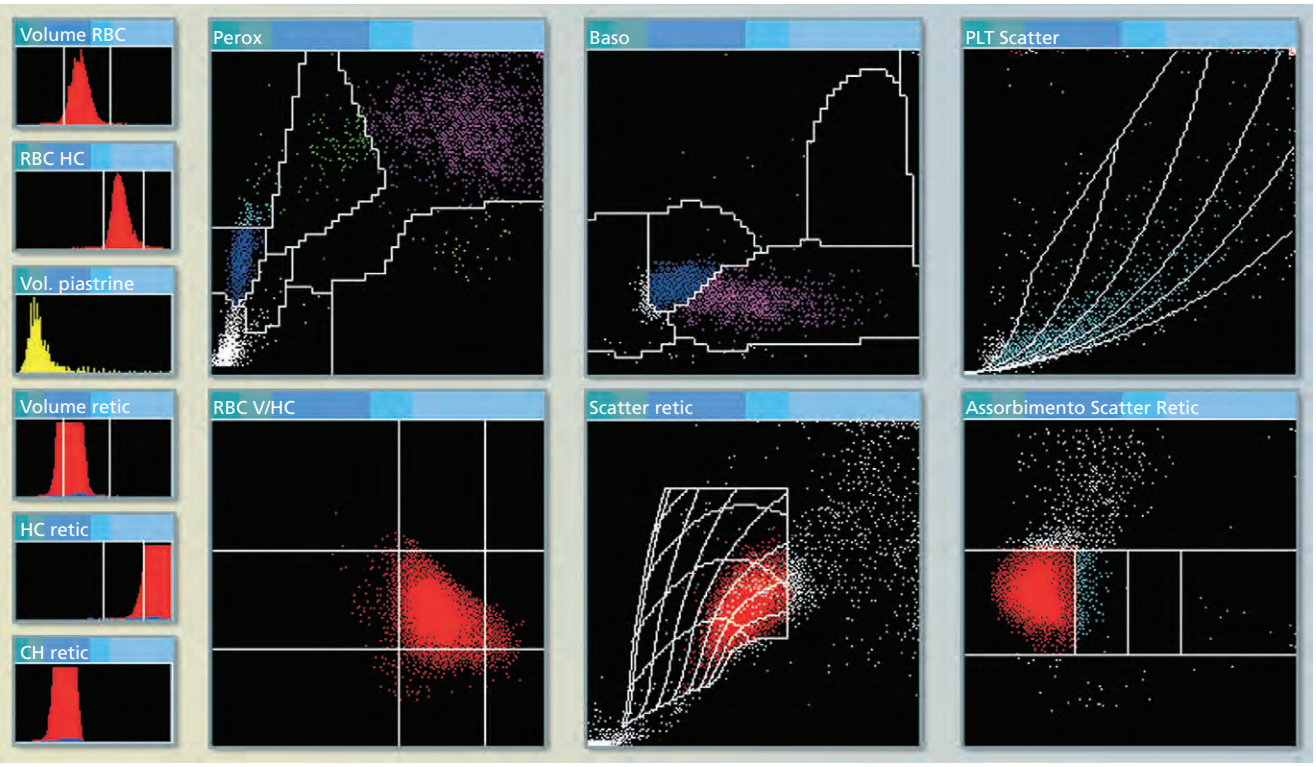

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 93 | g/l | |
| RBC | 3.73 | $\times 10^{12}$/l | |
| HCT | 0.291 | l/l | |
| MCV | 78.0 | fl | |
| MCH | 24.9 | pg | |
| MCHC | 319 | g/l | |
| CHCM | 316 | g/l | |
| RDW | 20.7 | % | Anisocytosis ++ |
| HDW | 4.36 | g/dl | Anisochromasia ++ |
| HYPO | 19.3 | % | Hypochromia +++ |
| HYPER | 2.2 | % | |
| MICRO | 11.6 | % | Microcytosis +++ |
| MACRO | 0.7 | % | |
| RBC fragments | 0.04 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 2.74 | % | RTCADA |
| RET | 102.2 | $\times 10^{9}$/l | |
| CHr | 27.4 | pg | |
| IRF (M+H) | 41.9 | % | |
| PLT | 45 | $\times 10^{9}$/l | |
| MPV | 7.7 | fl | |
| PDW | 19.4 | % | |
| Large platelets | 1 | $\times 10^{9}$/l | |
| MPC | 24.1 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 2.38 | * | |
| WBC Perox | 2.33 | * | |
| NEUT | 0.56 | 23.3 | * |
| LYMPH | 0.50 | 21.2 | * |
| MONO | 0.38 | 16.0 | * |
| EOS | 0.01 | 0.3 | * |
| BASO | 0.01 | 0.3 | * |
| LUC | 0.93 | 38.9 | * Atyp +++ |
| NRBC | | * | |
| LI | 1.79 | * B-NV | |
| MPXI | −0.7 | | |
| MN | | 64.1 | |
| PMN | | 35.2 | |
| BLASTS | | 1.1 | |
| Perox HPX | | 0.3 | |
| Perox SAT | | 0.2 | |

Anaemia with slight hypochromia and microcytosis, with erythrocyte morphology flags that are consistent with the erythrocyte indices. The two-dimensional RBC V/HC cytogram shows that the red cell population is expanded and occupies almost all the sectors, with the presence of hypochromic and some hyperchromic erythrocytes, as is characteristic of RARS.[97] In addition to anaemia and thrombocytopenia, with the MPV within normal limits, there is leucopenia with marked neutropenia. From the point of view of the differential white cell count, there is a marked increase in LUC, which has caused activation of the Atyp flag. Observation of the Perox cytogram shows in effect an elongated cluster of large peroxidase-negative elements, separated from the lymphocytes, which in part invade the monocyte area. The population of peroxidase-positive neutrophils is scanty and they are dispersed towards the right. In the Baso cytogram the PMN tail is small and not well defined and continues without interruption into the head of the monocyte cluster, causing a B-NV (Baso channel – no valley) flag and the presence of an asterisk on the LI; the sum of neutrophils and eosinophils in the Perox channel is 23.6%, in comparison with 35.2% PMN in the Baso channel (as if erythroblast nuclei were present, although this has not been signalled). The reticulocyte count is fairly high, with an increase of the IRF; there is a RECADA flag, indicating anomalous absorption.

**Morphology.** Neutrophil granulocytes 67%; lymphocytes 25%; monocytes 8%. Half of the neutrophils are severely dysplastic, with agranular cytoplasm and Pelger-type hyposegmented nuclei. The red cells show anisopoikilocytosis with basophilic stippling, Howell-Jolly bodies and polychromasia. Rare platelets are small and hyperchromatic. The population of LUC, separated from the lymphocytes in the Perox cytogram, is in fact caused by the presence of dysplastic agranular neutrophils which, with a specific cytochemical stain, are found to be two thirds peroxidase negative. These cells, because of the hyperdensity of the chromatin, fall on the threshold between MN and PMN in the Baso channel. It is also possible that the presence of erythrocyte inclusions of Howell-Jolly body type is interfering with the reticulocyte count, causing the flag for anomalous absorption. Microscopic examination of a bone marrow aspirate shows increased cellularity, with relative erythroid hyperplasia and trilineage dysplasia; the Perls reaction shows that bone marrow iron stores are well represented and there are more than 20% ring sideroblasts.

## REPORT 3.4 Myelodysplastic syndrome, classified as refractory anaemia with excess of blasts type 1



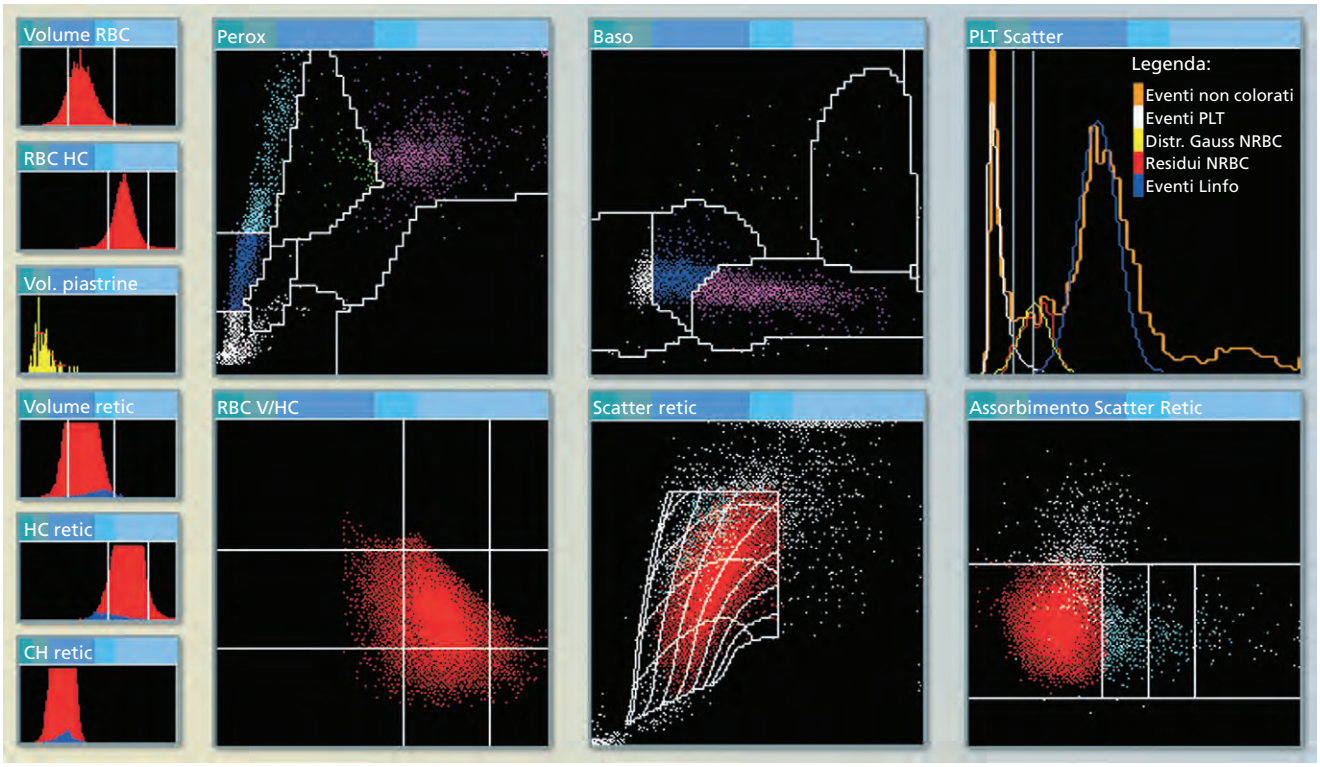

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 77 | g/l | |
| RBC | 2.82 | $\times 10^{12}$/l | |
| HCT | 0.231 | l/l | |
| MCV | 81.7 | fl | |
| MCH | 27.4 | pg | |
| MCHC | 335 | g/l | |
| CHCM | 339 | g/l | |
| RDW | 13.9 | % | |
| HDW | 2.70 | g/dl | |
| HYPO | 0.6 | % | |
| HYPER | 1.3 | % | |
| MICRO | 2.3 | % | |
| MACRO | 0.1 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 0.78 | % | |
| RET | 22.1 | $\times 10^{9}$/l | |
| CHr | 33.6 | pg | |
| IRF (M+H) | 5.5 | % | |
| PLT | 129 | $\times 10^{9}$/l | |
| MPV | 9.9 | fl | |
| PDW | 67.7 | % | |
| Large platelets | 7 | $\times 10^{9}$/l | |
| MPC | 23.1 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 2.60 | | |
| WBC Perox | 2.72 | | |
| NEUT | 1.51 | 58.3 | |
| LYMPH | 0.84 | 32.3 | |
| MONO | 0.11 | 4.1 | |
| EOS | 0.05 | 1.8 | |
| BASO | 0.01 | 0.5 | |
| LUC | 3.1 | 0.08 | |
| NRBC | 0.00 | 0.0 | |
| LI | 1.72 | | *B-NV, LSH ++ |
| MPXI | 16.8 | | |
| MN | | 56.6 | IG +++ |
| PMN | | 42.7 | |
| BLASTS | | 4.0 | |
| Perox HPX | | 7.0 | |
| Perox SAT | | 2.0 | |

Severe normocytic normochromic anaemia, without alteration of the red cell indices. The reticulocytes are reduced and poorly stained. Mild thrombocytopenia, with increased volumetric dispersion (PDW increased). The leucocyte count is reduced, with an absolute neutropenia. The neutrophils in the Perox cytogram are situated on the extreme right, indicating intense peroxidase activity, confirmed by an MPXI of 16.8. There is also an increase in HPX, with the presence of some very strongly positive cells (referred to as 'in saturation'). In the Baso cytogram the MN/PMN valley has disappeared, with an asterisked B-NV flag, reduced lobularity index and left shift (LSH), and an IG+++ flag; blast cells (signals in white to the left of the threshold in channel 8 on the x axis) are 4.0%.

**Morphology.** Neutrophils 58%; eosinophils 2.0; basophils 0.5; lymphocytes 35%; monocytes 3%; blasts 1.5%, with fine azurophilic granules. The neutrophils show anomalies of nuclear segmentation and cytoplasmic hypogranularity, with some being large with polylobated nuclei. No anomalies are evident in the erythrocyte and platelet lineages. In the Perox channel the blast cells are included in the percentage of peroxidase-positive cells (together with neutrophils and monocytes). Their number in the peripheral blood is not sufficient to trigger the specific Blast flag (in the absence of an increase in LUC the threshold is at 5%). The IG flag is activated because the difference between neutrophils plus eosinophils in the Perox channel exceeds the PMN in the basophil channel by more than 10%, because of the presence of mononuclear peroxidase-positive blast cells and dysplastic neutrophils. The bone marrow aspirate confirms dyserythropoiesis and reveals the presence of 9% granular, peroxidase-positive blast cells.

## REPORT 3.5 Myelodysplastic syndrome, classified as refractory anaemia with excess of blast type 2



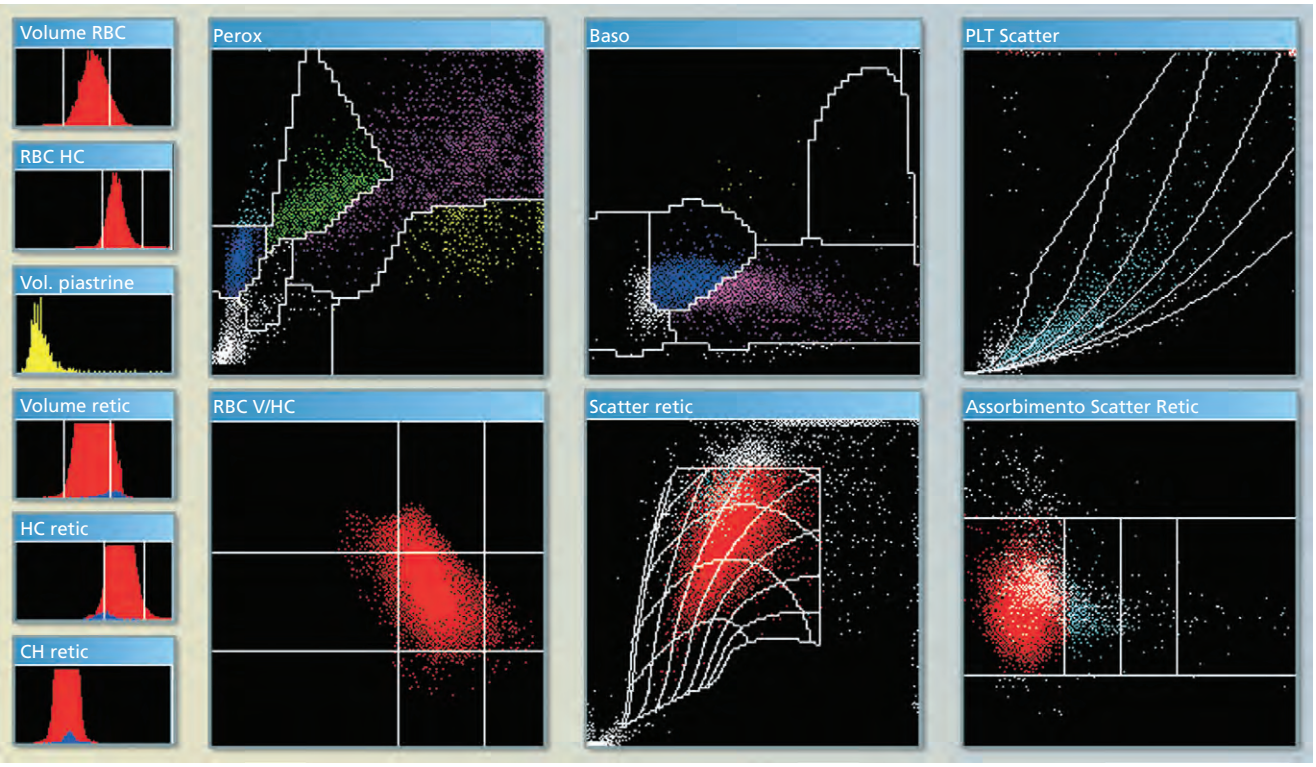

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 78 | g/l | |
| RBC | 3.11 | $\times 10^{12}$/l | |
| HCT | 0.243 | l/l | |
| MCV | 78.1 | fl | |
| MCH | 25.1 | pg | |
| MCHC | 322 | g/l | |
| CHCM | 332 | g/l | |
| RDW | 20.7 | % | Anisocytosis ++ |
| HDW | 3.53 | g/dl | Anisochromasia + |
| HYPO | 6.9 | % | Hypochromia + |
| HYPER | 1.5 | % | Microcytosis +++ |
| MACRO | 0.4 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 1.85 | % | |
| RET | 45.1 | $\times 10^{9}$/l | |
| CHr | 28.4 | pg | |
| IRF (M+H) | 22.6 | % | |
| PLT | 10 | $\times 10^{9}$/l | |
| MPV | 8.2 | fl | |
| PDW | 17.7 | % | |
| Large platelets | 0 | $\times 10^{9}$/l | |
| MPC | 20.6 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 3.27 | * | |
| WBC Perox | 3.18 | * PX-NV, NRPXNV | |
| NEUT | 1.08 | 33.1 | * |
| LYMPH | 1.20 | 36.6 | * |
| MONO | 0.09 | 2.6 | * |
| EOS | 0.00 | 0.1 | * |
| BASO | 1.1 | 0.04 | * |
| LUC | 0.86 | 26.4 | * Atyp ++ |
| NRBC | 0.14 | 4.4 | * NRBC + |
| LI | 1.64 | * B-NV, LSH | |
| MPXI | −4.7 | | |
| MN | | 58.6 | |
| PMN | | 39.5 | |
| BLASTS | | 9.3 | Blasts ++ |
| Perox HPX | | 1.6 | |
| Perox SAT | | 0.7 | |

Severe anaemia with anisocytosis, mild microcytosis and hypochromia. As a percentage, the reticulocytes are slightly more numerous than normal, but the absolute count is not increased, indicating an ineffective response to the severe anaemia. Thrombocytopenia is very marked. There is leucopenia with neutropenia and monocytopenia: in the Perox cytogram the population of neutrophils is slightly more dispersed than normal but the major anomaly is an increase in LUC, attributed to a cluster of peroxidase-negative signals generated by very large cells. In the Baso cytogram the PMN tail has its usual ovoid form, but it continues without a valley into the MN population (hence a B-NV flag with an asterisk). 9.3% of nuclear signals have spread beyond the channel 8 threshold on the x axis towards the origin, indicating the presence of cells with low density chromatin. Blast and Atypic flags have been activated. The NRBC histogram shows a small number of erythroblasts in the circulation.

**Morphology.** Neutrophils 35%; eosinophils 0.0%; basophils 0.5%; lymphocytes 44%; plasmacytoid lymphocytes 6%; monocytes 2.5%; agranular blasts 12% (peroxidase negative, CD34 positive and CD13 positive); orthochromatic erythroblasts 3 per 100 leucocytes. The neutrophils show nuclear hyposegmentation, well-represented granules and occasional Döhle bodies. There is anisocytosis with mild hypochromia. The bone marrow aspirate shows 18% of mainly peroxidase-negative blast cells. Megakaryocytes are markedly dysplastic and there is some dyserythropoiesis.

## REPORT 3.6 Leukaemic evolution of myelodysplastic syndrome, originally classified as refractory anaemia with excess of blasts type 1



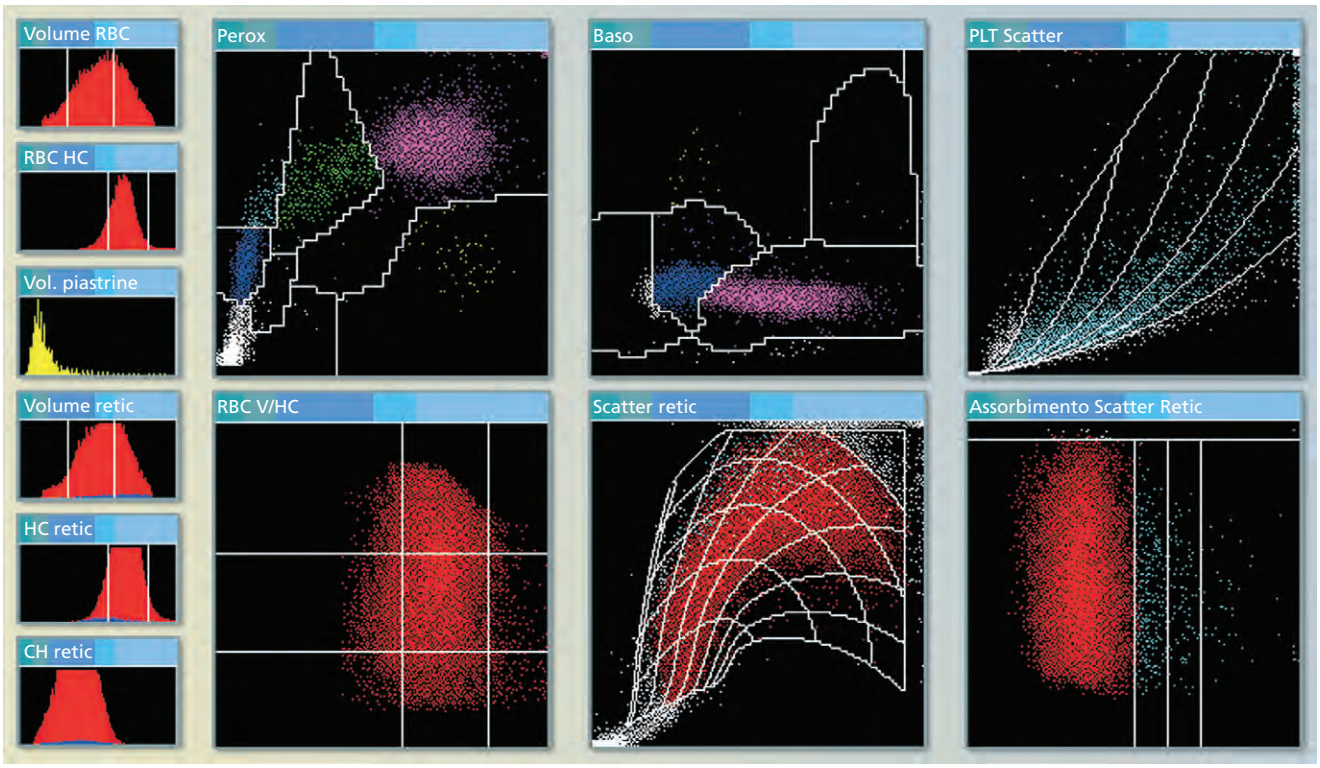

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 119 | g/l | |
| RBC | 3.62 | $\times 10^{12}$/l | |
| HCT | 0.368 | l/l | |
| MCV | 101.5 | fl | |
| MCH | 32.9 | pg | |
| MCHC | 324 | g/l | |
| CHCM | 328 | g/l | |
| RDW | 15.8 | % | Anisocytosis + |
| HDW | 2.77 | g/dl | |
| HYPO | 3.1 | % | |
| HYPER | 0.5 | % | |
| MICRO | 0.3 | % | |
| MACRO | 11.0 | % | Macrocytosis +++ |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 1.12 | % | |
| RET | 40.6 | $\times 10^{9}$/l | |
| CHr | 34.8 | pg | |
| IRF (M+H) | 13.9 | % | |
| PLT | 144 | $\times 10^{9}$/l | |
| MPV | 9.9 | fl | |
| PDW | 56.1 | % | |
| Large platelets | 6 | $\times 10^{9}$/l | |
| MPC | 21.2 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 5.42 | | |
| WBC Perox | 5.71 | | |
| NEUT | 2.11 | 39.0 | |
| LYMPH | 1.95 | 36.0 | |
| MONO | 0.92 | 17.0 | |
| EOS | 0.34 | 6.2 | |
| BASO | 0.02 | 0.4 | |
| LUC | 0.08 | 1.4 | |
| NRBC | 0.00 | 0.0 | |
| LI | 1.83 | * B-NV, | LSH |
| MPXI | 15.6 | | |
| MN | | 66.8 | IG +++ |
| PMN | | 32.6 | |
| BLASTS | | 11.7 | Blasts +++ |
| Perox HPX | | 14.3 | |
| Perox SAT | | 7.4 | |

There is mild anaemia for a woman, with macrocytosis and anisocytosis; reticulocytes are normal. The platelet count is preserved. The leucocyte differential count indicates relative monocytosis and mild eosinophilia, but there are significant flags for Blasts and IG. The blast count in the Baso channel is 11.6%, and a corresponding cluster is apparent in the cytogram, in which the head of the MN cluster appears deformed towards the left and downwards. The normal elongated ovoid of the PMN population is lost, as is the valley between MN and PMN (B-NV flag). The distribution of the leucocyte populations in the Perox channel is anomalous, such that the differential count cannot be accepted without microscopic review. Peroxidase-positive cells show increased dispersion; the cells classified as neutrophils form a dispersed wedge, with MPXI, HPX and SAT greatly elevated and invasion of the eosinophil and monocyte boxes. In the monocyte box there is also an ovoid cluster of cells spreading across the diagonal lower threshold, classified in part as monocytes (green) and in part as neutrophils (fuchsia). The area occupied by these cells shows analogies with the localisation of blast cells in some acute myeloid leukaemias with differentiation (see Report 4.1).

**Morphology.** Neutrophils 38%, dysplastic; lymphocytes 37%; monocytes 4%; blasts 21%, with azurophilic granules (peroxidase positive) and some Auer rods. Microscopy confirms the presence of blast cells, which have sufficient enzymatic activity to be localised with the monocytes in the Perox cytogram; the dysplastic neutrophils occupy the areas of greatest positivity in the neutrophil and eosinophil boxes. The IG flag results from the presence of the blast cells, which are classified as MN in the Baso channel and as peroxidase-positive cells in the Perox channel. The association of a Blast flag and an IG flag indicates the presence of granular blast cells.

## REPORT 3.7 Myelodysplastic syndrome associated with isolated del(5q)




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 78 | g/l | |
| RBC | 2.28 | $\times 10^{12}$/l | |
| HCT | 0.243 | l/l | |
| MCV | 106.4 | fl | |
| MCH | 34.3 | pg | |
| MCHC | 322 | g/l | |
| CHCM | 328 | g/l | |
| RDW | 29.0 | % | Anisocytosis +++ |
| HDW | 3.99 | g/dl | Anisochromasia + |
| HYPO | 11.6 | % | Hypochromia ++ |
| HYPER | 1.5 | % | |
| MICRO | 7.6 | % | Microcytosis ++ |
| MACRO | 33.4 | % | Macrocytosis +++ |
| RBC fragments | 0.06 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 1.74 | % | *, RTCADA |
| RET | 39.7 | $\times 10^{9}$/l | *, RTCINT |
| CHr | 36.3 | pg | * |
| IRF (M+H) | 31.3 | % | |
| PLT | 379 | $\times 10^{9}$/l | |
| MPV | 9.5 | fl | |
| PDW | 59.3 | % | |
| Large platelets | 14 | $\times 10^{9}$/l | LPLT ++ |
| MPC | 25.,5 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 6.51 | | |
| WBC Perox | 6.60 | | |
| NEUT | 4.64 | 71.2 | |
| LYMPH | 1.23 | 18.9 | |
| MONO | 0.43 | 6.6 | |
| EOS | 0.06 | 1.0 | |
| BASO | 0.03 | 0.5 | |
| LUC | 0.12 | 1.9 | |
| NRBC | | | |
| LI | 1.93 | | |
| MPXI | −0.9 | | |
| MN | | 27.1 | |
| PMN | | 72.1 | |
| BLASTS | | 1.5 | |
| Perox HPX | | 0.8 | |
| Perox SAT | | 0.3 | |

Marked anaemia with increased MCV and RDW. The deformation of the RBC Volume histogram and the two-dimensional red cell cytogram indicates a heterogeneous red cell population, which is invading all the sectors. Hypochromic microcytes are also present. The reticulocyte count and the IRF are high, indicating an attempt at an erythropoietic response to the anaemia, even if it is showing very little efficacy. The Absorption Scatter Retic is anomalous, this being confirmed by RTCADA and RTCINT flags, which suggest interference in measurement of light absorption. The scatter plots of the leucocyte populations are normal and there are no morphological flags.

**Morphology.** The white cell differential count confirms the instrument count. Examination of red cells shows macrocytosis with very marked anisopoikilocytosis (bizarrely shaped red cells, fragments, basophilic stippling, polychromasia and Howell-Jolly bodies). The platelets are heterogeneous in size and some are hyperchromatic. The bone marrow aspirate manifests dyserythropoiesis with megakaryocyte hyperplasia and dysplasia. Cytogenetic analysis reveals an isolated del(5q).

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CHAPTER 4

# Acute Myeloid Leukaemia

The multidisciplinary diagnostic approach to the classification of acute myeloid leukaemia (AML) has expanded in the last decade to the extent that this approach has become essential. Cytogenetic and molecular analysis at diagnosis are now indispensable, both for identifying specific genetic anomalies that distinguish subtypes of AML and for providing basic information that will later be necessary for the evaluation of remission and progression of the diseases. The striking developments in diagnostic sophistication have not, however, reduced the value of morphological analysis: rather they have confirmed that morphology is the solid base that underlies all these innovations. Although remaining grounded on microscopic evaluation of peripheral blood and bone marrow films stained by conventional methods, cytomorphology of leukaemia has in fact been enriched by refinements that have improved predictive capacity, specificity and sensitivity in relation to the underlying genetic lesions.[1]

AML is the result of a clonal neoplastic proliferation of haemopoietic cells that invade the bone marrow, the peripheral blood and, sometimes, other tissues. There is a marked heterogeneity – clinical, morphological, genetic and in responsiveness to therapy. The incidence is around 2.5–3 cases per 100 000 per year, with a median age of 65 years and a slight male predominance. Children under the age of 15 years represent about 20% of all cases of acute leukaemia.

AML is caused by a mutation in a single haemopoietic stem cell, the progeny of which become the leukaemic clone. Normal production of blood cells depends on the orderly occurrence of the processes of differentiation, controlled by delicate molecular mechanisms that direct the multipotent stem cell towards a specific lineage, with maturation subsequently occurring; this process is seen in the multiple phenotypic alterations that occur in the haemopoietic progenitors and their progeny. The genetic mutations that produce leukaemic transformation profoundly disturb these processes. Many of these genetic lesions are now known and involve the rearrangement of genes that encode transcription factors, the abnormal functioning of which causes aberrations in the process of myeloid differentiation (e.g. *PML-RARA* and other fusion genes resulting from translocations). Other genes that may be involved encode proteins that act by altering signal transmission, promoting proliferation and giving the mutated progenitor cells an advantage in terms of survival (e.g. *FLT3*, *NPM1*, *RAS* genes, *KIT*, *JAK2*). These genetic alterations often interact with each other in a multistep process. As a result of expanded knowledge, including knowledge of the potential clinical and therapeutic implications, the 2008 WHO classification has established a group of subtypes of AML that have specific recurrent genetic abnormalities: included in this group, in addition to the classical translocations and inversions, are several provisional categories that have molecular abnormalities in the absence of cytogenetic aberrations.

The acute leukaemias are primary bone marrow disorders. In the peripheral blood the signs of bone marrow failure are consistently observed, in the form of anaemia, neutropenia and thrombocytopenia. With the exception of AML with myelodysplasia-related changes and certain categories with recurrent genetic abnormalities, the mature cells do not usually show major deviations from normality. The leucocyte count can be reduced ('aleukaemic leukaemia'), normal or increased, sometimes greatly increased, because of entry of leukaemic blasts into the circulation (Fig. 4.1). The classical idea of the *hiatus leucaemicus*, by which one means the presence in the peripheral blood of blast cells and mature cells but with a lack of intermediate maturing forms, was in the past a basic criterion for the diagnosis of acute leukaemia, in contrast to chronic myeloid leukaemia (CML): this still has value, though not absolute, since some forms of AML can show a degree of maturation, albeit atypical. Bone marrow cellularity (Fig. 4.2) is almost always increased; the percentage of blast cells, which in acute lymphoblastic leukaemia (ALL) is often more than 90%, is more variable in AML, but by definition must be 20% or more. In 10% of cases the bone marrow is hypocellular, this being more common among subjects of an advanced age, and in those who have had a preceding haematological disorder or previous chemotherapy or radiotherapy. Hypocellularity is also more common in certain subtypes of AML associated with bone marrow fibrosis, such as acute megakaryocytic leukaemia. Hypocellularity does not appear to have any adverse

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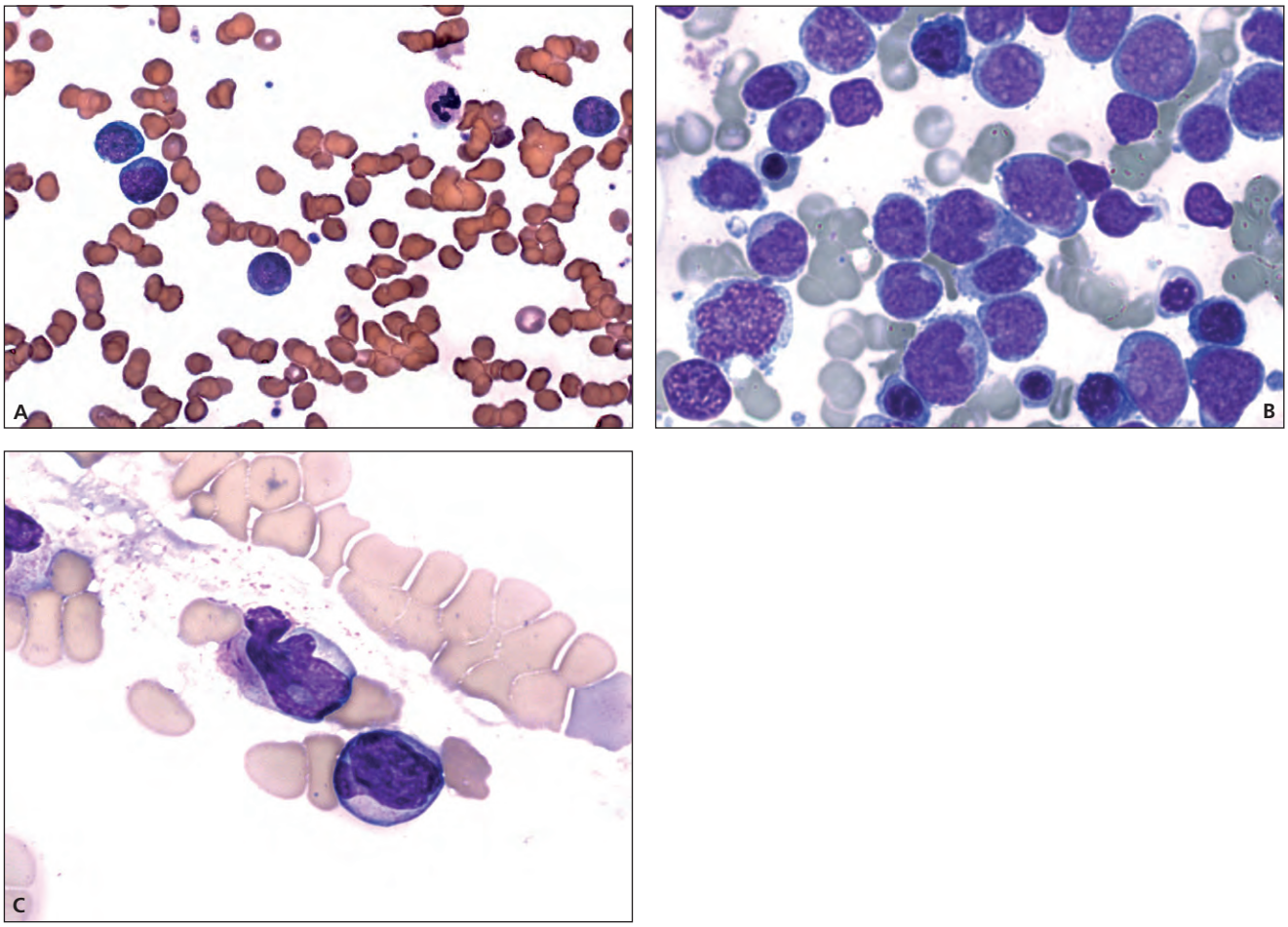



**Fig. 4.1** Peripheral blood film in three different cases of acute myeloid leukaemia (AML). (A) Four medium-sized to large blast cells without morphological signs of differentiation, with an increased nucleocytoplasmic (N:C) ratio and scanty, intensely basophilic cytoplasm. A neutrophil with minimal abnormalities (mild nuclear hypersegmentation and a Döhle body in the well-granulated cytoplasm), in the absence of precursors at intermediate stages of maturation, illustrates the concept of a *hiatus leucaemicus*. The red cells are forming rouleaux; bottom right, a polychromatic erythrocyte. The platelets are few in number, medium-sized to large and hyperchromatic. (B) Very marked leucocytosis with invasion of the peripheral blood by numerous blasts cells that are heterogeneous with regard to size and shape: small undifferentiated cells with scanty cytoplasm, no granules and the presence of a uropod creating a hand-mirror appearance; large cells with pale chromatin and nucleoli; others with some features of possible monocytic differentiation (notching and irregularity of the nucleus). The presence of erythroblasts is common in these cases with marked leucocytosis and reflects the disruption of the bone marrow-blood interface. (C) In films from leucopenic samples, observation of the edges and tail of the film, although the cytological details may not be as well preserved in these areas, sometimes permits the recognition of blast cells, which suggest the correct diagnosis, as in the case of these two small blast cells, one of which shows, in the left part of the pink cytoplasm, various Auer rods: this is the typical morphology of acute promyelocytic leukaemia (APL).

prognostic significance in AML.[2] These hypoplastic cases can, however, cause problems resulting from the difficulty in accurately evaluating the percentage and nature of the blast cells in the aspirate. Sometimes the peripheral blood shows leucopenia with undifferentiated blast cells and attempted aspiration does not yield any material. The differential diagnosis with aplastic anaemia and hypocellular myelodysplastic syndrome (MDS) must then rest on histological and immunohistochemical examination of biopsy sections, in order to document the presence of small focal accumulations of blast cells in a hypocellular marrow.[3]

## General criteria for diagnosis and classification

The aim of classification is the grouping together of conditions that have analogies with each other based on fundamental biological characteristics. These subdivisions are indispensable for comparison with clinical features, evaluating the results of various therapeutic regimes and making significant progress in research.

Haematologists have always considered it important to subclassify acute leukaemia as this condition shows striking bio-

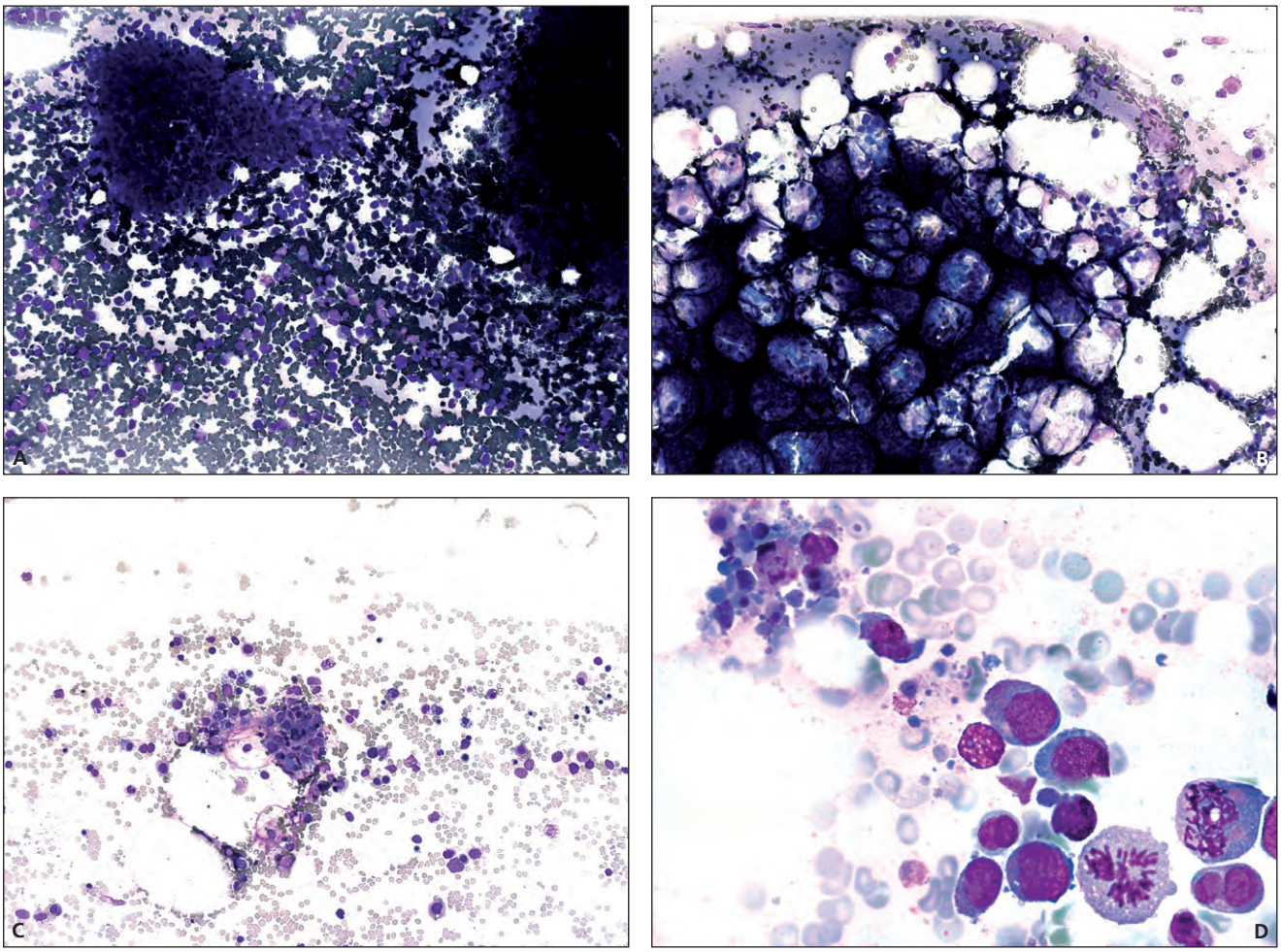



**Fig. 4.2** Bone marrow films showing various degrees of cellularity in patients with AML. (A) Hypercellular fragment. Even at low power one can appreciate the abundant cells that populate the tail of this film and also the marked monomorphism. No megakaryocytes are seen in this field, nor the dark nuclei of polychromatic or orthochromatic erythroblasts: all that is apparent is the presence of round, homogeneously violet nuclei mixed with erythrocytes. (B) Fragment of reduced cellularity. The optically empty spaces correspond to adipose cells, the cytoplasmic contents having been dissolved during fixation. Poorly defined cellular areas are seen only in the centre of the fragment. (C) In this small clump, in a hypocellular aspirate, some partly vacuolated blast cells are admixed with a few haemopoietic precursors and stromal cells, among which are adipocytes and osteoblasts. This appearance could suggest a tumour metastasis, from which it is distinguished, even at this power, by the heterogeneous cellular composition. (D) At the end of this trail in an aparticulate sample with very few cells one observes two macrophages laden with cellular debris, some granular blast cells, with large oval nuclei and plentiful intensely basophilic cytoplasm containing azurophilic granules, and two mitotic figures. Histological examination is essential in cases such as this.

logical and clinical variability. Almost all classifications have sought to subdivide cases in a manner related to normal haemopoietic lineages. At the end of the 19th century, following the observations of pioneers such as Virchow and Neumann, the leukaemias were subdivided into lymphatic, splenic and myelogenous forms.[4] In his manual of 1912, Ferrata distinguished, aligning himself conceptually with Paul Ehrlich, a lymphatic leukaemia and a medullary leukaemia, recognising for both the existence of acute and chronic forms. In the 1958 treatise of Ferrata and Storti, the subdivision was more complex and included the forms haemocytoblastic, lymphoblastic, myeloblastic and monocytic; a separate chapter was dedicated to erythraemia and the panmyeloses. Modern methods of classification are based on the ideas proposed in a 1975 article by two British haematologists, Galton and Dacie.[5] Their scheme was the basis of a classification generated by a French–American–British co-operative group known by the acronym FAB. The aim of the FAB group was to establish objective criteria for separating lymphoblastic leukaemia from myeloid leukaemia, and for further subdividing each category into subtypes. The first FAB classification recognised three types of lymphoblastic leukaemia and six types of myeloid: the latter, designated with the letter M followed by a number from 1 to 6, were identified according to two criteria:

1. The direction of differentiation to one or more lineages;
2. The degree of maturation.[6]

The original FAB classification was based exclusively on cytology and cytochemistry, because of the easy reproducibility and universal applicability of these modalities. The nomenclature introduced by the FAB group represented, for more than two decades, the reference classification and framework for any new biological, clinical and therapeutic information, including the introduction of new immunophenotypic and genetic markers. The FAB group subsequently extended, in further publications, the classification of AML,[7] introducing also criteria that were not exclusively morphological for the identification of certain subtypes.

The complex and variegated nature of leukaemia means that it is appropriate to investigate it by a variety of means, ranging from simple to sophisticated, the effectiveness of which has been steadily increasing during this era of biotechnology. In no other area has classical morphology both retained its primary role and also been enriched and amplified by support from cytochemical, immunological and cytogenetic techniques. This integration has permitted the identification of previously unknown morphological entities, associated with specific chromosomal alterations and individual antigen profiles. There is now only a small percentage of cases of acute leukaemia that remain genuinely unclassifiable.

On this basis, a quite large working group under the aegis of the World Health Organization (WHO), applying the principles initially defined in the REAL classification of lymphoproliferative disorders,[8] elaborated a system of classification based on the consensus of numerous experts and scientific societies. The aim remains that of identifying 'real' clinical entities that can be reliably and consistently diagnosed on the basis of the pathological features, using all the information available, from morphology to molecular genetics. The previous edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues included for the first time all lymphoid and myeloid neoplastic conditions.[9] Recently, a new, prestigious volume has provided an updated overview and has both included newly identified conditions and modified certain fundamental criteria, as a result of recent studies, both clinicotherapeutic and biological/genetic.[10] This updated version provides the basic framework of the descriptions that will be given in this chapter (Table 4.1).

The WHO classification departs from the simple approach of the FAB classification, which uses morphology to subdivide

**Table 4.1** The 2008 WHO classification of acute myeloid leukaemia (AML) (adapted from Swerdlow 2008,[10] with the permission of IARC)

1. AML with recurrent genetic abnormalities
   - A. AML with a defined recurrent cytogenetic abnormality and with a typical cytomorphological picture (diagnosis of AML can also be made with <20% blast cells)
     - A1. AML with core-binding factor (*CBF*) mutations
       - – AML with t(8;21), granulocytic maturation and Auer rods
       - – AML with t(16;16) or inv(16), myelomonocytic with abnormal eosinophils
     - A2. Acute leukaemia with t(15;17), acute promyelocytic leukaemia (APL)
       - – Hypergranular
       - – Microgranular
       - – With variant *RARA* translocations
   - B. Other AML with recurrent cytogenetic anomalies (diagnosis with at least 20% blast cells)
     - – AML with t(9;11), mainly monoblastic
     - – AML with t(6;9), with basophilia and dysplasia
     - – AML with t(3;3) or inv(3), with megakaryocyte dysplasia
     - – AML with t(1;22), megakaryoblastic
   - C. AML with gene mutation
     - – AML with *NPM1* mutation, myelomonocytic or monocytic
     - – AML with *CEBPA* mutation, without maturation
2. AML with myelodysplasia-related changes (AML with MRC)
3. Therapy-related myeloid neoplasms (including t-MDS and t-AML)*
4. AML, not otherwise specified (AML, NOS)
   - – With minimal differentiation
   - – Without (or with little) granulocytic maturation
   - – With granulocytic maturation
   - – Myelomonocytic
   - – Monoblastic and monocytic
   - – Acute erythroid
   - – Megakaryoblastic
   - – Basophilic
   - – Panmyelosis with fibrosis
5. Myeloid sarcoma
6. Myeloid proliferations related to Down syndrome
7. Blastic plasmacytoid dendritic cell neoplasm

*In the text and in the illustrations, the description of AML, NOS precedes that of therapy-related myeloid neoplasms to permit an orderly description of morphological features.

leukaemia into various types based on the lineage involved and the degree of maturation. The WHO classification makes use of all the diagnostic instruments available – morphological, cytochemical, immunophenotypic, genetic and clinical – in order to recognise and define in a reproducible manner pathological entities that are also of clinical significance. In this classification the term 'myeloid' is used to indicate all the haemopoietic cells belonging to granulocytic, monocytic, erythroid, megakaryocytic and mast cell lineages.[11] The first category of AML with recurrent cytogenetic abnormalities includes many conditions with a typical morphology, the specificity and predictivity of which has already been recognised: M2 AML with single Auer rods associated with t(8;21); acute promyelocytic leukaemias (APL) with t(15;17); M4 AML with eosinophilia with alteration of chromosome 16. What is new in the diagnostic approach is that the genetic alteration is the first defining feature in a case of suspected AML and also that the blast cell threshold for a diagnosis of AML has been reduced to 20%. Secondly, great relevance is given to associated myelodysplastic features and to a medical history revealing exposure to potentially mutagenic antitumour treatment. The use of the FAB abbreviations, M1 to M7, which has been found for many years to be very convenient, is discouraged by the WHO group in order to avoid oversimplification, which could hinder rapid molecular genetic stratification.

From the practical point of view, however, even though the diagnostic process is more straightforward with the availability of exceptionally efficacious diagnostic methods, the basic approach when a diagnosis of acute leukaemia is suspected has not altered. The key steps in the diagnosis of AML, which make possible the provision of effective therapy remain:

- Cytomorphological diagnosis of acute leukaemia, based on the recognition and quantification of more than 19% blast cells;
- The characterisation of the diverse types of myeloid and lymphoid leukaemia, by the application, depending on the type of leukaemia, of cytochemical, immunophenotypic and genetic analysis.

Morphology, moreover, must be used regularly and systematically to recognise the presence and the degree of myelodysplastic changes in the maturing cells that accompany the blastic population. This criterion determines the inclusion of a case in the specific category of AML with myelodysplasia-associated features; currently included in this category are many forms of AML with erythroid and megakaryocytic proliferation, which in the majority of cases are associated with multilineage dysplasia. Grouped together in a single separate category is therapy-related AML, and a final category accommodates the remaining cases under the designation AML, not otherwise specified (AML, NOS), within which there survives a subclassification that relates, implicitly, to the FAB approach.

## Morphological diagnosis: blast cells

The basic requirement for the recognition of acute leukaemia is the morphological recognition of blast cells in the bone marrow and the peripheral blood. The minimum limit for a diagnosis, already modified in the 2001 WHO classification, is 20% of bone marrow cells or of circulating leucocytes.

Blasts are recognised and defined in the first place morphologically; cytochemical and immunophenotypic techniques mainly give extra information that is relevant to their further characterisation. The blastic nature of a cell is recognised essentially from the nuclear morphology. The chromatin structure gives the most important information: the chromatin of blast cells in fine, dispersed and delicate; in the case of lymphoblasts and megakaryoblasts, there can be some chromatin condensation. Furthermore, one sometimes observes a difference in the thickness of the chromatin network in peripheral blood blast cells in comparison with those in bone marrow films. The presence of nucleoli is another useful indication of immaturity. The cytoplasmic criteria (azurophilic granules, Auer rods and basophilia) provide supplementary information, useful above all for giving information about the lineage to which the blast cells belong and their degree of maturation.

The 2008 WHO classification confirms that the blast cell count remains the fundamental diagnostic tool for classifying myeloid neoplasms and for judging their progression. It is established that their quantification must be based on counting a minimum of 200 cells in peripheral blood films and 500 cells in bone marrow films.[11] The morphological criteria adopted by the WHO group are those initially proposed by the International Working Group on Morphology of Myelodysplastic Syndromes (IWGM-MDS).[12] The count of CD34-positive cells is not recommended as a replacement for a count performed with a microscope, both because the bone marrow aspirate used for immunophenotypic analysis is often diluted with peripheral blood, thus lowering the blast percentage, and because not all blast cells express this marker. The evaluation of histological sections of bone marrow, supported by immunohistochemistry, is considered important, particularly when the bone marrow is hypocellular or fibrotic.

The following cells must be included in the blast percentage (Figs 4.3, 4.4 and 4.5):

- Agranular blast cells (corresponding to FAB type I blast cells): they have a large nucleus with finely dispersed non-condensed chromatin, a high nucleocytoplasmic (N:C) ratio, nucleoli that are often prominent and weakly basophilic agranular cytoplasm.
- Granular blast cells (corresponding to FAB type II blast cells, to which are added type III blasts): their characteristics are generally similar to those of agranular blasts, but they are distinguished by the presence in the cytoplasm of a variable number of delicate azurophilic granules; the N:C ratio may be slightly less than that of granular blasts and the chromatin may be somewhat more condensed.
- Blast cells showing evidence that they belong to a specific lineage: monoblasts and megakaryoblasts sometimes have undifferentiated morphology, without granules or particular nuclear features, and are then identifiable only by

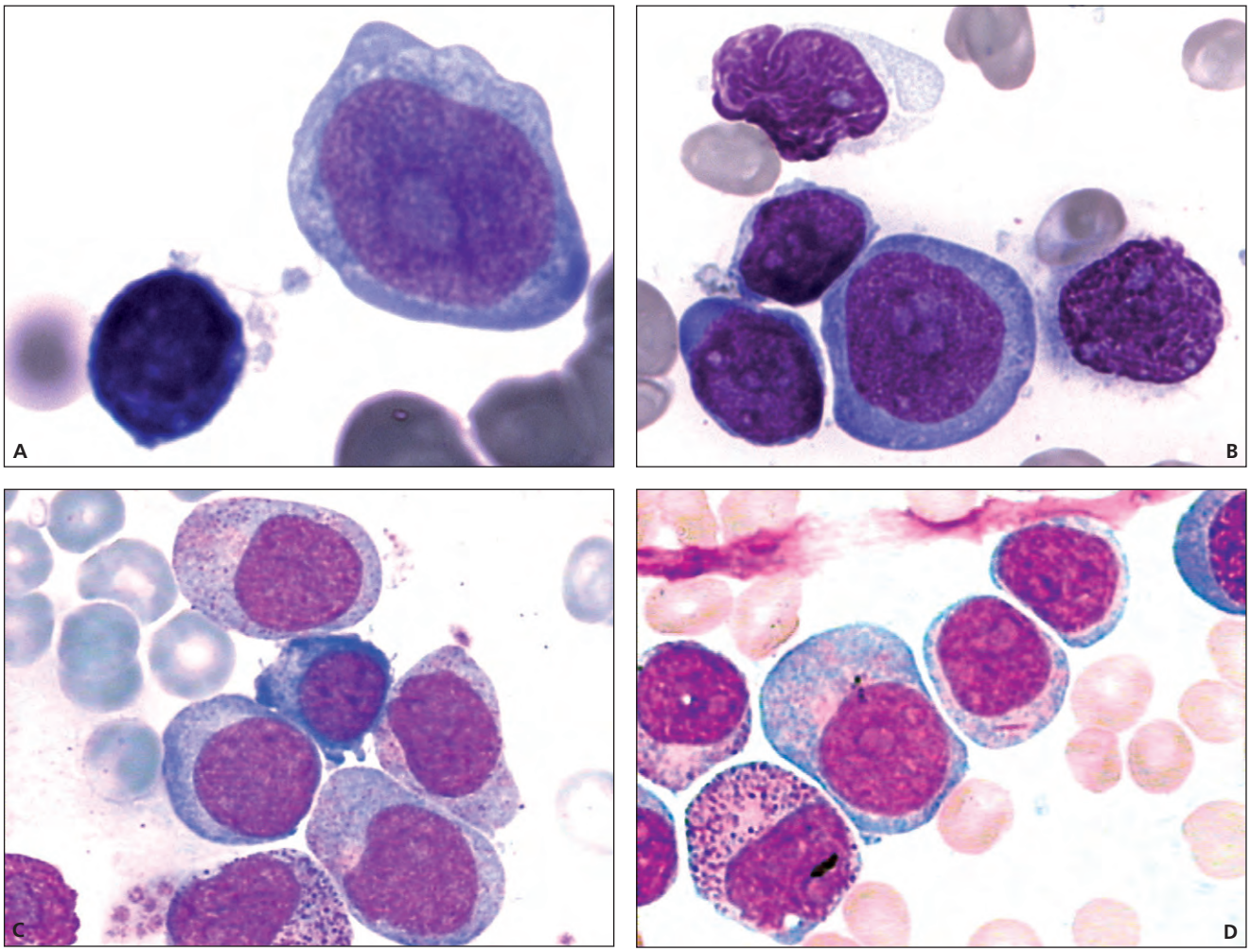



**Fig. 4.3** Leukaemic cells that must or must not be included in the blast count. (A) On the right, a large agranular blast cell with a fairly low N:C ratio. The nucleus is oval with some notches and angulation; the chromatin, which could be described as loose, granular, open or reticular, is condensed around an enormous prominent pale nucleolus. The cytoplasm is basophilic – a non-homogeneous opaque blue, particularly intense at the periphery – and is flattened around the nucleus. There are no granules or structure to the cytoplasm. On the left, a cell with a round and hyperdense nucleus and cytoplasmic projections is typical of a micromegakaryocyte (which should not be included in the blast count). (B) Agranular blast cells. In the centre at the bottom, a large immature cell with delicate chromatin; the plentiful cytoplasm is basophilic and agranular. To its left, two small blast cells with a high N:C ratio and dense chromatin with poorly visible nucleoli. A cytological distinction from a lymphoblast can be made only with the aid of cytochemistry or, with greater reliability, with immunophenotyping. The presence of angulation and straight segments of the nuclear outline, as in the larger blast cells, is often found in AML, but does not provide definite evidence of lineage. (C) Three granular blast cells from a patient with t(8;21): the cytoplasm is very plentiful and weakly basophilic with a pale paranuclear Golgi zone, and contains a variable number of granules, in part azurophilic and in part darker; the chromatin is granular. Cells of this type create problems with the cytological differential diagnosis with dysplastic promyelocytes, sometimes only resolvable with difficulty. In the centre, a proerythroblast with pale, very extensive arcoplasm, and a basophilic erythroblast in which the nucleus is eccentric because of compression by the surrounding cells: neither of these two cells should be included in the blast count. The proerythroblast is recognised as such because of the regularity of its nuclear outline, which is an almost perfect circle, without any of the angulation, notches or straight parts that distinguish the surrounding myeloblasts. (D) In this small microscopic field from another patient with the same cytogenetic abnormality, one can count three blast cells along the diagonal that starts in the top right corner. The small blast, at the top, appears to be agranular with fairly dense chromatin. The adjacent cell is very similar but contains a single Auer rod and fine azurophilic granules; it has a nucleus with more open chromatin and some nucleoli, one of which is very large and irregular. The third larger blast cell has chromatin than is looser and reticular, a giant nucleolus and two smaller ones; the N:C ratio is low since the cytoplasm is quite extensive; there is an obvious pale Golgi zone and numerous fine azurophilic granules. In the bottom left corner, to be excluded from the blast count, is a dysplastic promyelocyte in an advanced stage of maturation.

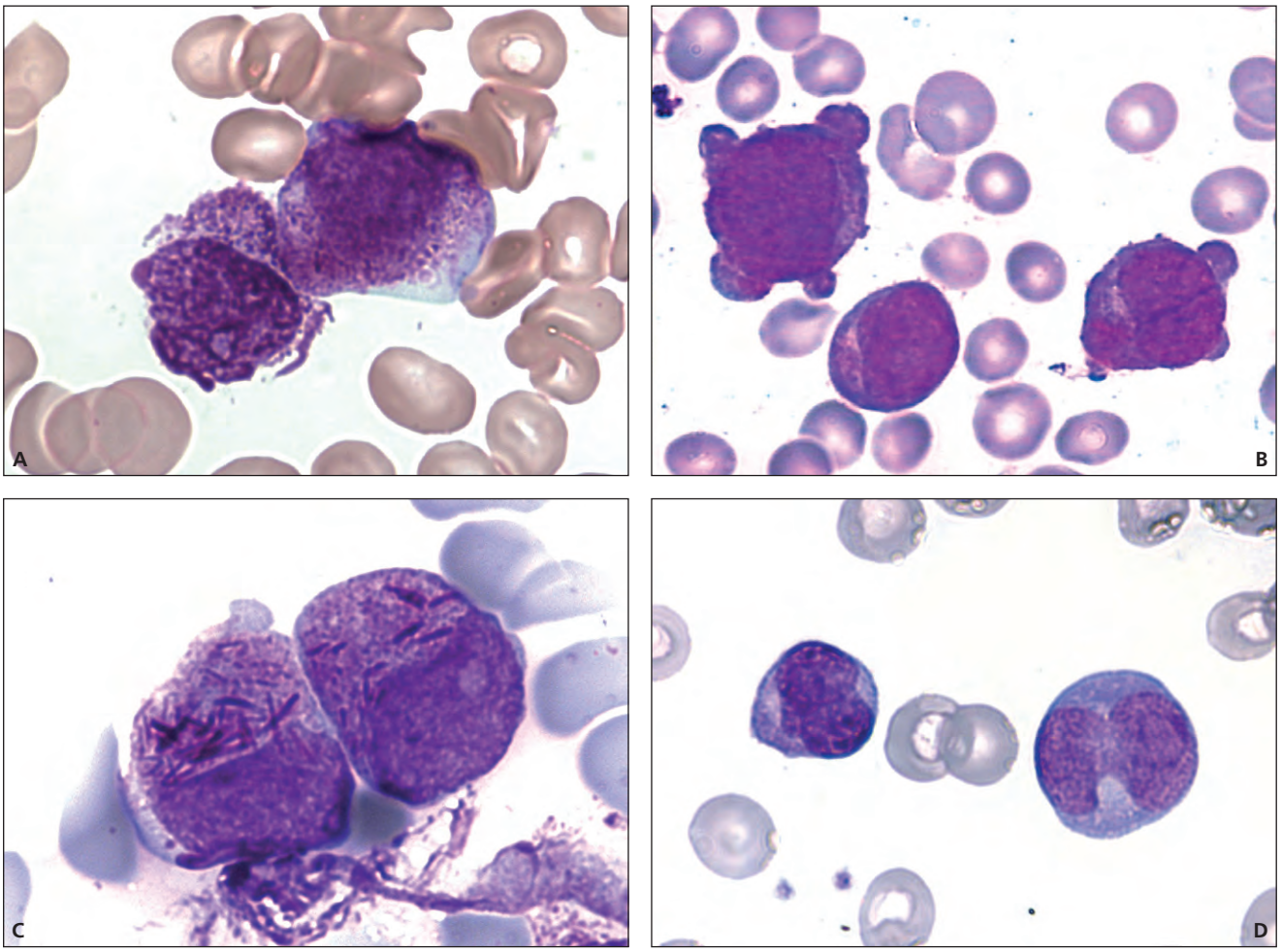



**Fig. 4.4** Various morphological features of blast cells in APL. (A) Two typical hypergranular promyelocytes. That on the right shows a characteristic very fine and gelatinous mass of granules, which surround and obscure the nucleus, leaving free a peripheral veil of weakly basophilic cytoplasm. (B) Leukaemic promyelocytes with intensely basophilic cytoplasm, violaceous and lacking in homogeneity because of the presence of granules, which extends into pseudopods. There is a suggestion of a Golgi zone and the nuclei show transverse furrows, sometimes producing, as in the cell on the right, the shape of a coffee bean. (C) Characteristic pathological promyelocytes with multiple Auer rods which, in the cell on the left, appear superimposed and form a loose bundle (a faggot cell). (D) Leukaemic cells in the peripheral blood of a patient with the microgranular variant of APL. While the smaller cell on the left resembles a monocyte, the large one on the right has morphology that is typical of this condition, with a bilobed nucleus with delicate chromatin and a giant nucleolus. To see how distinctive and characteristic this cell is, it can be compared with the cells in Fig. 4.48 from another patient with the same diagnosis.

cytochemical and immunological methods. Alternatively, they may have morphological features that suggest the lineage: typical monoblasts, for example, have a large round nucleus with delicate reticular chromatin that is very fine and homogeneous, with prominent nucleoli and ample, weakly basophilic cytoplasm; megakaryoblasts may have polyploid nuclei that are indented, notched or traversed by long grooves; they may have cytoplasmic features that suggest early platelet formation.

- Promonocytes: these cells are considered blast equivalents and thus when there is a monocytic component they are included in the blast count (on the cytomorphological plane, this is one of the more decisive innovations of the 2008 WHO classification): they have nuclear characteristics intermediate between those of monoblasts (chromatin that is fine and reticular with nucleoli possibly being present) and immature monocytes, with which they share characteristics such as the presence of notches and other irregularities that modify the nuclear outline. The distinction between monoblasts and promonocytes is not relevant for diagnosis, since both cell types are included in the blast count. However, it is very important to exclude from the blast count so-called atypical monocytes which are recognised in abundance in, for example, chronic myelomonocytic leukaemia[13]: the more condensed chromatin structure, the more irregular nuclear outline and the cloudy greyish cytoplasm are the features on which the differentiation is based.

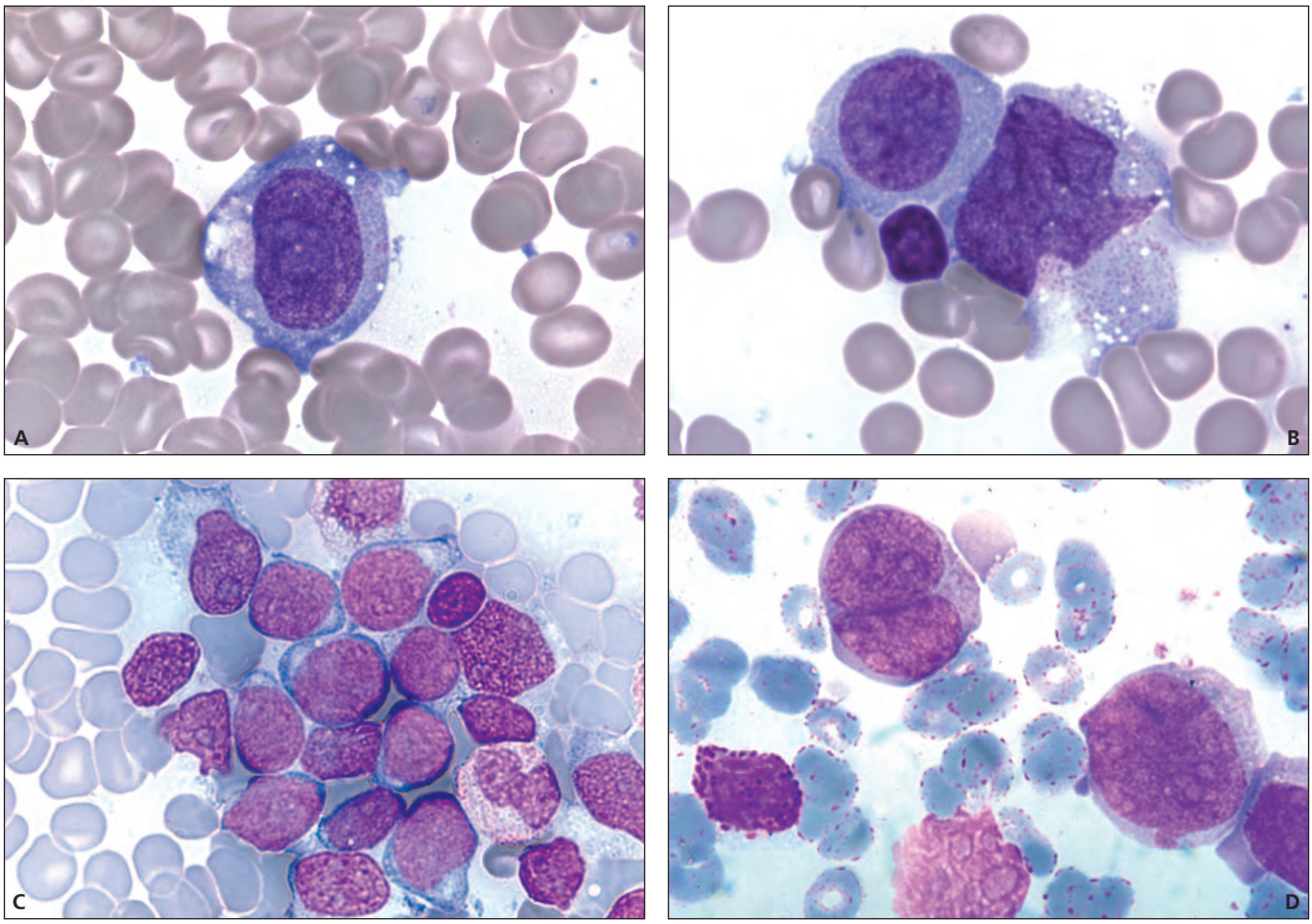



**Fig. 4.5** Blasts with features of specific lineages. (A) Monoblast: extremely delicate chromatin with visible, even if incompletely outlined, nucleoli; the nucleus can be oval, as in this case, but more often is rounded, as in (B), but with notches, folds and irregularity of the nuclear outline; the cytoplasm is basophilic and fairly abundant with vacuoles and fine dust-like granules (which can also be totally lacking). (B) Monoblast, on the left, with a rounded nucleus and abundant pale blue cytoplasm, which appears dusty though without visible granules. On the right, a typical promonocyte, with the nuclear chromatin still immature but with foci and linear areas of condensation; the nuclear outline is irregular with deep notches and folds; the cytoplasm is very plentiful, basophilic and vacuolated with numerous brick-red granules that are well separated from each other. (C) Apparently undifferentiated blasts. The chromatin is granular or stippled, the nucleus round and the cytoplasm basophilic, suggesting as one of a number of possible identifications, an erythroid cell; this was confirmed by immunophenotyping which showed this to be a case of pure erythroid leukaemia. (D) Megakaryoblasts in an acute leukaemia with megakaryocytic differentiation. It is not difficult to suspect the diagnosis in this case because of the large size and the nuclear morphology, with two lobes in the cell on the left, with condensation of the chromatin into small clumps, numerous nucleoli and grey-blue cytoplasm with scattered granules. On the left, a basophil.

- Erythroid blasts in the rare pure erythroid leukaemia, although these are not easily recognisable morphologically and are identified mainly from their immunophenotype.
- Promyelocytes with the specific abnormal morphological characteristics found in APL (previously FAB M3), including the microgranular variant.

The following cells must not be included in the blast count (Fig. 4.6):

- Dysplastic promyelocytes without the specific features of APL: these have more granules than granular blasts, and the granules can be coarse and atypical; the cytoplasm is more abundant, weakly basophilic with possible small areas that are non-staining or pink; a visible Golgi zone may be lacking; the morphology and distribution of the granules is irregular and disorganised; the nucleus tends to be central whereas in a normal promyelocyte it is eccentric. The features used for the cytological differentiation between granular blasts, dysplastic promyelocytes and normal promyelocytes have already been delineated in Chapter 3, dedicated to the MDS: the criteria for normality are based essentially on the orderly and harmonious distribution of the cellular components, with eccentricity of the nucleus and a pale circumscribed Golgi zone located in the nuclear indentation, around the outside of which are accumulated numerous granules[12]; the difficulty in making a distinction between normal and hypogranular dysplastic promyelocytes is greater.

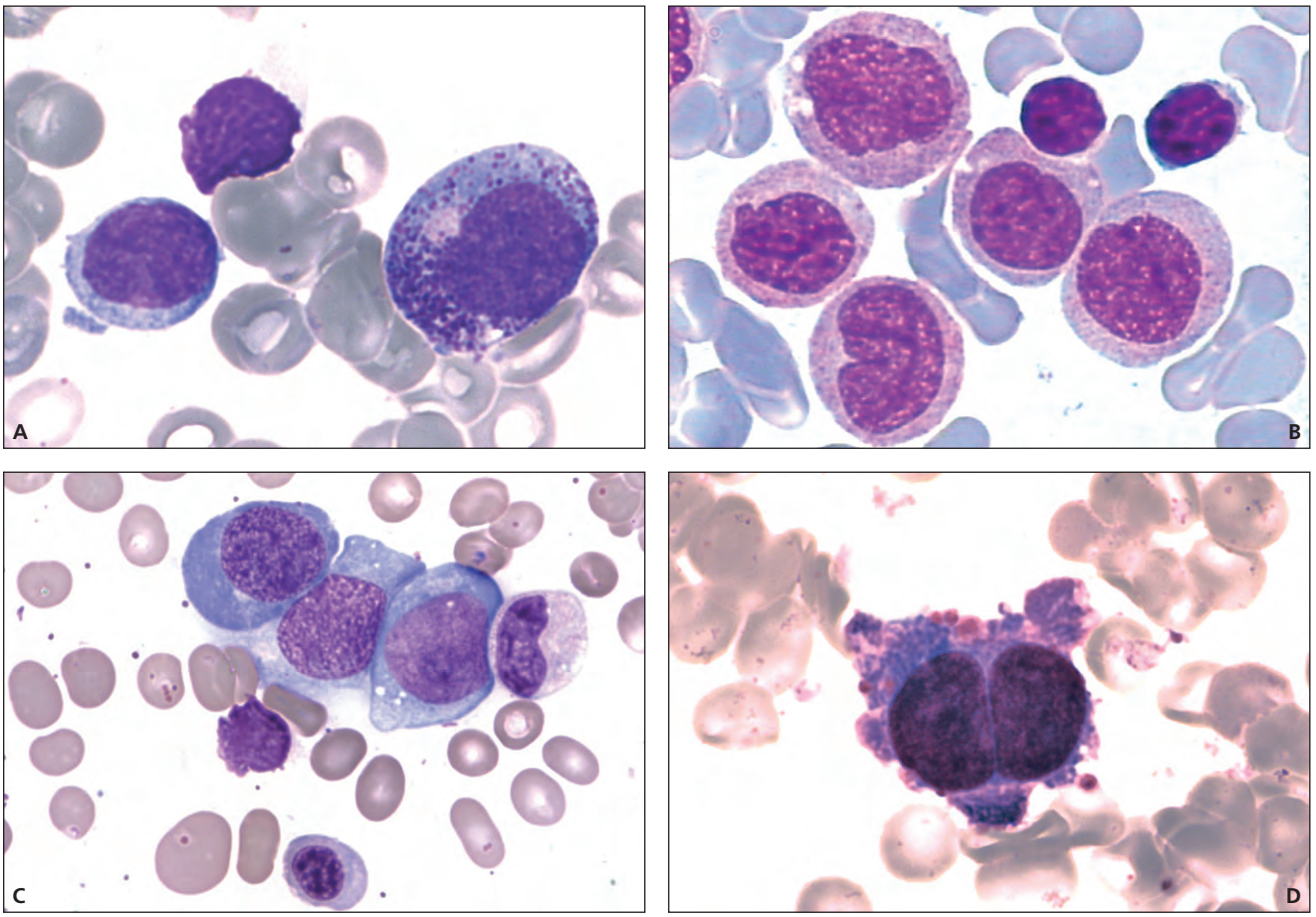



**Fig. 4.6** Immature or atypical cells that must not be included in the blast count when applying the criteria of the 2008 WHO classification. (A) On the right, a dysplastic promyelocyte, with condensed chromatin, numerous granules and asynchronous maturation of the cytoplasm, which shows a clear Golgi zone and many granules scattered in a disorderly manner, crowded together mainly at one pole, within the intensely basophilic cytoplasm. (B) Five atypical and dysmorphic monocytes: the nuclei are convoluted or reniform, with condensed chromatin which has a glassy appearance; there is extensive greyish cytoplasm with a violet tinge and a ground-glass, dusty appearance attributable to the presence of numerous fine granules that are not individually visible. (C) Promegaloblast and basophilic megaloblasts in the bone marrow aspirate of a patient with pernicious anaemia. The delicate chromatin network and the blue colour of the cytoplasm create the risk that this cell will be considered a blast cell, whereas it is the result of a reduced rate of synthesis of DNA. (D) Small dysplastic promegakaryocyte with two apparently separate nuclei, very condensed chromatin and a straight nuclear outline where the nuclei are in contact. The cytoplasm is basophilic and ragged where there is attempted, but ineffective, platelet production.

- Proerythroblasts, both normal and megaloblastic, or dysplastic, except in the case of pure erythroid leukaemia.
- Micromegakaryocytes and other types of promegakaryocyte, and dysplastic megakaryocyte.
- Atypical monocytes, recognised by their chromatin condensation and their cytoplasmic characteristics.

The classification of blast cells is rendered more difficult by the co-existence of myelodysplasia, by the presence of precursors with asynchrony of nuclear and cytoplasmic maturation, by atypical nuclear shapes and by hypogranularity. Sometimes, morphological doubts can be clarified by demonstrating cellular markers of immaturity, such as terminal deoxynucleotidyl transferase (TdT) and the CD34 antigen.

A good quality film is an indispensable requisite for the cytological recognition of blast cells. Cells that are insufficiently spread out, in a part of the film that is too thick, can appear to have more compact, hyperchromatic chromatin and the nucleoli may disappear: this leads to an underestimation of the number of blasts. Conversely, the incomplete rupture of fragile cells, such as some lymphocytes, can cause the chromatin to have an appearance similar to the chromatin of blast cells.

With regard to the significance of the number of blast cells, the FAB group originally proposed a blast plus promyelocyte count above 50% as a minimum criterion for a diagnosis of acute leukaemia and for its differentiation from the MDS with a blastic component.[6] In subsequent documents this was

changed to 30% blast cells, expressed as a percentage of all the nucleated bone marrow cells.[14] The 2001 WHO classification reduced this threshold to 20%, based on prognostic considerations that led to the inclusion of most of the previous RAEB-T group of MDS in the AML category. This minimum of 20% blasts, recognised either in the bone marrow or peripheral blood or both, now represents one of the universally accepted criteria for the diagnosis of AML. There are a few exceptions. Firstly, the diagnosis is accepted with a lower blast count in patients with the translocations t(8;21), t(15;17) and t(16;16) or with inversion of chromosome 16, and with the fusion genes characteristic of these chromosomal rearrangements, in which the typical bone marrow features occur with a blast count below 20%. Also excepted are some AML, NOS with erythroid differentiation, with more than 50% of erythroid cells, in which the blast count is not related to all cells but to non-erythroid cells (NEC) only, which excludes lymphocytes and plasma cells in addition to morphologically recognisable erythroblasts. Finally, the diagnosis of AML is made not only in acute megakaryoblastic leukaemia in infants with Down syndrome, but also in transient abnormal myelopoiesis seen in neonates with Down syndrome.

There exist cases in which the percentage of blast cells in the peripheral blood is higher than the percentage in the bone marrow[15]: in these so-called peripheral leukaemias, the threshold value may be exceeded only in the peripheral blood. The count of bone marrow blast cells presents notable difficulties in the case of hypoplastic leukaemia, in AML associated with fibrosis and in the form typified by marked trilineage dysplasia. In uncertain situations of this type, in which there are numerous features that favour a diagnosis of AML but the diagnostic blast threshold is not reached, it is useful if the morphological features are clearly described and an interpretation of these findings is offered; from this report a clinical judgement can be made. The final diagnosis and therapeutic decision must be based on the full range of information available (clinical history, severity of the peripheral cytopenia, age of the patient, clinical and morphological evolution of the disease over time).

Usually it is not difficult to make a generic diagnosis of acute leukaemia on the basis of morphology. In the lymphoid form, the distinction from lymphoblastic lymphoma can be difficult, but can be made on the basis of the degree of bone marrow infiltration and peripheral blood involvement. On occasion diagnostic doubts can arise between conditions that are fundamentally similar, such as acute monocytic leukaemia and malignant histiocytosis or acute megakaryoblastic leukaemia and acute myelofibrosis; however, there can also be diagnostic doubt between pathological processes that are quite different and have quite different therapeutic and prognostic implications, such as erythroleukaemia and pernicious anaemia. Sometimes, metastatic cells can diffusely infiltrate the bone marrow producing a picture that is very similar to acute leukaemia. One is dealing here with tumours that have a tendency to invade the bone marrow and that are composed of immature cells, with very dispersed chromatin and basophilic, often vacuolated, cytoplasm, which infiltrate as single cells admixed with haemopoietic cells rather than forming cohesive masses and aggregates that evoke an extramedullary origin; these include Ewing sarcoma, neuroblastoma, rhabdomyosarcoma, small cell carcinoma of the lung and very undifferentiated neoplasms of other origins (see Chapter 11). In these cases, confirmation of a suspected diagnosis may require cytochemistry, immunophenotyping, histology and immunohistochemistry. Finally, in some cases of multiple myeloma, the neoplastic population is so immature that it is not possible to recognise their plasma cell nature, and acute undifferentiated leukaemia is thus simulated: generally, these cells express cytoplasmic immunoglobulin with monotypic light chain and are recognisable from their immunophenotype.

The differentiation between AML and ALL nowadays is based on the assembly of diagnostic information, which provides multiple markers that are highly efficacious, specific and sensitive. Morphology establishes a positive diagnosis of a myeloid leukaemia on panoptically stained films in a good percentage of cases in which the blasts show some maturation. It is not possible, however, to establish a positive diagnosis of ALL solely on the basis of morphology and cytochemistry. Similar considerations apply to undifferentiated AML, pure erythroid leukaemia and megakaryoblastic leukaemia, which in the past were not uncommonly diagnosed as ALL on the basis of morphological and cytochemical characteristics.

The morphological criteria for identifying a population of cells as myeloid derive in the first place from cytoplasmic characteristics, in particular from the presence of typical granulation. Primary azurophilic granules identified in the cytoplasm are sufficient to define a cell as belonging to a non-lymphoid lineage, provided that their nature is confirmed by cytochemical demonstration of peroxidase activity. Rarely, peroxidase-negative granules are observed, e.g. in rare cases of ALL, usually with a common ALL immunophenotype, in natural killer (NK) cell leukaemia and in acute basophilic leukaemia. In ALL, the granules correspond to very large (peroxidase-negative) lysosomes.

Auer rods (Figs 4.3C and 4.4C) provide positive, non-equivocal evidence of the granulocytic nature of the cells that contain them: they are the only morphological marker with this absolute significance, equal to that of cytochemical evidence of peroxidase activity. Even a single peroxidase-positive Auer rod in a single cell of any type provides reliable evidence of a myeloid neoplasm (AML or refractory anaemia with excess of blasts type 2 [RAEB-2]). Fundamental to the differential diagnosis is the rare formation of crystals that appear similar to Auer rods, but which are negative for peroxidase, chloroacetate esterase (CAE) and Sudan black B, in neoplastic or reactive plasma cells[16] and in lymphoproliferative disorders and B-cell lymphomas.[17–19] Auer rods, described paradoxically in 1906 by the author from whom they derive their name in a leukaemia that was consid-

ered to be lymphoblastic,* are linear azurophilic structures, in the form of a needle, a rod or an elongated diamond, with their thickness and number being very variable in different morphological contexts: they are derived from primary granules by a process of lining up, coalescence and anomalous crystallisation, which can happen at the moment of their formation in the cisternae of the Golgi apparatus or subsequently, by fusion of already formed granules within autophagic vacuoles. On electron microscopy, which permits the recognition of the various phases of their formation, they appear to be membrane bound and have an internal structure of parallel filaments with a periodicity of 8–12 nm. Their lysosomal nature is confirmed by strong cytochemical positivity for peroxidase, CAE, Sudan black B and acid phosphatase. Auer rods are needles and crystals with very pointed ends: large rounded and oval formations that form by coalescence of abnormal granules in some cases of AML, particularly when there is granulocytic or myelomonocytic differentiation, have an analogous significance but the term 'Auer rod' should not be applied to them. By definition, Auer rods are never found in AML, NOS with minimal differentiation; in acute monoblastic and megakaryoblastic leukaemias they are very rare and their presence indicates involvement of the granulocytic lineage in the leukaemic process; they are observed with a variable frequency, between 30% and 70%, in the forms with granulocytic differentiation, in erythroleukaemia, in MDS and, very rarely, in blast crisis of CML. Other than in blast cells, they are sometimes observed in maturing cells of neutrophil lineage. Several classical studies have demonstrated that the presence of Auer rods in AML has a favourable prognostic significance in terms both of the response to induction chemotherapy and long-term survival.[20–22]

Other morphological criteria are much less specific, but nevertheless useful if evaluated globally. Myeloid blasts generally have an angulated nuclear outline and a more or less eccentric nucleus, which is sometimes notched or indented. The specific characteristics of chromatin are another characteristic that suggests the monoblastic, erythroid or megakaryoblastic nature of a blast cell. The Golgi zone is rarely pronounced in myeloid blast cells, an exception being AML with t(8;21).

Other morphological information on the nature of a leukaemic population can be derived not from the features of the blast cells but from the characteristics of more differentiated cells that co-exist with them in the blood and marrow. For example, the presence in an untreated patient of clear trilineage dysplasia in maturing cells together with undifferentiated blasts cells suggests that the blasts are myeloid. Particular value attaches to the recognition of dysplastic mature neutrophils. Anomalies of maturation in the monocyte, erythroid and megakaryocyte lineages suggest that these are involved in the leukaemic process and direct the diagnosis towards AML. The presence of eosinophilia does not have the same diagnostic value: increased eosinophils in the peripheral blood and bone marrow may be seen in AML, but also in blast crisis of CML, in a significant proportion of cases of ALL and in multiple myeloma. Consequently, it is important to seek evidence of clonality of the eosinophil component (see Chapter 6).

## Characterisation: cytochemistry

Cytochemical confirmation remains important in the initial diagnostic approach in AML.[23] According to the recommendations of the International Council for Standardization in Haematology (ICSH),[24] an initial cytochemical evaluation of acute leukaemia is based on three methods:

- Peroxidase;
- Monocytic esterases;
- Chloroacetate esterase (CAE), which is generally specific for neutrophils (but is also positive in the abnormal eosinophils associated with inversion of chromosome 16).

The most important and effective technique is a peroxidase reaction (Figs 4.7, 4.8 and 4.9). The most widely used method employs as a substrate diaminobenzidine or 4-chloro-1-naphthol, which precipitate in the cytoplasm when oxidised by nascent oxygen that is released from hydrogen peroxide in primary granules or lysosomes. The utility of the method is increased by the use of a Giemsa counter stain, which permits the optimal morphological definition of positive and negative cells. These techniques identify not only myeloperoxidase (MPO), which is seen as strong clear staining in the neutrophil series and as fine scattered granules in monocytes, but also eosinophil peroxidase.

According to the FAB criteria, and also in the AML, NOS category of the WHO classification, the diagnosis of AML with granulocytic differentiation requires the presence in the bone marrow of at least 3% of peroxidase-positive blast cells. This criterion takes account of the possible persistence of a small proportion of normal myeloblasts in the bone marrow of patients with ALL. Ultrastructural examination has confirmed that peroxidase activity of myeloid blast cells can be localised in granules of less than 250 nm in diameter, below the level of resolution of the light microscope. In any event, it is possible to diagnose AML with total peroxidase negativity in various circumstances:

- In AML with minimal evidence of differentiation in which the myeloid nature can be demonstrated by more sensitive methods, such as ultrastructural cytochemistry, immunophenotyping with anti-MPO or with other monoclonal antibodies directed at specific antigens of the myeloid series or by investigation for messenger RNA for MPO[25];
- In acute monoblastic leukaemia;
- In pure erythroid leukaemia;
- In AML with megakaryocyte differentiation;
- In individuals with an inherited peroxidase deficiency or acquired peroxidase deficiency (as can occur in neutrophil

*Auer also indicated that the first reference to these rod-like cytoplasmic inclusions had been made by T. McCrae (Seymour SF. 101 years of McCrae's (not Auer's) rods. Br J Haematol 2006, 133:690).

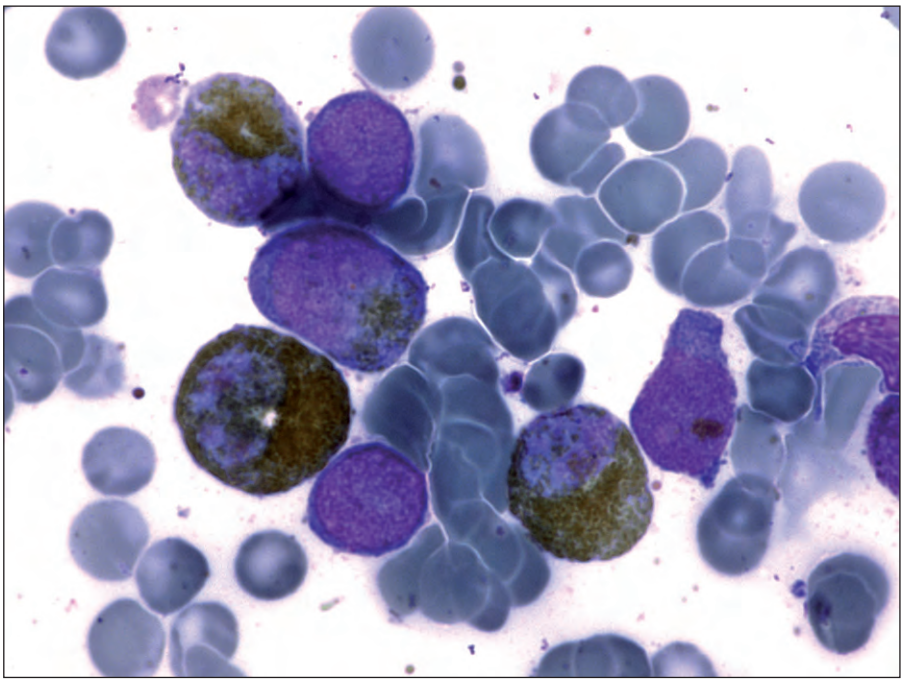


**Fig. 4.7** Peroxidase stain in AML. There is a diffuse dark golden yellow, almost blackish precipitate in the most mature cells, which express this enzymatic activity strongly, but focal activity in the centre of the cytoplasm in the less differentiated blast cells.

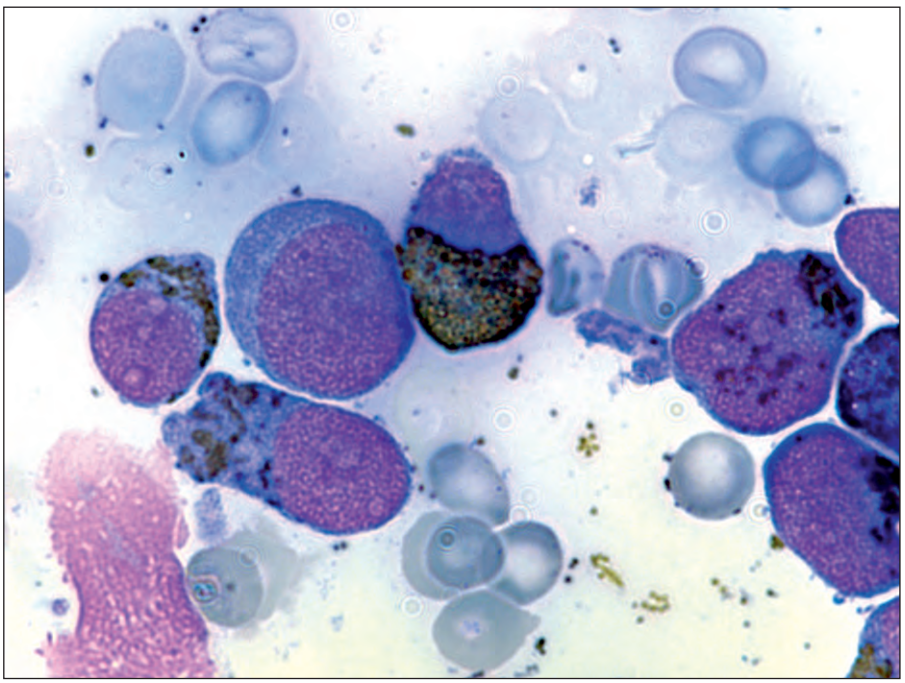


**Fig. 4.8** Granular peroxidase activity in a case of acute myelomonocytic leukaemia (AMML). The cell with large distinct golden-yellow granules, some very dark, which crowd the cytoplasm and completely cover the nucleus, is an eosinophil myelocyte. The other cells are blasts, one of which is peroxidase negative.

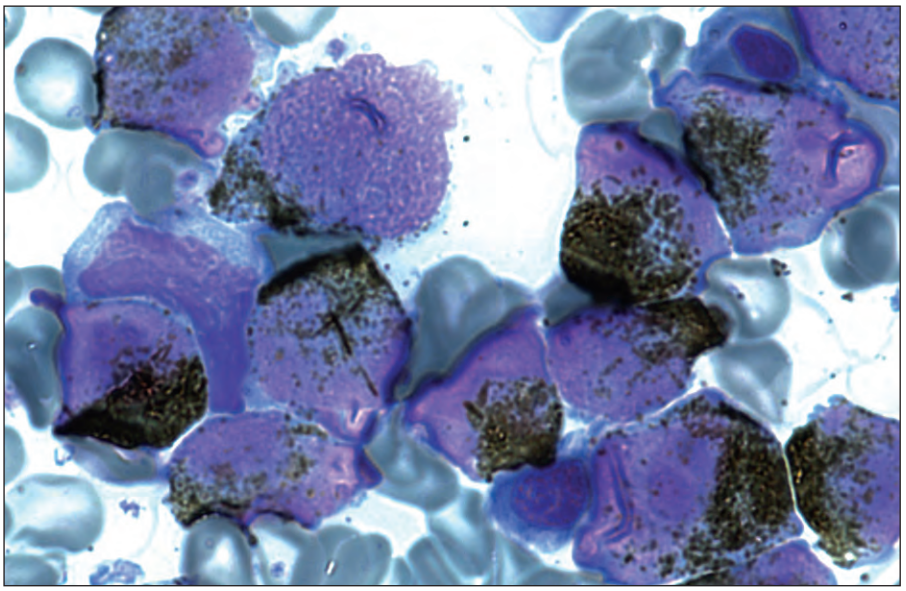


**Fig. 4.9** Peroxidase reaction showing very intense positivity in APL. Auer rods are always very strongly positive and are very well seen when they are superimposed on the nucleus.

dysplasia): in these cases Sudan black B can be useful or, preferably, the flow cytometric demonstration of MPO protein with monoclonal anti-MPO antibodies.

Esterases are enzymes that hydrolyse aromatic and aliphatic esters. They are used principally to demonstrate that a cell belongs to the monocyte lineage: they are, however, present in cells of numerous lineages, with the specificity of the reaction being very variable, depending on the substrate used. The use of sodium fluoride, which selectively inhibits monocyte esterase, increases the specificity. The cytochemical demonstration of esterase activity is based on the activity on various synthetic esters of naphthyl, which permits subdivision into four categories:

- Alpha-naphthyl acetate esterase (ANAE): gives strong evidence of monocyte lineage with its specificity being increased by demonstration of inhibition by fluoride; this is considered the technique of choice by the ICSH; megakaryocytes also have a positive reaction with focal positivity in leukaemic megakaryoblasts;
- Alpha-naphthyl butyrate esterase (ANBE): more specific for the monocytic lineage, to the extent that it can be used without fluoride inhibition; it is less sensitive than ANAE (there exist monocytic leukaemias that are ANAE positive ANBE negative, even by electron microscopy) (Fig. 4.10); megakaryocytes are negative;
- Naphthol AS-D acetate esterase (NASDE), positive in monocytes, neutrophils and other haemopoietic cells; it requires sodium fluoride inhibition, but its diagnostic use is not advised because of limited specificity;
- Naphthol AS-D chloroacetate esterase (CAE) is specific for cells of neutrophil lineage, which give a strongly positive, brilliant red reaction: it is less sensitive than a peroxidase reaction because it appears later in myeloblast maturation, being also positive in promyelocytes; it is also positive in mast cells and bone marrow histiocytes.

From the point of view of AML classification, the monocyte esterases, such as ANAE inhibited by sodium fluoride, and ANBE, are optimal markers for a leukaemia that originates, in whole or in part, from cells belonging to the monocyte lineage. Positivity is moderate or strong; either diffuse or a mixture of granular and diffuse. Sensitivity is high but not absolute: between 10% and 20% of cases of AML that are certainly of monocyte lineage are negative for all esterase reactions. Specificity is high, but there are certain exceptions that one must be aware of. In acute hypergranular promyelocytic leukaemia the ANAE reaction can give a positive reaction, either fine granules or diffuse activity, resulting from increased expression of non-specific esterase; there is a variable resistance to sodium fluoride inhibition. Similar observations can be made in some cases of the microgranular variant, which can at times lead to some diagnostic difficulty in the differential diagnosis with acute monocytic leukaemia. In AML with granulocytic differentiation, reactions are usually negative, with rare cases of granular or focal positivity in the Golgi area. ANAE is positive in megakaryocytes: a leukaemia with blasts that are ANAE positive and ANBE negative may be megakaryoblastic. Esterase positivity has been reported in leukaemic erythroblasts and in T-ALL (with a typical focal or polar positivity).

CAE is useful for defining the neutrophil lineage of an AML. Specificity is high, even if aberrant positivity has been reported in rare cases of ALL (particularly when the lymphoblasts have granules), in erythroblasts of erythroleukaemia and in some pure monoblastic leukaemias.[26] In acute myelomonocytic leukaemia (AMML) a double esterase stain is particularly useful,

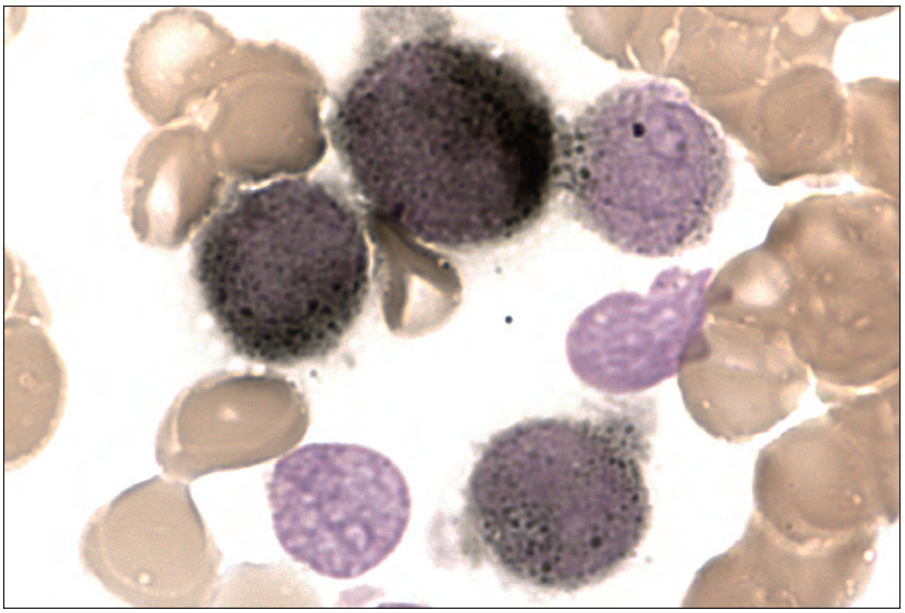


**Fig. 4.10** Cytochemical stain for alpha-naphthyl acetate esterase (ANAE) in a case of acute monocytic leukaemia. The quantity of the blackish precipitate tends to correlate with the maturity of the cells: monoblasts usually have a granular reaction of only moderate intensity, while promonocytes and even more differentiated cells have cytoplasm that is completely positive so that the nuclear outline and structure are obscured.

combining ANBE (or ANAE with inhibition) with CAE, with the aim of demonstrating from a single slide the double nature of the leukaemic population. With this method, the cells of the neutrophil lineage are bright red, while the monocytes and their precursors appear blue or black, depending on the substrates and the chromogens used (Fig. 4.11).

Among the other methods used in classical haematological cytochemistry are Sudan black B, which stains various types of molecule, particularly lipids (neutral fats, phospholipids, sterols), giving results parallel to those of peroxidase. It is, however, less specific, being able also to stain pathological cells in a small number of cases of ALL and other lymphoid neoplasms. Nevertheless, Sudan black B gives strong positivity, localised or diffuse, in blasts of granulocyte lineage and fine scattered granules in those of monocyte lineage. Some authors, in contrast with the low value given to Sudan black B in the ICSH document, maintain that positivity with this cytochemical reaction constitutes an important criterion for distinguishing less differentiated from well-differentiated AML, with complete remission being more likely in those with a higher percentage of Sudan black B-positive cells.[27] The periodic acid-Schiff (PAS) reaction shows, with an elegant red or deep red staining, the presence in cells of carbohydrate, glycogen, mucopolysaccharide, glycoproteins and glycolipid. It is normally positive in neutrophils and, weakly, in monocytes, platelets and megakaryocytes. It is negative in eosinophils, erythroblasts and lymphocytes, in which, however, there may be fine granular positivity. According to traditional ideas, PAS permits the distinction of ALL (granular or block positivity that surrounds the nucleus like a rosary on a clear cytoplasmic background) from AML (positive granules on a background of diffuse pink). The diagnostic value of a PAS stain is, however, modest because of its lack of specificity. The most useful information that it gives is when it shows abnormal positivity in erythroblasts in erythroleukaemia and in eosinophils in AMML with aberrations of chromosome 16. Acid phosphatase is present in almost all nucleated cells and also in platelets. It therefore has a very low level of specificity. In T-ALL there is often a typical strong focal positivity, which was diagnostically useful before the wide availability of immunophenotyping. Other cytochemical techniques can be used in specific contexts, such as metachromatic staining with toluidine blue, alcian blue or astra blue, which permits the recognition of basophils and mast cells (the latter also being CAE positive); Oil Red O staining for lipids, which stains strongly the vacuoles of circulating Burkitt lymphoma cells; methyl green pyronine, which demonstrates the presence of RNA in cytoplasm and stains plasma cells and Burkitt cells intensely. Prussian blue staining for the demonstration of iron is useful not only for assessing iron stores, but also for the demonstration of ring sideroblasts and is therefore useful in the diagnosis of MDS and erythroleukaemia and AML associated with or secondary to myelodysplasia.

## Characterisation: immunophenotyping

The nature of leukaemic cells can be even more precisely demonstrated with the use of monoclonal antibodies directed at antigens specific for individual haemopoietic lineages.[28,29] Immunophenotyping has had less diagnostic impact in AML than in ALL because of the availability of valid morphological and cytochemical criteria. It does however have considerable

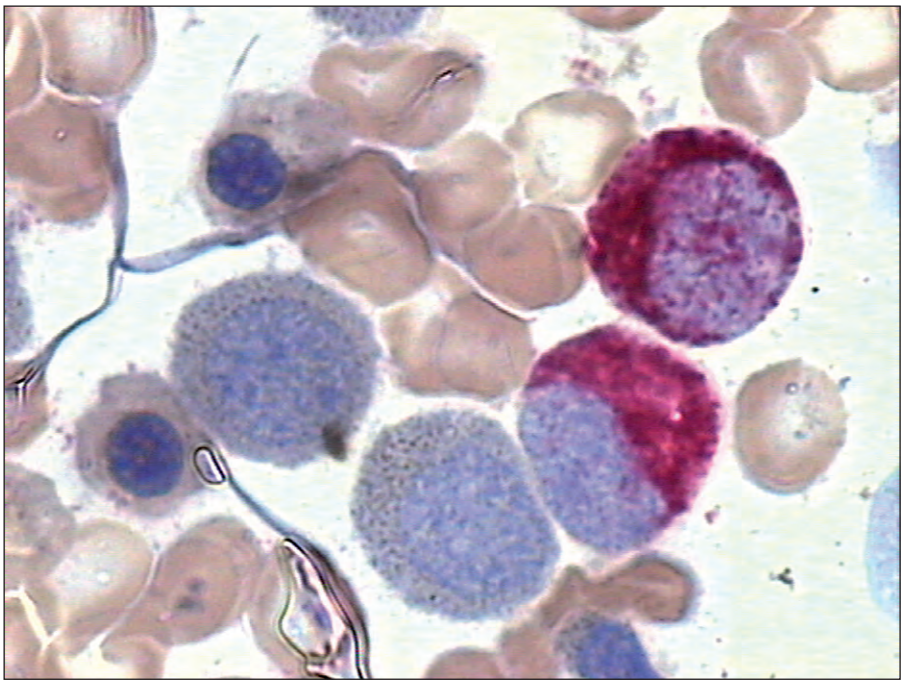


**Fig. 4.11** A double esterase reaction provides very significant information in myelomonocytic leukaemia in which the blasts and maturing cells of granulocytic lineage are red, indicating chloroacetate esterase activity, while the blasts and maturing cells of monocyte lineage display a black precipitate, indicating ANAE activity.

value in AML with undifferentiated blasts and in AML with erythroid or megakaryocytic differentiation. Multiparameter flow cytometry is very precise and can analyse entire populations simultaneously for a large number of antigens, using monoclonal antibodies bound to various different fluorochromes. Immunophenotypic analysis of myeloid neoplasms requires large antibody panels in order to recognise the lineage to which the blast cells belong, detect the expression of aberrant markers of other lineages and diagnose mixed lineage and ambiguous acute leukaemias. In some cases, the investigation of cytoplasmic antigens is fundamental, e.g. to demonstrate MPO positivity. Very important information, which completes the microscopic evaluation of sections of bone marrow biopsies, is obtained by immunohistochemistry; it is valuable for the semiquantitative assessment of CD34-positive blast cells, particularly in hypocellular AML and AML with associated fibrosis, and for demonstration of specific lineages, such as erythroid or megakaryocytic.

Flow cytometric immunophenotyping provides useful complementary information in all cases:

- The position of blast cells on the two-dimensional cytogram using CD45/SSC (side scatter): myeloid blasts show more scatter than lymphoblasts, because of the presence of cytoplasmic granules;
- Demonstration of non-specific markers of haemopoietic progenitors (CD34, CD38, HLA-DR, CD117), some of which may be lacking in APL;
- Demonstration of lineage-specific antigens indicating differentiation, which appear progressively in the various phases of haemopoietic maturation and are found in various associations in corresponding myeloid neoplasms:
  - Granulocytic series: CD13, CD33, CD15, CD16, CD65, MPO
  - Monocytic series: CD11b, CD11c, lysozyme, CD4, CD14, CD64
  - Erythroid series: CD71 and CD36 (non-specific), CD235 (glycophorin A)
  - Megakaryocyte series: CD41, CD61, CD42;
- Demonstration of aberrant expression on myeloid blasts of lymphoid markers: CD2, CD7, CD19, CD56.

Characteristic antigen profiles, which will be described below, have been described in certain AML with recurrent genetic abnormalities, including APL and AML associated with t(8;21): these data are particularly useful for indicating the need for genetic-molecular analysis with fluorescence *in situ* hybridisation (FISH) or reverse transcriptase-polymerase chain reaction (RT-PCR).

At the clinical level, the presence of certain markers, such as aberrant expression of CD7, can identify particular subgroups of AML that show significant prognostic differences. The demonstration of particular antigens, such as CD33, can be very valuable in relation to the use of specific monoclonal antibody therapy. Finally, immunophenotyping is fundamental for the identification of minimal residual disease in patients who are in remission following treatment.

### Further characterisation: cytogenetic and molecular analysis

Cytogenetic and molecular analysis is being increasingly applied in AML, both because of its capacity to recognise specific entities that are now included as WHO categories and for its value in indicating certain mutations that are relevant to prognosis and indicate the need for specific forms of therapy.[30]

The great progress in cytogenetics has made it possible to demonstrate chromosomal anomalies in 70–85% of cases of AML and to identify genetic rearrangements associated with specific morphology and clinical evolution.[31,32] Cytogenetic analysis is essential in new cases of AML and must also be repeated during the course of the illness in order to detect evidence of disease evolution.

Seeking genetic alterations with specific probes using FISH or real-time quantitative polymerase chain reaction (RQ-PCR) is very valuable when indicated by the results of morphological, cytochemical or immunophenotypic investigations. From the point of view of practical diagnostic applications, these new methods have established an important link between the study of the genome, morphology and clinical evolution. In some cases, the transcripts of fusion genes represent a specific molecular marker of the illness. Investigation by quantitative PCR is also useful for following the response to treatment.

Molecular diagnosis,[33,34] by gene sequencing, allele-specific PCR and other techniques, permits the identification of mutations in single genes, which, in some cases (e.g. *KIT*, *FLT3*, *NPM1* and *CEBPA*) are important for diagnosis and prognostic stratification. Genetic anomalies are now identifiable by new and complex technologies, such as genetic profiling and study of single nucleotide polymorphisms by microarray analysis, measurement of the expression of microRNA or of DNA methylation and proteomic profile.[35,36]

## Acute myeloid leukaemia with recurrent genetic abnormalities

The first category that the WHO classification identifies in the AML group is the form in which recurrent specific clonal genetic abnormalities have been identified, generally resulting from a balanced translocation. The structural rearrangement of the chromosomes results in the formation of a fusion gene that encodes a chimaeric protein, this being considered a fundamental event, even if it is neither the only event nor in itself sufficient for leukaemic transformation. In the majority of cases, the genes involved have a role in haemopoietic differentiation.

The value of identification of this type of leukaemia is not limited to the biological and aetiopathological planes. These subcategories constitute homogeneous clinicopathological entities, often already diagnosable from morphology, with particular immunophenotypic characteristics and a prognostic significance that is sometimes favourable, as in the form associated with t(8;21), and sometimes unfavourable, as in the form associated with t(9;11).

In this category one can recognise, relating morphological diagnosis and genetic abnormalities, three subgroups. The first includes three entities that have a sufficiently distinctive and consistent clinical and morphological picture that the recurrent genetic abnormality can often be predicted.[1] These had already been described in detail in the most recent FAB classification[7]:

- AML with maturation, associated with t(8;21);
- AMML with anomalous eosinophils, associated with t(16;16) or inv(16);
- APL, associated with t(15;17).

The first two of these have related molecular abnormalities, comprising cytogenetic rearrangement involving genes that encode the subunits of core binding factor (CBF).

These three entities are so characteristic that, exceptionally in the WHO classification, the diagnosis of AML can be made even if the blast percentage is less than 20% in both the peripheral blood and the bone marrow.

The second group in this category of leukaemia, associated with other recurrent genetic abnormalities of which the most common is t(9;11), are not diagnosed as AML with a blast count below 20%, the molecular data in this circumstance not being considered sufficient to make such a diagnosis.

The third group, more recently and incompletely identified, is constituted of AML in which specific alterations of single genes are identified.

### Acute myeloid leukaemia with characteristic morphology and mutation of core binding factor genes

These two forms of AML have different morphological features, but they are often grouped together in clinical studies because of their related molecular mechanisms and their shared good prognosis. The frequency of complete remission with standard induction chemotherapy is, in fact, 90% and the incidence of relapse is low.[37–39] CBF is a heterodimeric transcription factor, the correct synthesis of which is essential for the normal harmonious processes of normal haemopoiesis. It is constituted of:

- A DNA-binding CBFα subunit, which is one of three possible subunits (encoded by *RUNX1*, *RUNX2* or *RUNX3*, homologues of the runt gene that is involved in segmentation in Drosophila);
- A CBFβ subunit, which can bind to RUNX1, RUNX2 and RUNX3, but not to DNA; it protects RUNX1 or one of its homologues from proteolysis.

The RUNX1 subunit, also called AML1, binds to promoter sequences of DNA and its union with CBFβ protects it from proteolysis.

The translocation t(8;21)(q22;q22) results in the fusion of part of *RUNX1* (*AML1*), which is found at 21q22, with the *RUNX1T1* gene (also called *ETO*, from eight-twenty-one), with the formation of a chimaeric *RUNX1-RUNX1T1* (*AML1-ETO*) fusion gene, typically associated with leukaemic transformation with granulocytic differentiation. Inversion of chromosome 16,

inv(16)(p13q22), or the translocation t(16;16)(p13;q22) leads to fusion of part of *CBFB*, which is found at 16q22, with part of the gene for the heavy chain of smooth muscle myosin, *MYH11*. The chimaeric *CFB-MYH11* encodes a protein that has a negative effect on the transcriptional activity of *RUNX1*.

The identification of transcripts of these two chimaeric genes, by means of RT-PCR or FISH, permits the diagnosis of AML despite there being less than 20% blast cells.

The genetic data, furthermore, have a highly specific diagnostic value when detected by karyotypic analysis and, even more so, when detected by molecular techniques such as RT-PCR or FISH, which are more rapid and sensitive and can identify cryptic rearrangements that are not visible with standard cytogenetic techniques. These techniques can also serve to demonstrate, during or following treatment, the absence of the fusion gene or fusion transcript during complete remission.

Karyotypic analysis also permits the identification of secondary chromosomal abnormalities, which are frequent in the CBF-leukaemias: loss of a sex chromosome and deletion of the long arm of chromosome 9 in cases with t(8;21); trisomy of chromosome 8, 21 or 22 in the form associated with inv(16).[40] In a third of cases of both subtypes, mutations have recently been described in the *KIT* gene, which encodes a tyrosine kinase-type receptor, implicated in cellular proliferation and survival, and interesting because it is associated with a less favourable prognosis, and because of the possible therapeutic application of tyrosine kinase inhibitors.[41,42]

### Acute myeloid leukaemia with t(8;21)

The translocation t(8;21)(q22;q22) was described in 1973, but only ten years later was its specific association with a subtype of AML, with characteristic morphological and clinical aspects, recognised.[43] It is a common form of AML, representing around 7–9% of *de novo* cases and 10–30% of the FAB M2 category. It is slightly more common in men and in younger patients. It can present with solid extramedullary tumours (myeloid sarcoma) or with a central nervous system localisation. It is distinguished by a good prognosis, a high probability of complete remission and often an enduring remission. Less common, but not rare, is the therapy-related form of this leukaemia: in this instance the prognosis is less favourable.[44] This form of AML is hardly ever associated with trilineage myelodysplasia (being on the contrary associated with dysplasia affecting maturing granulocytes of the leukaemic clone). Rare karyotypic variations have been described, such as complex translocations that involve also a third chromosome.

Peripheral blood leucocytosis is common (see Report 4.1) and bone marrow infiltration is often conspicuous (Figs 4.12 and 4.13). The morphological picture is so characteristic that it often suggests the presence of the aberration before cytogenetic analysis has been done,[45] and in addition can suggest the presence of this genetic abnormality in cases that are not detected by standard cytogenetic analysis, but that can be confirmed by PCR.[46] The predictive power of morphology is similar to that observed in chronic granulocytic leukaemia, in relation to the presence of *BCR-ABL1*. The blast cells have clear signs of granulocytic differentiation (Figs 4.14 through to 4.23). They are fairly large, with the N:C ratio not being very high; the nucleus is often more or less deeply indented, with pale open chromatin in a delicate network, possessing one or two evident nucleoli. There is asynchrony between the very immature nucleus and the abundant cytoplasm, characterised by clear signs of abnormal

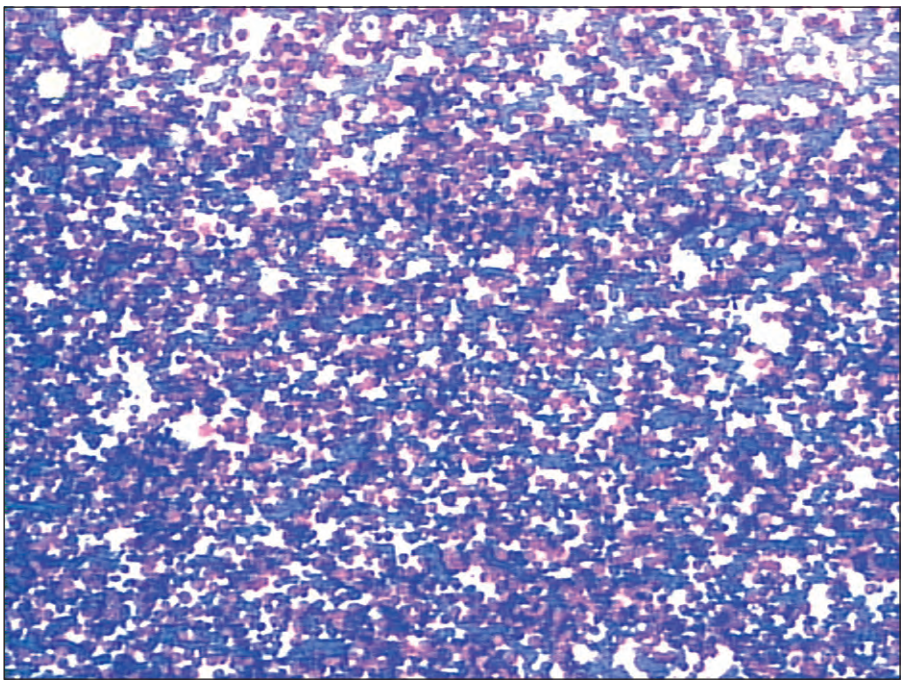


**Fig. 4.12** Acute myeloid leukaemia with the t(8;21) recurring genetic abnormality. Even at low power the bone marrow film shows massive monomorphic infiltration by cells with large nuclei that almost outnumber the red cells in the background.

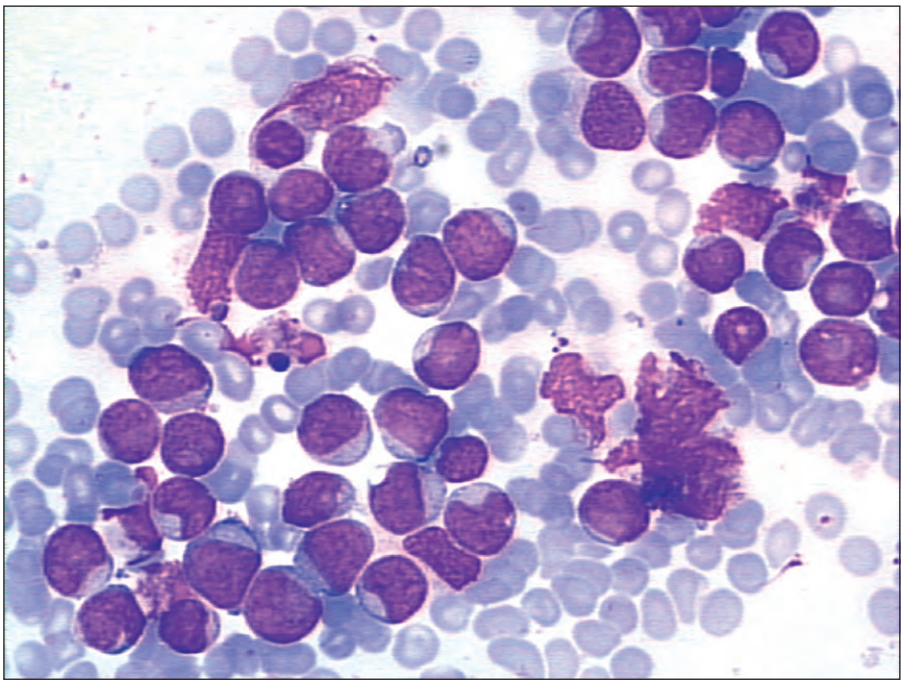


**Fig. 4.13** At higher power, same film as Fig. 4.12, one can confirm the morphological uniformity of the cellular population that is occupying almost all the available space, apparently obliterating any residual normal haemopoiesis. The blast cells are medium sized to large but with a normal N:C ratio because of the presence of large eccentric nuclei, some of which are notched, surrounded almost always on only one side by a moderate amount of basophilic cytoplasm; some of the cells give the impression of granularity. However, blastic infiltration is not always as total as this and often blast cells with these morphological features are accompanied by dysmorphic granulocyte progenitors and morphologically normal erythroblasts.

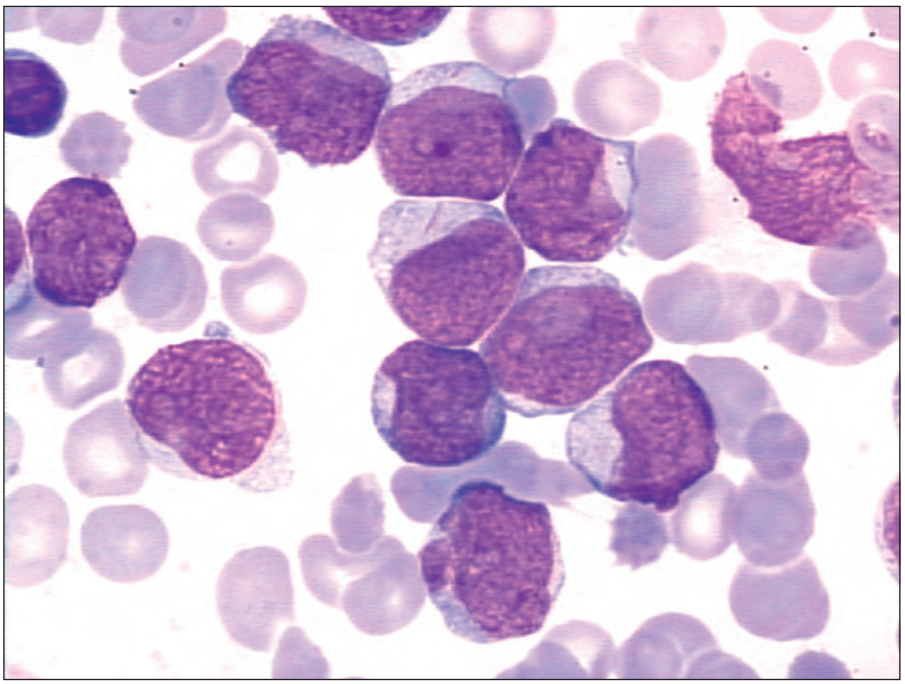


**Fig. 4.14** Same case as Figs 4.12 and 4.13, at higher magnification and in a thinner part of the film. The blasts have fairly homogeneous and repetitive morphological features. The nuclei tend to be oval or polygonal, with angulation, small notches and straight sections. The chromatin is open, immature, reticular or loose; one can see nucleoli, but they are not prominent. A cell centre right shows a large pale nuclear hollow. The cytoplasm is moderately abundant, weakly basophilic with a sky-blue or cerulean colour in the smaller more compact cells; a cell at the top contains fine isolated granules. In the large blast cell in the centre, a long thin Auer rod, the cytological correlate of this genetic subtype of leukaemia, can be seen in the absence of any apparent granulation.

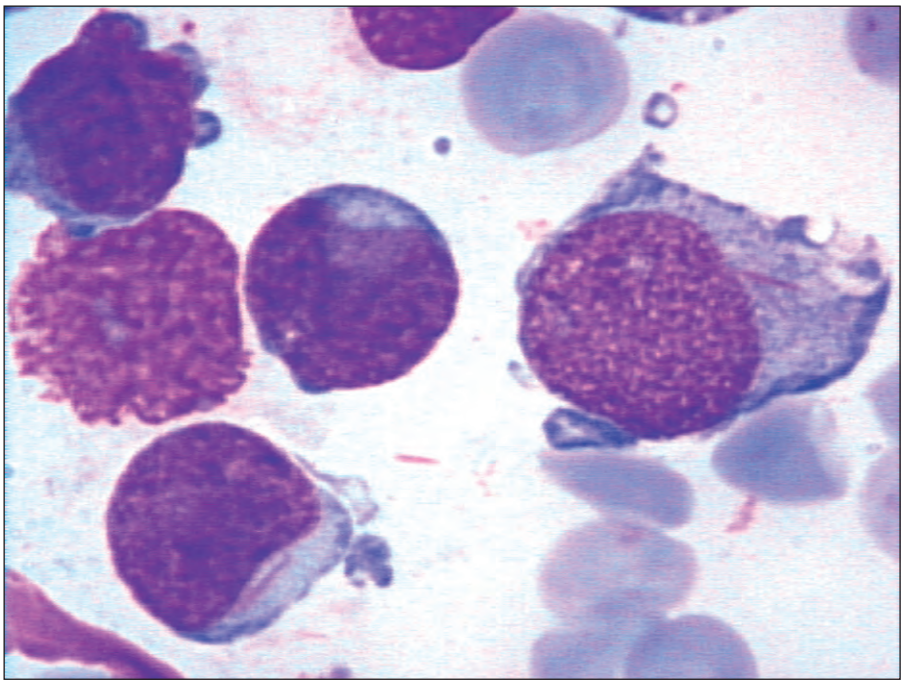


**Fig. 4.15** AML with t(8;21); bone marrow aspirate. On the left are two small blast cells with compact chromatin and a high N:C ratio: the scanty cytoplasm is consistently basophilic and is to one side of the eccentric nucleus, which is touching the cytoplasmic membrane. A third blast cell, on the right, is larger and has an almost completely round nucleus with granular cytoplasm. The pale clear cytoplasm of the blast cells in this subtype of leukaemia permits an appreciation of the presence, shape and wine-red colour of the Auer rods. In this image one sees three long pointed Auer rods, one of which is extracellular as the result of perforation of the cytoplasmic membrane of another cell; perhaps it belongs to the torn nucleus that is seen close to the left edge of the image.

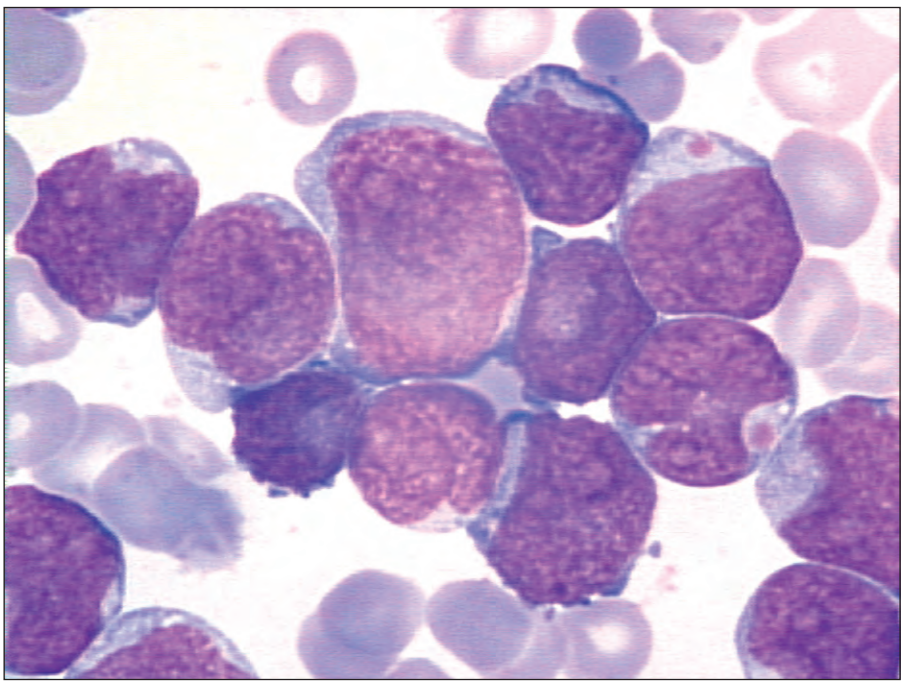


**Fig. 4.16** AML with t(8;21); bone marrow aspirate. The blasts in this patient have a more irregular outline and are more variable in size; the chromatin is light and homogeneous; there are small dark foci of condensation and frequent, not very prominent, nucleoli. One of these cells has a large tetraploid nucleus. Two cells show a single giant granule formed by coalescence.

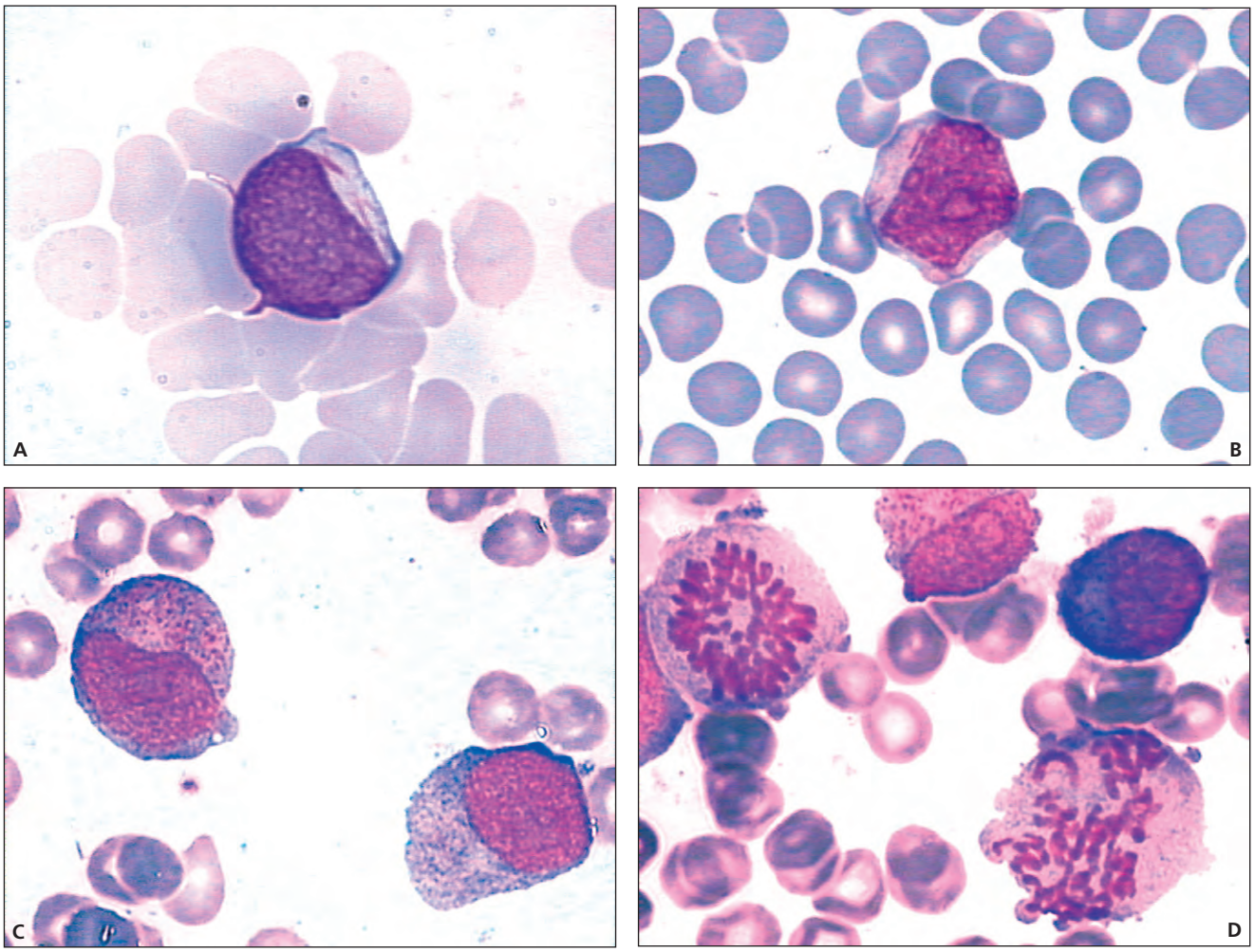



**Fig. 4.17** The morphology of AML with t(8;21) is very characteristic and includes certain key features which in part are summarised here. (A) The nucleus, with homogeneous but not particularly loose chromatin, is eccentric, sometimes touching the cellular membrane. Its outline is angulated with some straight sections in the area facing the cytoplasm; the cytoplasm is moderately abundant, weakly basophilic and contains granules and Auer rods, the latter more often single but sometimes, as in this case, double or triple. (B) Nucleoli are often present, sometimes prominent and irregular, as in this cell, which has cytoplasm that is in part pink and contains a pair of Auer rods that are blunter than normal. (C) The cell on the left, with a Golgi zone and mature granules creating a 'rising sun' appearance in the blue of the very basophilic cytoplasm, is a dysplastic promyelocyte. The cell on the right is clearly a myeloblast, in which the very fine granules, even though they cannot be individually recognised, alter the colour of the cytoplasm to a variable extent. (D) In this subtype of leukaemia, mitotic figures are usually fairly orderly, like that seen top left, and are not infrequent.

maturation: beginning from a circumscribed focal area of pallor, corresponding to the Golgi zone that nestles in an indentation in the nucleus, there is propagation of a variable number of azurophilic granules that appear around its periphery; these are bright red and stand out from the basophilic background, which is more intense peripherally where granules are lacking; sometimes there is some vacuolation. Auer rods are the most characteristic feature of these blast cells: they are usually single, rarely double or triple; they are always very long, often thin, sometimes fusiform and sometimes needle-shaped with a pointed tip. One can also see giant, coalescent globular granules, which resemble those of the Chédiak-Higashi anomaly. In some patients, the blast percentage is low, but in this instance the diagnosis is confirmed by demonstration of the molecular abnormality.

The tendency of these blasts to differentiate is reflected in the rarity of severe neutropenia and the presence in the bone marrow of promyelocytes and myelocytes, which are often dysplastic. Morphological abnormalities in mature neutrophils are also common: hypolobated nuclei, reduced granulation, anomalous granules and even, rarely, Auer rods (Fig. 4.24). These signs of granulocytic dysplasia are present in 90% of cases: in contrast, erythroid and megakaryocyte dysplasia are absent. In general, there is no monocytic component. In around 5% of cases there

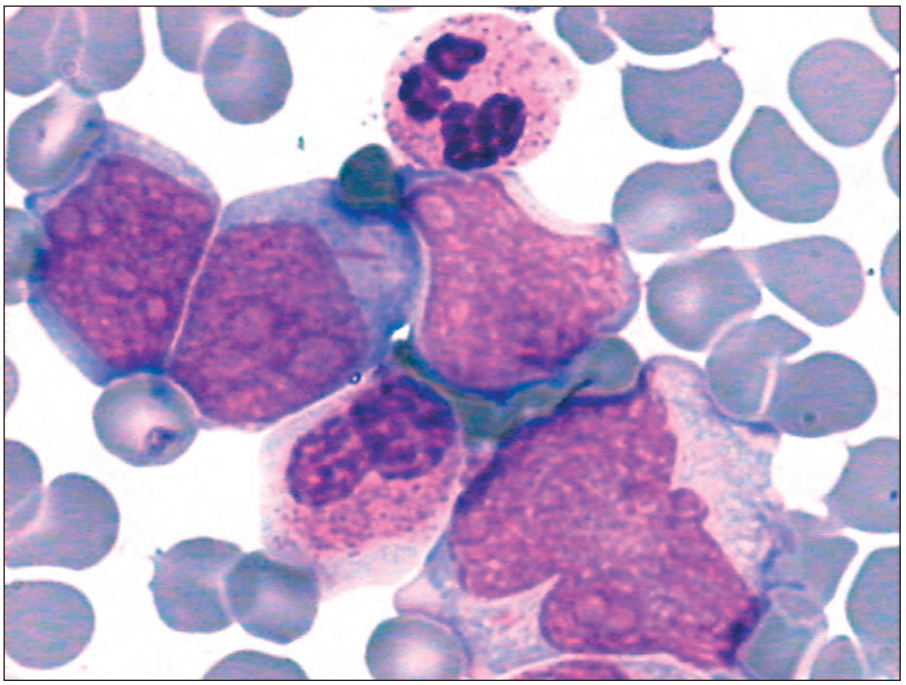


**Fig. 4.18** Other variations in the morphology of AML with t(8;21); bone marrow aspirate of a patient with FISH positive for the *RUNX1-RUNX1T1* fusion gene. On the left, two blasts that are very similar to each other with large angulated nuclei. As is typical in this type of leukaemia, there are nucleoli, which are often multiple and clearly seen; their colour differs little from that of the surrounding chromatin, which shows thin and often incomplete circles of perinucleolar condensation. The cytoplasm of these two cells shows intense basophilia, which tends to obscure the pink of the granules, but does not manage to hide the large paranuclear Auer rod that is present in the larger of these two cells. At the top and near the bottom, a neutrophil granulocyte and a small metamyelocyte show signs of dysplasia, which is not a rare observation in this type of leukaemia with a significant proportion of maturing granulocytes; however, erythroid and megakaryocytic dysplasia are not seen. Bottom right, a large granular blast cell with a deeply notched tetraploid nucleus.

is an increase in eosinophils, with dysplasia in this lineage being less marked than, although in part analogous to, that in AMML associated with inv(16) or t(16;16): rather than the granules being purple-red, large and irregular, the eosinophil precursors in these patients have granules that are cloudy, glassy, blue-green.[47] Peripheral eosinophilia can be marked and exceptionally Auer rods are seen in mature eosinophils, giving evidence that this lineage is part of the leukaemic clone.

The cytochemical pattern in this subtype of AML is characteristic, with peroxidase positivity, often intense, and CAE positivity: the Auer rods are strongly positive and show a typical central non-staining cavity. The eosinophils are peroxidase positive and CAE negative, as is normal for this lineage, but they can show aberrant PAS positivity. The immunophenotype is also distinctive: in addition to the strong expression of CD34, HLA-DR, MPO and CD13 (with CD33 less frequently expressed and also weaker), a significant proportion of cases show asynchronous co-expression of antigens typical of immature and mature cells (CD34 and CD15 respectively) and the aberrant expression of lymphoid antigens, particularly CD19 and, less often, the NK antigen, CD56.

The morphological suspicion, reinforced by the cytochemical and immunophenotypic data, must be confirmed by cytogenetic analysis and methods such as FISH or RT-PCR, which show specifically the *RUNX1-RUNX1T1* rearrangement.

### Acute myelomonocytic leukaemia with eosinophilia and inv(16) or t(16;16)

This variant, which is not rare, represents 3–5% of cases of AML and 15–20% of cases of AMML. It is associated with involvement of the eosinophil lineage, the presence of a highly specific cytogenetic and molecular abnormality and a favourable clinical response with good long-term survival.[48,49]

In the peripheral blood there is usually leucocytosis with blast cells and possibly increased monocytes, which are often atypical or immature (Fig. 4.25); peripheral eosinophilia is absent or moderate. In the bone marrow (Figs 4.26 through to 4.29) the blast cells have ample basophilic cytoplasm, with clear signs of monocytic differentiation. Auer rods may be present, either in myeloblasts or, more rarely, in more mature neutrophil granulocytes; they have not been described in the eosinophils. There is no erythroid or megakaryocytic dysplasia.

The most characteristic morphological feature is the presence of eosinophils in various stages of maturation, from promyelocytes to mature granulocytes, usually more than 5% but varying from 1% to 30%. Their morphology shows various peculiarities.

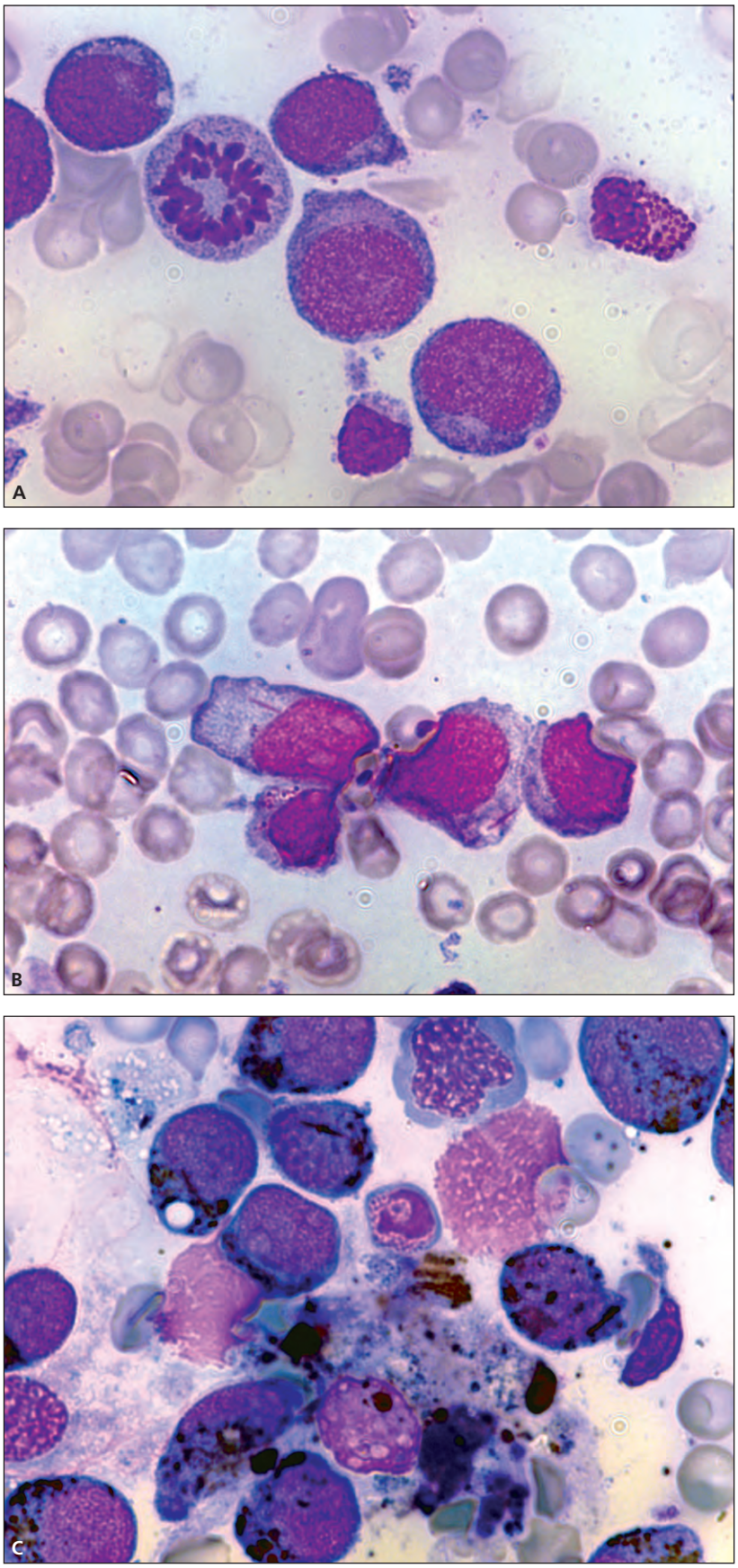



**Fig. 4.19** Another three cases of AML with t(8;21); bone marrow aspirates. (A) Five blast cells, two small and two large, together with a fifth in telophase, a small lymphocyte at the bottom and, on the right, an eosinophil with a non-segmented nucleus and immature granules. The two larger blast cells with particularly granular chromatin, a regular nuclear outline and intensely basophilic cytoplasm could be confused with proerythroblasts. However, the presence of a pale Golgi zone, which in the cell at the bottom is arising in relation to an indentation in the nucleus, confirms the myeloblastic nature of these cells (and can be compared with the arcoplasm of the proerythroblast in the centre of Fig. 4.3C). (B) Three morphologically unmistakable blast cells, with polygonal eccentric nuclei, granular chromatin, nucleoli and plentiful cytoplasm, which in two of the three cells contains long sharp Auer rods. (C) Peroxidase stain. The intense positivity of the cytoplasm of the blast cells is seen as large blackish granules and straight lineage structures that represent Auer rods. At the bottom is the pale nucleus of a macrophage, the cytoplasm of which is packed with cellular debris, which is in part peroxidase positive, indicating that the debris is derived from myeloblasts or degenerating granulocytes. It is not rare for granules that are only seen with difficulty on panoptic stains to be shown much more clearly by a peroxidase reaction.

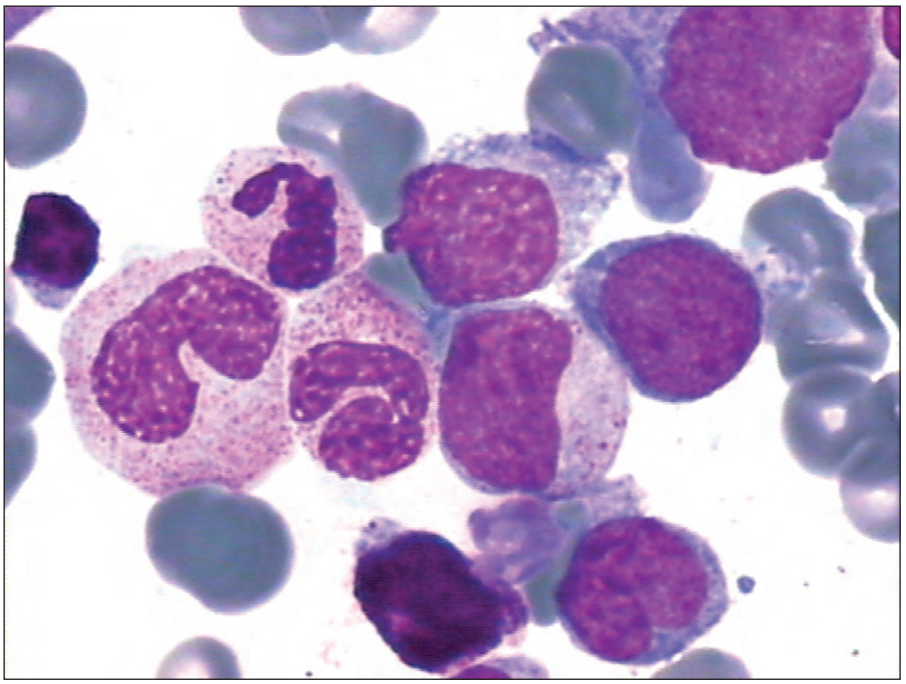


**Fig. 4.20** Moderate neutrophil dysmorphism in the blood film of the same patient as Fig. 4.3C. From the left, a small lymphocyte, a large metamyelocyte that is probably tetraploid, a small neutrophil with prepyknotic chromatin at the top and another with two nuclear lobes and a more open chromatin pattern at the bottom: all are fairly well granulated. On the right, a hypogranular myelocyte followed by a small blast cell with scanty basophilic cytoplasm.

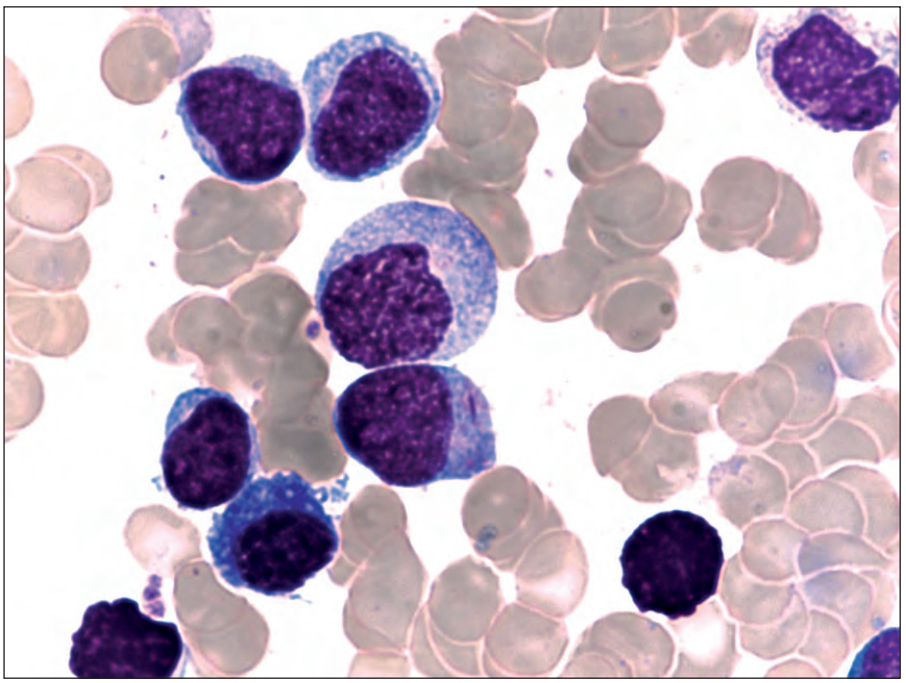


**Fig. 4.21** Bone marrow aspirate from a patient with AML with t(8;21): the blast cells show the typical cytological features with an eccentric nucleus, basophilic cytoplasm, delicate granules and an Auer rod. Bottom left, a lymphocyte and a plasma cell.

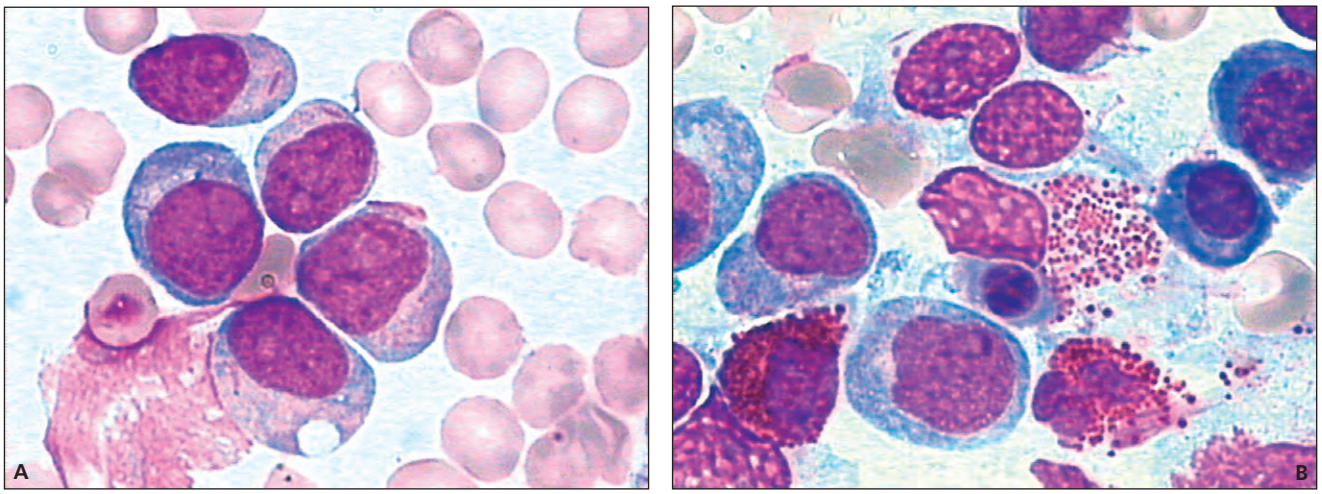



**Fig. 4.22** Another case, in a female, of AML with t(8;21). (A) Peripheral blood film. These myeloblasts, forming a small group, repeat all the typical morphological characteristics previously described; these features have considerable predictive value for the underlying molecular abnormality. In the lowest cell, the cytoplasm contains lakes of pinkish hyaline material, produced by fusion and coalescence of granules. (B) Bone marrow aspirate. In this field are two blast cells with characteristic morphology (a third is cut off at the left edge of the image); three eosinophils, one of which is damaged and has liberated its granules into the surrounding area; a polychromatic erythroblast, a plasma cell to the right and a basophilic erythroblast cut off by the right edge. Centre top, an apparently free Auer rod, probably derived from the damaged cell below it, the flattened nucleus of which shows a clearly outlined nucleolus.

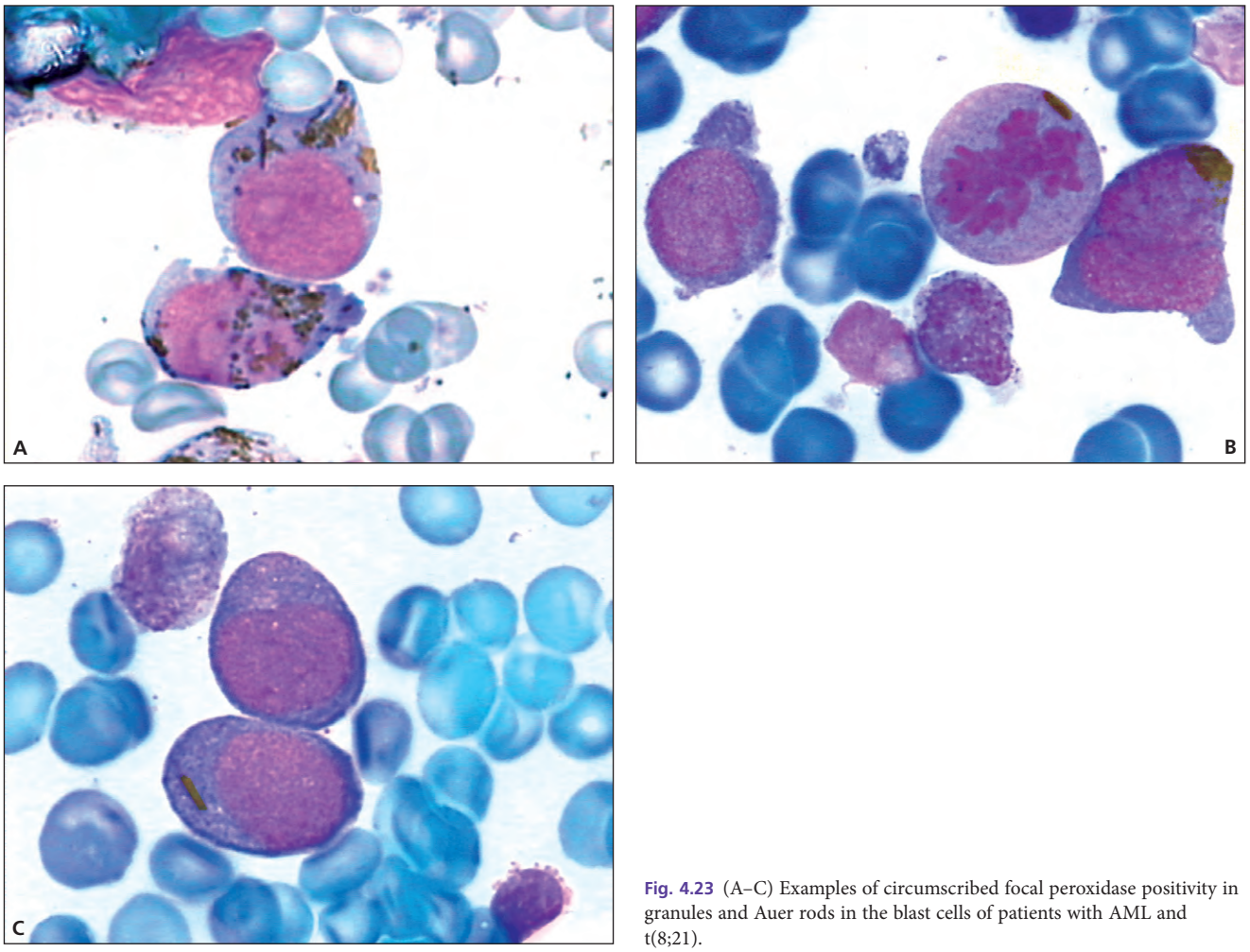



**Fig. 4.23** (A–C) Examples of circumscribed focal peroxidase positivity in granules and Auer rods in the blast cells of patients with AML and t(8;21).

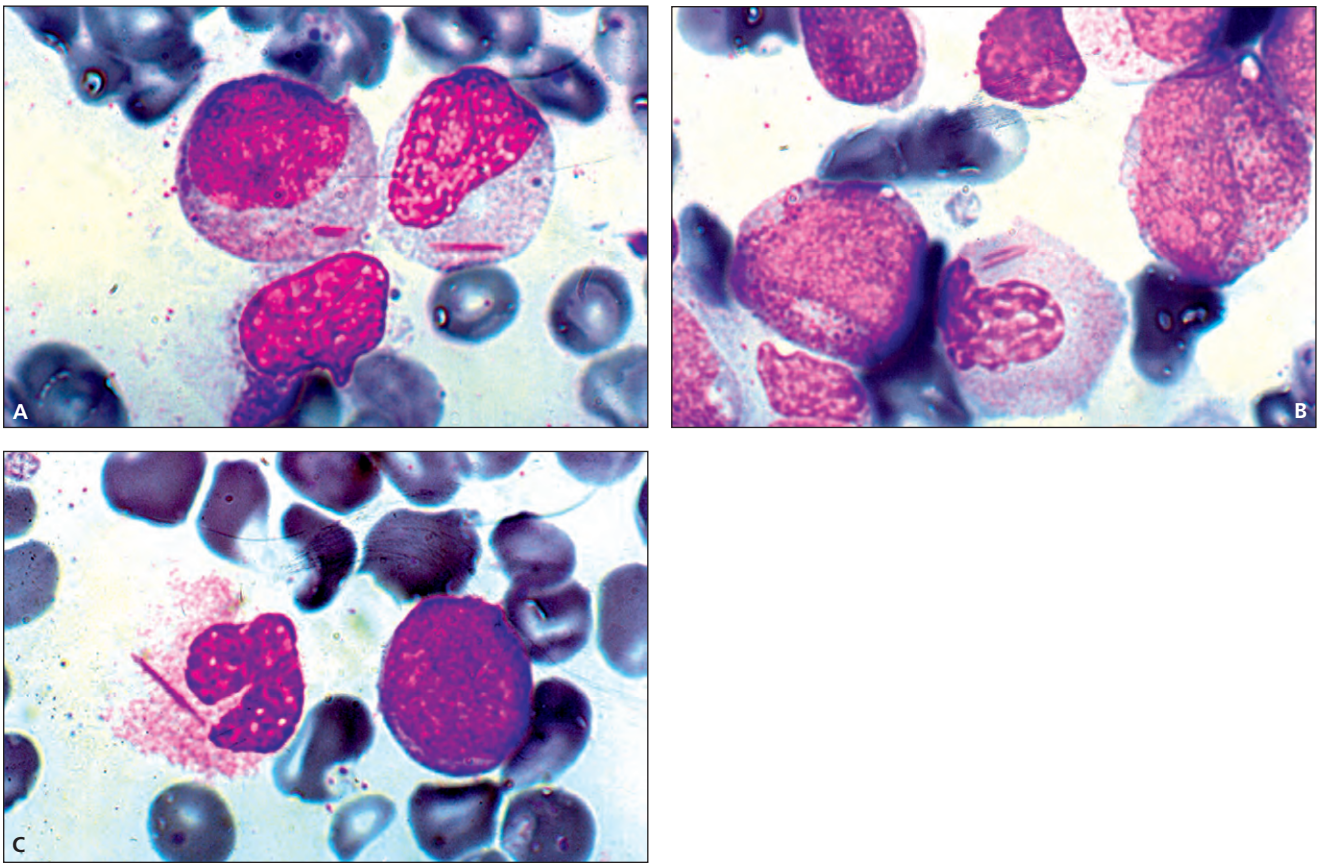



**Fig. 4.24** Persistence of Auer rods with granulocytic differentiation in AML with t(8;21). (A) Bone marrow aspirate. Two blasts with characteristic morphology. That on the right with a long pointed Auer rod has worm-like nuclear chromatin. (B) Bone marrow aspirate. Two blast cells have cytoplasm containing many azurophilic granules, which are denser near the pale Golgi zone. The cytoplasmic background, visible in the less granular areas, is basophilic. The nuclei are eccentric with granular chromatin and some prominent nucleoli. At the top, partly hidden at the upper edge, a smaller hypogranular blast cell. In the centre, a dysplastic neutrophil metamyelocyte with a large Auer rod that appears hollow in the centre: cells such as this indicate clearly that the blasts have undergone spontaneous maturation. (C) Peripheral blood of the same patient: a blast showing little differentiation with a thin rim of basophilic cytoplasm and a neutrophil band form containing a long Auer rod.

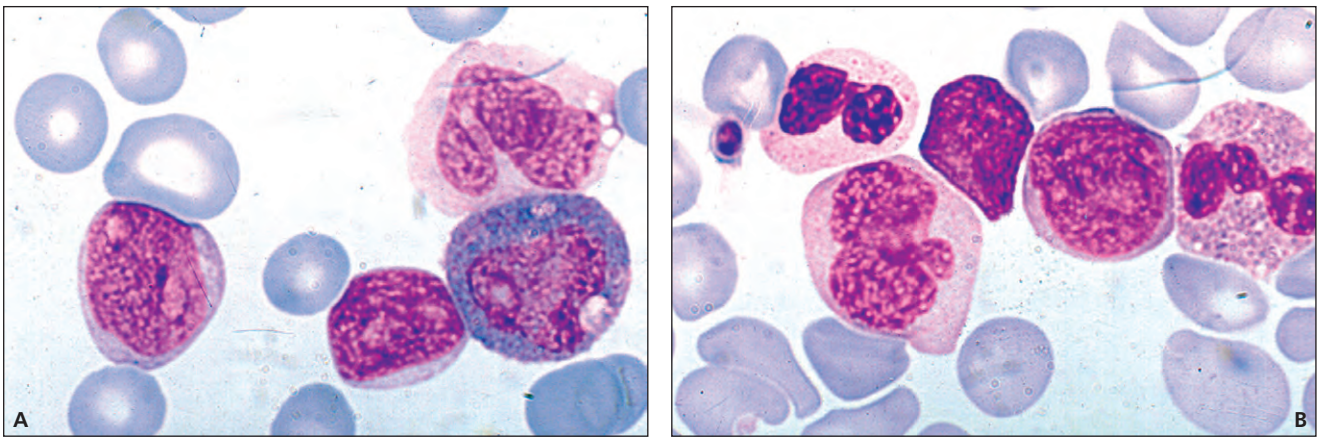



**Fig. 4.25** AML with recurrent genetic abnormality with inv(16); peripheral blood film. (A) On the left, two undifferentiated myeloblasts with a high N:C ratio and open chromatin, with focal condensation alternating with delicate pale spaces; the nucleoli, two per cell, are particularly prominent in the cell further to the left. On the right, an eosinophil myelocyte with a triangular nucleus and cytoplasm packed with greyish granules; immediately above, an immature monocyte with a convoluted nucleus, granular or worm-like chromatin condensation and plentiful grey-pink cytoplasm with some vacuoles. (B) From the right, towards the left, a trilobed eosinophil; a blast cell with a high N:C ratio and fairly dense heterogeneous chromatin with ill-defined nucleoli and without clear signs of differentiation; a bare, damaged nucleus; a large immature monocyte; a bilobed poorly granulated neutrophil; a strange, very small fragment containing a sphere of chromatin, probably detached from a cell undergoing apoptosis (not to be confused with a platelet or a fungal spore).

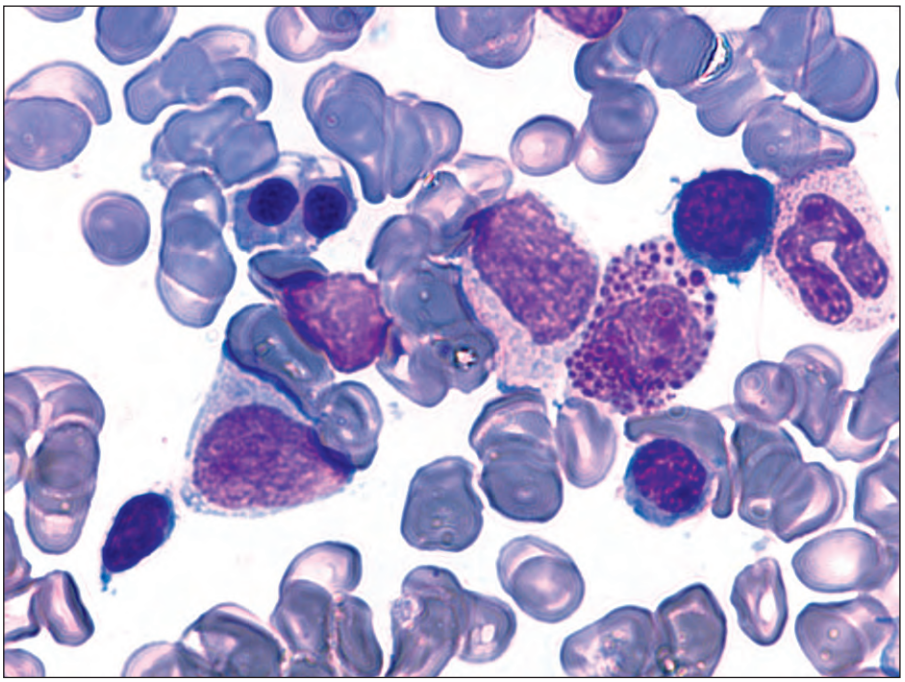


**Fig. 4.26** Bone marrow aspirate from a patient with AML with recurrent genetic abnormality with t(16;16). In the centre, two blast cells without clear signs of differentiation and, to their right, an eosinophil myelocyte with dysmorphic features: its ample cytoplasm, revealed in the upper part of the cell, appears to be still basophilic and contains granules of variable size, the largest of which are very dark, the colour of an aubergine. The other cells are erythroblasts in various stages of maturation, a neutrophil band form and a lymphocyte.

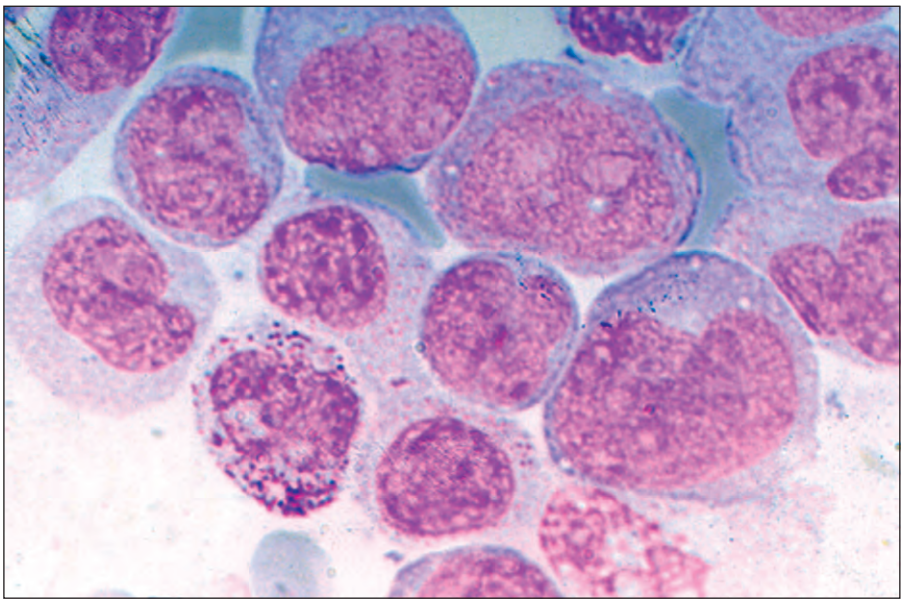


**Fig. 4.27** AMML with eosinophilia and inv(16); bone marrow aspirate. On the right, three large hyperdiploid cells, which according to the WHO classification must be designated promonocytes because of the notched and irregular nuclear outline and the very delicate reticular chromatin pattern with visible nucleoli. These must be included in the blast percentage. Their cytoplasm is plentiful and basophilic and, in the promonocyte in the centre, shows fine azurophilic granules; the latter cell also has particularly well-developed nucleoli, one of which appears stamped out (because of the peripheral reinforcement by chromatin condensation). The other cells are mainly immature monocytes and are not included in the blast count. The cell lower down to the left, however, is an eosinophil precursor which does not have specific granules, only proeosinophilic granules within cytoplasm that appears half empty. On its right, a small hypogranular neutrophil myelocyte.

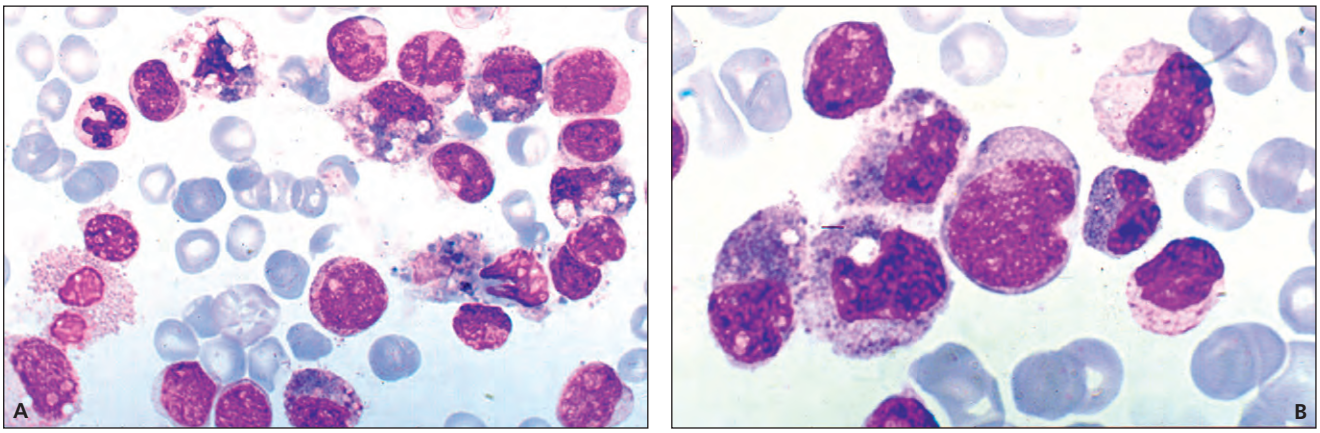



**Fig. 4.28** AMML with eosinophilia and inv(16); bone marrow aspirate. (A) There are blast cells that are either undifferentiated or show myeloblast features (Golgi zone, indented nucleus, azurophilic granules). Top left, a neutrophil and bottom left, adjacent to a blast with two nucleoli, an eosinophil with abnormally distended cytoplasm that is filled with mature orange granules. The other cells are mainly eosinophil myelocytes and metamyelocytes with very vacuolated cytoplasm, variegated and greyish because of the mixture of more or less mature granules on a background that is still weakly basophilic. In the eosinophil myelocyte visible only in part in the centre of the lower edge, some large dark red proeosinophilic granules. Half way up, immediately to the right of a nucleolated blast cell, the cytoplasm of a debris-laden macrophage that is in part sea-blue. (B) In the same film, top left, a small undifferentiated blast cell and, in the centre, a large promonocyte with finely perforated basophilic cytoplasm. The two cells further to the right are neutrophil myelocytes with pale, partially hypogranular cytoplasm: between them is a mature eosinophil, which has three superimposed nuclear lobes and numerous granules that are normal with regard to colour and size. In the left part of the image, three eosinophil myelocytes stand out because of their vacuolated cytoplasm, full of poorly defined granules, blurred and indefinite against the still basophilic background; looking closely one can distinguish single dark granules, with their colour between mahogany and chocolate.

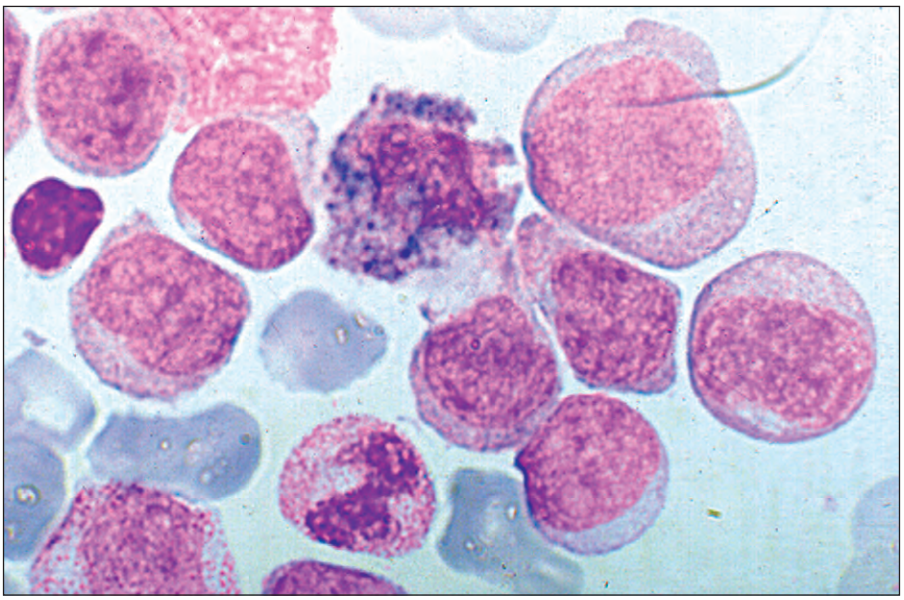


**Fig. 4.29** Heterogeneity of the maturing monocytic component from another patient with AMML with eosinophilia and inv(16); bone marrow aspirate. In the centre at the top, an atypical eosinophil myelocyte with the cytoplasm filled with a mixture of dark granules and orange granules that are rendered opaque because of the cytoplasmic basophilia. On its left, an undifferentiated agranular blast cell; on its right, a large monoblast characterised by the delicacy of the open, reticular chromatin; it has a number of nucleoli and the basophilic cytoplasm contains very fine, pale red granules. The three cells immediately beneath are immature monocytes (which are not included in the blast count), with more condensed chromatin. The fourth cell, further down, has a round nucleus and fairly dense chromatin, and must be included in the blast count because of the presence of a large prominent nucleolus. To the left, a neutrophil band form, a promonocyte and a lymphocyte.

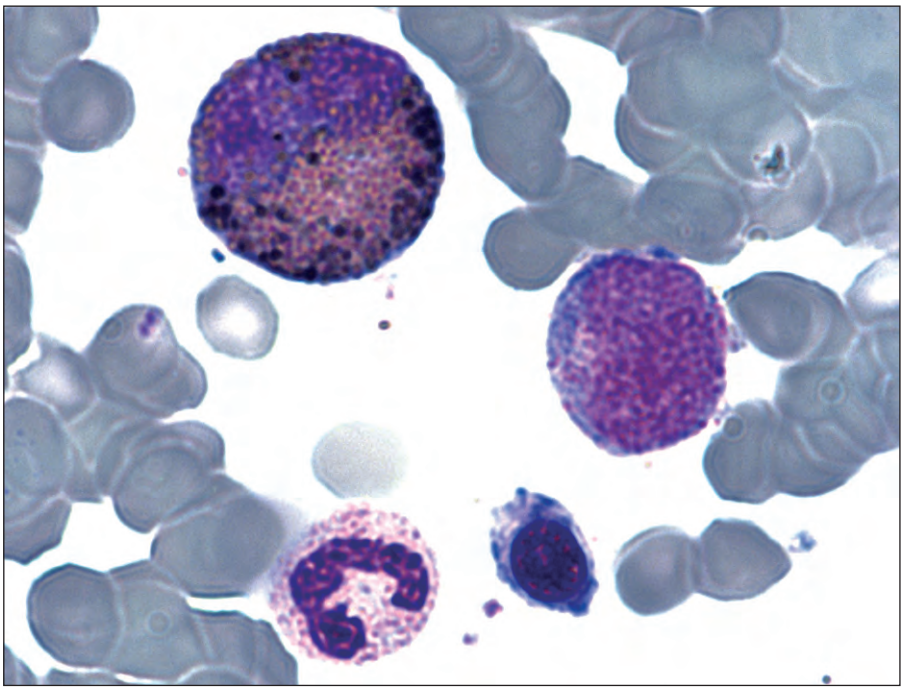


**Fig. 4.30** AMML with inv(16); bone marrow aspirate. At the top, a large immature and atypical eosinophil, which can be classified as an eosinophil myelocyte; the nucleus is displaced, markedly eccentric, with the convex part of its outline in close contact with the inner surface of the upper half of the cytoplasmic membrane; its concave surface is towards the centre of the cell where there is a myriad of almost mature granules, which are developing an orange colour, although they do not stand out very clearly from the blue background; the much larger and darker proeosinophilic granules are displaced to the periphery, in part covering the nucleus. To the right, a granular myeloblast with some protrusions of the basophilic cytoplasm. Lower down, a polychromatic erythroblast and a non-segmented neutrophil with a Döhle body in its cytoplasm.

Particularly in the less mature precursors (promyelocytes and myelocytes), there are frequent proeosinophilic granules, of a colour ranging from purplish-red to deep violet, plum or aubergine, not to be confused with the granules of basophils (these proeosinophilic granules are consistently negative with toluidine blue); in general, these granules are admixed with mature orange-coloured eosinophil granules, the proportion of which increases progressively in the more mature cells (Figs 4.30 and 4.31). These bizarre staining characteristics have led to these cells being called 'harlequin cells'.[39] This appearance cannot be considered specific: numerous proeosinophilic granules can also be seen in severe reactive eosinophilia, in the idiopathic hypereosinophilic syndrome and in some cases of chronic granulocytic leukaemia. In AMML with inv(16) or t(16;16), however, the proeosinophilic granules are frequent, irregularly shaped and often very large; the eosinophil precursors may show other dysplastic features: left shift of the maturation curve, anomalies in nuclear segmentation (either increased or decreased) and cytoplasmic vacuoles. When eosinophils are numerous, Charcot-Leyden crystals may be recognised in the intact or disrupted cytoplasm of bone marrow macrophages. These eosinophils are also cytochemically atypical: they are consistently, although not strongly, positive for CAE and show granular staining with PAS. Positivity for CAE is unique and specific for this condition and is not observed in normal eosinophils or in those of other leukaemias with hypereosinophilia. With PAS, however, normal eosinophils show a diffuse background positivity with negative granules, while granule positivity has been described in AML with t(8;21) and in reactive eosinophils. In a minority of cases of AML with this specific genetic alteration, the eosinophils are neither increased nor cytologically abnormal[50]; at times the blast cells show granulocytic or monoblastic differentiation.

The blast cells are immunophenotypically heterogeneous, with a proportion of immature cells that are positive for CD34 and CD117, mixed with cells showing monocytic differentiation (positive for CD14, CD4, CD11b, CD11c, CD64) and others showing granulocytic differentiation (positive for CD13, CD33, MPO, CD15). It is particularly frequent for there to be aberrant positivity for the T-lymphoid marker, CD2.

The morphology, with the support of cytochemistry and immunophenotyping, directs the diagnosis strongly towards this subtype of AML with recurrent genetic abnormality: confirmation requires cytogenetic analysis or techniques such as FISH or RT-PCR, which show specifically *CBFB-MYH11*.

## Acute promyelocytic leukaemia

APL comprises 5–8% of cases of AML.

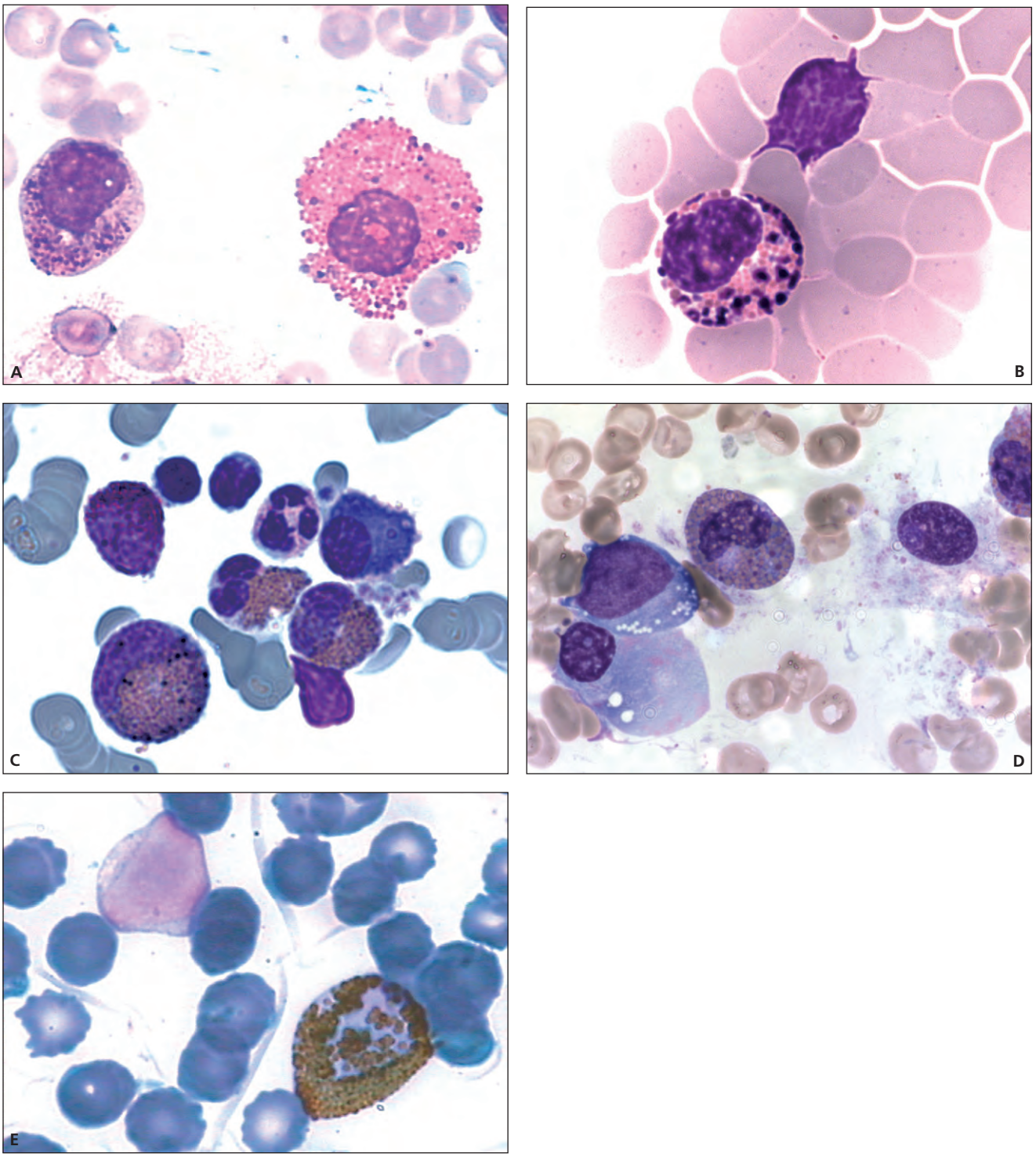



**Fig. 4.31** Morphology of eosinophils in AMML with recurrent cytogenetic abnormality of chromosome 16. (A) On the left, an eosinophil promyelocyte: the prominent nucleolus stands out from a mass of condensed chromatin containing very small vacuoles; the cytoplasm is greyish and in large part occupied by dark granules that stand out on an orange background composed of mature granules that are not individually recognisable; there is a paranuclear vacuole. On the right, an eosinophil with a ring-shaped nucleus, condensed chromatin and extensive chromatin brimming with mature granules, with a small number of dark proeosinophilic granules. The cytoplasmic membrane appears irregular, perhaps damaged, but the granules remain compact, surrounding the nucleus and visible even in an intranuclear vacuole. (B) An eosinophil metamyelocyte with a nucleus shaped like a figure of eight: the proeosinophilic granules are large and dark, close to the membrane, being pushed to the periphery by a mass of mature orange granules which are smaller and packed together. (C) Top left, a basophil; at the bottom, proceeding anticlockwise, a large metamyelocyte, a neutrophil and a small eosinophil metamyelocyte, some agglutinated platelets, a plasma cell, a neutrophil and two lymphocytes. (D) From left to right: a plasma cell with plentiful hypertrophic cytoplasm, with basophilia that weakens towards the periphery and which alternates with small flaming circular areas; a blast cell with delicate homogeneous chromatin and numerous small cytoplasmic vacuoles; an eosinophil metamyelocyte with N:C asynchrony; and a histiocyte/macrophage with a characteristic spongy oval nucleus and plentiful ragged granular blue cytoplasm with indistinct margins. (E) Peroxidase stain. At the top, a completely negative undifferentiated blast cell: the outline of the nucleus, angular and with straight areas, tends to exclude a monoblastic lineage. On the right, an eosinophil myelocyte in which all the granules are individually distinguishable and intensely positive.

More than any other type of AML, this subtype has highly distinctive biological, morphological and clinical features, and specific therapeutic requirements. It was recognised as a clinicocytological entity only at the end of the 1950s, when it was associated with a severe haemorrhagic syndrome resulting from disseminated intravascular coagulation.[51] In the classic form of APL, the leukaemic cells that infiltrate the bone marrow are mainly abnormal promyelocytes, which are packed with granules and often also contain multiple Auer rods that cross over each other and are gathered together in bundles. APL is part of this category of AML with recurrent genetic abnormalities because of the presence of a consistent genetic alteration, t(15;17)(q22;q12), first described over 30 years ago,[52] in which part of the retinoic acid receptor alpha gene, *RARA*, at 17q12 fuses with part of the *PML* gene at 15q22, which encodes a protein with a regulatory function in the nucleus. In rare cryptic cases, in which this translocation is not apparent, there is a variant complex translocation that also has 17q12 and 15q22 breakpoints and generates a PML-RARA transcript demonstrable by molecular methods. The normal counterpart of these pathological promyelocytes is a myeloid stem cell that has the potential for granulocytic differentiation.

The breakpoint on chromosome 17 is in a 12-kb region, in which the *RARA* gene is found. The breakpoints on chromosome 15 are grouped together in the *PML* gene, with three possible locations that give rise to three *PML-RARA* transcripts, designated bcr1, bcr2 and bcr3. RARA is a member of the superfamily of hormonal nuclear receptors: after interaction with retinoic acid, its natural ligand, it binds to the promoters of target genes and stimulates transcriptional activity. PML belongs to a family of proteins implicated in the suppression of tumours, in the control of apoptosis and in genomic instability.

The hybrid protein PML-RARA acts as a receptor for retinoic acid in an aberrant manner, altering the DNA binding properties, and becoming a potent suppressor of transcription signals furnished by retinoic acid itself. This repression blocks differentiation of the leukaemic cells. This block can be overcome by the use of pharmacological doses of retinoic acid in the form of all-*trans*-retinoic acid (ATRA), which reactivates transcription, re-establishes the capacity for cellular maturation and favours the differentiation of the pathological cells.[53] Using anti-PML monoclonal antibodies for immunofluorescence, it is possible to recognise, in normal subjects, the localisation of PML in the nucleus in the form of structures designated 'nuclear bodies'; in APL these typical bodies have disaggregated into a microgranular distribution, attributable to the action of PML-RARA; this is the basis of a rapid, specific diagnostic method.[54]

The hybrid fusion gene is the true genetic marker of APL. Its quantification by RT-PCR permits assessment of the response to therapy with ATRA and arsenic trioxide, which nowadays are able to cure this most feared of leukaemias, and permits also monitoring over time of maintenance of complete remission at a molecular level.[55]

In APL a high frequency has been found, about 45% of cases, of mutation in the gene encoding the FLT3 receptor, which leads to its constitutive activation; this is found particularly in the form of the disease with a high white cell count and a *PML-RARA* fusion transcript of bcr3 type[56]: this observation raises the possibility of a therapeutic approach based on FLT3 inhibitors.

Even if the usual picture is highly characteristics, APL is not a homogeneous entity[57]: together with the classical hypergranular form, which is a good deal more frequent, various atypical forms have been recognised, the most characteristic of which is the so-called microgranular form.

### Hypergranular promyelocytic leukaemia

Hypergranular promyelocytic leukaemia strikes at a younger age than other forms of AML, particularly in adolescents and young adults. Its incidence is higher in autumn and spring. In the peripheral blood in the majority of cases there is leucopenia (see Report 4.2), with reduced neutrophil numbers; the atypical cells are absent or present in only low numbers. The neutropenia is the result of lack of maturation of the pathological promyelocytes, which are blocked in their differentiation. In other cases there is a moderate leucocytosis. Promyelocytes in the peripheral blood can be easily recognised (Figs 4.4, 4.32 and 4.33), but it is not rare for them to be quite scanty (Fig. 4.1C). Furthermore, the circulating leukaemic cells may be morphologically different from those in the bone marrow: in some cases, for example, they tend to be less granular, with a monocytoid appearance; or they are small with scanty intensely basophilic cytoplasm. It is always a good idea in these cases to search a preparation that has been enriched in white cells, for more typical hypergranular forms with Auer rods, which are hardly ever absent. Thrombocytopenia is consistently present and is severe: it is the result of insufficient bone marrow production and disseminated intravascular coagulation that is a result of the procoagulant activity of the promyelocyte granules. In promyelocytic leukaemia at diagnosis there is no erythroid or megakaryocytic dysplasia.

Preparation of bone marrow films can be difficult because the aspirate tends to clot very rapidly. Often, as a result, the aspirated material is hypocellular. The morphology of the leukaemic cells is, however, so typical that the observation of only small numbers is likely to be sufficient to suggest the correct diagnosis (Figs 4.34 through to 4.45). The atypical promyelocytes of acute hypergranular promyelocytic leukaemia are generally of intermediate size with a relatively low N:C ratio. The nucleus may be in large part hidden by the granules, obscuring its outline, which is rounded or, more often, indented, kidney-shaped, folded on itself or irregular. The cytoplasm has unmistakeable features: the background colour, variable basophilic or pink, is usually inapparent because of the numerous large azurophilic granules, which are a vivid red or purple and often quite dark. The granules are so tightly packed that they often completely obscure the nucleus, making it impossible to appreciate the

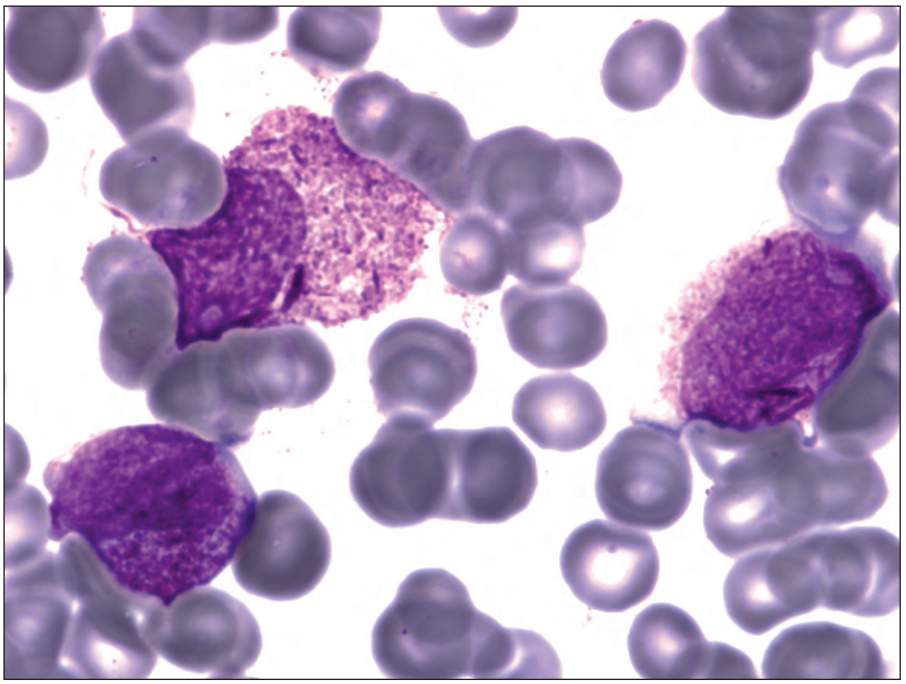


**Fig. 4.32** APL; peripheral blood film. This image summarises different morphological features that are typical of this type of leukaemia. Bottom left, a typical small, hypergranular promyelocyte: the nucleus is oval and eccentric with dense chromatin without visible nucleoli, and appears to be traversed diagonally by a furrow. The cytoplasm, visible only in the small area that is lacking granules, is intensely and homogeneously basophilic. The very numerous variably sized granules vary also in colour, from dark violet to blackish; they form a dense compact mass close to the nucleus. On its left edge, this cell has an oval pseudopod with hardly any granules; the other apparent excrescences are actually just the result of pressure from erythrocytes. The promyelocyte adjacent to the right margin has a nucleus with open filamentous and worm-like chromatin with a nucleolus with an irregular sinuous outline evident at the top. At the top this promyelocyte has an agranular area of basophilic cytoplasm, similar to that in the cell that has just been described. The cytoplasm on the left of the nucleus is pinkish with very delicate granules and microvacuoles, while on the right it contains a number of Auer rods that vary in size and morphology: some are thin and cross over each other; one is short and darkly staining and is in part superimposed on the nucleus. The promyelocyte seen top left has a nucleus with dense chromatin clumps alternating with pale spaces and a prominent blue-violet nucleolus. A large almost rhomboidal Auer rod is lying adjacent to the nucleus; another thinner Auer rod is seen on the right, mixed with dark granules in cytoplasm that is partly pink and partly colourless. The alterations in cell shape result from pressure of the surrounding red cells and indicate that despite their internal structure, these leukaemic cells are fragile and deformable.

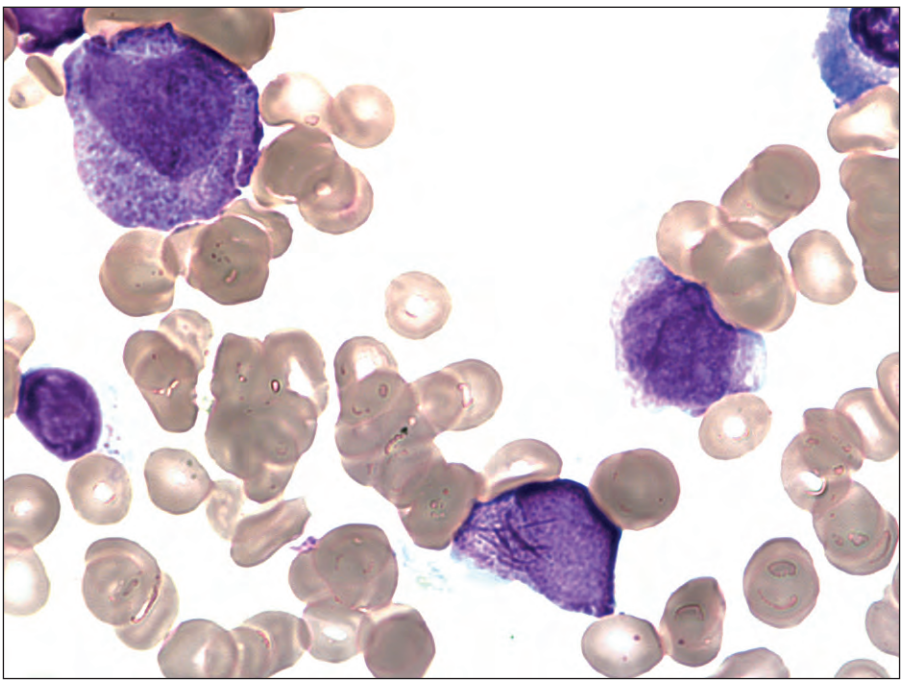


**Fig. 4.33** APL; peripheral blood film from the same patient as Fig. 4.32. Top left, a large hypergranular promyelocyte with an eccentric nucleus: the granules are diffusely and fairly evenly spread through the ample basophilic cytoplasm, to which they convey a violaceous tone. The cell seen centre right is a smaller promyelocyte, with a small amount of irregularly basophilic cytoplasm containing very fine violaceous granules that are tending to cluster within the cytoplasm; the nuclear outline is in part obscured; the nucleus contains some nucleoli, which are not very prominent. The promyelocyte at the bottom has a nucleus that is in close apposition to the cytoplasmic membrane, delicate homogeneous chromatin and numerous Auer rods of variable length and thickness, which are crossing over each other within the granule-rich cytoplasm and in part are superimposed on the nucleus.

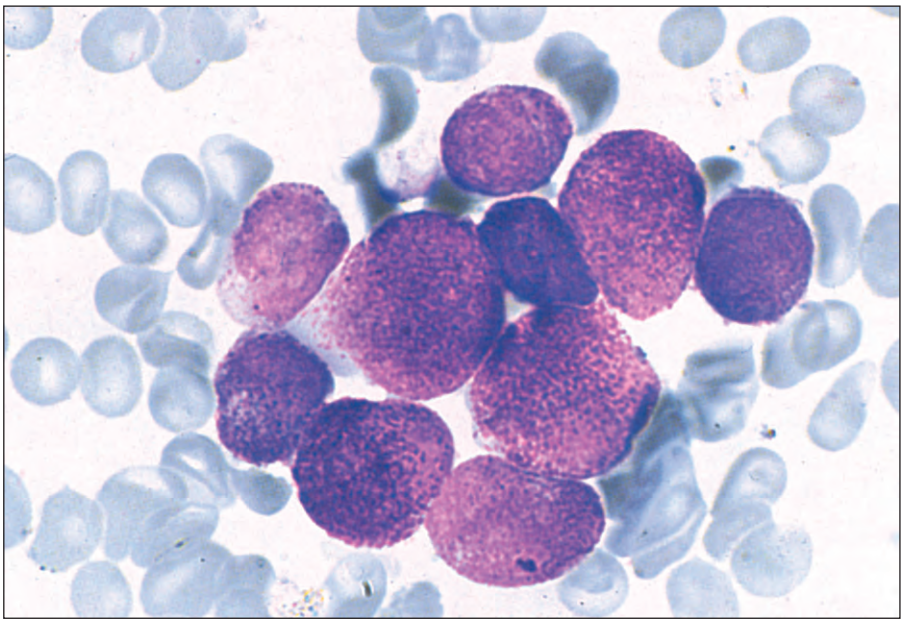


**Fig. 4.34** Promyelocytes loaded with granules in a bone marrow aspirate. The appearance of these cells is unmistakable: there can be no doubt about the diagnosis of the hypergranular form of APL, even though the trails are hypocellular, the specimen is partly clotted and no cells with typical multiple Auer rods are seen. It is impossible to ascertain the features of the nucleus, which consistently occupies an eccentric position; there is no visible Golgi zone and none of the orderly arrangement of the various cellular components that characterises normal promyelocytes. The cells vary in size with some smaller cells being less granular, with cytoplasm that is weakly or intensely basophilic.

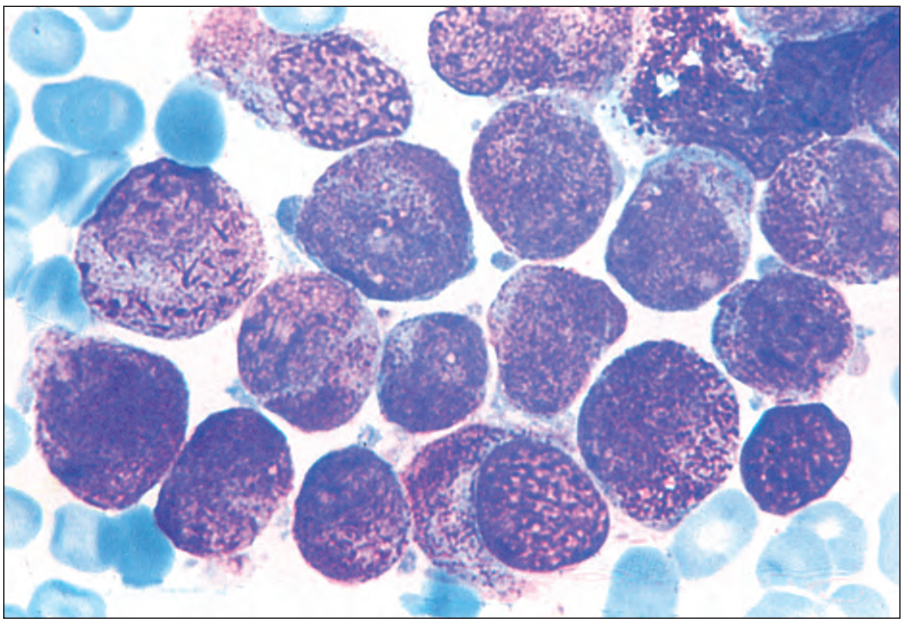


**Fig. 4.35** APL; bone marrow aspirate. Heavy infiltration of the bone marrow by a heterogeneous population of hypergranular promyelocytes, with almost complete replacement of normal haemopoietic tissue and disappearance of maturing granulocytes. In a less granular promyelocyte on the left there are numerous short thick Auer rods, like lightning bolts, crossing over each other in various directions. Some cells high up in the centre and towards the right are smaller and have the appearance of blast cells, being less rich in granules and with basophilic cytoplasm. The form of APL in which these cells predominate is more difficult to diagnosis if there are no cells with bundles of Auer rods.

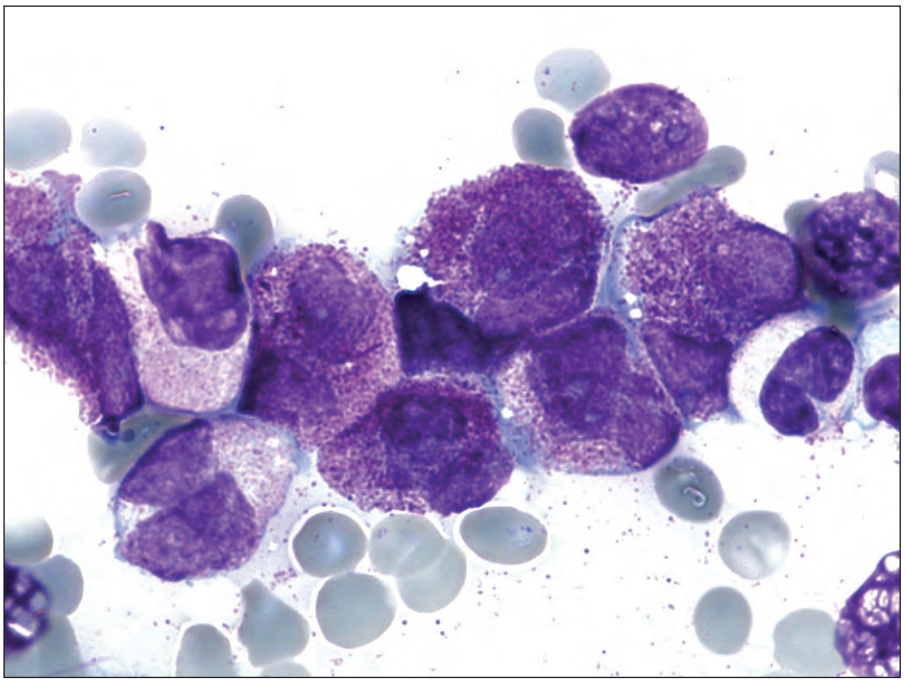


**Fig. 4.36** Pathological promyelocytes massed together in the trail of a fragment in a hypocellular aspirate. In the five large typical cells in the centre, the nuclei are irregular, tending to be bilobed; the chromatin is fairly condensed, but the structure is difficult to evaluate; there appear to be numerous pale blue nucleoli, sometimes with peripheral reinforcement by a circle of condensed chromatin. The cytoplasm is laden with azurophilic granules, which in some cells (on the left) have the appearance of fine violet dust, and in other cells (on the right) appear tightly packed, although individually recognisable. In the few spaces not occupied by granules, the cytoplasm can be seen to be cobalt blue. A distinct Auer rod is visible in one of the promyelocytes; to the left and below it, a hypogranular promyelocyte with a bilobed nucleus; above the latter is a granulocyte with liquefied chromatin resulting from abnormal differentiation or apoptosis of a leukaemic promyelocyte; the same has occurred in the case of the small agranular band neutrophil at the extreme right. The overall picture suggests the presence of the *PML-RARA* fusion gene.

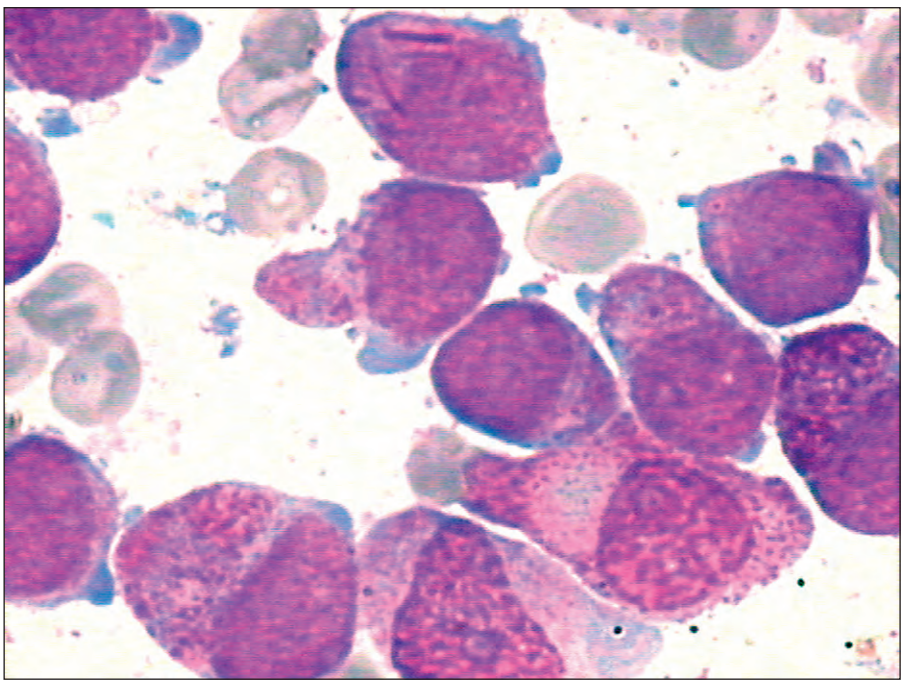


**Fig. 4.37** APL; bone marrow aspirate. Some of these promyelocytes are morphologically quite characteristic, such as the cell at the top with multiple Auer rods, the cell packed with dark granules cut off at the right border of the image and that bottom left with masses of reddish granules and a polygonal nucleus with fine chromatin and no visible nucleoli. Other cells have fewer, large thick granules and have nuclei that do not differ from those that are found in other types of AML. The cytoplasm appears intensely basophilic in the areas where it is free of granules and in many cells is tending to form projections that vary in size and shape. Two promyelocytes at the bottom have chromatin that appears worm-like, perhaps the effect of flattening.

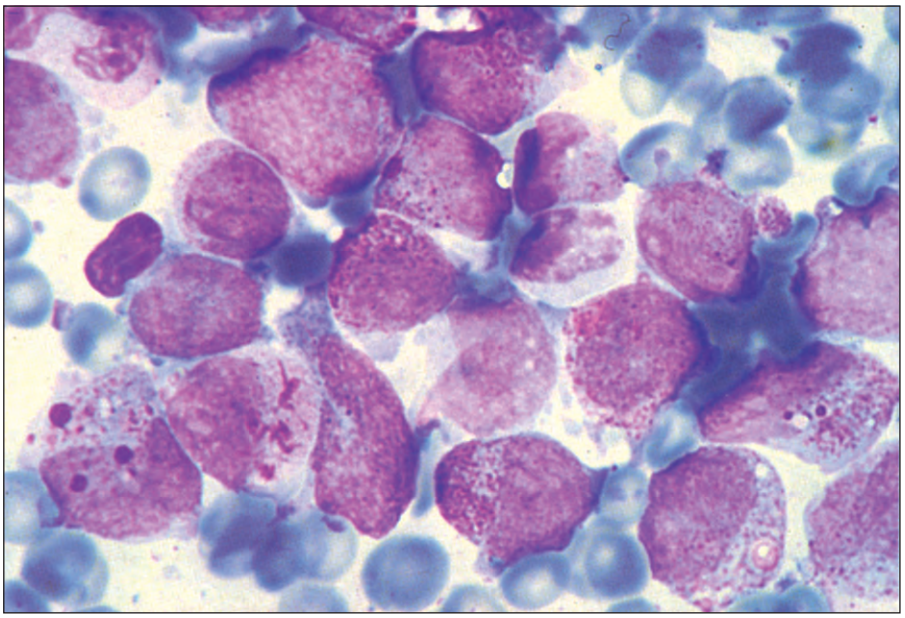


**Fig. 4.38** Acute hypergranular promyelocytic leukaemia; bone marrow aspirate. In this case, the hypergranular blastic population that is diffusely infiltrating the bone marrow is characterised by marked heterogeneity. There are some cells that are crowded with delicate azurophilic granules and others with very large, coalescent pseudo-Chédiak-Higashi granules, such as the cell seen bottom left. Adjacent to the latter is a typical leukaemic promyelocyte with multiple short thick Auer rods. Other cells have plentiful basophilic cytoplasm containing few visible granules or apparently no granules, such as the cell beneath the centre with a lobulated nucleus and chromatin that is so delicate that it appears transparent.

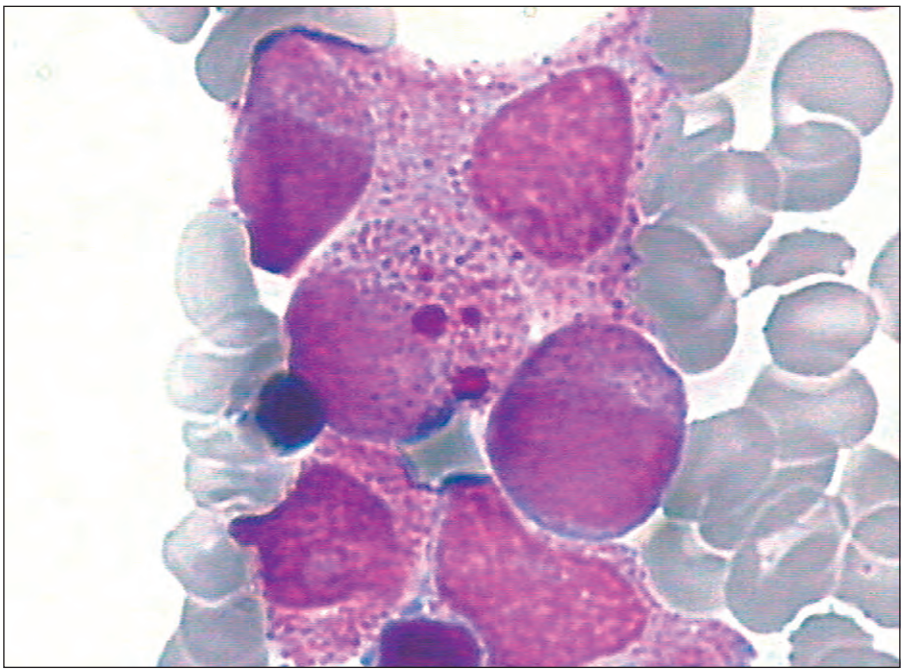


**Fig. 4.39** Acute hypergranular promyelocytic leukaemia; bone marrow aspirate. The cell in the centre of this film from another patient has numerous granules, some of which are giant as a result of coalescence; they look like giant dark red droplets, the typical morphology of pseudo-Chédiak-Higashi granules.

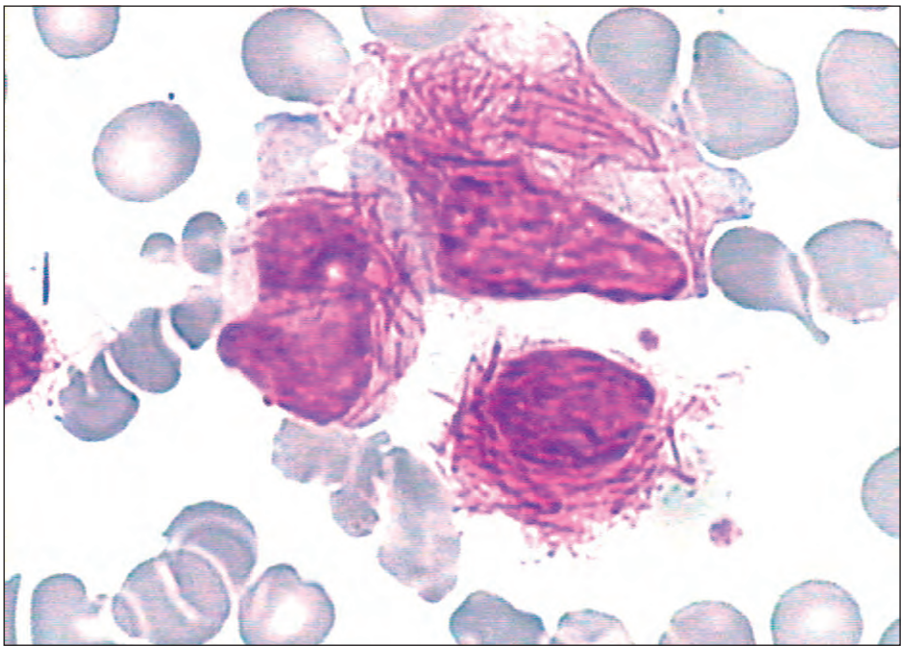


**Fig. 4.40** These three promyelocytes, from the same patient as Fig. 4.39, have particularly condensed chromatin and bunches and bundles of long straight Auer rods, with weak cytoplasmic basophilia in the few areas that are free of granules. In the smaller cell, the Auer rods appear curiously interwoven and form a nest in which the nucleus is lying, like a giant dark red egg. The tips of the Auer rods of the leukaemic promyelocytes are generally less sharp than those that are seen in AML with t(8;21).

chromatin structure or visualise the nucleoli. In some cells the granules coalesce into gross rounded or irregular forms of a pseudo-Chédiak-Higashi type, while in other cases they are so densely packed that they seem to merge with the nucleus. In yet other cases, they are so delicate that they give the appearance of red dust. Sometimes the distribution of granules is disorderly, with some areas of cytoplasm totally agranular and others with densely packed granules. Only rarely does one observe a pale area that corresponds to the Golgi apparatus.

The true morphological marker of APL is the presence of multiple Auer rods, packing the cytoplasm, a feature that is present in more than 50% of cases: their presence, although extremely characteristic, is not essential for the morphological diagnosis. In some cells the Auer rods can be so numerous as to be gathered together in bundles or 'faggots': they can be long and thin or, more often, short and thick, or splinter-like, with a characteristic russet colour; sometimes, when the cytoplasm is plentiful, they cross and superimpose themselves on each other in an irregular and disorganised manner. Often the cell membrane of the leukaemic promyelocyte ruptures, perhaps because of the needle-like points of the Auer rods, which may then be seen free, admixed with numerous granules, in the background of the film, mixed with intact cells or superimposed on them.

On electron microscopy the Auer rods of APL are different from those seen in other types of AML, with an internal structure of hexagonal tubules with a periodicity of 22–25 nm, in comparison with the laminar structure with a periodicity of 6–20 nm in other types of AML.

Despite this general overall appearance, there are almost always present a minority of cells that are apparently agranular or that have very small opaque rust-coloured granules. In yet other cells, one sees, instead of granules, veritable lakes of hyaline rose-coloured material (Fig. 4.39). In some infrequent cases, there is a proportion of small blast cells, with a small amount of cytoplasm that is intensely basophilic and in general contains few granules, with pseudopod-like cytoplasmic protrusions (Fig. 4.45); these cells sometimes resemble small megakaryoblasts and micromegakaryocytes.[58]

APL has a typical cytochemical profile, with intense positivity for peroxidase (Fig. 4.43) and CAE. Sudan black B staining is also strong, while PAS staining shows fine diffuse positivity, which gives a reddish dusty appearance to the cytoplasm. ANAE can sometimes be positive, albeit weakly with sensitivity to sodium fluoride inhibition, which can cause a certain confusion with monocytic leukaemia: such positivity for monocytic esterases is not seen in normal promyelocytes. In rare cases, a variable percentage of cells, but usually less than 10%, have been found to show metachromatic staining with toluidine blue, which renders the granules dark red.[59]

From the point of view of the cytomorphological differential diagnosis, the conditions that can be confused with the hypergranular form of APL are not numerous. The most frequent and

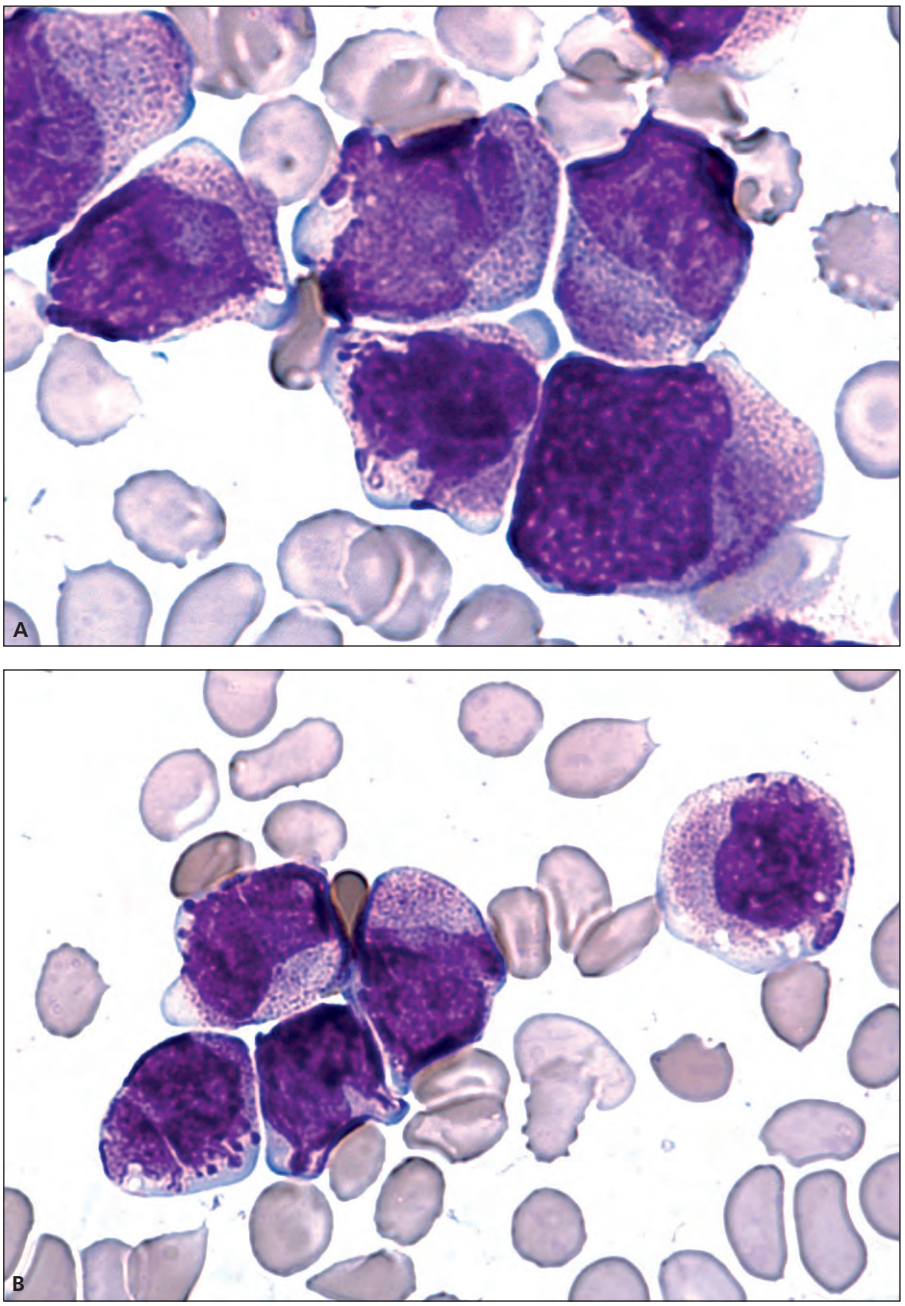



**Fig. 4.41** Hypergranular promyelocytic leukaemia; bone marrow aspirate. (A) The pathological promyelocytes in this patient have plentiful basophilic cytoplasm full of very fine granules: sometimes this looks almost like a pink jelly surrounded by a rim of azure cytoplasm, as is seen well in the larger cell bottom right. Other cells have very irregular, folded and convoluted nuclei; they are tending to form violaceous projections similar to multiple drumsticks, one of which has a pale centre. The chromatin is particularly condensed in these cells, without visible nucleoli. (B) In this field can be seen all the morphological features just described. The two cells bottom left have particularly numerous nuclear appendages, but few granules. The larger cells are more granular and have more homogeneous paler chromatin. Some of the promyelocytes have vacuoles that vary in size and position.

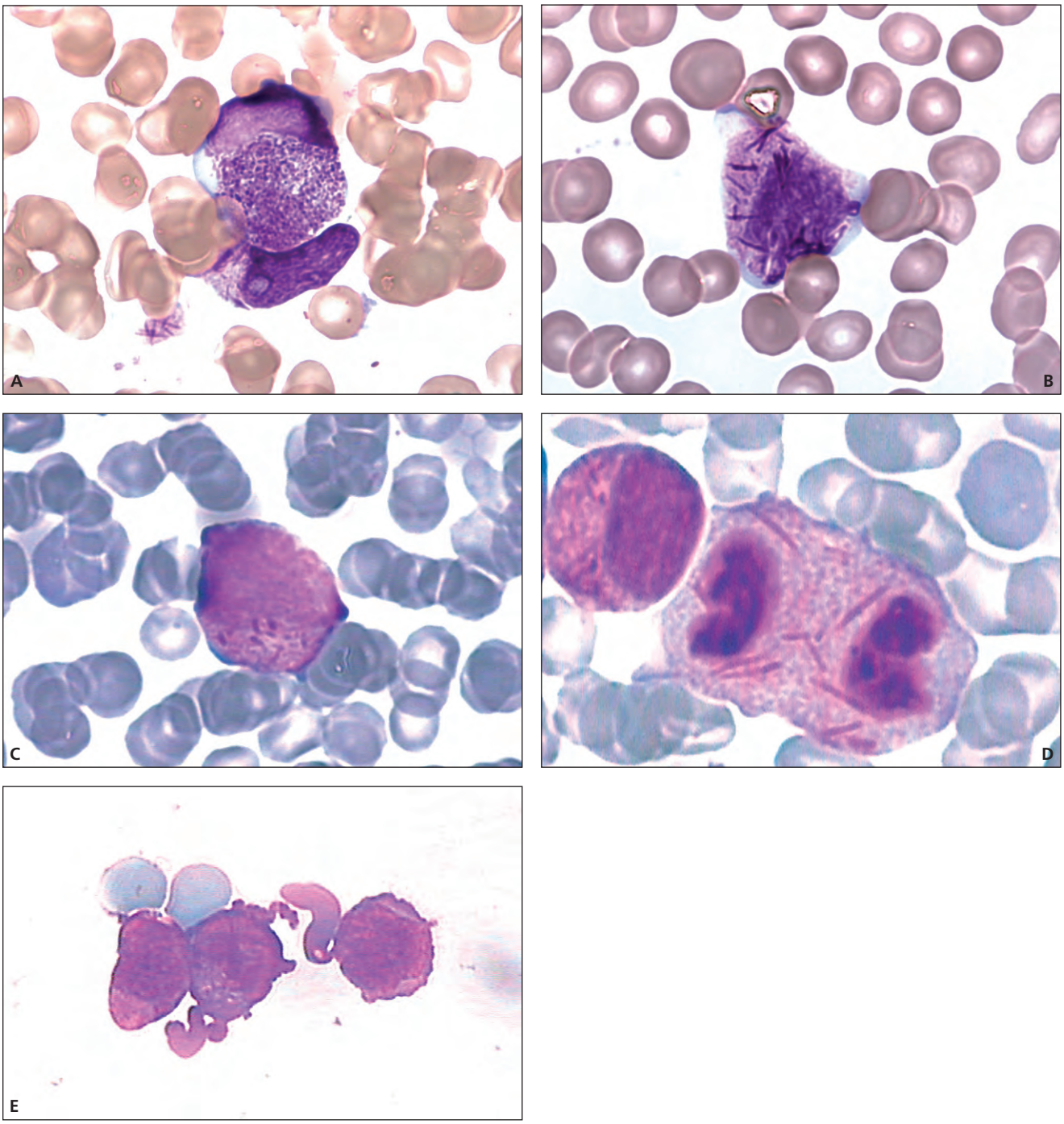



**Fig. 4.42** Heterogeneity in morphological features of leukaemic promyelocytes. (A) At the top, a large promyelocyte with a very low N:C ratio, a compact nucleus with no nucleolus and, flattened at one side of the cell, weakly basophilic cytoplasm that is almost completely filled with masses of tightly packed granules; at the bottom, a small promyelocyte with a very high N:C ratio, vermicular chromatin with a prominent nucleolus and rose-coloured cytoplasm containing Auer rods of variable length. Bottom left, a cytoplasmic fragment of the same colour, stuffed with Auer rods, the result of clasmacytosis. (B) Promyelocyte with an angulated nucleus; it has a straight edge on the left and two nuclear appendages on the right. The cytoplasm is plentiful with a low N:C ratio, and appears to be composed of two zones – a central zone occupied by powdery and gelatinous pink microgranules plus a conspicuous dozen or so short thick Auer rods, and a peripheral zone, clearly delimited also on its inner surface, of pale blue basophilic cytoplasm. (C) In this cell, which has a large eccentric nucleus and a high N:C ratio, the cytoplasm appears to be occupied by granules and very short Auer rods, like splinters, which stand out even above the nucleus. (D) The presence of multiple Auer rods admixed with fine azurophilic granules makes the morphology of leukaemic promyelocytes unmistakable, even when a cell is in mitosis, as seen in this cell in telophase; this cell has not yet undergone definitive cytoplasmic division and the chromosomes are 'frozen' into two masses of pre-apoptotic chromatin. €(E) Promyelocytes in a cytocentrifuge preparation of cerebrospinal fluid from a patient with meningeal localisation of APL.

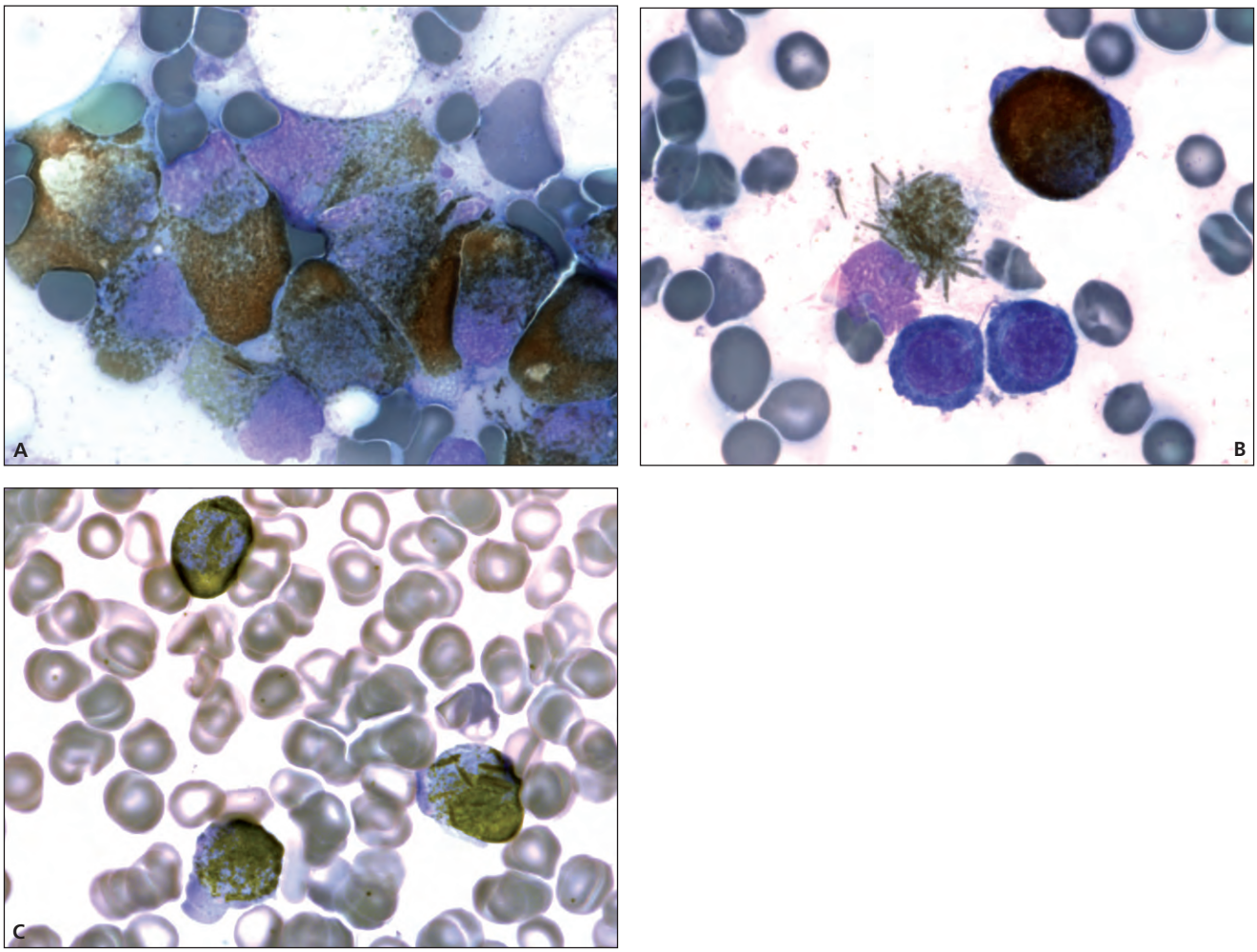



**Fig. 4.43** Intense peroxidase activity in hypergranular APL. (A) The greenish-yellow precipitate has accumulated as both granular and diffuse staining of the entire cytoplasm of these promyelocytes. (B) The torn cytoplasm of this cell lets the morphological details of the peroxidase staining be appreciated; there are large granules massed together and individually identifiable Auer rods in a loose bunch. The cell at the top has its nucleus totally obscured by peroxidase-positive granules. (C) In this promyelocyte the positivity for peroxidase is granular and occupies almost all the cytoplasm; the positive reaction of the numerous Auer rods in the cell on the right is appreciated better when they overlie the nucleus.

important is the appearance that the bone marrow aspirate may have during recovery of granulopoiesis after transient neutropenia of any origin. In these cases the granulocytic series can show a clear reduction or arrest of maturation at the promyelocyte stage with a high percentage of promyelocytes laden with azurophilic granules.[60] A similar picture can be seen in agranulocytosis or, more often, after induction chemotherapy for acute leukaemia. Particularly during treatment of AML and, above all, APL, one may harbour doubts as to whether the promyelocytes that are repopulating the marrow after aplasia induced by chemotherapy should be regarded as normal or leukaemic. In many cases, only repeating the aspirate after several days eliminates the doubt. Rarely, diagnostic perplexity is with regard to MDS with a large proportion of dysplastic promyelocytes (see Fig. 3.69). A useful cytomorphological criterion is based on the recognition of the orderly structure of the normal promyelocyte, which has an eccentric nucleus, ample cytoplasm, an easily seen pale paranuclear zone and a regular distribution of azurophilic granules. In any event, the differential diagnosis is completed with cytogenetic and molecular investigation.

APL is also distinguished by a specific immunophenotypic picture[61–63]:

- Positivity for CD117;
- Strong and homogeneous positivity for CD33 (narrow-based histogram);
- Heterogeneous positivity for CD13 (broad-based histogram);
- Frequent absence or low expression of HLA-DR and CD34;
- Expression of CD9;
- Absence (or very low expression) of CD7, CD11a, CD11b, CD14, CD18, CD66b and CD66c;

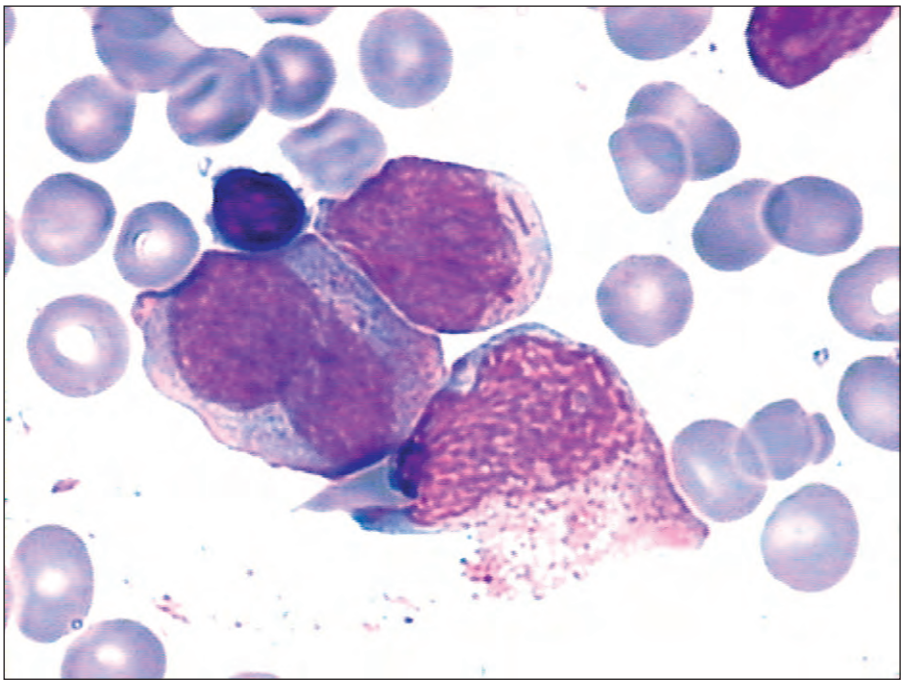


**Fig. 4.44** Less characteristic morphological aspects in a case of hypergranular APL; bone marrow aspirate. There is a large promyelocyte with a bilobed nucleus, similar to those that are seen in the microgranular variant, with plentiful basophilic cytoplasm, which is granular only in some areas, while other areas contain coalescent hyaline material. Higher up is a promyelocyte with a high N:C ratio, a compact nucleus with a prominent nucleolus and other incomplete nucleoli, and scanty, mainly pink cytoplasm with one Auer rod clearly seen top right and others superimposed on the nucleus.

- Absence (or very low expression) of markers of granulocytic differentiation, CD15 and CD65;
- Low expression of the proteins associated with multidrug resistance (MDR), such as P-glycoprotein (PGP);
- Co-expression of CD2, particularly in the microgranular variant.

CD56, an adhesion molecule of neural cells, is positive in 20% of cases and has been associated with an unfavourable prognosis and, together with the aberrant expression of CD2, has been associated with a higher frequency and greater severity of coagulopathy.[64,65]

Recently, an antigenic profile common to all APL has been described, regardless of the genetic lesion and the morphological subtype: this is characterised by the absence of a distinct blastic population on the two-dimensional CD45/SSC cytogram, by the presence of CD11b-negative myeloid cells localised in the granulocyte region, by the lack of HLA-DR and CD11c in all the myeloid cells and by the expression of CD117.[66]

The morphological and immunophenotypic features of APL have such a high degree of specificity that it is almost always possible to predict the specific, unique cytogenetic abnormality that characterises it. The *PML-RARA* fusion can be recognised both by FISH and by RT-PCR. It is present, at least at the molecular level, in more than 95% of cases (being replaced in the remaining infrequent cases by variant *RARA* translocations; see below) and is never found in other types of leukaemia.

All-*trans*-retinoic acid induces rapid terminal differentiation of the leukaemic promyelocytes. The therapeutic efficacy of high doses of this drug is attributable to its ability to overcome the maturation block of the leukaemic cells, which leads to characteristic morphological alterations, principally the appearance of cells of neutrophil lineage with a variable degree of dysmorphism and apoptosis, with the remains of Auer rods in mature neutrophils (Fig. 4.46). Arsenic trioxide, also quite efficacious, induces early apoptosis in leukaemic promyelocytes and only secondarily favours differentiation.[67]

In some patients treated with ATRA there is an acute syndrome called the retinoic acid syndrome, characterised by fever, acute pulmonary insufficiency with pulmonary infiltration, massive neutrophil leucocytosis and renal insufficiency. The aetiology of this condition, which is not yet completely clarified, relates to the liberation of inflammatory cytokines by the leukaemic promyelocytes under the influence of ATRA.[68]

### Microgranular variant of acute promyelocytic leukaemia

Some case of APL have morphological features that differ from those of the classical form; there are large blast cells with an irregular nucleus and ample cytoplasm containing few granules.[69,70] In general, these patients have a more marked leucocytosis (30–50 $\times$ $10^9$/l) (see Report 4.3). The majority of the leukaemic cells, particularly in the peripheral blood (Figs 4.47 and 4.48), are medium sized to large with plentiful pale

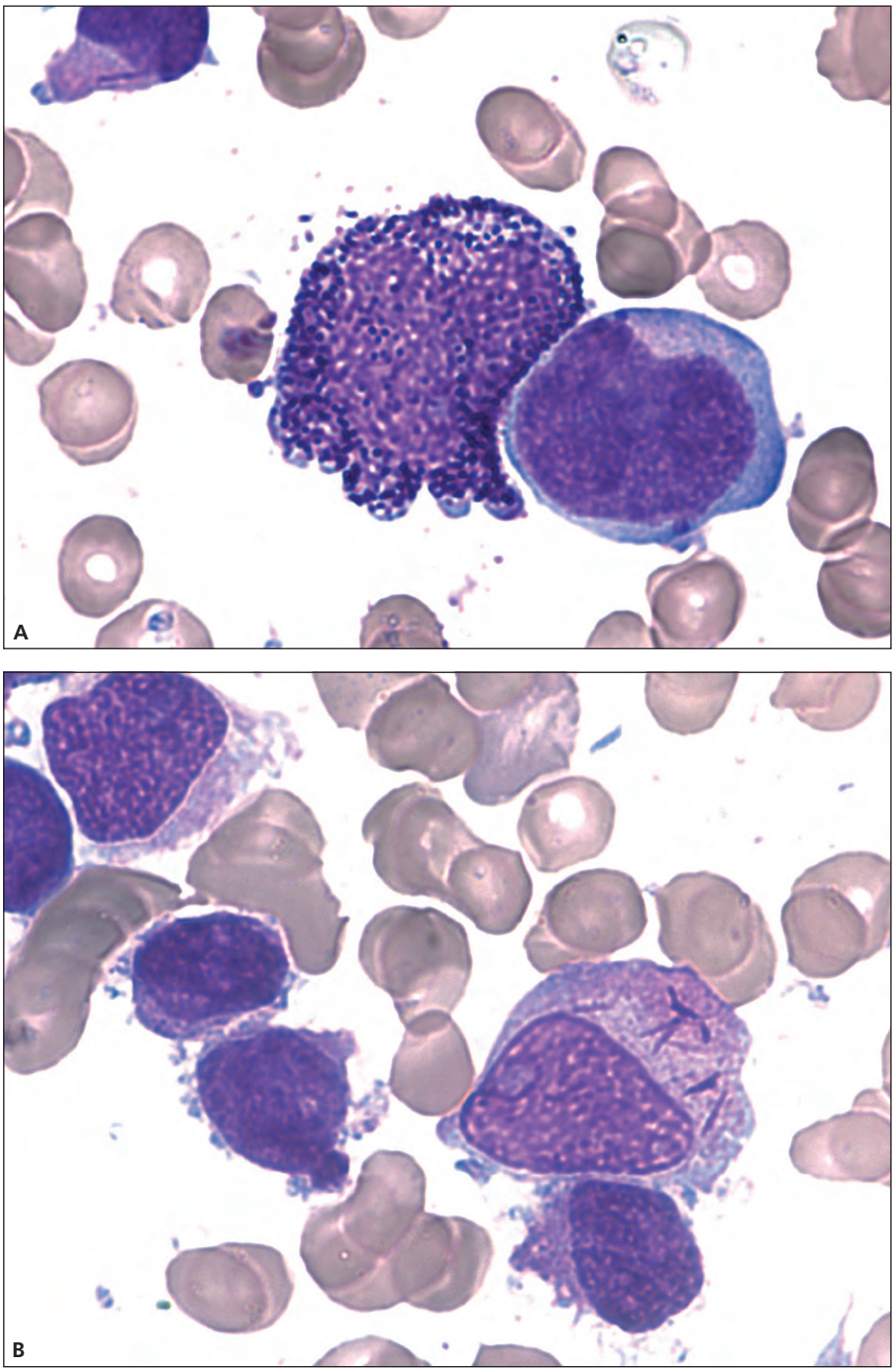



**Fig. 4.45** A case of APL with morphology that is not completely typical; bone marrow aspirate. (A) In the centre, a promyelocyte laden with very numerous dark granules that are individually identifiable and spread throughout the entire cell, covering both the nucleus, obliterating its outline, and the cytoplasm. The cytoplasm appears ragged at the bottom. On the right, a cell with intensely basophilic cytoplasm containing fine granules that occupy, particularly, the visible Golgi zone. The basophilia is accentuated peripherally. The nucleus has granular, mainly homogeneous chromatin, with areas of condensation, and has an indentation beneath the Golgi zone, from which there seems to extend a longitudinal sulcus which is partially dividing the nucleus into two large lobes. From the upper left corner there projects outwards half of a small promyelocyte with scanty blue and violet cytoplasm occupied by a long thick Auer rod. (B) The large cell on the right is undoubtedly a typical leukaemic promyelocyte, with a flattened nucleus that shows with great clarity both a large prominent nucleolus and the thick dark nuclear membrane that surrounds the triangular nucleus; the cytoplasm is basophilic and rich in fine granules, in this case particularly abundant at the periphery; there are also numerous Auer rods. On the left, a pair of small promyelocytes with condensed chromatin and scanty, very basophilic cytoplasm with numerous projections that recall megakaryoblasts and micromegakaryocytes.

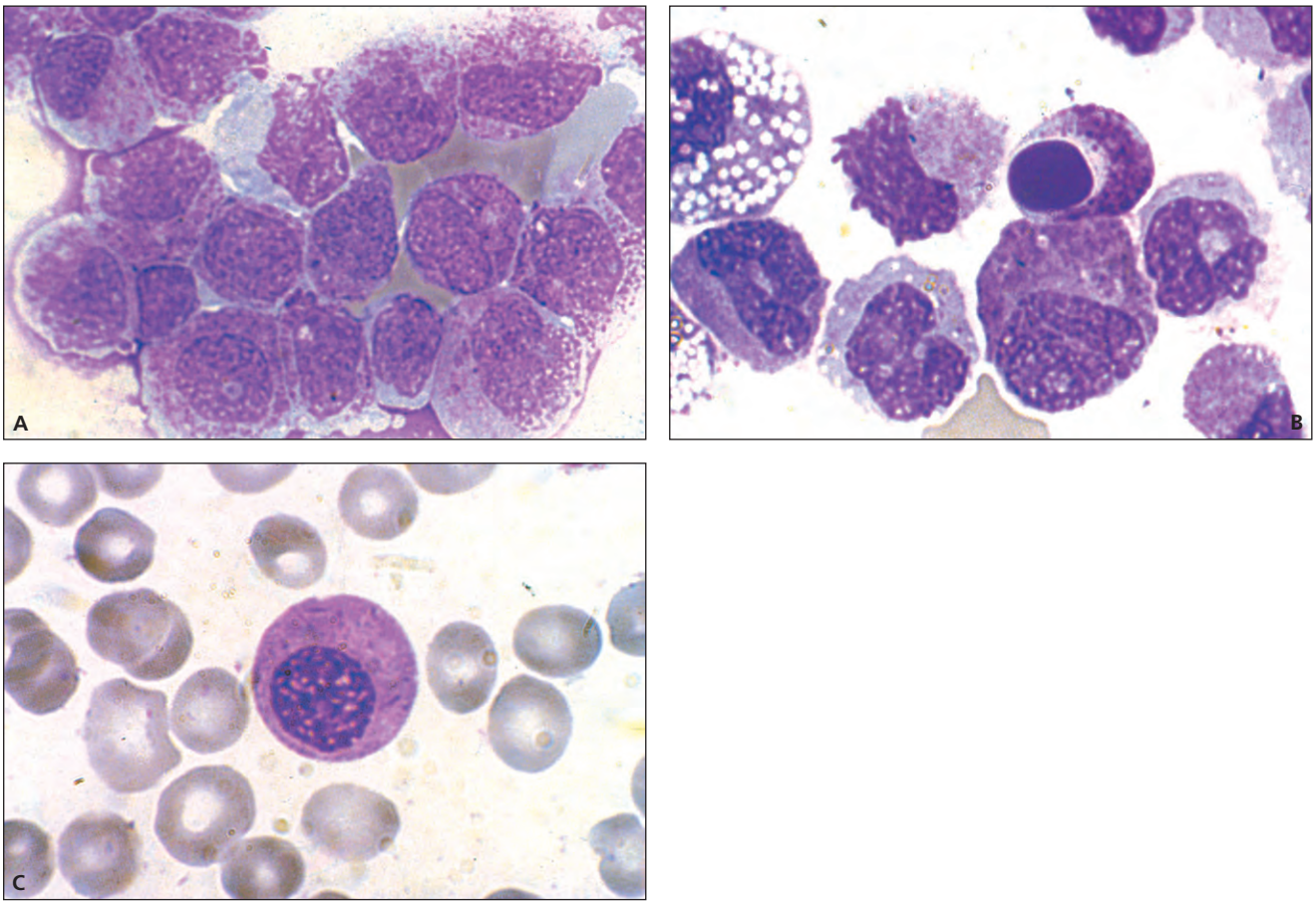



**Fig. 4.46** The effect of retinoic acid on the morphology of APL. (A) Hypergranular promyelocytes on the first day of culture in the presence of retinoic acid. The cells are hypergranular blast cells with nuclei that have open chromatin and prominent nucleoli. (B) The appearance of the same culture after eight days. Retinoic acid has induced the maturation of the promyelocytes, which have differentiated into myelocytes, metamyelocytes and band neutrophils. Near the centre, an apoptotic cell with a small round nucleus, which has completely homogeneous chromatin and is separated by a clear halo from the cytoplasm with its indistinct granules. On the left, a degenerating cell with vacuolated cytoplasm. (C) Peripheral blood of a patient with APL who is receiving all-*trans*-retinoic acid. This neutrophil has a non-segmented pseudo-Pelger nucleus and granular cytoplasm containing the splinter-like remains of Auer rods. Cells of this type are the result of the maturation that occurs when pharmacological doses of this drug overcome the maturation block.

cytoplasm of a blue-grey colour, either appearing totally agranular or having scattered fine azurophilic granules and rare Auer rods. The nuclear configuration is distinctive: the nucleus is clearly visible because of the lack of granules and can be reniform, bilobed, convoluted or in the shape of an hourglass, a butterfly, a handlebar or a mushroom (Figs 4.47, 4.49 and 4.50). The most individual shape resembles the handset of an old telephone, with two rounded extremities that are wider than the elongated central portion and are folded in on the same side. The first impression on examining the film is of a leukaemic population with atypical monocytic features. This diagnostic uncertainty is resolved by three types of observation:

- The recognition of some typical hypergranular promyelocytes with multiple Auer rods, which are consistently present, even if in only very low numbers; one may also observe small hyperbasophilic promyelocytes with cytoplasmic extensions;
- The appearance of the bone marrow aspirate, in which the overall picture is often closer to the classical form with a larger proportion of promyelocytes that are rich in granules and Auer rods;
- Recourse to cytochemistry: the peroxidase and CAE reactions have the same positivity as in the hypergranular form, although with the reaction sometimes being weaker.[71]

On electron microscopy the cells appear rich in primary granules with dimensions varying between 100 and 400 nm (in comparison with 120–1000 nm in the case of typical APL promyelocytes), in large part less than 250 nm and thus below the level of resolution of the light microscope. These granules are asymmetrical and positive for peroxidase at an ultrastructural level.

The morphological distinction between the classical and the microgranular forms of APL is not clear-cut: sometimes it can

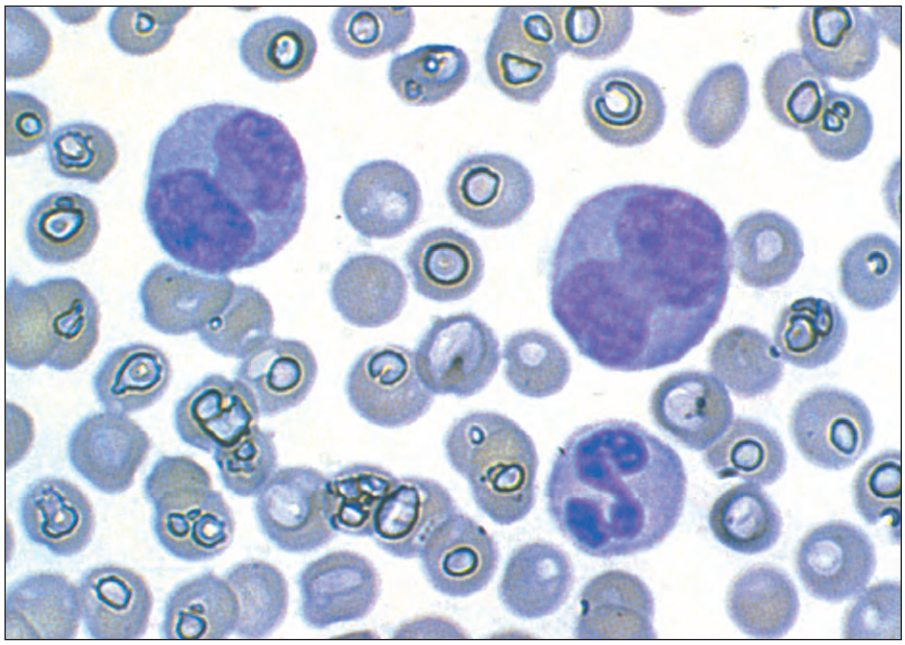


**Fig. 4.47** APL, microgranular; peripheral blood film. At the bottom, a hypogranular neutrophil. The two immature leukaemic cells have a low N:C ratio because of the presence of plentiful basophilic cytoplasm, in which granules are not seen. The nuclei are characteristic because of their bilobed form, resembling an hour glass, a dumbbell or a mushroom; a particularly suggestive feature is the apparent reduction of the chromatin density in the narrow, central area where the nucleus is thinner. Despite the vague similarity to cells of monocyte lineage, the morphology of these cells is unmistakable and diagnostic of this subtype of leukaemia.

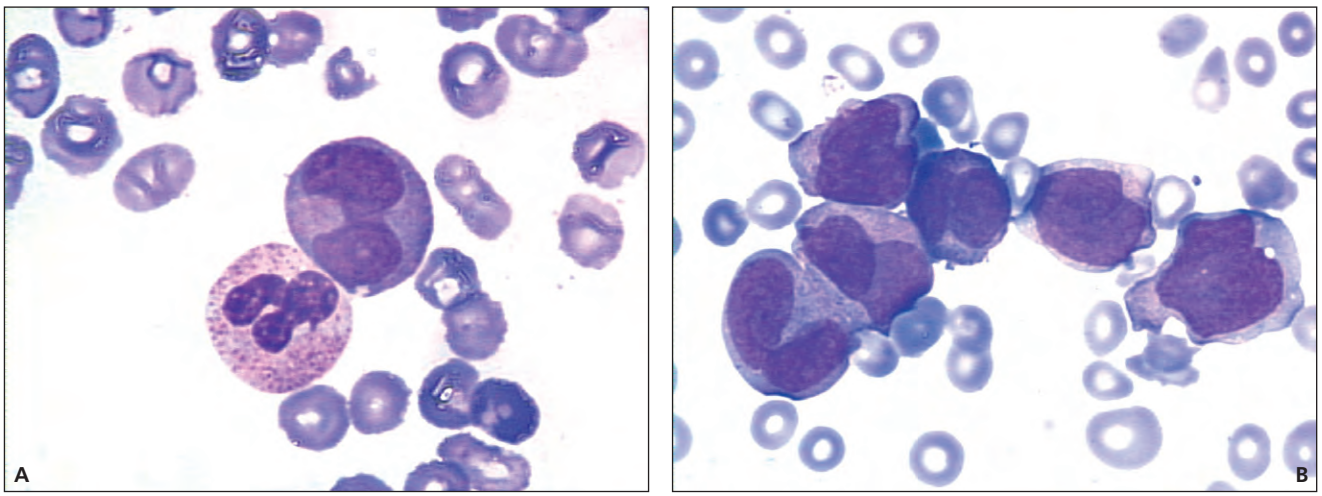



**Fig. 4.48** A case of APL, microgranular variant, in a 44-year-old patient with marked leucocytosis (WBC 62 × $10^9$/l), anaemia and moderate thrombocytopenia; peripheral blood films. The presence of the *PML-RARA* fusion gene was confirmed by RT-PCR. (A) Beside a normally granulated mature neutrophil there is a large immature leukaemic cell with a N:C ratio that is low because of the abundant greyish-lilac cytoplasm; the latter has a ground-glass appearance because of the mixture of the background basophilia, greater at the periphery, with fine dots that represent a myriad of fine azurophilic granules that are not individually recognisable. The nucleus is bilobed with fairly fine and homogeneous chromatin, with some areas of condensation, particularly in the upper lobe, and the outline of a nucleolus in the lower lobe. The hollowed out isthmus between the two lobes gives a suggestive morphology to the nucleus (like the handset of a telephone or a dumbbell) indicating that this is the microgranular variant of APL. (B) The immature leukaemic cells have a monotonous appearance, with minor variations: a large bilobed nucleus, reniform or notched, only apparently monocytoid, with delicate homogeneous chromatin; plentiful basophilic cytoplasm with a lilac dusting of very small granules, sometimes almost invisible, as in the cell on the right. These cells also are variant promyelocytes. The cell bottom left has the typical saddlebag nuclear conformation.

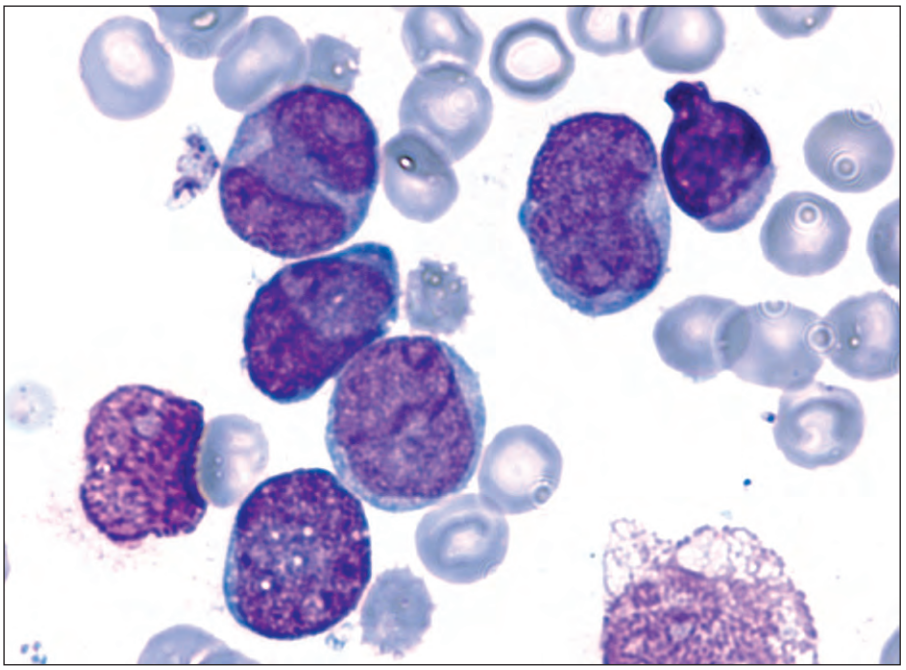


**Fig. 4.49** Bone marrow aspirate, same case as Fig. 4.48. It is not rare for the immature leukaemic cells that infiltrate the bone marrow, largely replacing normal haemopoiesis, to be less immediately evocative of the microgranular variant of APL. The only cell in this image that is really characteristic is the bilobed cell top left, in which there is notable delicacy of the chromatin, a deeply carved out isthmus and a giant nucleolus in the upper lobe. The other cells have a similar chromatin structure, finely granular, with a notched outline and plentiful basophilic cytoplasm or, sometimes, more scanty cytoplasm as in the cell at the bottom with nuclear vacuoles. The smallest cell, top right, could be a small blast cell with shiny condensed prepyknotic chromatin.

be difficult to classify a case as one or the other. There are intermediate forms with a variable proportion of hypogranular cells (more numerous in the blood) and hypergranular forms (more numerous in the bone marrow) (Figs 4.51 and 4.52). When the cells of the hypogranular form are cultured *in vitro*, they can show an increase in granulation. It is also possible for the hypergranular form to relapse as the microgranular variant. The fact that one is dealing with two morphological variants of what from various points of view is a single entity is demonstrated by: cytogenetic and molecular identity (consistent presence of the t(15;17) or the *PML-RARA* fusion gene), consistent clinical features (disseminated intravascular coagulation) and the same therapeutic response (sensitivity to ATRA).

The immunophenotypic profile of the microgranular variant can be similar to that of the hypergranular variant; however, it is more common for there to be expression of CD34, HLA-DR and, sometimes, CD2 and CD56.[72] The expression of the T-lymphoid antigen, CD2, has been described in cases with microgranular morphology and a high white cell count at diagnosis.[73]

A rare form of AML, often but not always showing morphological analogies with APL, has a unique immunological profile characterised by positivity for CD33 and, variably for CD13, associated with CD56 expression (characteristic of NK cells) and negativity for CD34 and HLA-DR[74]: the leucocyte count tends to be high and the morphology of the blasts resembles that of the microgranular variant with peroxidase positivity. Auer rods are rare and do not occur in bundles. The translocation t(15;17) is absent and there is no response to ATRA.

### Acute myeloid leukaemia with variant translocations involving RARA

AML with translocations involving *RARA* and a partner gene other than *PML* have been described.[75] They are often morphologically somewhat different from classical APL. For example, one can observe:

- The translocation t(11;17) with a *ZBTB16-RARA* fusion gene. The second gene involved, at 11q23, is also known as the promyelocytic zinc finger gene (*PLZF*). In this variant, the blasts have atypical intermediate characteristics, with a mixture of promyelocytes and poorly granulated blast cells (Fig. 4.53), strongly positive for peroxidase and CD56; Auer rods are absent in the majority of cases, but not in all; the mature neutrophils often have a pseudo-Pelger nucleus and hypogranularity[76]; in other cases there is involvement of the *NUMA* gene at 11q13.
- The translocation t(5;17), which involves the gene encoding nucleophosmin and has an *NPM1-RARA* fusion gene, in

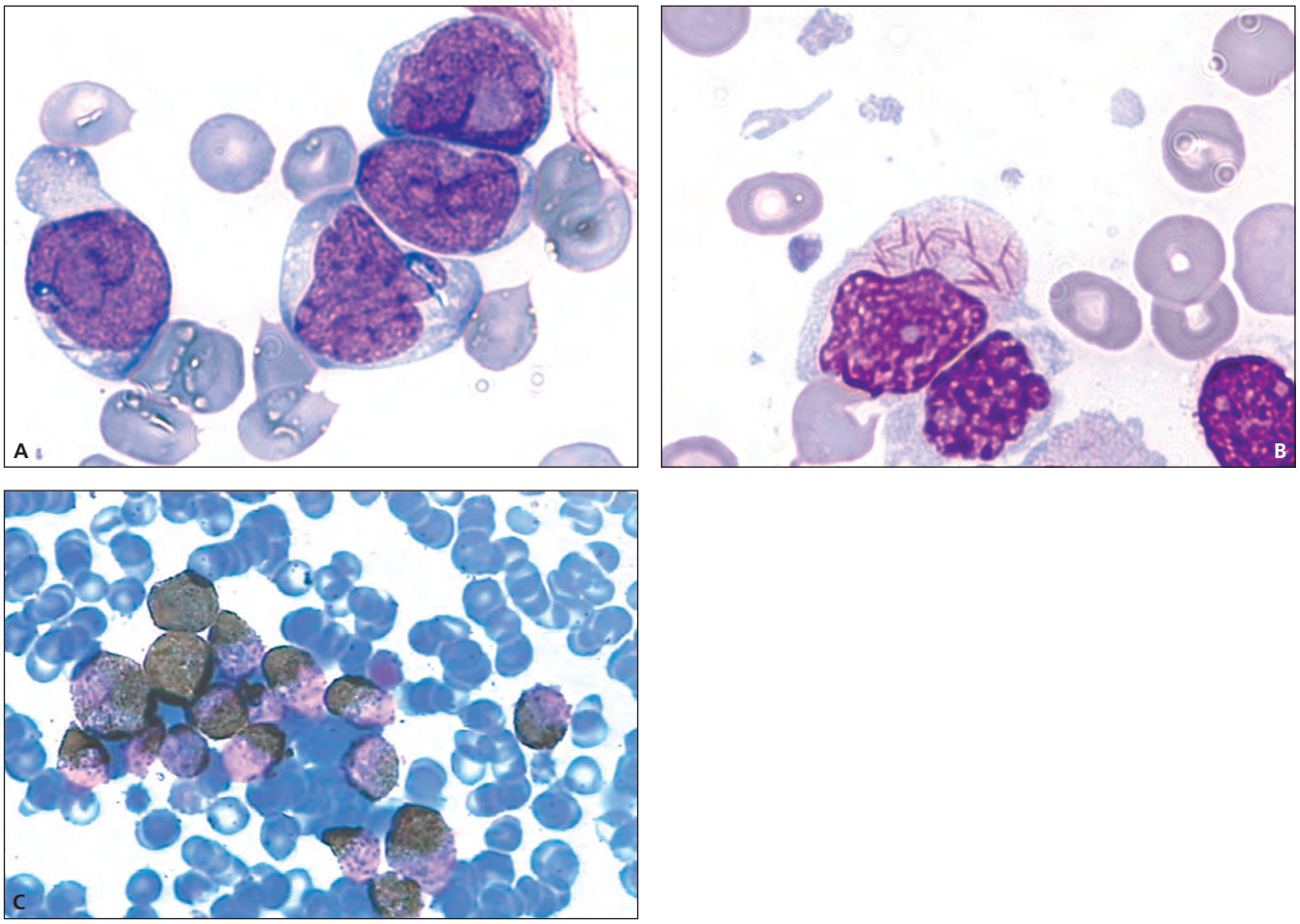



**Fig. 4.50** Microgranular variant of APL; bone marrow aspirate. (A) In this case, the nuclei appear very convoluted, with open granular chromatin, with deep valleys and gigantic nucleoli: such nuclear structure evokes an almost tactile sensation of softness, plasticity or sponginess. The plentiful cytoplasm is pale near the nucleus and intensely basophilic, cobalt blue, at the periphery: there are no granules that are either visible or alter the tinctorial characteristics of the cytoplasm; there may be a small Auer rod in the promyelocyte on the left. The likely diagnosis is less immediately obvious than in the case in Fig. 4.49. (B) In the microgranular variant one almost always finds rare hypergranular promyelocytes or, as in this case, promyelocytes laden with Auer rods, which facilitate the morphological diagnosis. This promyelocyte has been flattened during the spreading of the film so that it is easy to see, even though the cell is still intact, that the cytoplasm is full of red rods and that the nucleus has a vermicular chromatin structure and a nucleolus. (C) The apparent absence of cytoplasmic granules observed in (A) is belied by the intense positivity of all the pathological cells in this myeloperoxidase stain. In addition to the tightly packed granules, one can also see rare Auer rods, as in the larger promyelocyte on the left.

which there is a heterogeneous population of hypergranular and hypogranular promyelocytes without Auer rods.

According to the 2008 WHO classification, these rare forms are not classified as APL, but as AML with variant *RARA* translocations. Some of these subtypes, in particular that with t(11;17), are not sensitive to ATRA.

## Other acute myeloid leukaemias with recurrent genetic alterations

Only one type of AML in this second group of chromosomal translocations and inversions had already been included in the previous edition of the WHO classification: that characterised by blast cells with mainly monocytic morphology and the translocation t(9;11).[9] The other three subtypes, with diverse morphological characteristics, have been recognised more recently as distinct cytogenetic entities and have been included only in the fourth edition of the WHO classification, that of 2008[45]: they are fairly rare (each with a frequency of 1–3%, or less, of cases of AML) and are aggressive, characterised by a short survival; allogeneic transplantation is the only effective treatment.[77]

The genetic lesions can be investigated by cytogenetic analysis, FISH or RT-PCR. However, even when the relevant genetic or cytogenetic abnormality has been detected, the WHO classification requires that the blast count must be above 19% in the blood or bone marrow for a diagnosis of AML to be made. It has not been excluded, however, that in the future these genetic

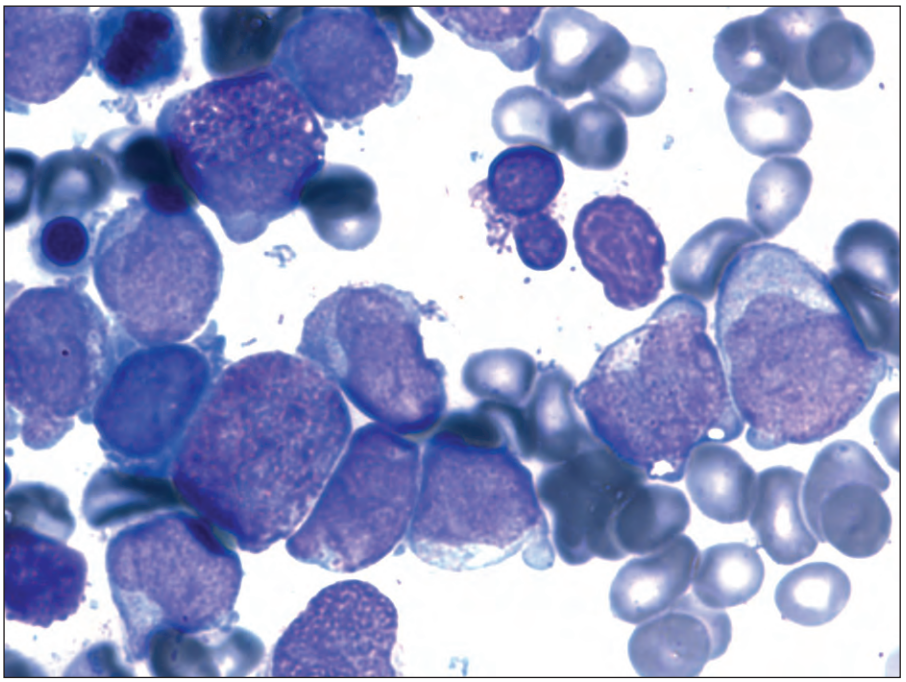


**Fig. 4.51** APL with morphological features intermediate between the typical hypergranular form and the microgranular variant; bone marrow aspirate. In this heavily infiltrated marrow there is a prevalence of blast cells with plentiful basophilic agranular cytoplasm and convoluted folded nuclei with pale granular cytoplasm, as seen in the cell towards the right. Nuclear folding is visible also in the blast cells on the extreme left, beneath the single orthochromatic erythroblast. Other blasts have a less complex nuclear conformation and less abundant cytoplasm, sometimes with some granules. There are other cells that are typical hypergranular promyelocytes, such as the cell that is well seen top left, in which there is an impressive mass of dark granules superimposed on the nucleus, which is found in the lower part of the cell, obscuring its edge; the cytoplasm appears intensely basophilic in the area that is free of granules and in the large pseudopodial projection emerging from the lower pole. Also, the large cell straight below is a hypergranular promyelocyte. The diagnosis was confirmed by cytogenetic and molecular analysis.

abnormalities might be considered in themselves sufficient for a diagnosis of AML.

### Acute myeloid leukaemia (monoblastic) with t(9;11)(p22;q23)

This entity was included in the previous edition of the WHO classification under the designation of AML with abnormality of 11q23/*MLL*. It occurs more often in the paediatric age range and represents 2% of cases of AML in adults. Presentation can be with disseminated intravascular coagulation or with extramedullary disease, either myeloid sarcoma or diffuse infiltration of skin and gums.

This genetic aberration is associated almost always with a monoblastic phenotype with bone marrow infiltration by monoblasts and monocytes (Figs 4.54 and 4.55). The authors of the 2008 WHO classification describe monoblasts as 'large cells with abundant cytoplasm which can be moderately to intensely basophilic and may show pseudopod formation'. The cytoplasm may contain fine azurophilic granules and vacuoles. The nucleus is usually round with delicate lacy chromatin with one or more large prominent nucleoli. Promonocytes, which must be included in the blast count, are characterised by a nuclear configuration that is more irregular and delicately convoluted; the cytoplasm is usually less basophilic and sometimes clearly granulated, occasionally with large azurophilic granules and vacuoles.[45] MPO may be negative in monoblasts (Fig. 4.56), but shows weak positivity in promonocytes. Monoblasts are usually positive for non-specific esterase, with stronger reactions seen in promonocytes (Fig. 4.57). The immunophenotype is characterised by strong expression of HLA-DR, CD4, CD11b, CD11c, CD33, CD64, CD65 and lysozyme. Positivity for markers of immaturity, CD34 and CD117, is absent or weak.

The *MLL* (mixed-lineage leukaemia) gene functions as a positive regulator of gene expression in early embryonic development and in haemopoiesis. Its translocation and rearrangement with *MLLT3* is the most frequent abnormality in acute monoblastic leukaemia, with the *MLLT3-MLL* fusion gene encoding a fusion protein that is responsible for aberrant transcriptional activation.[78] The *MLL* gene can also contribute to fusion genes with other partners, with the production of other fusion proteins and sometimes with a distinct clinicomorphological picture (e.g. ALL). These variant translocations, recognised by sensitive molecular methods, must be specified in detail in the diagnosis.

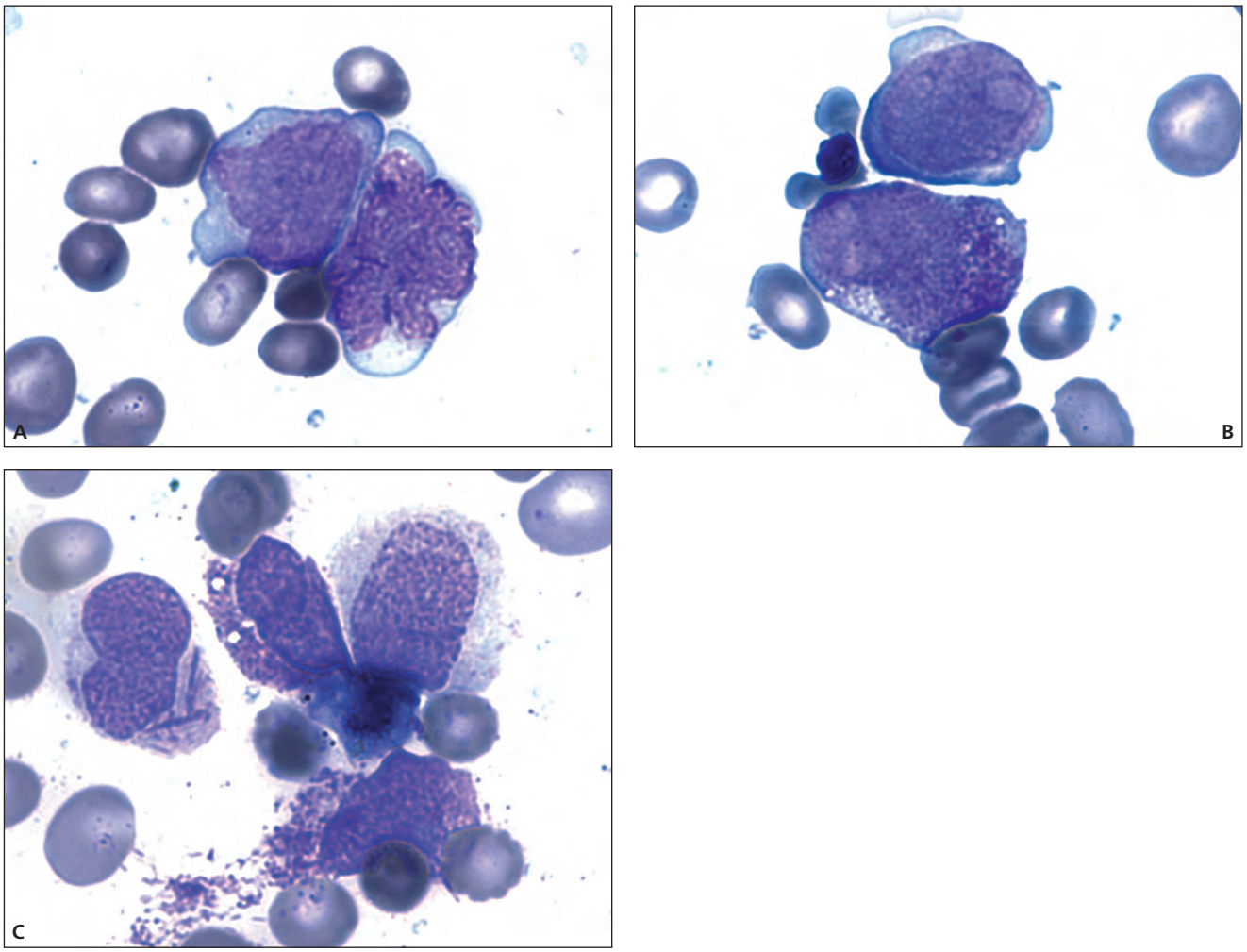



**Fig. 4.52** APL, same case as Fig. 4.51; bone marrow aspirate. (A) Two blast cells that appear to be agranular, with very convoluted nuclei. The chromatin is loose and expanded in the cell on the left and vermicular, possibly premitotic, in the cell on the right. The cytoplasm is homogeneously basophilic if one excepts the pale area at the bottom of the right-hand cell; no granules of any type are seen. (B) The blast at the top has a moderate amount of intensely basophilic cytoplasm with pale areas and a tendency to form broad pseudopodia; the nucleus is oval without any folding, with fine homogeneous chromatin with small dark foci, a prominent nucleolus and other smaller incomplete nucleoli. The lower blast has a different appearance, since the nucleus is reniform and, particularly, since the cytoplasm is packed with dark wine-red granules that obscure the right edge of the nucleus. (C) On prolonged examination of the film, some typical hypergranular promyelocytes were found, one of which contained two enormous dark Auer rods and others that were more delicate.

### Acute myeloid leukaemia with t(6;9)(p23;q34), basophilia and multilineage dysplasia

The blood count in this type of AML shows anaemia, thrombocytopenia and, often, neutropenia. The leucocyte count can be reduced or increased, without there being extreme leucocytosis: the average count reported in the literature is around $12 \times 10^9$/l.[79] The bone marrow is usually very cellular, but there is no specific morphological picture: the blast population may be heterogeneous, with granulocytic differentiation, with cytoplasmic granules and sometimes Auer rods, or myelomonocytic (Figs 4.58 and 4.59A). The most characteristic feature is an increase in basophils, which account for more than 2% of leucocytes in the circulation in around half of cases (Fig. 4.59B). In addition, dyserythropoiesis and dysgranulopoiesis are common (67%),[80] while megakaryocyte dysplasia is only occasionally seen. The immunophenotypic profile reflects the morphological heterogeneity, with the frequent expression of CD34, of granulocytic markers and, sometimes, of CD9 and CD64. The prognosis is very poor, with remission occurring in less than half of cases and survival of less than a year.

Diagnosis requires cytogenetic analysis and molecular analysis by RT-PCR. The translocation leads to fusion of part of *DEK*, located on chromosome 6, with part of *NUP214* (previously known as *CAN*), which encodes nucleoporin: the *DEK-NUP214*

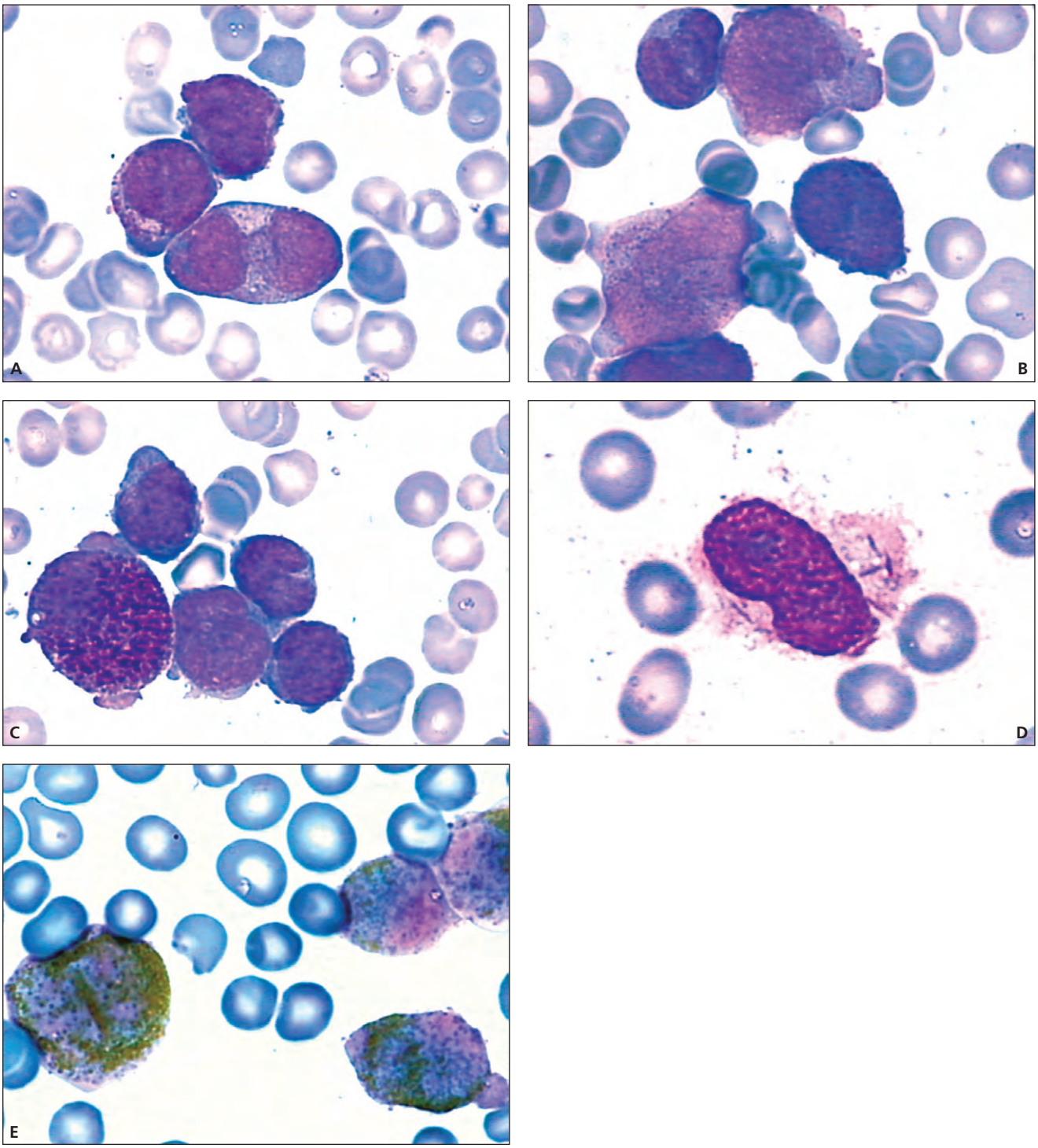



**Fig. 4.53** AML with the variant translocation, t(11;17), and the *ZBTB16-RARA* fusion gene. This subtype of AML is not included, according to the 2008 WHO classification, in the category of APL with recurrent cytogenetic abnormality, particularly because it does not show the same response to treatment with retinoic acid. This case, characterised by leucocytosis ($46 \times 10^9$/l), has heterogeneous morphological features, not dissimilar to those seen in Fig. 4.52. (A) Peripheral blood film. A large blast with a dumbbell-shaped nucleus with two large lobes similar to the cap of a mushroom joined to a carved out isthmus. On the left, a granule-rich blast cell with a rounded nucleus and, at the top, a third blast with a convoluted nucleus and scanty blue cytoplasm. (B) Bone marrow aspirate. The two large blast cells have pale chromatin, folded nuclei and very plentiful cytoplasm, which is sky-blue or lilac as a result of the admixture of the background basophilia with the azurophilia of the fine granules, some of which stand out because they are so dark. The two small blasts have convoluted nuclei with condensed chromatin and very blue cytoplasm. (C) In addition to four blast cells, which variably recapitulate the characteristics just described, there is a typical, large hypergranular promyelocyte, in which the granules, varying in colour from cherry red to dark prune, overflow and cover the eccentric nucleus, the dense chromatin of which can be seen only in the upper left part of the cell. The basophilic cytoplasm can be seen only in the agranular polar projections. (D) In the same film there are occasional promyelocytes, such as this torn example, with numerous small Auer rods. (E) A peroxidase stain shows granular reactivity in all cells, very strong in some cells, moderate in others.

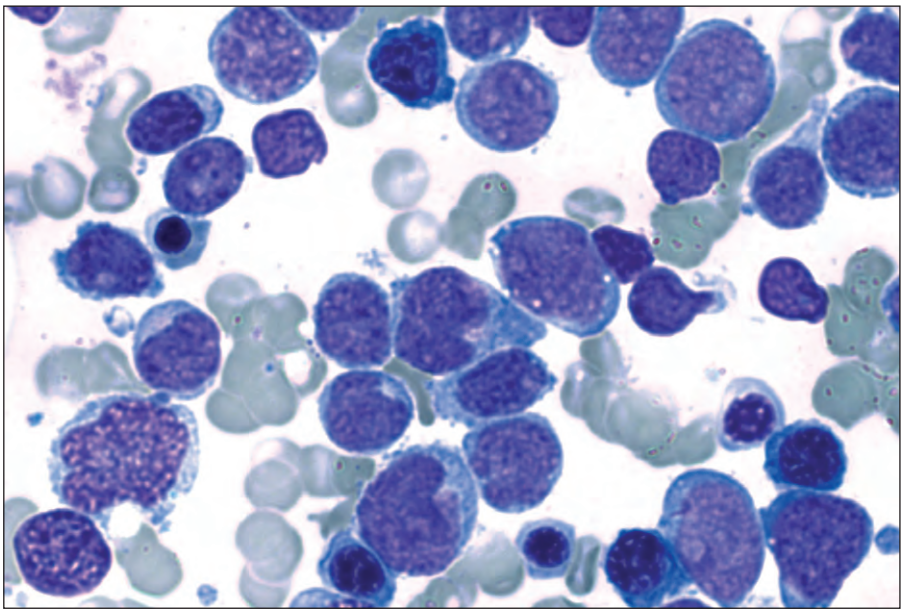


**Fig. 4.54** AML with a recurrent genetic abnormality, t(9;11); bone marrow aspirate. The bone marrow is infiltrated by large blast cells, with large nuclei that have a loose, reticular and fairly homogeneous chromatin structure. The variable amount of cytoplasm is basophilic and agranular. Some blasts appear to show little differentiation, such as the smaller cells top left. Others, such as the two cells in the top right corner and another two bottom right, are typical monoblasts with round nuclei and clearly visible nucleoli. Yet others, with an indented or irregular nuclear outline and slightly denser chromatin, are promonocytes. There are also a number of small erythroblasts.

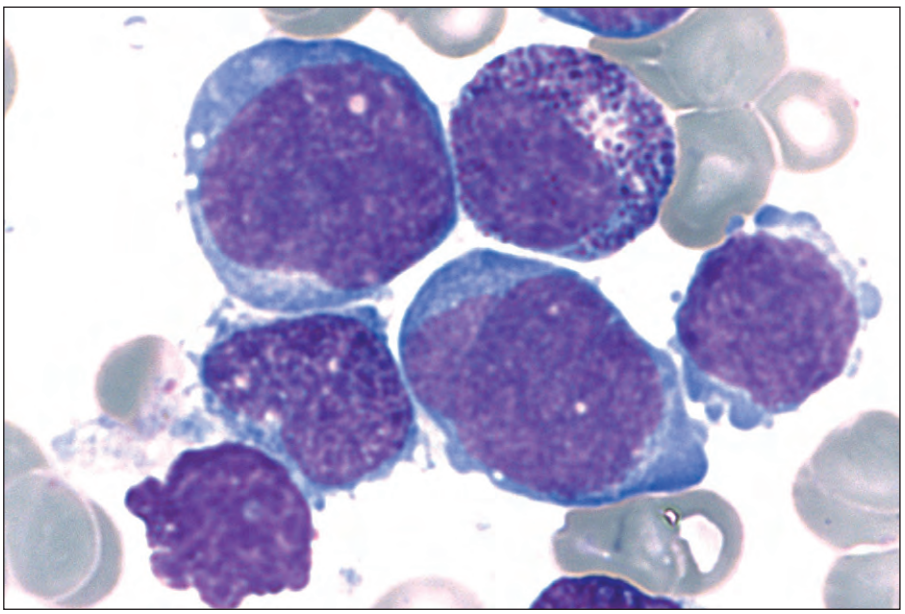


**Fig. 4.55** AML with a recurrent genetic abnormality, t(9;11); same case as Fig. 4.54; bone marrow aspirate. At higher power, two large promonocytes, with minimal indentations and folding of the nuclear outline and homogenous, delicate but compact chromatin with barely visible nucleoli. The two smaller cells are morphological undifferentiated blasts. Top right, an intermediate granulocyte precursor which appears to be an early eosinophil promyelocyte.

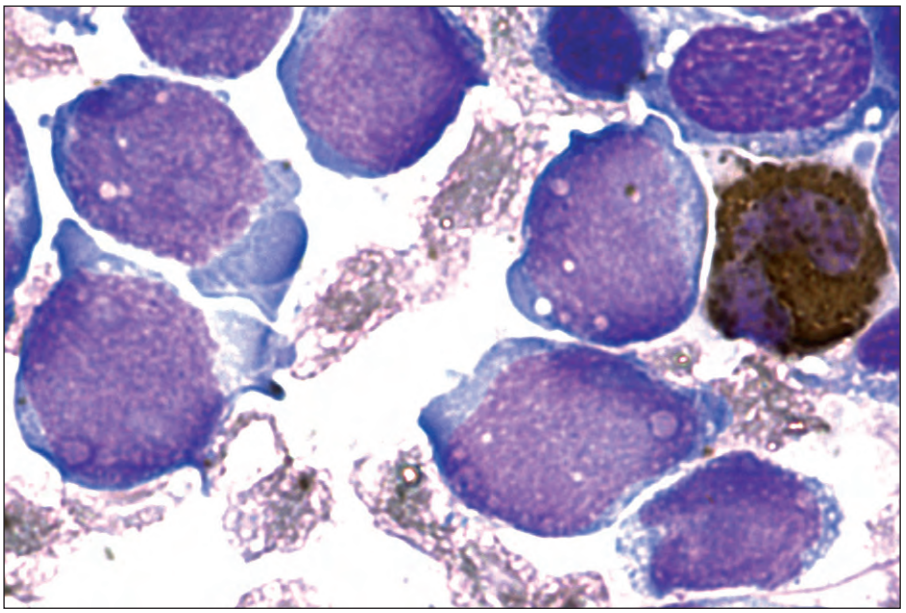


**Fig. 4.56** AML with a recurrent genetic abnormality, t(9;11); same case as Fig. 4.54; bone marrow aspirate, peroxidase reaction. There is strong diffuse positivity only in a mature segmented granulocyte. All the blast cells are completely negative.

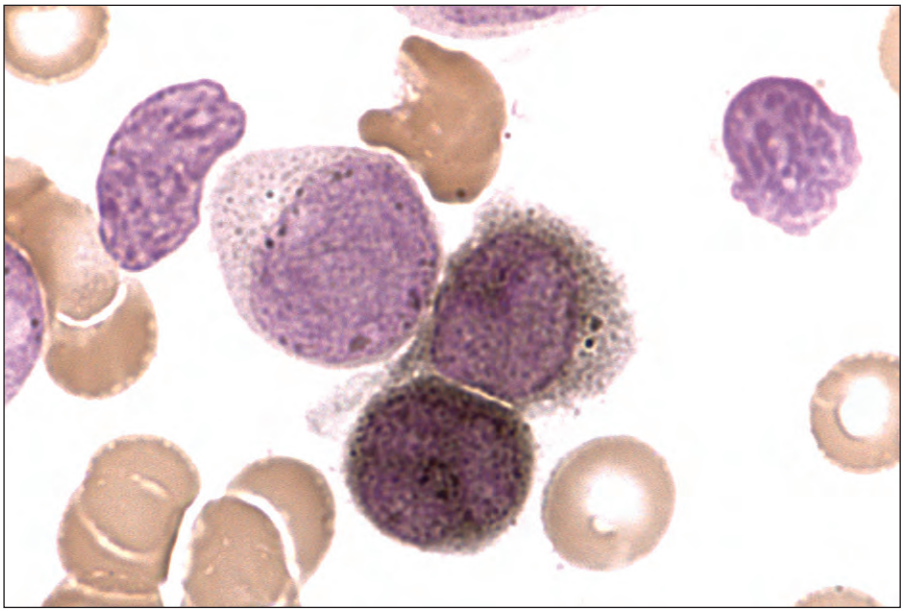


**Fig. 4.57** AML with a recurrent genetic abnormality, t(9;11); same case as Fig. 4.54; bone marrow aspirate, ANAE reaction. A large monoblast shows weak granular positivity. A promonocyte, on its right, has stronger positivity that is both granular and diffuse. The third cell, at the bottom, is more intensely positive and seems to have more condensed nuclear chromatin: it is certainly a more differentiated monocyte.

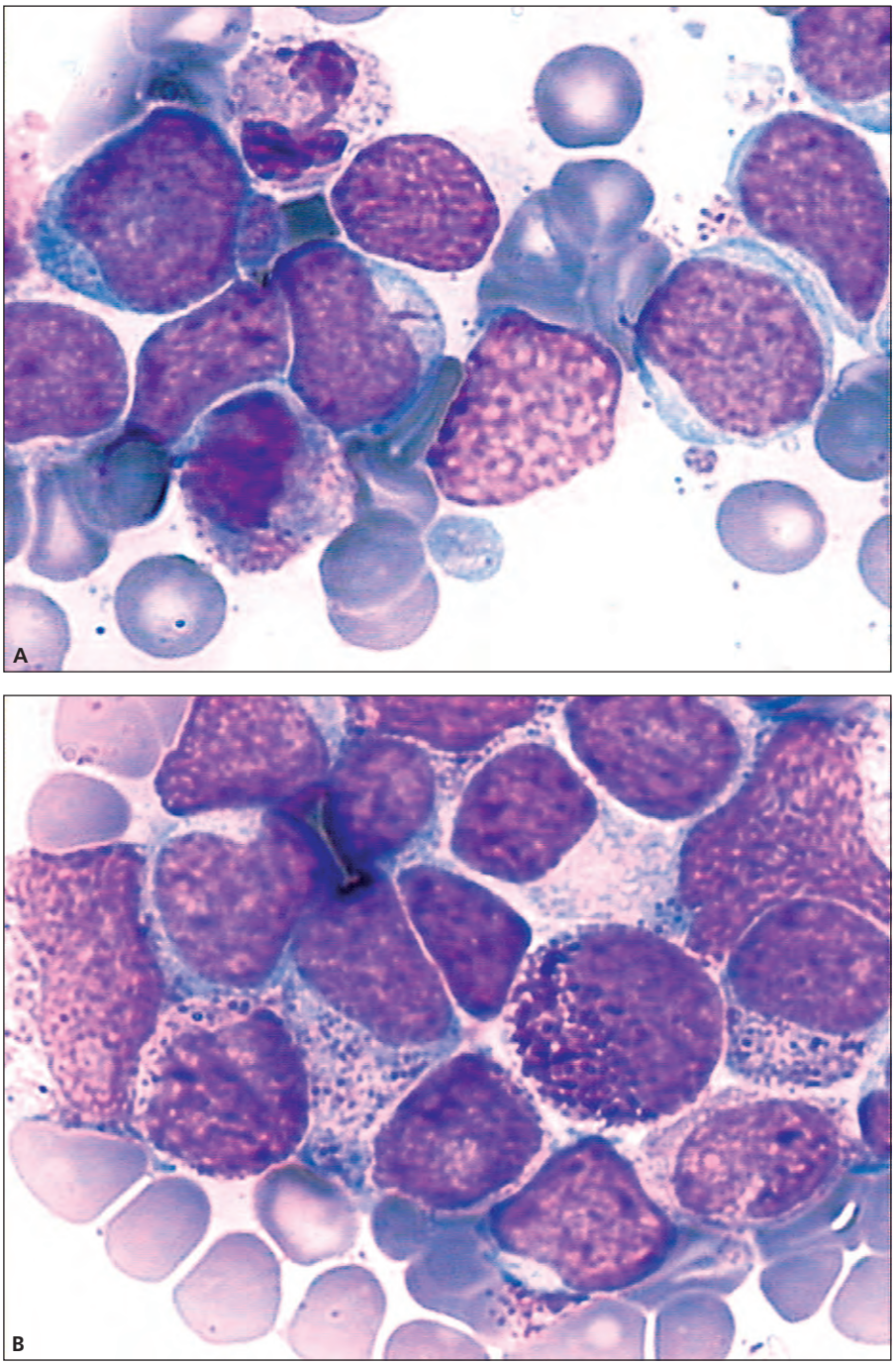



**Fig. 4.58** Bone marrow aspirate of a patient with AML with recurrent genetic abnormality, t(6;9). The blood count showed marked anaemia and thrombocytopenia; the white cell count was 23.4 × $10^9$/l, with 54% blast cells. Bone marrow blasts were 44%. (A) Part of a microscopic field that had numerous blast cells. Four of these are seen; they have the morphology of myeloblasts with a high N:C ratio, an angulated nucleus, irregularly condensed chromatin and barely visible nucleoli. The cytoplasm is basophilic and does not show any clear granules; very obvious, however, in a cell just to the left of centre, is an Auer rod with one pointed end and the other end being superimposed on the nucleus. Just below is a cell of granulocyte lineage with a mature ovoid nucleus, the outline of which consists of segments of circles, with relatively plentiful greyish cytoplasm containing bright red granules that tend to cluster at one pole of the cell; this cell could be a basophil precursor. (B) Cells of granulocyte lineage in various stages of maturation. In the middle of the right edge of the image is a dysplastic promyelocyte, with an eccentric nucleus and chromatin similar to that of the blast cells seen in (A), but less basophilic cytoplasm with numerous variably sized granules, some large and dark, arranged in a disorderly manner. Immediately to the left, another larger promyelocyte with an eccentric nucleus and extensive cytoplasm that is completely packed with large, very dark granules, occupying various levels in the cell and superimposed on each other. It can be suspected that this cell also belongs to the basophil lineage. Still further to the left, a myelocyte with a deeply cleft nucleus and a small number of large dark granules.

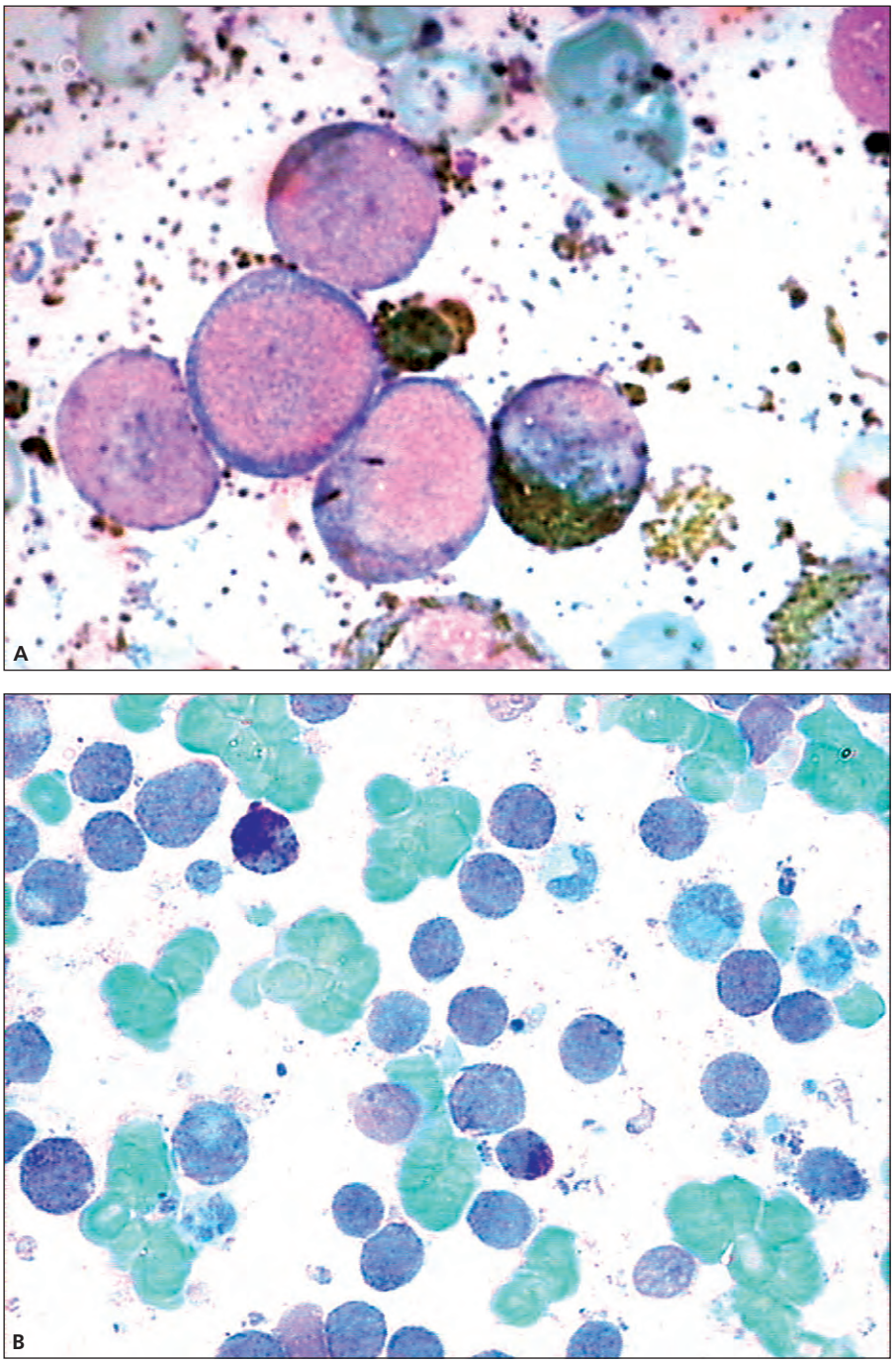



**Fig. 4.59** Cytochemical stains of the same bone marrow aspirate as Fig. 4.58. (A) The blast cells appear negative for peroxidase, with the exception of positive staining by two Auer rods, appearing almost black, that are present in the cell low in the centre. On its right, a cell of neutrophil granulocyte lineage, perhaps a myelocyte, that is intensely peroxidase positive. (B) Toluidine blue staining gives a strongly metachromatic reaction in two cells that appear to be packed with reddish-black granules, confirming that cells of basophil lineage are part of the leukaemic population that has infiltrated the bone marrow. The blue granules present in many other cells, however, are not metachromatic.

fusion gene on chromosome 6 encodes a protein that favours leukaemic transformation.

Mutation of *FLT3* is also frequent; this additional mutation is associated with a higher leucocyte count and higher bone marrow blast percentage, and with an even lower probability of complete remission.[81]

### Acute myeloid leukaemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)

These anomalies of chromosome 3q are associated with *de novo* AML and with AML secondary to MDS.[82] There is no specific morphological picture, with blast cells similar to those of other types of AML (with the exception of APL). The most notable peculiarity is the rarity of thrombocytopenia and the presence of trilineage dysplasia, with variable fibrosis and increased megakaryocytes, which can be small with a non-lobated or bilobed nucleus[83] (Figs. 4.60 and 4.61). The immunophenotype does not have any specific features.

The structural alteration of chromosome 3 involves the *RPN1* and *EV1* genes with transcription of *RPN1-EVI1*. The involvement of *EVI1* has a recognised role in leukaemogenesis also in rare patients in whom the translocation involves a gene other than *RPNI*, to the extent that some authors have proposed the designation 'AML with hyperexpression of EVI1' to indicate these conditions, all characterised by an unfavourable prognosis. It is not rare for there to be associated aberrations involving chromosomes 5 and 7.

### Acute myeloid leukaemia (megakaryoblastic) with t(1;22)(p13;q13)

This subtype is characterised by megakaryocytic differentiation. It is very rare and occurs particularly in girls in the first few years of life (not having a predilection for children with Down syndrome). Bone marrow cellularity is normal or increased. Blast cells may appear undifferentiated, resembling lymphoblasts, or may show differentiation similar to that described below for AML, NOS, megakaryoblastic (Fig. 4.62). Immunophenotyping shows the expression of platelet antigens (CD41 and CD61); CD34, HLA-DR and CD45 can be negative. Peroxidase is negative by cytochemistry and flow cytometry.

The *RBM15-MKL1* fusion gene explains the abnormal deregulation of transcriptional activity in haemopoietic precursors.[84]

## Acute myeloid leukaemia associated with gene mutation

In this subgroup are included two new provisional entities, distinguished by genetic alterations that are not the result of translocations or inversions, but of mutations specific to a particular gene. These are mutations in *NPM1* or *CEPBA*, which lead to the particular subtype of leukaemia described.

A non-specific mutation in AML that differs from these is that of *FLT3*, a gene localised on the long arm of chromosome 13 (13q12), which encodes a receptor tyrosine kinase implicated in the processes of stem cell proliferation and differentiation. *FLT3* mutation has been found in 20–40% of cases of AML of all types, but more frequently in APL, in the subtype with t(6;9) and in cases with a normal karyotype. Mutation in the *KIT* gene (4q11-12) is present in a large proportion of cases of AML and can be associated with an unfavourable prognosis.

### Acute myeloid leukaemia with *NPM1* mutation (myelomonocytic or monocytic)

The *NPM1* gene encodes nucleophosmin, a nucleolar protein with many functions among which are the stabilisation of tumour suppressor proteins, the regulation of ribosomal biogenesis and the control of centrosome duplication. *NPMI* is mutated in 30% of all cases of AML and in 50–60% of cases with a normal karyotype; mutation is more frequent in adults. The haematological picture in AML with *NPM1* mutation is distinguished by a marked peripheral blood leucocytosis and a bone marrow that is generally hypercellular (Figs 4.63 and 4.64); the blast cell morphology is monocytic or myelomonocytic; there is multilineage dysplasia in a quarter of cases.[85] Extramedullary localisation can occur. The immunophenotypic profile, in addition to expression of myeloid and monocytic markers, is strongly characterised by negativity for CD34. Diagnosis is based on identification of the mutation by molecular analysis or by immunohistochemical analysis of paraffin-embedded trephine biopsy sections, which show aberrant cytoplasmic expression of nucleophosmin. The prognosis is relatively favourable.

### Acute myeloid leukaemia with mutation of *CEBPA* (without maturation)

The *CEPBA* gene encodes a transcription factor, CCAAT/enhancer binding protein alpha, which plays an important role in the control of haemopoietic stem cells: its mutation favours their proliferation and blocks their differentiation along myeloid maturation pathways.[86]

The mutation is observed in 10% of AML with a normal karyotype. Usually the blast cells do not show any morphological evidence of differentiation but, more rarely, they have monoblastic and promonocytic morphology. The immunophenotype is characterised by expression of CD34, HLA-DR and, not rarely, CD7. The diagnosis is based on the molecular identification of the mutation, which has a favourable prognostic significance.

## Acute myeloid leukaemia with myelodysplasia-related changes

A high percentage of cases of AML, equivalent to a third or more in elderly patients, have increased bone marrow blast cells accompanied by dysplastic features in differentiated haemopoietic precursors and in mature cells in the peripheral blood.[87]

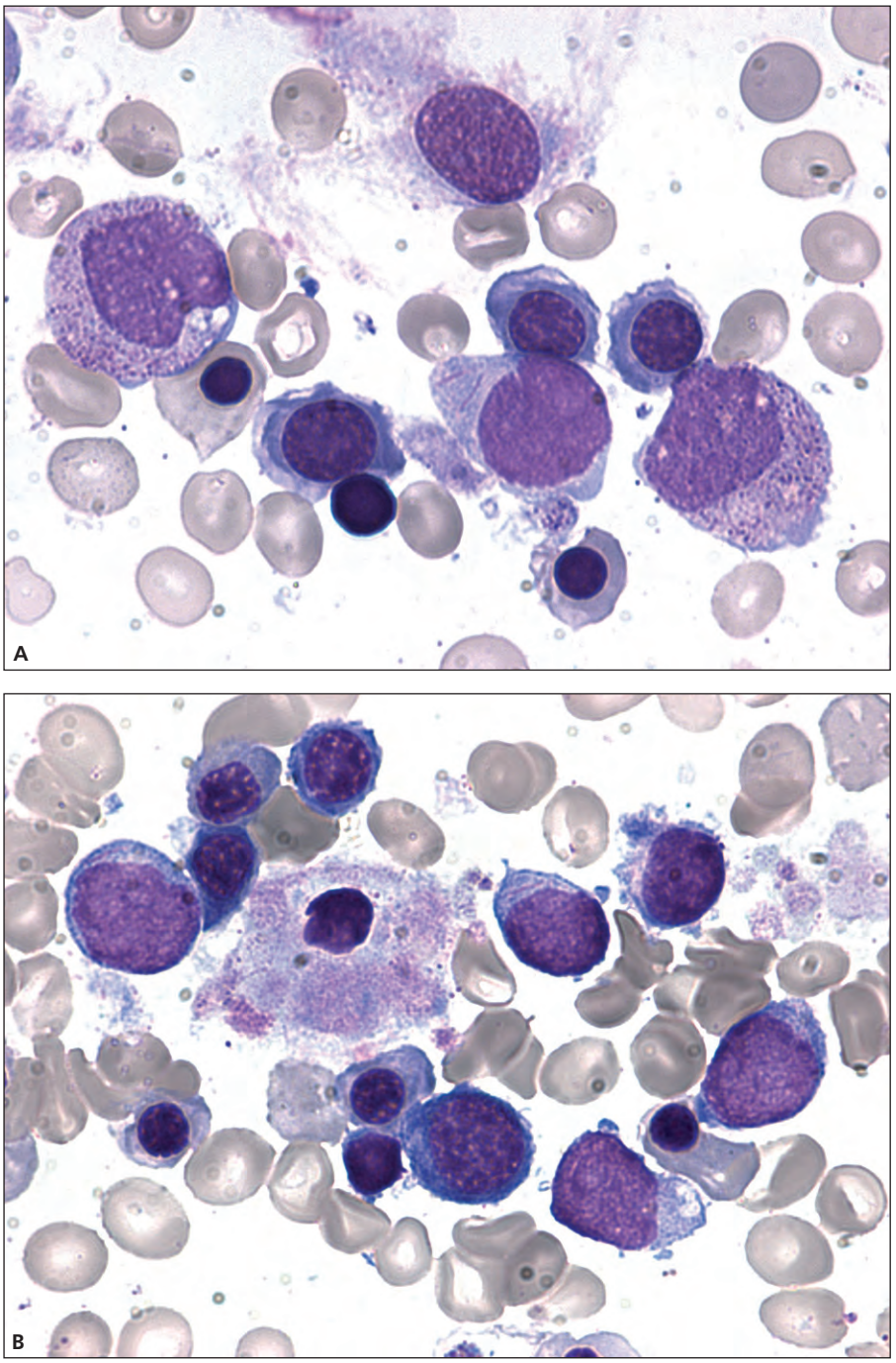



**Fig. 4.60** Bone marrow aspirate of a patient with AML with recurrent genetic abnormality, t(3;3). (A) In the centre, a myeloblast with plentiful basophilic cytoplasm containing two long thin Auer rods; the nucleus has fine, homogeneous, stippled chromatin, with a non-prominent nucleolus at 1 o'clock. The two large granule-rich cells adjacent to the right and left edges respectively can be classified as dysplastic promyelocytes, according to the criteria of the 2008 WHO classification, in part because of the greater degree of chromatin condensation. Around the central blast cells are five erythroblasts showing some defect in haemoglobinisation and moderately asynchronous maturation, a rounded pyknotic bare nucleus that has just been expelled and, on the left, a red cell with basophilic stippling. Centre top, a macrophage which has ragged cytoplasm but no evidence of phagocytosis. (B) Four blast cells, two of which, at the top, contain long thin Auer rods, six erythroblasts with features of dyserythropoiesis and a lymphocyte. A little to the left and above the centre, a micromegakaryocyte with a small pyknotic nucleus and plentiful cytoplasm with peripheral extensions that represent attempts at platelet formation. Further to the right, a small syncytial carpet of variably granulated, dysmorphic platelets.

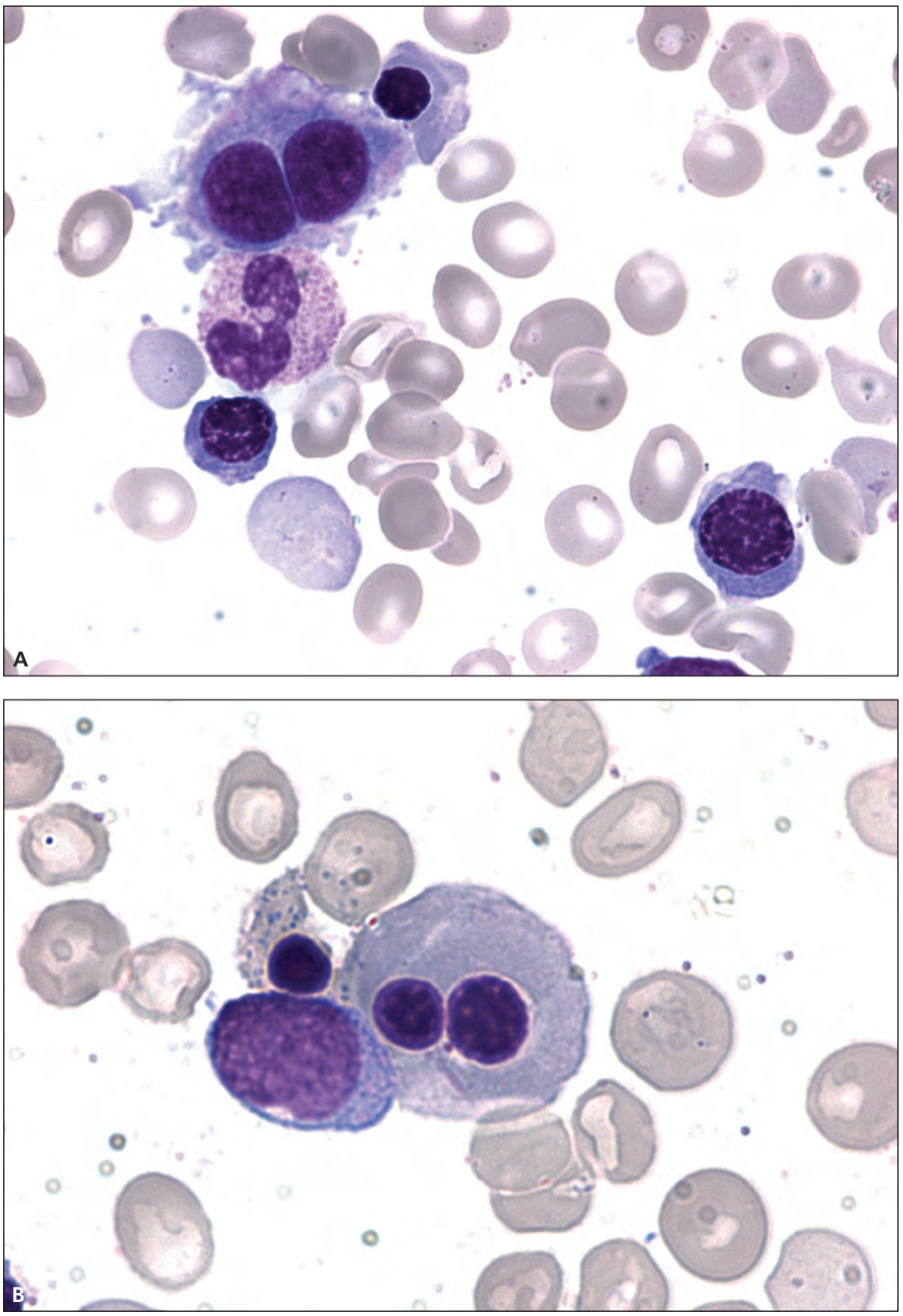



**Fig. 4.61** Bone marrow aspirate of the same patient as Fig. 4.60, with AML with t(3;3). (A) A small dysplastic binucleated megakaryocyte, three erythroblasts and a neutrophil band form. At the bottom, a large polychromatic erythrocyte. (B) A blast cell with an eccentric nucleus, homogeneously condensed chromatin and basophilic cytoplasm in which there seem to be some fine azurophilic granules. A small orthochromatic erythroblast with cytoplasm containing heavy basophilic stippling and a giant polychromatic erythroblast with two nuclei confirm the presence of dyserythropoiesis. A classification as AML with recurrent genetic abnormality, however, takes precedence over a classification as AML with myelodysplasia-related changes (AML with MRC).

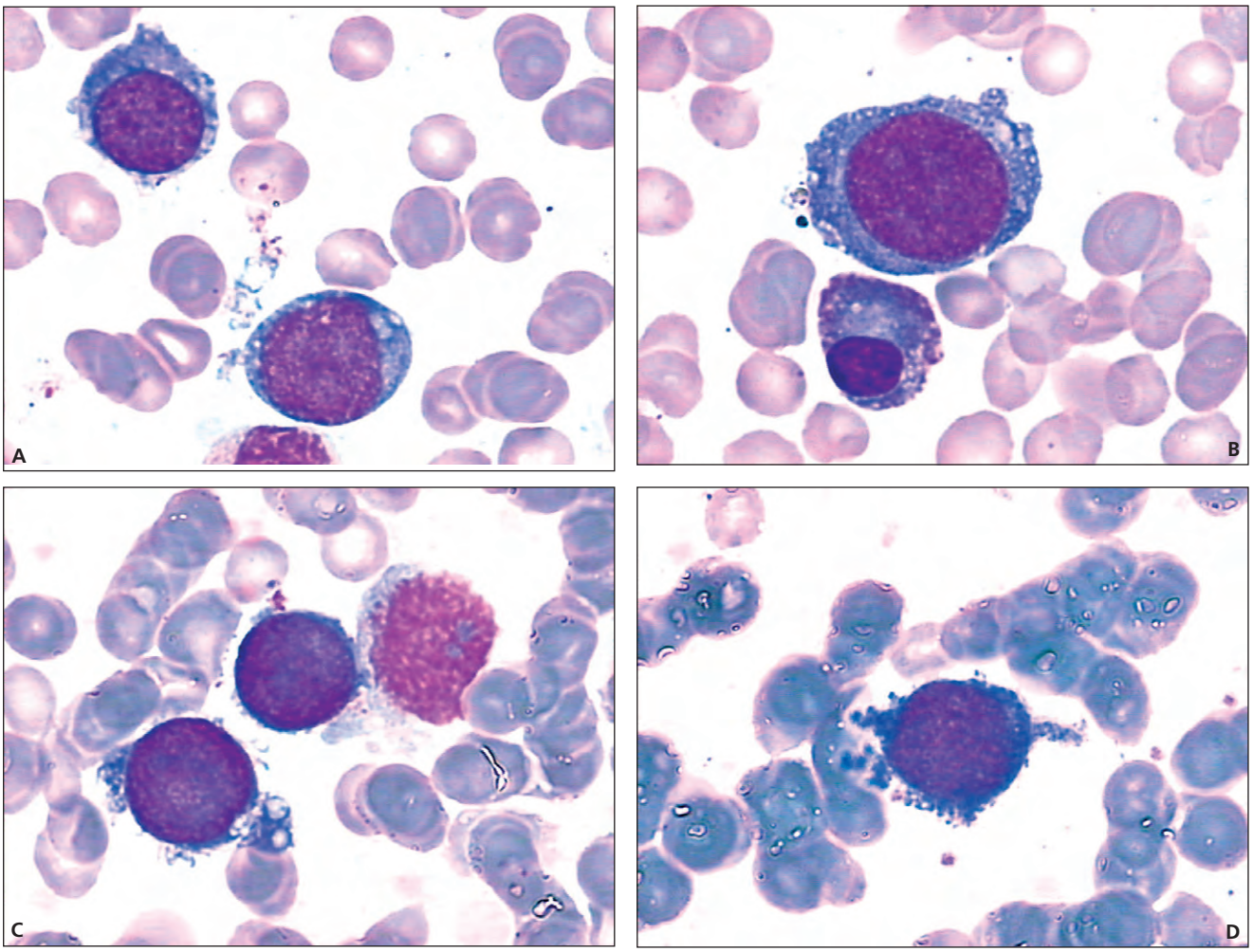



**Fig. 4.62** Acute megakaryoblastic leukaemia with the recurrent genetic abnormality, t(1;22); peripheral blood film. An attempted bone marrow aspirate was a 'dry tap'. The lineage of the cells was confirmed by positivity on flow cytometry for CD41 and CD61. (A) At the bottom, a blast cell with open, finely granular chromatin without visible nucleoli; the nucleus is polygonal with some straight sections, thus differing from the nucleus of a proerythroblast; the cytoplasm is fairly abundant and basophilic with vacuoles. The cell at the top is similar although smaller, with an eccentric round nucleus and a thick, regular nuclear membrane so that it resembles a basophilic erythroblast or an immature plasma cell. (B) At the top, a blast cell with a very large, slightly oval nucleus with dense, homogeneous, stippled chromatin in which there are a number of not very prominent nucleoli; the nuclear margin is regular and clearly outlined. The cytoplasm is plentiful, intensely basophilic and finely vacuolated; at the periphery there are fine pseudopodial projections. Beneath it is a plasma cell. (C) The morphology of these two blast cells with a high N:C ratio is particularly suggestive of megakaryocytic differentiation because of both the typical nucleus and the irregular cytoplasmic projections. (D) Another circulating megakaryoblast with abnormal platelet formation.

This category, previously known as AML with trilineage myelodysplasia and now designated AML with myelodysplasia-related changes (AML with MRC), has been redefined in the 2008 WHO classification on the basis of morphological or cytogenetic features and a rigorous clinical history. The category has not been enlarged to include any AML with generic or limited dysplastic features, but includes only those cases of AML with more that 19% of blast cells in the peripheral blood or bone marrow in which:

- There is bilineage or trilineage dysplasia, meaning more than 50% of cells of a lineage being dysplastic; or
- There is evolution from a preceding MDS or myelodysplastic/myeloproliferative neoplasm (MDS/MPN); or
- There is one of a defined group of cytogenetic anomalies that is associated with MDS, such as deletion or monosomy of chromosome 5 or 7.[88]

Recurrent cytogenetic abnormalities, as defined above, must be absent, and the clinical history must not reveal previous

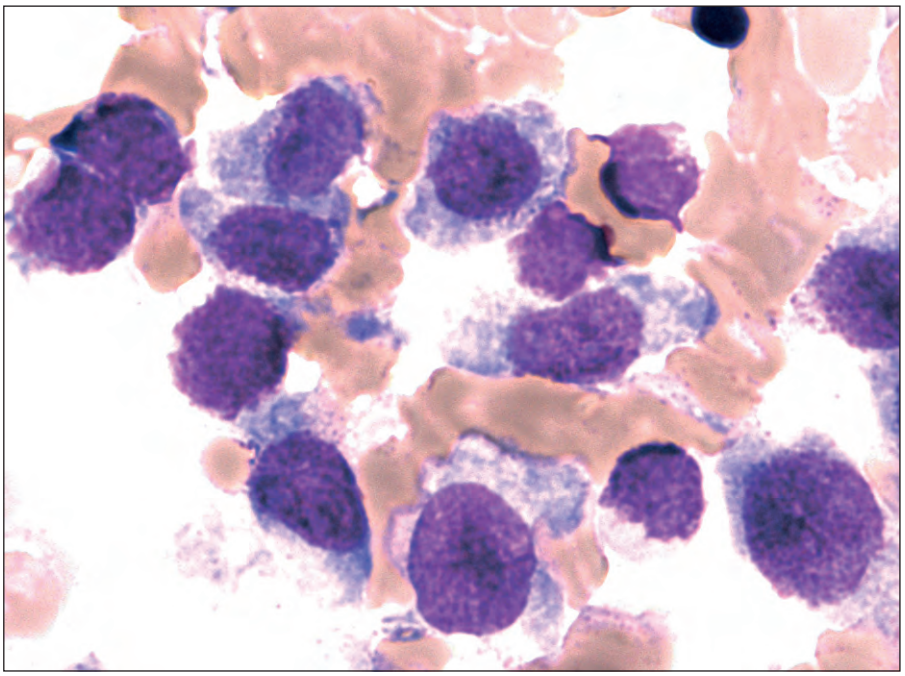


**Fig. 4.63** AML with mutation of *NPM1*. The blood count showed severe pancytopenia with neutropenia and no circulating blast cells. The bone marrow aspirate, at diagnosis and repeated a number of times, showed massive blastic infiltration, which could not be further defined because of distortion and fuzziness resulting from gelatinous transformation and because of very rapid clotting of the aspirate in the syringe.

exposure to leukaemogenic agents, which would take priority and lead to classification as therapy-related AML.

Some cases currently classified as AML with MRC, with between 20% and 30% of bone marrow blast cells, would have been assigned, according to the FAB classification, to the MDS category of refractory anaemia with excess of blasts in transformation (RAEB-T): these sometimes show a slow clinical course, with relative stability of the blood count and blast numbers. Conversely, other cases, with more than 50% erythroid cells and more than 19% of non-erythroid cells being blast cells would have been classified as FAB M6 AML: in fact, these cases are often associated with multilineage dysplasia, which in the WHO classification takes precedence over classification based solely on the lineage involved. In the presence of more than 19% blast cells, in fact, the recognition of dysplasia in 50% or more of cells in at least two haemopoietic lineages now leads to a diagnosis of AML with MRC rather than AML, NOS with erythroid differentiation.

In AML with MRC, the most frequent peripheral blood observation is of pancytopenia, often quite marked, with the possible presence of blast cells. Cases with leucocytosis, however, are not rare (see Report 4.4). One can observe erythroid dysmorphism, morphologically abnormal platelets and abnormalities of nuclear segmentation and cytoplasmic granulation in neutrophils. The bone marrow aspirate shows variable cellularity, often increased. Blast cells are 20% or more and have a variable morphology and degree of maturation (undifferentiated, granular, erythroid, megakaryoblastic), but with the exception of APL features. Dysplastic changes in maturing cells are the same as those described in Chapter 3 (Figs 4.65 through to 4.71). The frequency of multilineage dysplasia is greatest, in order, in the forms with erythroid, megakaryoblastic and granulocytic maturation. It is important to note also that a diagnosis of AML with MRC, according to the 2008 WHO classification, is now possible in the absence of dysplastic features, when a specified cytogenetic abnormality is present or when the clinical history shows evolution from an MDS or MDS/MPN that was diagnosed according to WHO criteria. The cases that present as *de novo* AML with MRC are characterised by a higher platelet count and a higher percentage of blast cells; those that evolve from pre-existing MDS, on the other hand, more often have neutrophils showing pseudo-Pelger morphology, micromegakaryocytes and multinucleated erythroblasts[89]; an example of this type of case, in which a neutrophil anomaly predominates, is that associated with deletion of 17p[90,91] (Fig. 4.71).

Still under discussion is the effect of dysplasia on the frequency of complete remission and survival. According to studies based on the FAB classification, isolated dyserythropoiesis and peroxidase deficiency do not have any impact on the prognosis which, however, is worse in the presence of other types of dysgranulopoiesis and worse still in the presence of megakaryocyte dysplasia.[92] In general terms, recent multivariate analysis seems to indicate that AML with MRC according to the 2008 WHO classification has a worse prognosis than AML, NOS, with an

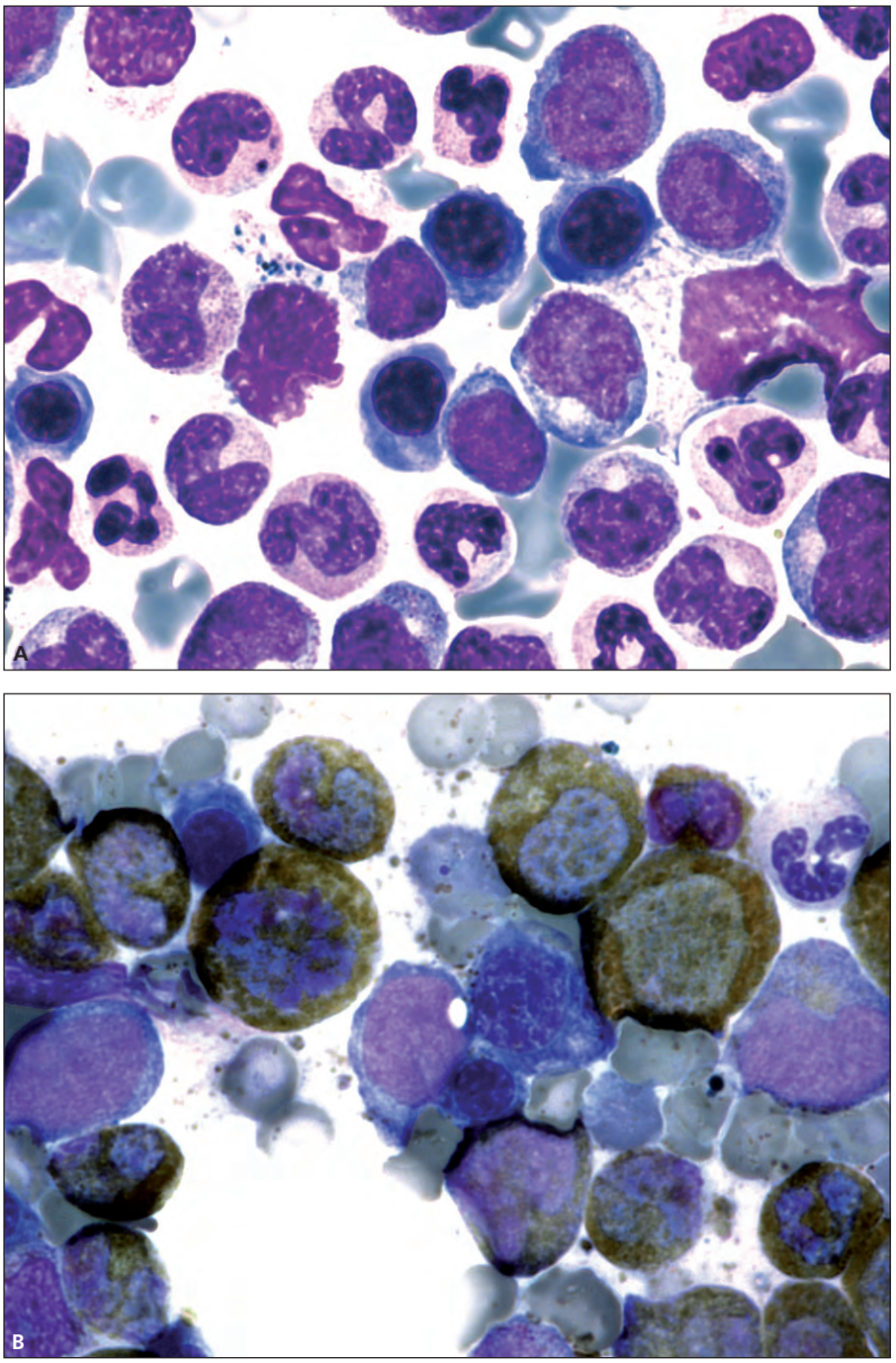



**Fig. 4.64** Bone marrow aspirate four months after diagnosis of AML with mutation of *NPM1*; same patient as Fig.4.63. (A) Remission induced by treatment is only partial. There are numerous neutrophils and dysplastic precursors, sometimes with nuclear appendages (at the bottom in the centre) and nuclear fragments within the cytoplasm (top left), four erythroblasts and some blast cells (top right), which have fairly condensed chromatin, basophilic cytoplasm and sometimes a pale Golgi zone. (B) In the same bone marrow aspirate, a myeloperoxidase (MPO) stain permits the observation, along the midline, of two blast cells that are completely peroxidase negative and a third, on the right, with weak focal paranuclear activity. The maturing granulocytes show normal intense positivity, with the exception of a mature neutrophil, on the right, which is completely negative, thus confirming the presence of dysgranulopoiesis.

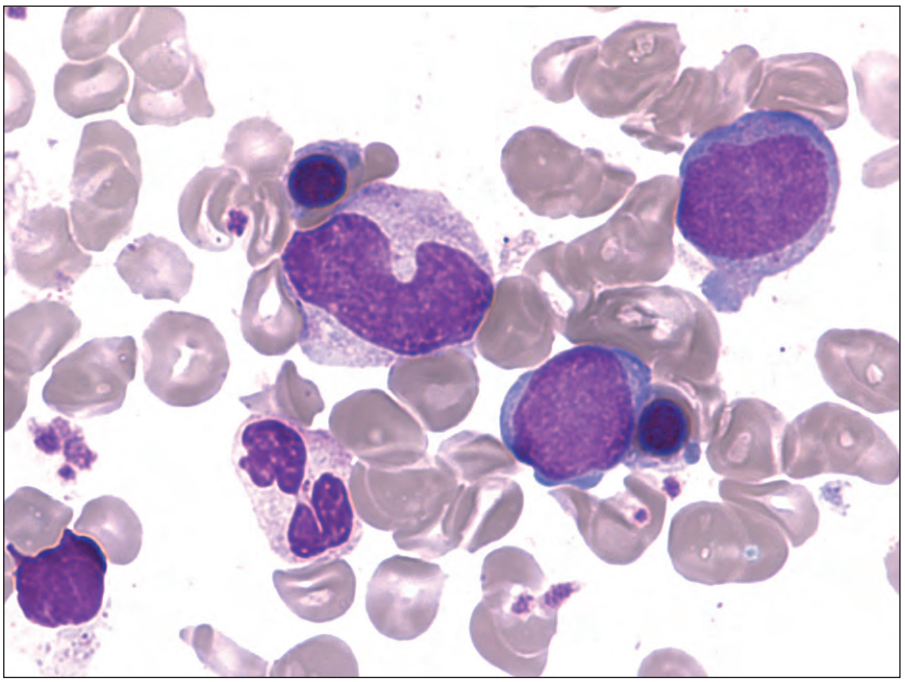


**Fig. 4.65** AML with MRC; bone marrow aspirate. Immediately to the right of the centre, a blast cell with intensely basophilic agranular cytoplasm and an indented nucleus with pale homogeneous chromatin is in contact with a small orthochromatic erythroblast with a defect in haemoglobinisation. Top right, a larger blast cell, which also has basophilic cytoplasm, but contains some azurophilic granules. Towards the left, a giant hypogranular metamyelocyte, a polychromatic erythroblast and, at the bottom, a hypogranular neutrophil. There are a number of hyperchromatic platelets.

overall shorter survival regardless of the age and cytogenetic alterations.[93] Other authors maintain, on the contrary, that an unfavourable prognosis, although being seen overall in the total group of AML with MRC, actually relates only to those AML with MRC cases that have specified cytogenetic alterations or have evolved from previous MDS.[94] The prognostic value of MRC associated with AML remains, thus, under debate. Cases in which a *FLT3* mutation is found, however, are characterised by a worse clinical course.

AML with MRC can enter remission with induction chemotherapy and subsequently relapse as MDS, with or without an excess of blast cells.[95]

## Acute myeloid leukaemia, not otherwise specified

Assigned to this category are all the cases of AML that do not satisfy the criteria for other categories of the 2008 WHO classification.[96] One is thus dealing, according to the definitions of the authors of this classification, with a residual 25–30% of cases that have neither the specified genetic abnormalities for AML with recurrent genetic abnormality (including the provisional categories with mutations of *NPM1* and *CEBPA*) or AML with MRC, nor multilineage dysplasia, nor a history of preceding MDS or MDS/MPN, nor previous exposure to leukaemogenic agents. The diagnosis is thus still based, fundamentally, on the criteria defined by the FAB group (but with alteration of the blast threshold required for diagnosis): the identification of the type and degree of differentiation towards one or more of the myeloid lineages, using morphological analysis of the peripheral blood and bone marrow films, with the support, sometimes indispensable, of immunophenotyping and histology (Table 4.1).

An AML, NOS category that maintains, in the absence of genetic markers or relevant history, the morphological basis of the FAB classification means that the FAB classification has thus survived, to some extent and not without controversy, the rigidity of the criteria of the 2008 WHO classification. On one hand, there has been no certain demonstration that assignment to AML, NOS has a prognostic value, or indicates the need for a specific treatment choice (even if an unfavourable outcome is fairly usual in the megakaryocytic and erythroid forms). On the other hand, there is the evident opportunity to maintain a traditional knowledge base, which will help to validate new genetic or therapeutic knowledge in the context of morphological features that have been recognised and habitually used over time. The diagnosis of certain subtypes, such as erythroleukaemia, must be based still in large part on a morphological and immunophenotypic evaluation. Finally, a less technological approach must remain available in all the situations in which advanced genetic methodology is not available, as in less developed countries or when the initial diagnostic assessment is carried out in peripheral sites that are not highly specialised.[97]

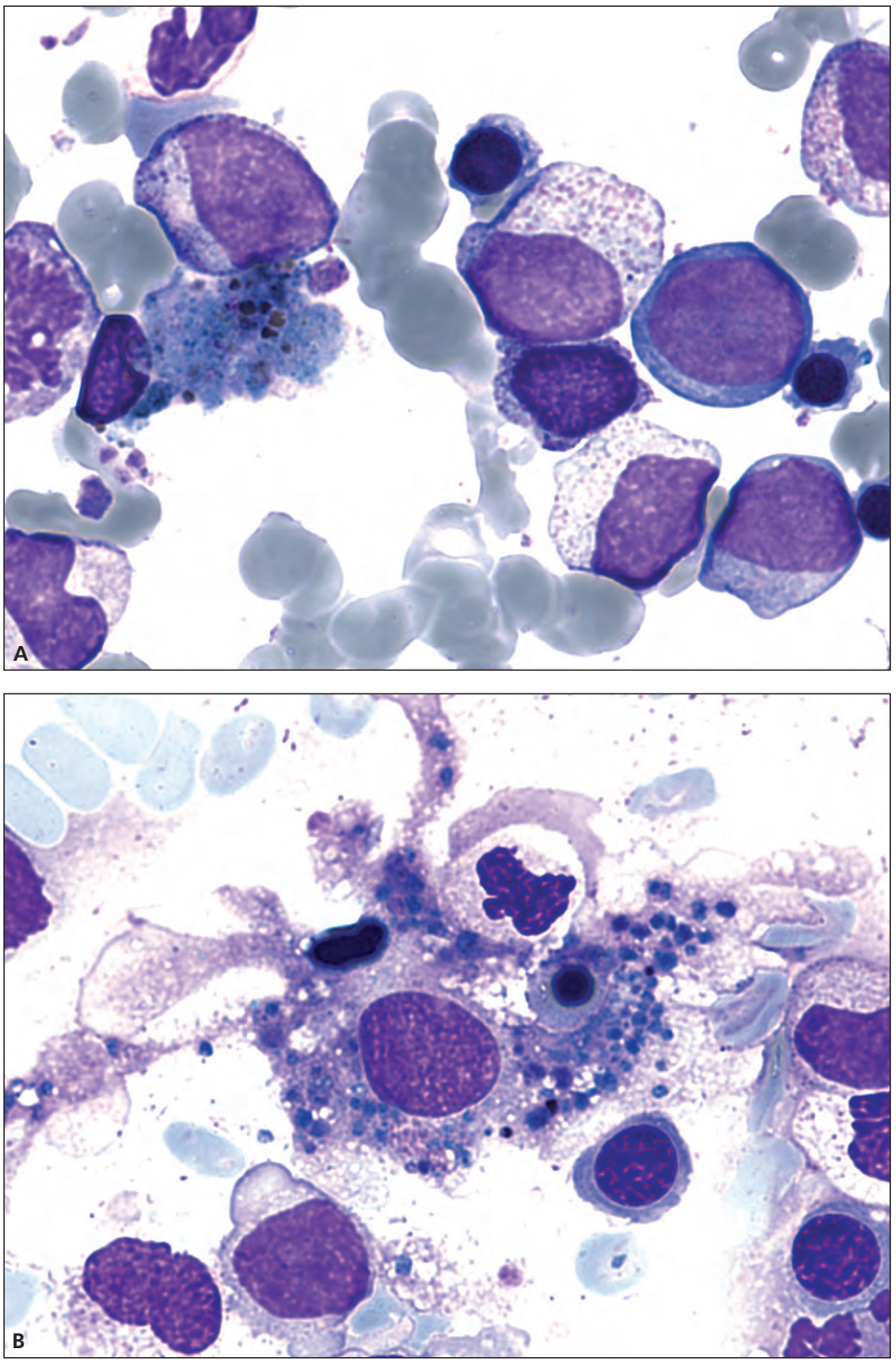



**Fig. 4.66** AML with MRC; same case as Fig. 4.65. (A) In the bottom right corner, an agranular blast cell with a nucleus that has a polygonal outline without visible nucleoli, above which is a small, almost orthochromatic erythroblast and a large proerythroblast, recognisable particularly because of the perfectly oval nucleus, the intensity of the basophilia and the presence of pale arcoplasm. On its left, two myelocytes, probably dysplastic eosinophil myelocytes, with hypogranular cytoplasm that is full of small vacuoles; between them is a crushed cell that is not really recognisable and, above, a polychromatic erythroblast. In the area to the left, a granular blast cell partly superimposed on a small macrophage with sea-blue cytoplasm, the nucleus of which is in contact with a mitotic figure that is cut off at the left-hand edge of the image. (B) At the bottom, towards the left, a blast cell with a polygonal nucleus and plentiful pale cytoplasm; on the right, two polychromatic erythroblasts with N:C asynchrony. In the centre, a large macrophage, with a large pale nucleus with spongy chromatin, extends its cytoplasmic projections to engulf still recognisable cells (a neutrophil and an orthochromatic erythroblast), debris derived from other cells and numerous blue globules, that have resulted from the degeneration of lipid-rich structures that have been phagocytosed. Increased phagocytosis correlates with the presence of myelodysplasia and ineffective erythropoiesis, with the intramedullary death of precursors that fail to mature.

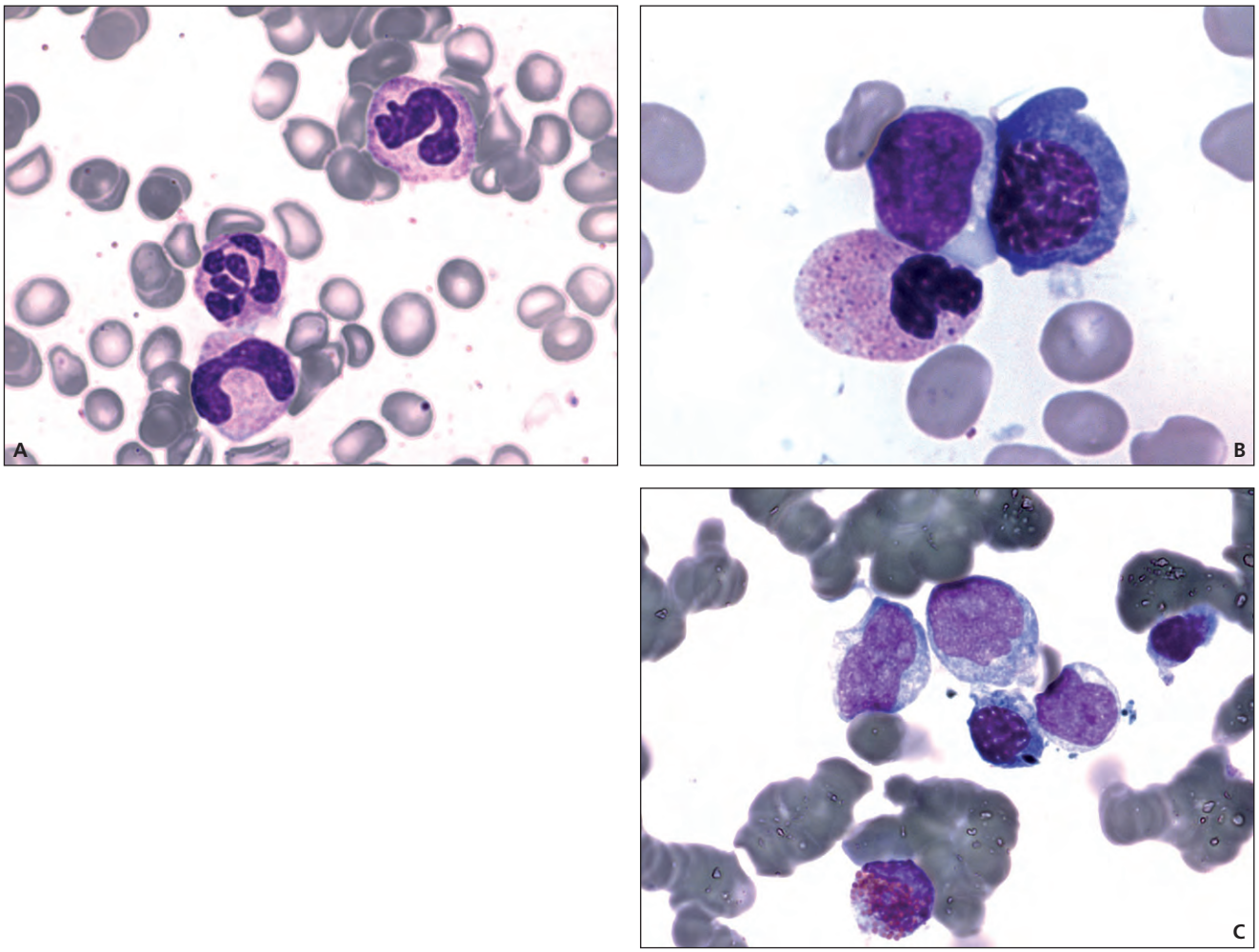



**Fig. 4.67** Another case of AML with MRC. (A) Peripheral blood film. The neutrophil granulocytes show quite variable alterations in nuclear segmentation, with either a failure of segmentation or hypersegmentation. Cytoplasmic granulation appears normal. (B) Bone marrow aspirate. A small agranular blast cell, a basophilic erythroblast and a dysplastic granulocyte with an eccentric, non-lobated, hyperchromatic nucleus that is almost pyknotic; the plentiful cytoplasm contains numerous fine pink granules and a few large dark granules, on a blue-grey cytoplasmic background, which is visible at the pole opposite the nucleus. (C) Bone marrow aspirate. Three agranular blast cells, two of which have an irregular nucleus with a visible nucleolus. A poorly haemoglobinised polychromatic erythroblast completes the central small group. On the right, a very small cell that resembles a micromegakaryocyte. At the bottom, a small eosinophil metamyelocyte, which is also dysmorphic.

The new 2008 WHO classification has radically altered the terms used in classification, cancelling completely in a type of *damnatio memoriae*, the FAB shorthand designations of M0 to M7, the immediate intelligibility of which has contributed in past decades to standardisation and widespread use of the FAB diagnostic criteria; these criteria prepared the way for the acquisition of new genetic and molecular knowledge, permitting the correlation of specific chromosomal translocations with a particular morphological picture. While attempting to avoid oversimplification, it appears opportune to note that the subclasses of the AML, NOS category correspond fairly closely to the most recent version of the FAB classification[7,98,99] and also to the previous WHO classification,[9] with the two fundamental exceptions of the lowering of the blast percentage to 20% of total cells and the inclusion of promonocytes in the blast percentage.[11]

By definition, in AML, NOS recurrent cytogenetic and molecular genetic alterations of the types discussed earlier are absent. It is not rare, on the other hand, to recognise non-specific mutations such as those in *RUNX1* or *FLT3*.

### Acute myeloid leukaemia with minimal differentiation (similar to FAB M0)

The FAB papers of 1976 and 1985 required the percentage of peroxidase-positive bone marrow blast cells to be at least 3% to classify a morphologically undifferentiated leukaemia as myeloid. With the development and widespread use of immu-

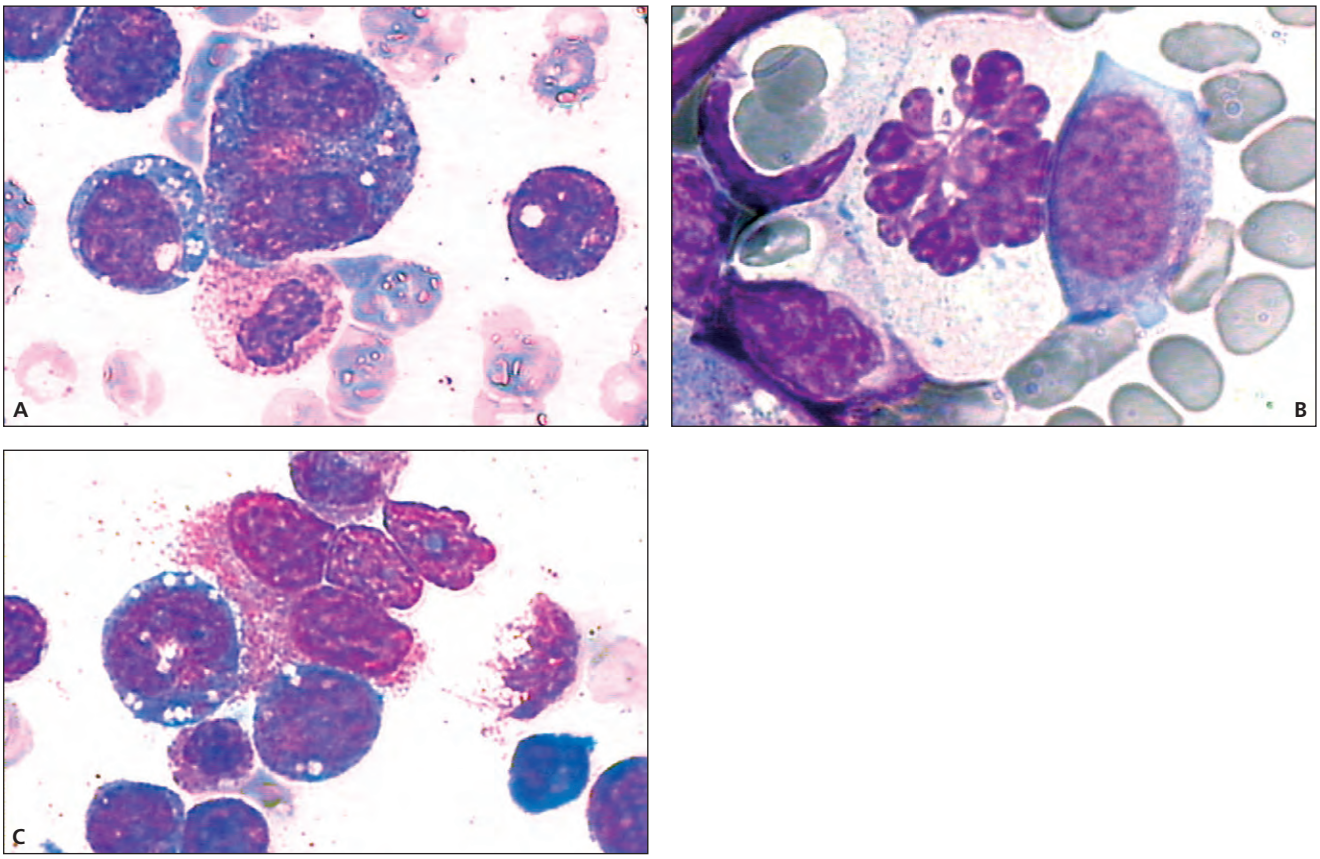



**Fig. 4.68** This case of AML shows quite marked dysmorphism both of blast cells and of maturing granulocytes, which show a tendency to binuclearity and tetraploidy. (A) From the left, a small agranular blast cell that is very vacuolated and, immediately beside it, a giant binucleated blast cell with intensely blue cytoplasm; the latter is also vacuolated and is rich in azurophilic granules that are condensing as a reddish mass between the two nuclei, in an area that certainly corresponds to the Golgi zone. (B) On the right: a blast cell with a few blurred granules; an enormous neutrophil granulocyte with a central nuclear mass that has 20 or so lobes joined in the centre by filaments, and pale completely agranular cytoplasm; two agranular dysplastic granulocytes that have phagocytosed intact erythrocytes, which are seen within optically empty vacuoles. (C) Two vacuolated granular blast cells, a giant neutrophil with enormous lobes, which has been torn during the spreading of the film and, beneath it, a neutrophil with a non-segmented pseudo-Pelger nucleus.

nophenotyping techniques, the availability of sensitive and early markers of myeloid differentiation has led to the discovery of new varieties of acute leukaemia: these are characterised by the presence of morphologically undifferentiated blast cells, without any lineage-specific attributes such as granules or Auer rods and peroxidase negative by conventional cytochemistry, the myeloid nature of which is revealed by the expression of immunophenotypic myeloid markers detected with monoclonal antibodies such as anti-MPO, anti-CD13 or anti-CD33.[99–101] A minimal peroxidase activity can also be demonstrated by ultrastructural cytochemistry.

This subtype represents 2–5% of AML. In the peripheral blood there are obvious signs of haemopoietic insufficiency: the white cell count can be reduced or very high, with circulating blast cells. From the morphological point of view, bone marrow cells are usually replaced by apparently undifferentiated agranular blast cells, of medium size with a high N:C ratio (Figs. 4.72A and 4.73). The nucleus is round or indented; chromatin is dispersed or moderately condensed; in general there are one or two nucleoli. The cytoplasm is agranular with variable basophilia and often no visible Golgi zone. The general appearance at first glance is of an ALL. Certain morphological clues may suggest the involvement of a myeloid stem cell: these can include, for example, the total lack of precursors showing granulocytic maturation and a lack of mature neutrophils in the peripheral blood; or the presence of some dysplastic neutrophils or other signs, albeit not frequent, of dysplasia.

Cytochemical stains are negative for peroxidase or positive in less than 3% of blast cells, and do not provide any useful information. In some cases there may be weak positivity for ANAE or ANBE: then again, these patients sometimes relapse with the development of a monocytic component.

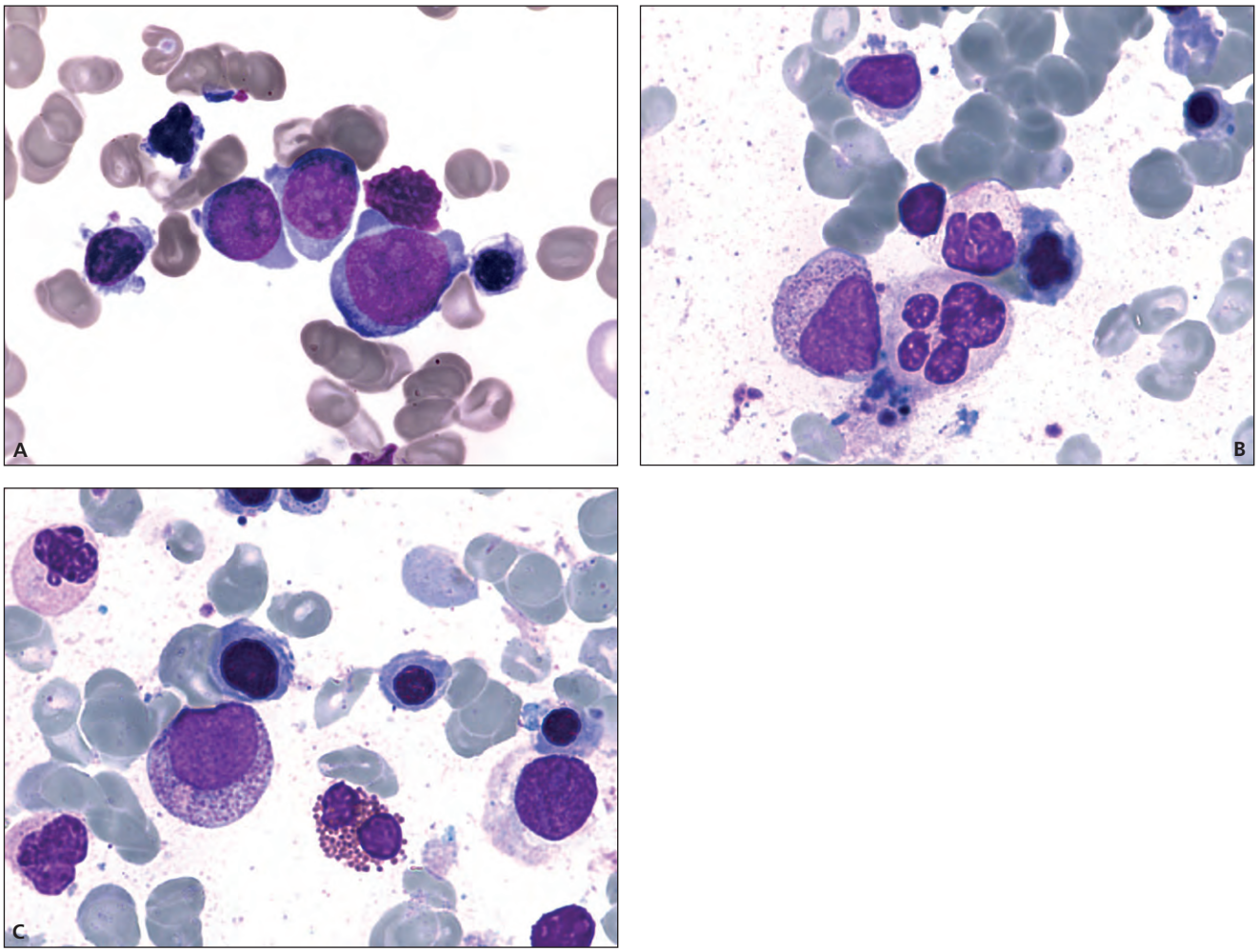



**Fig. 4.69** Hypocellular bone marrow aspirate from a case of refractory cytopenia in which the blast count rose above 20% eight months after diagnosis. (A) In the centre, three undifferentiated blast cells with basophilic, agranular cytoplasm. The chromatin is open and homogeneous in the larger cell, which has a large nucleolus, and more condensed in the other two. The two small cells on the left could be micromegakaryocytes. On the right, a polychromatic erythroblast with poorly haemoglobinised cytoplasm. (B) Marked dysplasia, particularly in the agranular neutrophil with asymmetrical nuclear segmentation low down in the centre. Two small erythroblasts, one of which has a nuclear outline composed of segments of circles. At the top, a lymphocyte. (C) Other features of dysgranulopoiesis and dyserythropoiesis in a hypocellular film. When the aspirate is of low cellularity, the diagnosis of leukaemic evolution often requires the help of histology and immunohistochemistry.

By definition, in AML with minimal differentiation at least one immunophenotypic myeloid markers must be positive (CD13, CD117, CD33), this often being associated with markers of immaturity such as CD34, CD38 and HLA-DR; aberrant expression of CD7 has been described in more than a third of cases.

The differential diagnosis includes ALL, leukaemic phase of lymphoma and peroxidase-negative AML (monoblastic, erythroid, megakaryoblastic), and is essentially based on immunophenotyping.

### Acute myeloid leukaemia without (or with little) granulocytic maturation (similar to FAB M1)

In this subgroup, equivalent to 5–10% of AML, the blast cells are at least 90% of NEC and show limited signs of granulocytic differentiation; maturing granulocytic cells, from myelocytes to neutrophils, plus any monocytic component are less than 10% of NEC. The blasts show variable morphological features (Figs 4.72B, 4.74 and 4.75): sometimes they appear undifferentiated, without granules and with scanty weakly basophilic cytoplasm, indistinguishable from lymphoblasts. More often, cases show a leukaemic population that is heterogeneous with a variable proportion, albeit a minority, of granular blasts: these are larger with a lower N:C ratio, more open chromatin, more numerous and easily visible nucleoli and, above all, some azurophilic granules and rare Auer rods. The granular blasts, however, are not taken as evidence of maturation: they are thus not included in the count of maturing granulocytes, in which, however, dysplastic promyelocytes must be included.

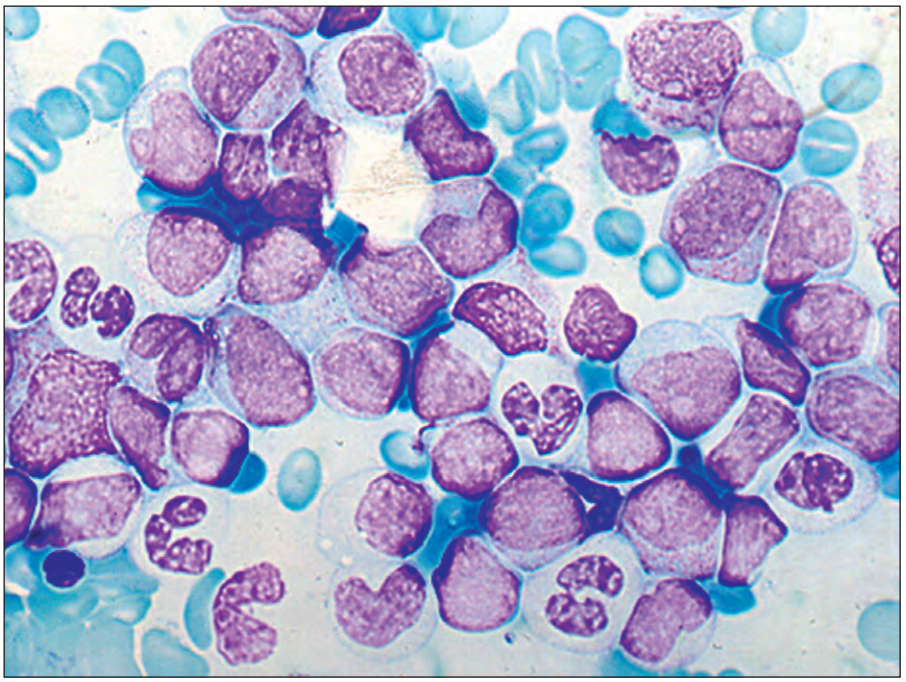


**Fig. 4.70** AML with MRC as evolution of a pre-existing myelodysplastic syndrome (MDS). Massive bone marrow infiltration by granular and agranular blast cells with pale open chromatin and a number of nucleoli. There are also numerous mature neutrophil granulocytes with completely agranular cytoplasm and nuclei that are anomalous both with regard to hyposegmentation and also the structure of the chromatin, which has very obvious pale spaces.

Peroxidase activity (or positivity for Sudan black B) is present in 3% or more of blast cells; CAE is negative in the cases in which non-granular blast cells predominate and positive in other cases.

The immunophenotypic profile more often includes expression of CD34, CD13 and/or CD33. CD7, typical of the T lymphoid lineage, may show aberrant expression in this and other types of AML, NOS, being co-expressed with myeloid antigens – MPO, CD13 and/or CD33 – and markers of immaturity. The T-cell receptor gene is in germline configuration and CD3 is not expressed. It is not rare for there to be, together with CD7, expression of monocytic antigens such as CD14, which is indicative of a worse prognosis. From the clinical point of view, CD7-positive AML is distinguished by frequent hepatosplenomegaly, central nervous system involvement, a very high leucocyte count and an unfavourable prognosis.[102–104]

### Acute myeloid leukaemia with granulocytic maturation (similar to FAB M2)

This subtype is common, including as many as 10% of all cases of AML. The peripheral blood shows leucocytosis, which is usually marked, plus anaemia and thrombocytopenia. Blast cells make up more than 19% of all nucleated cells (ANC). Maturing cells of granulocyte lineage, from promyelocytes to mature granulocytes (neutrophils, eosinophils and basophils), make up at least 10% of cells. Monocytes and their precursors make up less than 20% (a characteristic essential for making a distinction from AMML). In general, the bone marrow is hypercellular (Figs 4.76 through to 4.82). The blast cells are characterised by a lower N:C ratio than in the preceding form; the nucleus is often indented or angulated; the cytoplasm is more or less intensely basophilic and there may be a pale paranuclear Golgi zone. Cytoplasmic granules are present in a very variable percentage of cells; Auer rods are frequent.

The maturing granulocyte population may show dysplastic features, particularly asynchronous maturation and reduced granulation. In some cases, maturation is predominantly or exclusively to the eosinophil lineage, without evident morphological anomalies. In other cases, there are giant granular blast cells, with double sized or hyperdiploid nuclei (Fig. 4.79): these are particularly numerous in the variant described by various authors as near-tetraploid AML[105–107] which, however, sometimes falls into the category of AML with recurrent genetic abnormality, particularly associated with t(8;21),[108] or into the category of AML with myelodysplasia-related changes.

Cytochemical stains show that the cells are strongly and variably positive for peroxidase, Sudan black B and CAE: the positivity may be focal, being confined to the Golgi zone within a nuclear indentation. In rare cases with dysplasia, both the blast cells and the maturing progeny are peroxidase negative. Particular attention must be paid to monocyte esterase reactions, which are negative or very weak.

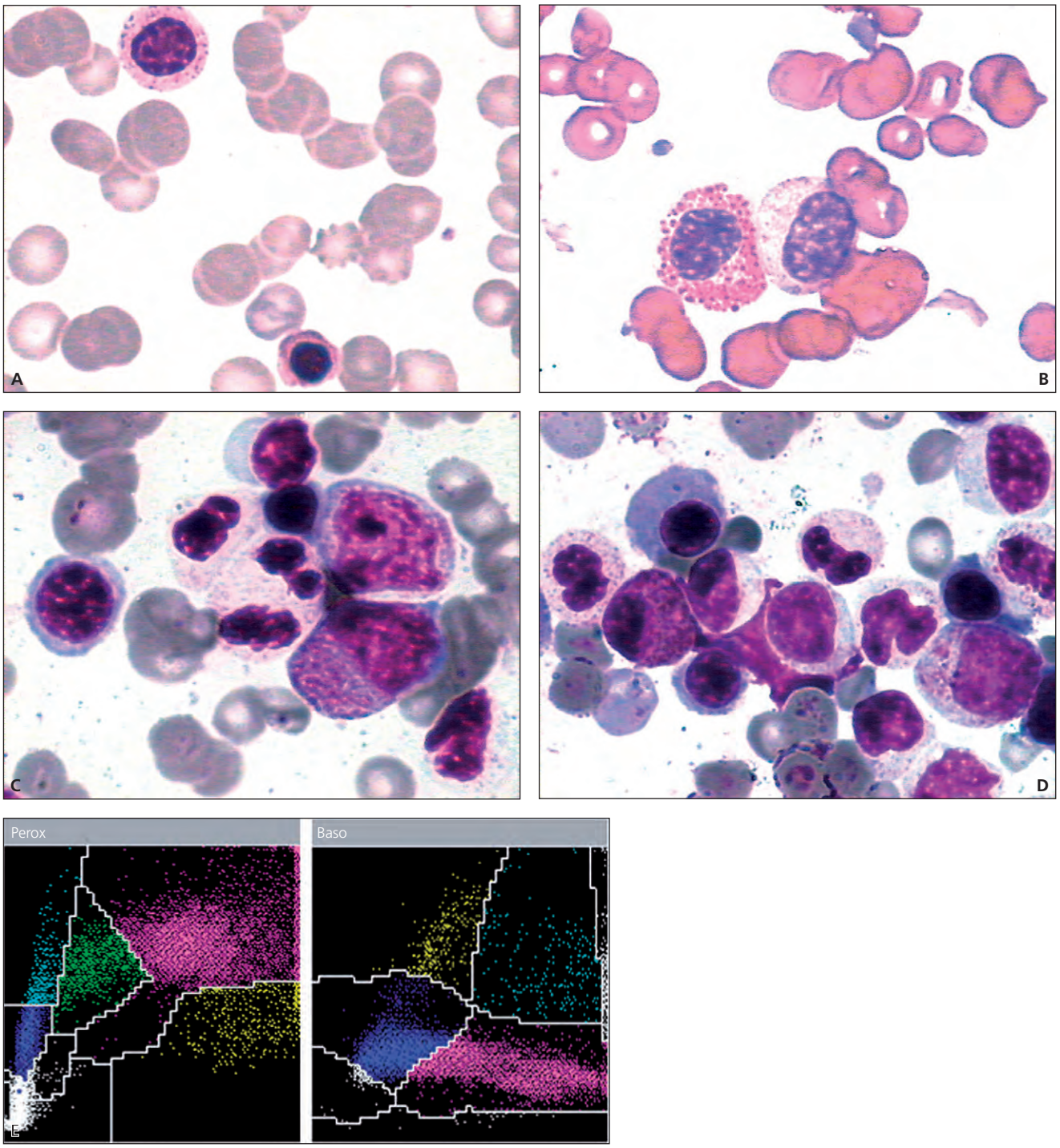



**Fig. 4.71** Evolution into AML with MRC in a patient with myelodysplasia and del(17p). (A) Peripheral blood film. The neutrophils often have spherical, completely non-segmented, pseudo-Pelger nuclei. (B) Peripheral blood film. An eosinophil with a non-segmented nucleus and cytoplasm packed with granules, adjacent to a hypogranular neutrophil with the same nuclear anomaly. (C) A follow-up bone marrow aspirate from the same patient six months after diagnosis confirms dysgranulopoiesis, but also shows 23% blast cells. There is a pseudo-Pelger neutrophil and another binucleated neutrophil, both of which are hypogranular; a blast cell is seen towards the top right and a dysplastic promyelocyte immediately beneath it. (D) In the same film as (C): an agranular blast cell in the centre, a granular blast cell on the right, an agranular myelocyte in the top right corner and a number of hyposegmented dysplastic neutrophils. A hypergranular cell, on the left, could be a mast cell or a degenerating granulocyte. (E) Morphology of the cytograms of the ADVIA system on the same sample as (B). In the Perox channel, the dysplasia has caused spreading of the neutrophil population towards the area of high peroxidase activity, with an increased HPX and an increase of cells in saturation. In the Baso channel, their pseudo-Pelger nuclei with hyperdense chromatin are localised to the bottom and right of the PMN zone, in an area usually occupied by erythroblasts. There was no Blast flag and the absence of blasts was verified on the blood film.

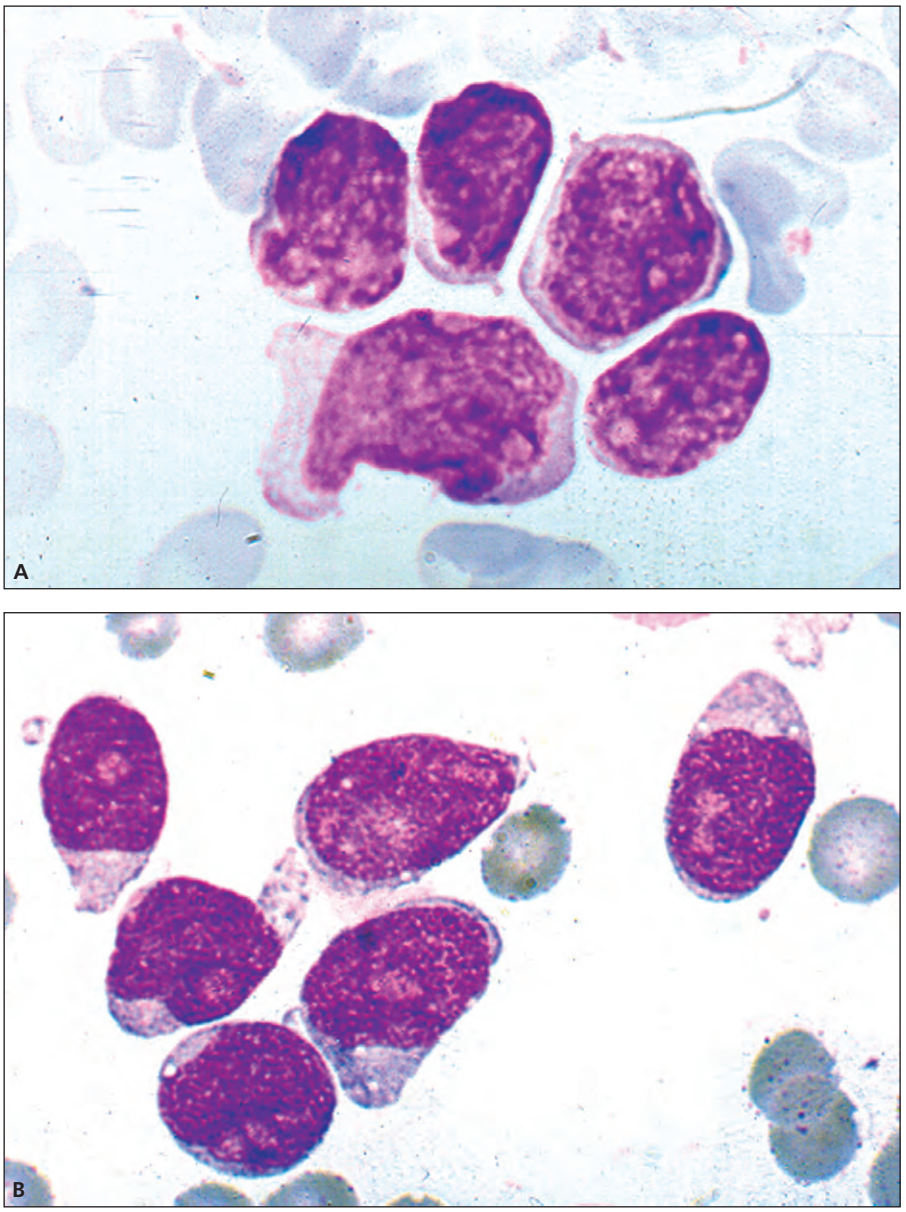



**Fig. 4.72** AML, not otherwise specified (AML, NOS); examples of blast cells with absent or only minor signs of differentiation. (A) AML with minimal differentiation; peripheral blood film. The blasts are heterogeneous in size, with a prevalence of small and medium-sized forms; the N:C ratio is very high, but tends to be lower in the larger cells. The nuclear morphology is variable, sometimes being irregular with notches; the chromatin is compact in some cells, while in others there is alternation of pale spaces and dark clumps. Three cells, of the five present, have a single nucleolus with partial perinucleolar chromatin condensation. The scanty cytoplasm is weakly basophilic and has neither granules nor Auer rods. Peroxidase-positive blast cells in the marrow were below 3%. The diagnosis was made on the basis of immunophenotypic evidence of granulocytic differentiation. (B) AML without maturation; bone marrow aspirate. Haemopoietic cells have been completely replaced by apparently undifferentiated, medium-sized blast cells with a high N:C ratio. The nucleus is eccentric with the cytoplasm being well represented at one pole of the cell and in some cells it is prolonged into pseudopodia that create a racquet or hand-mirror appearance. The general impression is of a lymphoid leukaemia, but the chromatin has a granular, fine and homogeneous structure; there is at least one clearly evident nucleolus in each nucleus. In the cytoplasm of the cell top right, fine azurophilic granules. According to the WHO criteria, there must be at least 3% of peroxidase-positive blast cells for inclusion in this category and the percentage of maturing cells must not exceed 10%. Also in these cases, the support of immunophenotyping can be very useful.

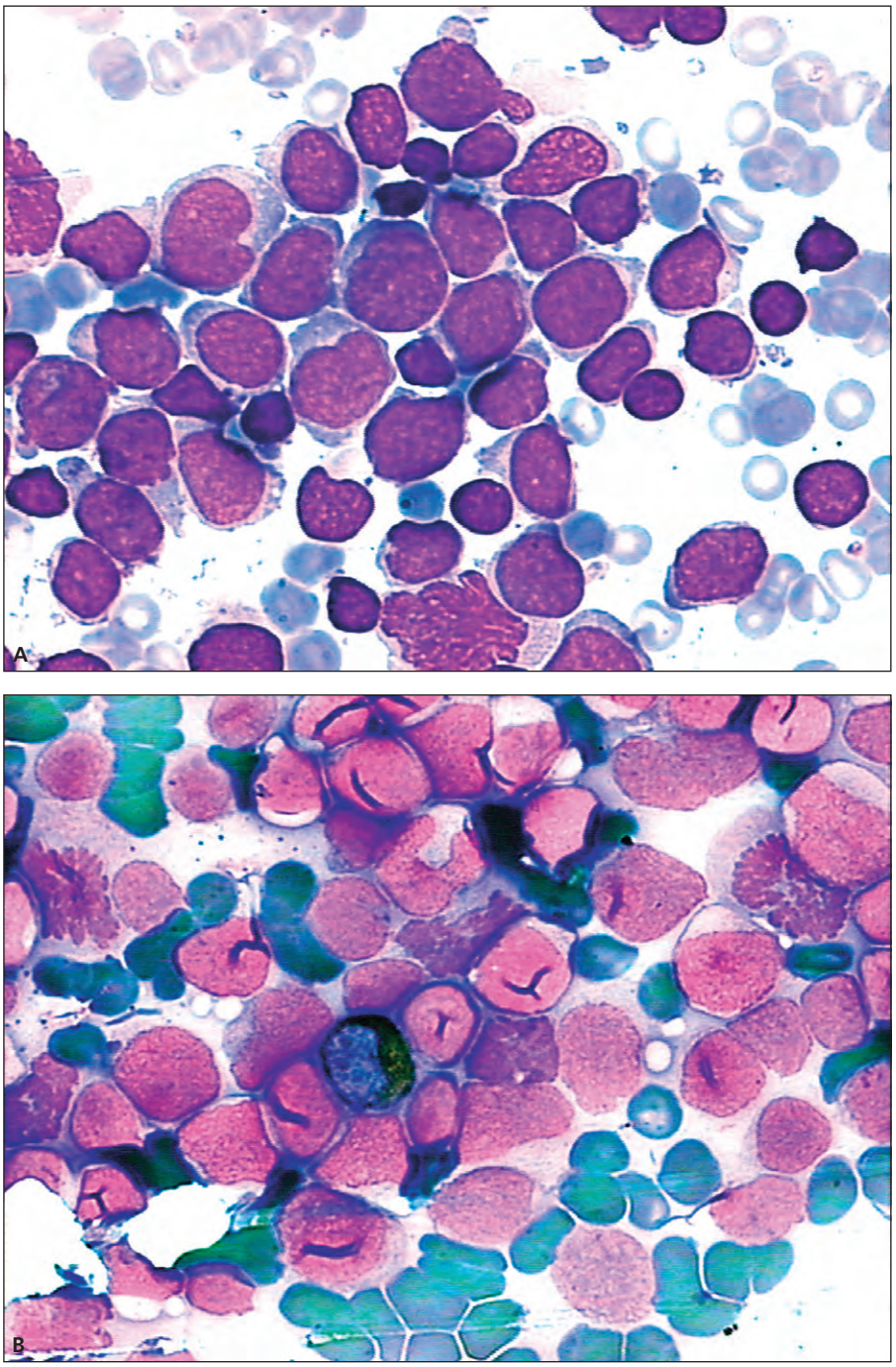



**Fig. 4.73** AML with minimal differentiation; bone marrow aspirate. (A) The bone marrow is cellular with virtually all the cells being blast cells of varying sizes with a high N:C ratio, a large rounded nucleus with compact chromatin without nucleoli, and scanty basophilic cytoplasm. A few cells have more plentiful cytoplasm and a notched nucleus with a small area of paranuclear pallor. There are numerous small compact apparently bare nuclei, corresponding to apoptotic blasts. (B) The same bone marrow aspirate as (A), peroxidase stain. The only positive cell is certainly a neutrophil myelocyte; the blastic population is totally negative. On the left and right respectively, two mitotic figures.

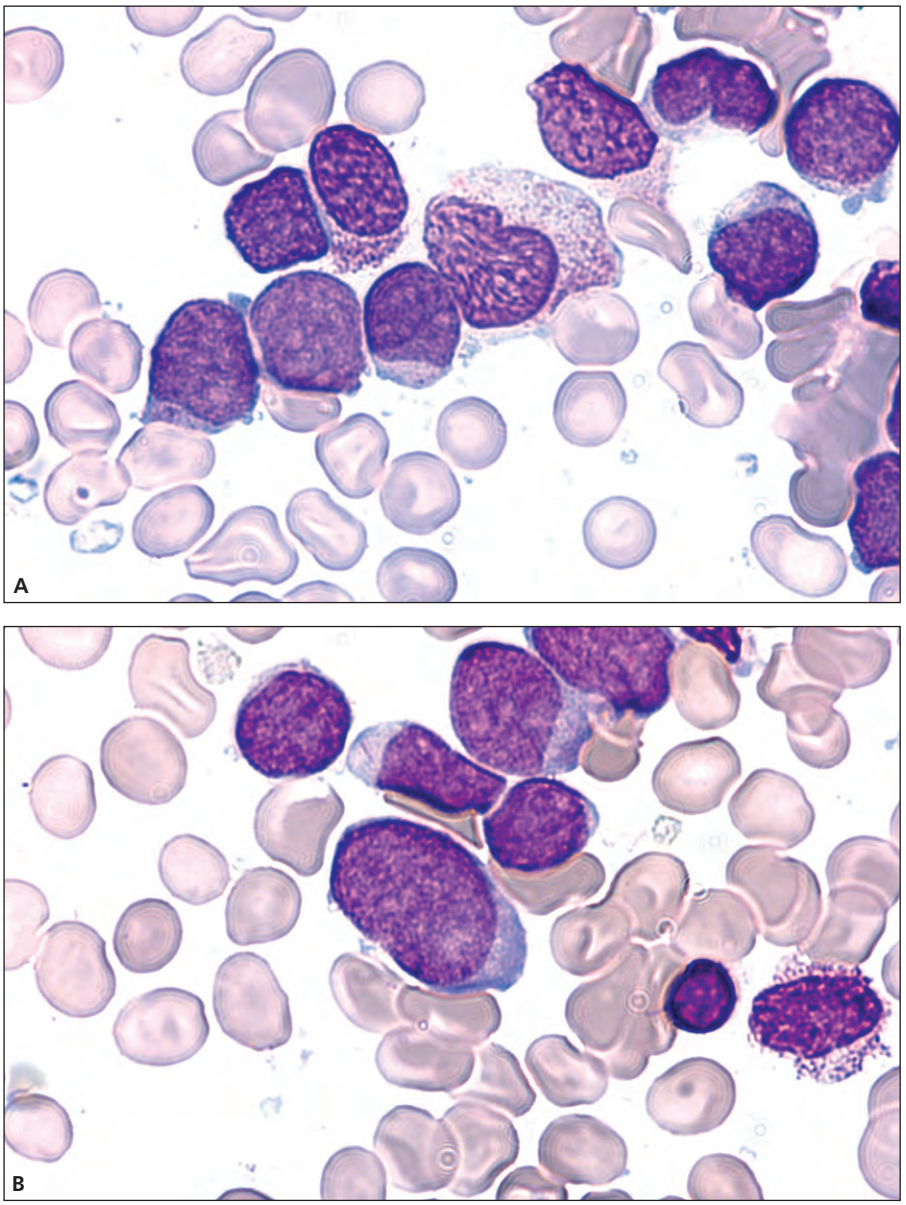



**Fig. 4.74** AML without maturation; bone marrow aspirate. (A) There are seven blast cells, mainly small, with scanty cytoplasm and round or oval nuclei, some of which are notched, with reticular, granular chromatin (in the three cells in a straight line from the left, which also have visible nucleoli) or with more condensed and compact chromatin (in the two cells on the right). The cell furthest to the left shows an angulated nucleus and some cytoplasmic granules. In the centre, a dysplastic maturing granulocyte with a distended nucleus with vermicular chromatin and very plentiful, basophilic cytoplasm, rich in granules that are scattered in a disorderly manner (the appearance of the cytoplasm is that of a dysplastic promyelocyte and this cell should not be included in the blast count). On its left, at the top, another cell of granulocyte lineage with an oval nucleus and a fringe of granule-rich cytoplasm. (B) In the same film as (A), one of the blast cells contains an Auer rod. Another large blast cell has a hyperdiploid nucleus and intensely basophilic cytoplasm. Bottom right, another small myelocyte with scanty granular cytoplasm. The inclusion of the case in this WHO category is because the number of maturing granulocytes does not reach 10% of cells.

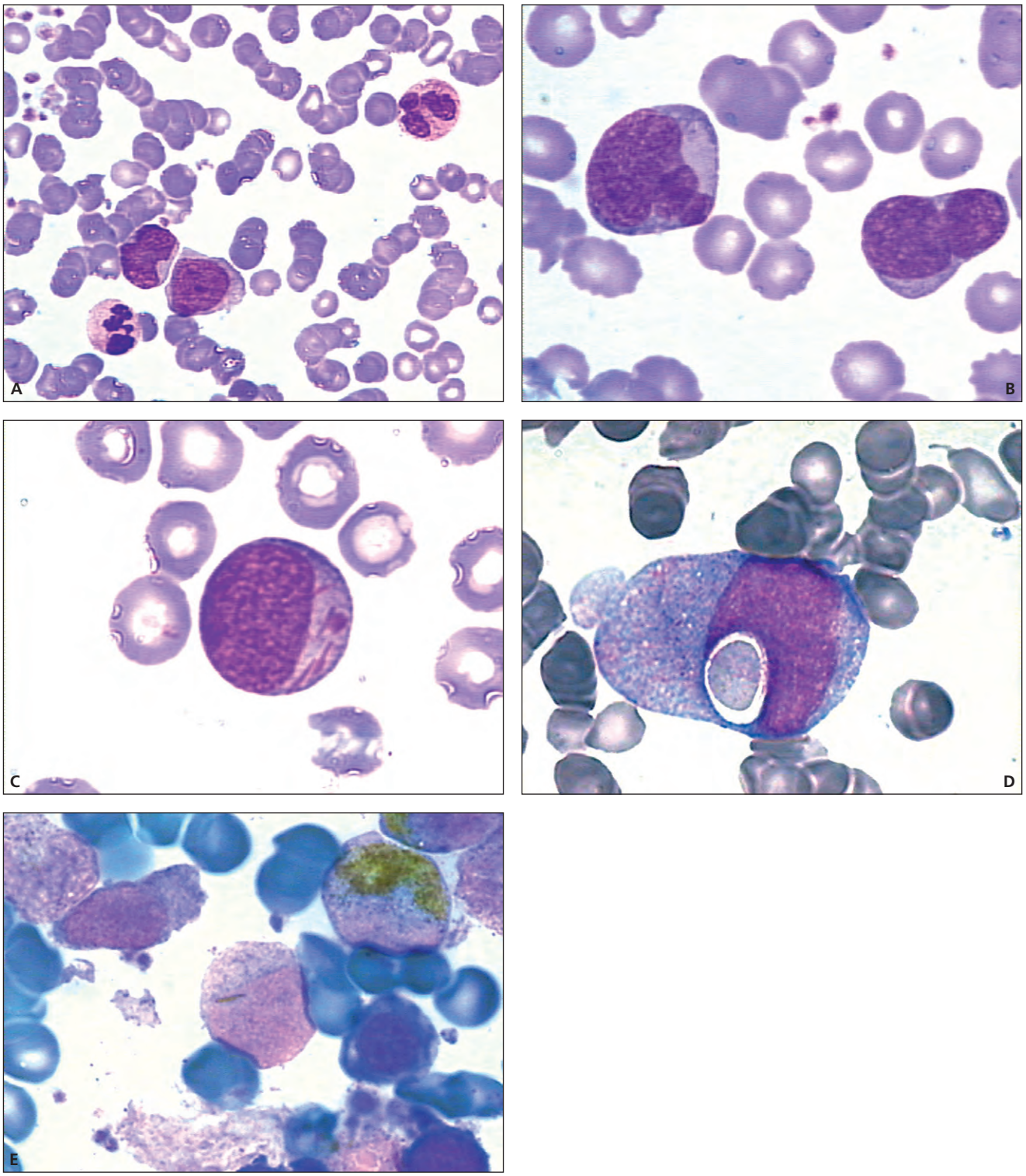



**Fig. 4.75** Examples of blast cells in cases of AML without maturation. (A) Peripheral blood film. Two blast cells with fairly plentiful cytoplasm, an irregular or polygonal, angulated nucleus, granular chromatin and, in the cell on the right, a number of nucleoli. Two neutrophil granulocytes with dysplastic features: hypogranularity in the cell on the left, anomalies of nuclear segmentation in both. (B) Peripheral blood film. Two blast cells have very marked irregularity of the nuclear outline. The chromatin is granular and nucleoli are only in part identifiable. The cytoplasm is plentiful and basophilic, with some paler areas. (C) Peripheral blood film. A blast with a nucleus that has granular cytoplasm without visible nucleoli; the cytoplasm contains at least four Auer rods and a large coalescent granule. (D) Bone marrow aspirate. A blast has plentiful intensely basophilic cytoplasm with fine azurophilic granules, some very small vacuoles and, on the left, a cytoplasmic projection, probably representing early clasmacytosis. The nucleus is deformed by a large autophagic vacuole that contains a fragment of granular cytoplasm. (E) Bone marrow aspirate, peroxidase stain. On the left, a peroxidase-negative blast cell; in the centre, another blast with positivity limited to a single Auer rod; on the right, a third blast with intense granular and diffuse positivity.

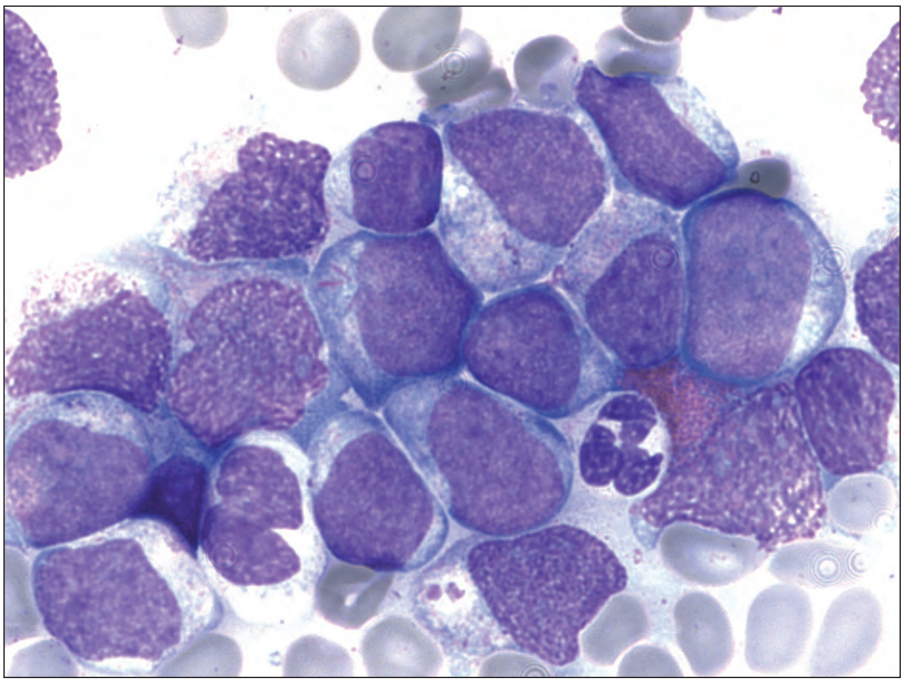


**Fig. 4.76** AML with maturation; bone marrow aspirate. The blast cells are large with a fairly low N:C ratio because of the presence of plentiful basophilic cytoplasm, often with a pale, irregularly shaped Golgi zone, fine granules and, sometimes, Auer rods that may be multiple. The chromatin is delicate and homogeneous and nucleoli can be faintly seen. In the lower part of the image, three dysplastic cells of granulocyte lineage: from the left, a myelocyte, a metamyelocyte and, further to the right, a segmented neutrophil, the cytoplasm of which is pale and completely devoid of granules. This case, however, was not classified as AML with MRC because the dysplastic features were present only in one lineage, the granulocytic.

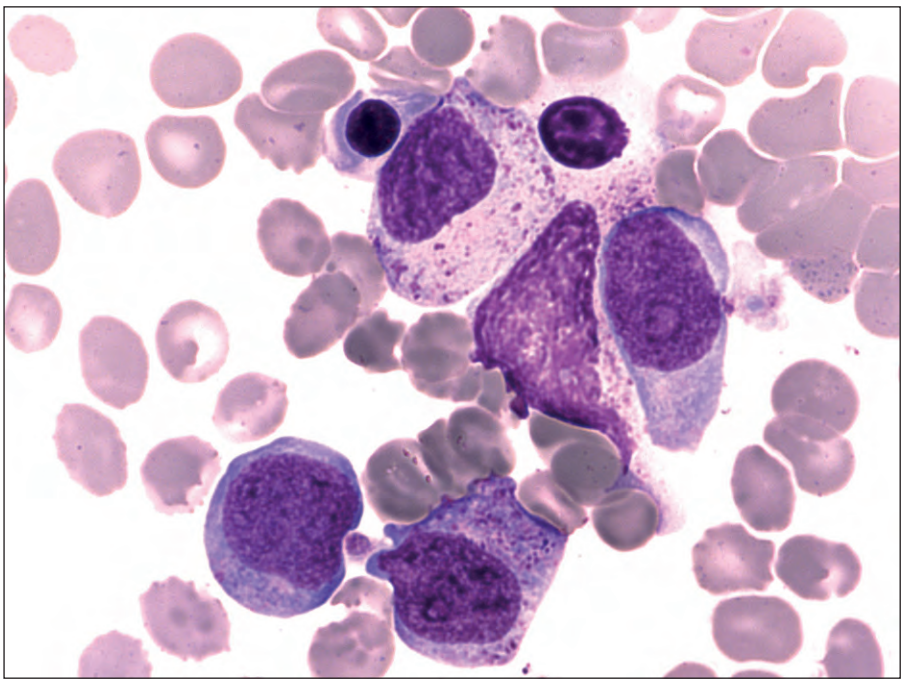


**Fig. 4.77** AML with maturation; bone marrow aspirate. From bottom left, anticlockwise: a blast with fine azurophilic granules, a dysplastic promyelocyte, another blast with a large prominent nucleolus, a dysplastic myelocyte and a polychromatic erythroblast.

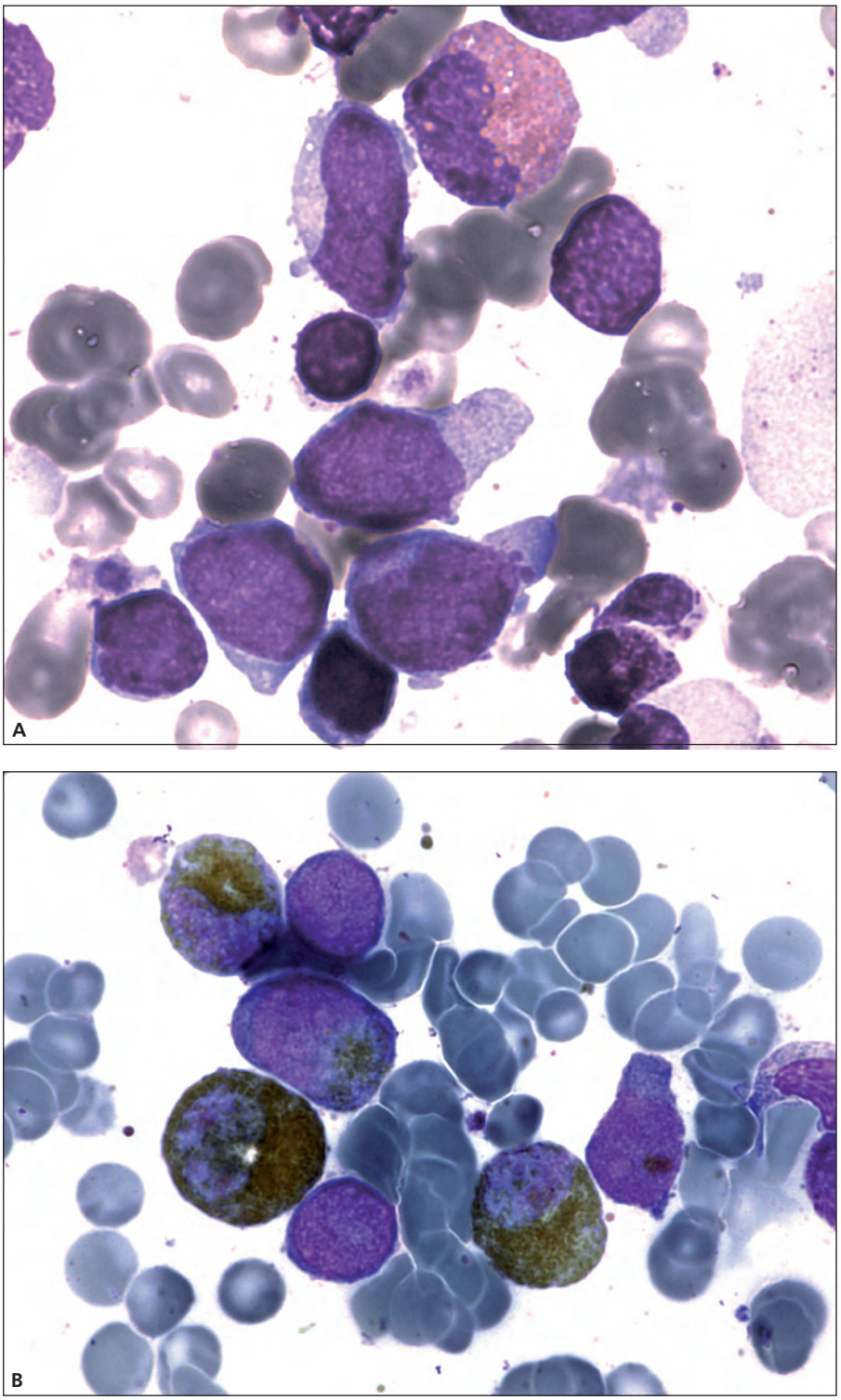



**Fig. 4.78** AML with maturation; bone marrow aspirate, same case as Fig. 4.77. (A) Bottom left, a small undifferentiated blast cell with a nuclear notch in relation to which one can see the scanty basophilic cytoplasm. Another four blast cells are larger with more abundant cytoplasm, with only the two further to the top showing signs of differentiation in the form of fine granules. The blast in the centre at the bottom has small nuclear protrusions. The only two maturing cells are an eosinophil myelocyte, at the top, and a distorted basophil, bottom right. (B) A peroxidase stain shows three small, undifferentiated blast cells with scanty cytoplasm and a negative peroxidase reaction. There is very clear focal granular paranuclear positivity in the larger blast to the left of centre, between a metamyelocyte (at the top) and an intensely positive myelocyte (at the bottom). At the bottom, another cell with plentiful peroxidase-positive cytoplasm and an eccentric nucleus which must certainly be a promyelocyte.

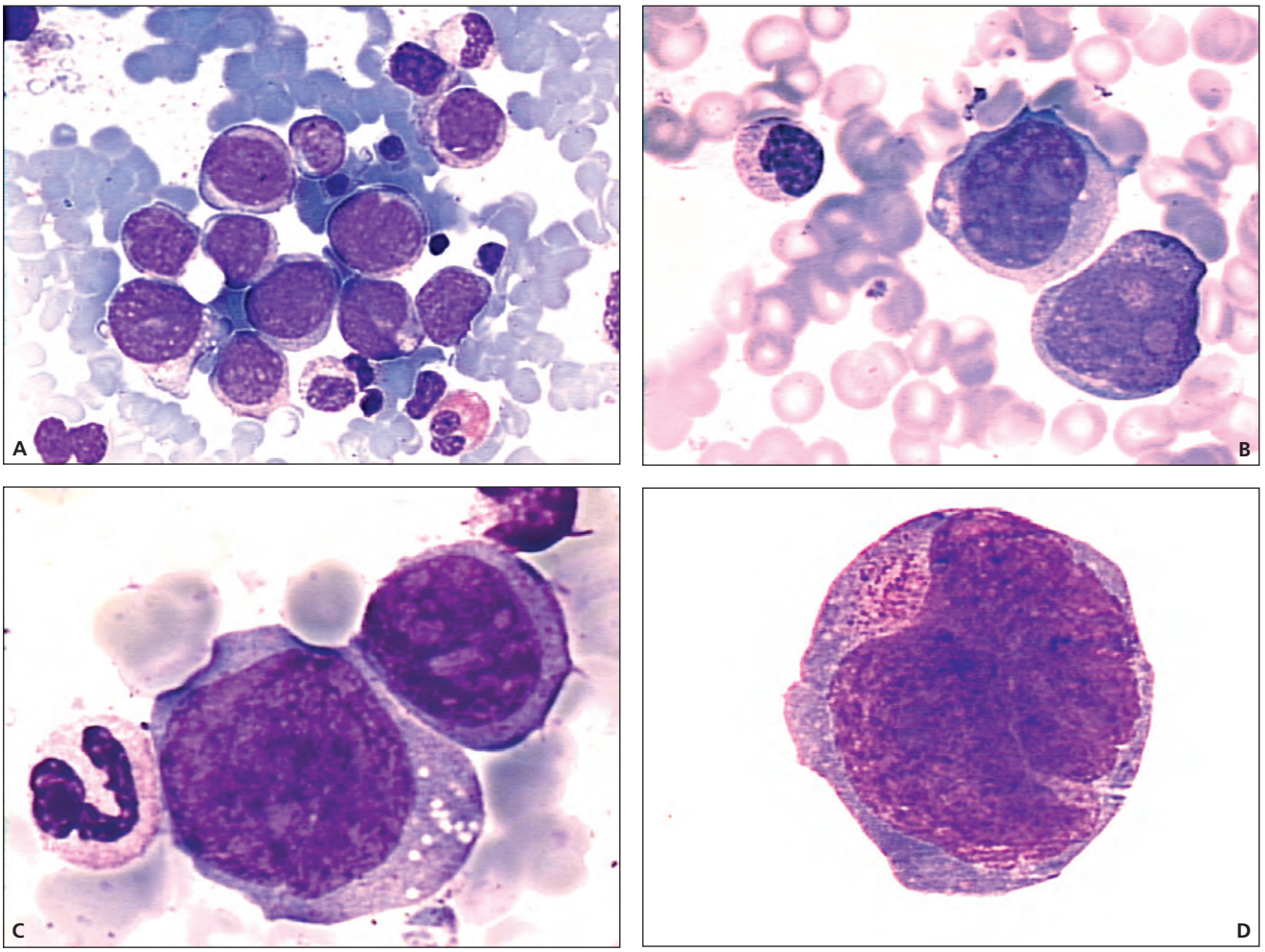



**Fig. 4.79** AML with maturation, with gigantic (near-tetraploid) blast cells; bone marrow aspirate. (A) Massive bone marrow infiltration by granular blast cells with hyperdiploid nuclei. There are also some dysplastic granulocytes and an eosinophil. (B) On the left: a hyposegmented neutrophil; a large blast with an enormous nucleus, of the type that has been described as having a near-tetraploid complement of chromosomes; another similar cell with two enormous nucleoli, one of which shows peripheral chromatin reinforcement, and plentiful granule-rich cytoplasm. (C) A large blast cell of near-tetraploid type, with a giant nucleus with heterogeneous chromatin showing areas of condensation and a number of indistinct nucleoli; the cytoplasm appears violet, because of the presence of very fine azurophilic granules on a basophilic background, and contains numerous vacuoles. The massive size of this cell can be appreciated by comparing it with the non-tetraploid myeloblast, above and to the right (which otherwise has analogous characteristics, though the chromatin is more condensed and the nucleoli more evident), and with the neutrophil on the left. (D) Giant hyperdiploid myeloblast in a cytocentrifuge preparation of the same patient.

Myeloid markers such as MPO, CD13, CD33, CD15 are usually positive; expression of CD34 and other markers of immaturity is variable, often being seen in only a low percentage of blast cells; CD7 may be positive. Immunophenotyping may provide relevant information in cases in which the direction of maturation is not evident from morphology and cytochemistry.

The difference between AML with granulocytic maturation and myelomonocytic maturation was, in the FAB period, a major problem, being based mainly on morphological and cytochemical criteria. In any event, in the absence of recurrent genetic abnormalities, therapeutic choices do not differ. The differential diagnosis with RAEB, on the other hand, is important in cases of AML with a low blast percentage and must be based on an accurately performed myelogram.

### Acute myelomonocytic leukaemia (similar to FAB M4)

Previous classifications always recognised the existence of acute leukaemia with mixed myelomonocytic differentiation, sometimes known as 'Naegeli type', and pure monocytic differentiation, sometimes known as 'Schilling type'. In leukaemia with a monocytic component, more in monoblastic than myelomonocytic, there is often increased lysozyme in the serum and urine and extramedullary localisation of disease with infiltration of

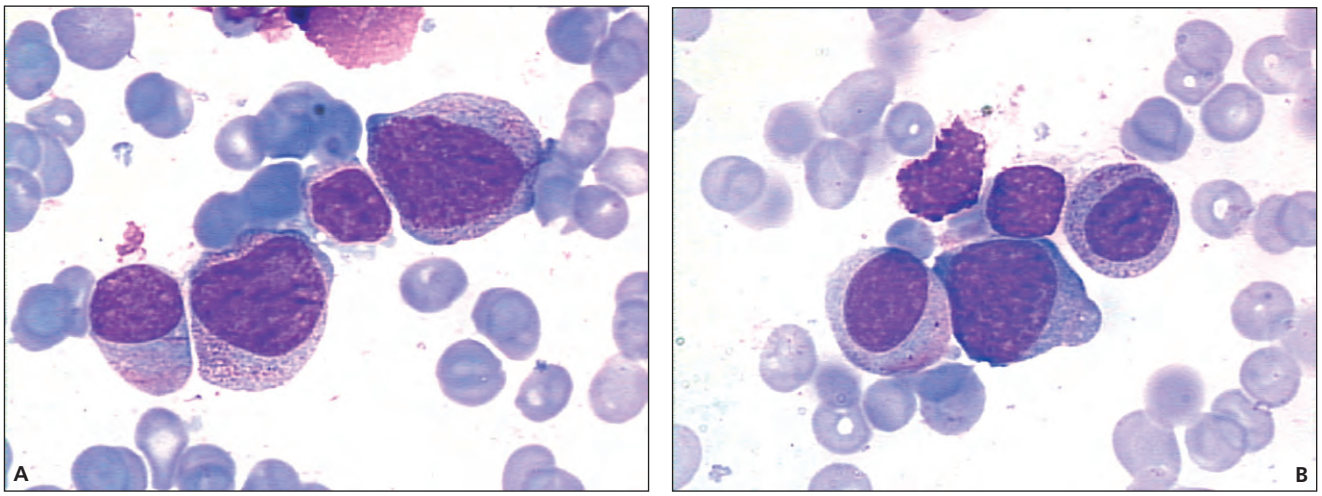



**Fig. 4.80** Another patient with AML with maturation; bone marrow aspirate. (A) From the left towards the right: a myeloid blast with plentiful basophilic cytoplasm, fine granules and two Auer rods that differ in length; a dysplastic promyelocyte with marked N:C asynchrony; a lymphocyte; another dysplastic promyelocyte. (B) A large blast with plentiful, intensely basophilic cytoplasm in which one can just detect granules. At the two sides are maturing dysplastic granulocytes: the cell on the left is certainly difficult to classify because of the difficulty in seeing the numerous fine granules (two of which, however, are hypertrophic), the weakly basophilic cytoplasm and the compact chromatin of the perfectly oval nucleus; the cell on the right is a neutrophil myelocyte.

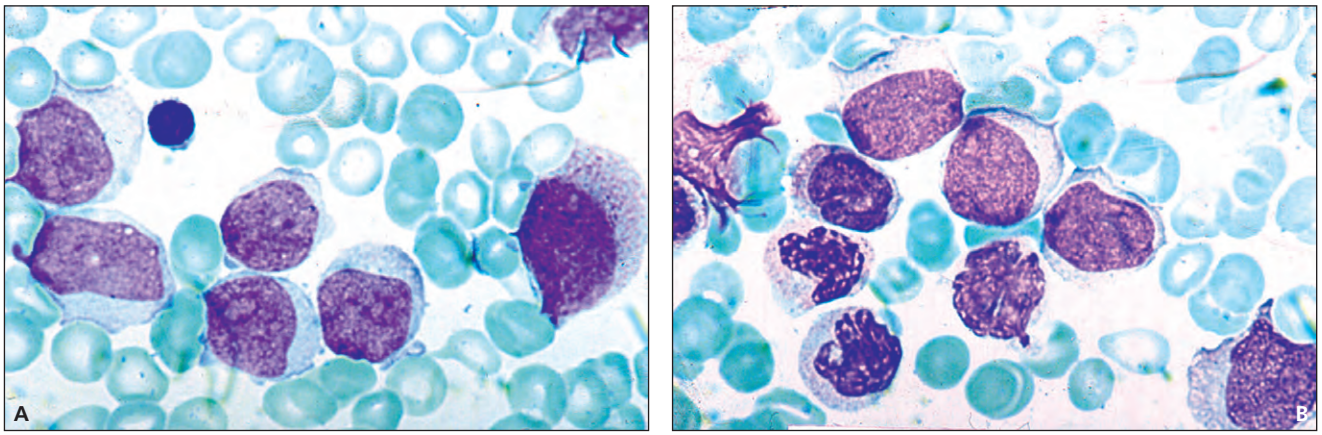



**Fig. 4.81** AML with maturation; bone marrow aspirate from another patient with marked dysplasia confined to the granulocyte lineage. (A) Five blast cells with plentiful weakly basophilic cytoplasm without any apparent granules. On the right, a dysplastic promyelocyte. (B) Four blasts and four dysplastic granulocytes. To classify cases of AML, NOS according to the degree of maturation, using the 2008 WHO criteria, the signs of differentiation in blast cells (granules or Auer rods) are not considered, the presence of 10% or more maturing granulocytes being what is required.

lymph nodes, liver, spleen, skin, gums, central nervous system and serous cavities.

AML, NOS, with myelomonocytic differentiation is not rare (5–10% of all AML). As indicated by the name, it is characterised by simultaneous differentiation of the leukaemic blasts to granulocytic and monocytic lineages.[109] Promonocytes must be included in the blast count.[11] As in other types of AML, NOS, the percentage of blast cells in the peripheral blood or bone marrow aspirate must be equal to or greater than 20% (with <50% of erythroblasts in the bone marrow). The definition also requires that there are at least 20% of cells showing granulocytic differentiation (neutrophils and their precursors) and at least 20% showing monocytic differentiation (monocytes and their precursors).

Leucocytosis is very common in AMML (Fig. 4.83). Microscopic evaluation of the blood film is of diagnostic importance in all leukaemias with monocytic differentiation, since often features indicative of monocytic maturation (nuclear morphology and cytoplasmic colour) are more evident in the peripheral blood than in the bone marrow. A peripheral blood monocyte count above $5 \times 10^9$/l is common, there often being a larger proportion of mature monocytes than in the bone marrow.

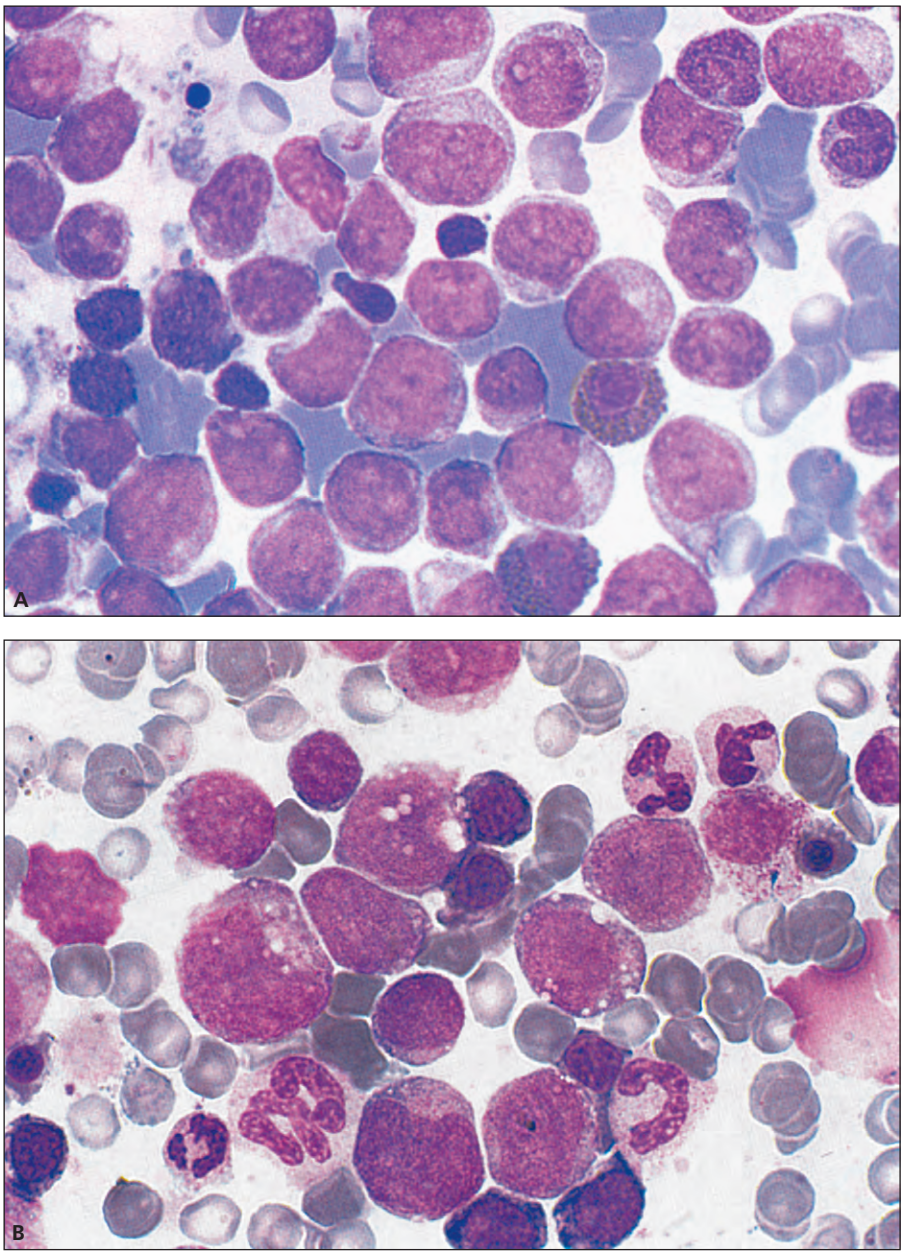



**Fig. 4.82** Bone marrow aspirate in two different cases of AML with maturation. (A) Marked hypercellularity. The percentage of blasts is high (70%): they are mainly cells with fine azurophilic granules within fairly plentiful cytoplasm; the nucleus is oval and has delicate chromatin with visible nucleoli. In the larger cells, there is a nuclear notch or indentation that is related to the Golgi zone. Near the top left corner, beneath a pyknotic nucleus that has been expelled from an orthochromatic erythroblast, is an Auer rod that appears to be within the cytoplasm of a macrophage. On the left, a small group of basophilic erythroblasts. In this field there are no neutrophil granulocytes, but there are two eosinophils towards bottom right, one of which has a spherical non-lobulated nucleus. There are a number of lymphocytes admixed with blast cells in the central area. (B) The marrow of this patient shows increased cellularity with a heterogeneous mixture of cells and with moderate dysgranulopoiesis. In this microscopic field, one can see three types of cell: blasts, mature neutrophils and erythroblasts. The blasts show clearly their myeloid nature, with plentiful granular cytoplasm in which there is an impression of a Golgi zone pushing the nucleus to one side; there are frequent vacuoles. Some neutrophils show reduced granulation and anomalies of nuclear segmentation; the largest, at the bottom just to the left of centre, seems to have two band-shaped nuclei. Top right, a dysplastic myelocyte.

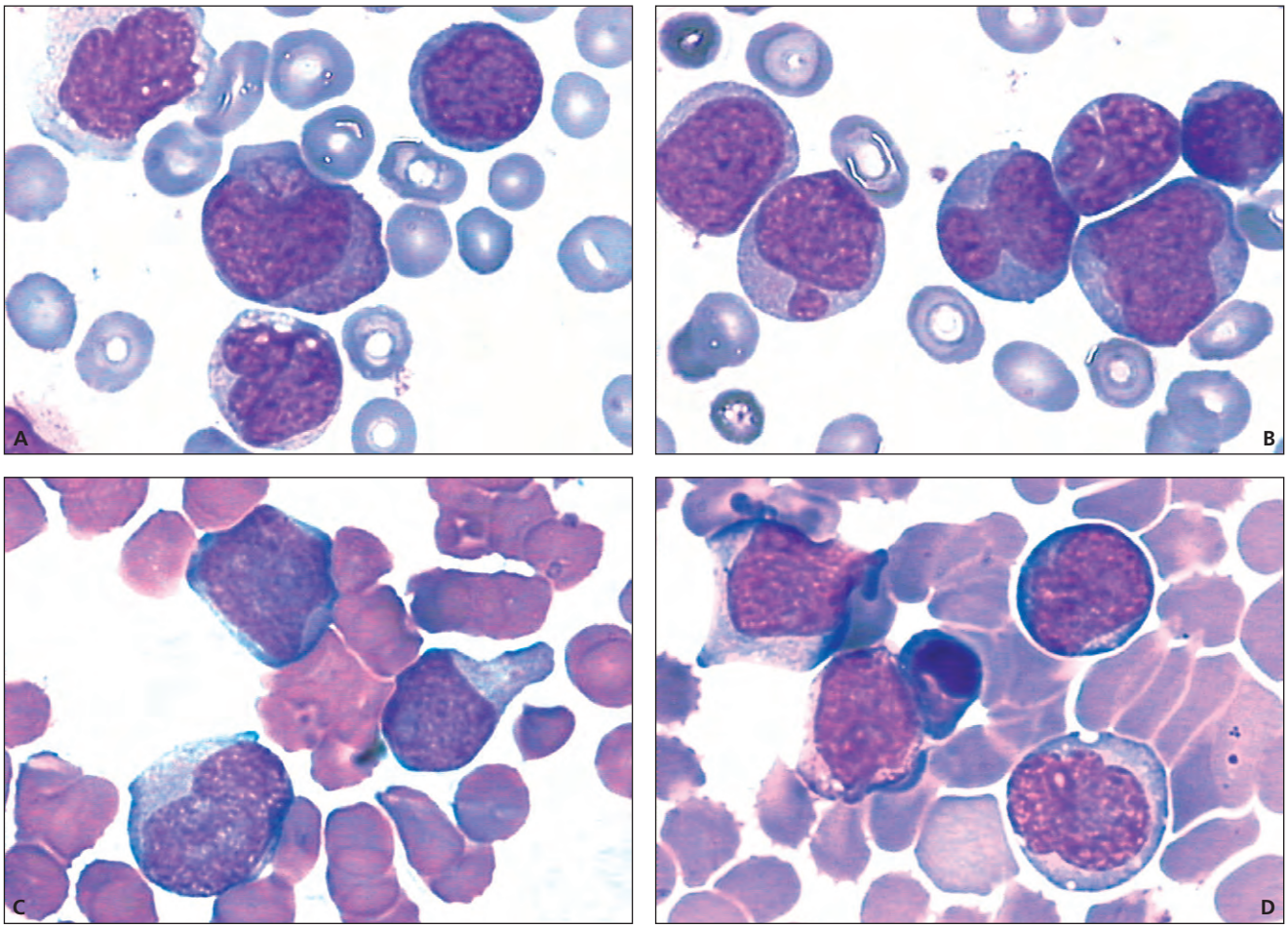



**Fig. 4.83** AML; peripheral blood. Morphological heterogeneity of circulating blast cells. (A) Top right, a blast with a high N:C ratio and a rounded nucleus with heterogeneously clumped chromatin and small nucleoli; the cytoplasm does not show any signs of differentiation. In the centre, a large myeloblast with a N:C ratio favouring the cytoplasm, which is plentiful, intensely basophilic and rich in very fine granules, in the upper part of the cell, tending to coalesce; the nucleus has a concavity and the chromatin is heterogeneously condensed. The other two cells are immature monocytes, which are not included in the blast count. (B) From the same case as (A). Six blast cells with heterogeneous morphology, which are not easy to assign to a specific lineage. The nuclei are sometimes polygonal (on the left) or rounded with compact chromatin (on the right), but more often they show notches, incisions, constrictions and a tendency to form lobes. The chromatin is sometimes fine and homogeneous, letting the nucleoli be seen, as in the cell on the right with plentiful basophilic cytoplasm; in other cells there is partial, incomplete chromatin condensation, as in the cell on the left with a small nuclear pseudo-lobe and plentiful cytoplasm that appears to be very finely granular. (C) Also in this patient, one observes the co-existence of blasts that appear undifferentiated, such as that at the top, with others that have more specific features, such as the cell at the bottom, which has the appearance of a promonocyte. On the right, a myeloblast with non-homogeneous chromatin condensation and a cytoplasmic tail containing some azurophilic granules. (D) From the left, anticlockwise: an undifferentiated blast cell with a polygonal angulated nucleus and condensed chromatin; a myelocyte with scanty hypogranular cytoplasm; a promonocyte with spongy cytoplasm and a nuclear outline including segments of circles; another blast, in the top right corner, with an indented nucleus, a large non-prominent nucleolus and intensely basophilic cytoplasm. In the centre, a cell (probably belonging to the leukaemic population) in apoptosis: the nucleus is liquefied into a blackish, structureless mass and the cytoplasm is intensely blue and opaque.

The bone marrow is often very cellular (Figs 4.84 through to 4.88). There is co-existence of agranular blasts, granular blasts, monoblasts and promonocytes. The first and second of these have rounded or oval nuclei or a notched or indented nuclear outline. The chromatin is delicate and reticular and nucleoli are often evident. The cytoplasm sometimes contains azurophilic granules and Auer rods, usually single. Monoblasts have round nuclei with very fine homogeneous and reticular chromatin, containing one or more large prominent nucleoli; the cytoplasm usually contains granules and is weakly basophilic or greyish. Promonocytes have a similar chromatin structure, but the nuclear outline is irregular, suggestive of the monocytic lineage; the cytoplasm may be basophilic, blue or grey, not dissimilar to that of monoblasts, from which they are distinguished by the irregular shape of the nucleus and by the presence of azurophilic granules, which are sometimes larger and more obvious than those of mature monocytes.[13] Granulocytic differentiation is generally neutrophilic; the eosinophil lineage is increased and

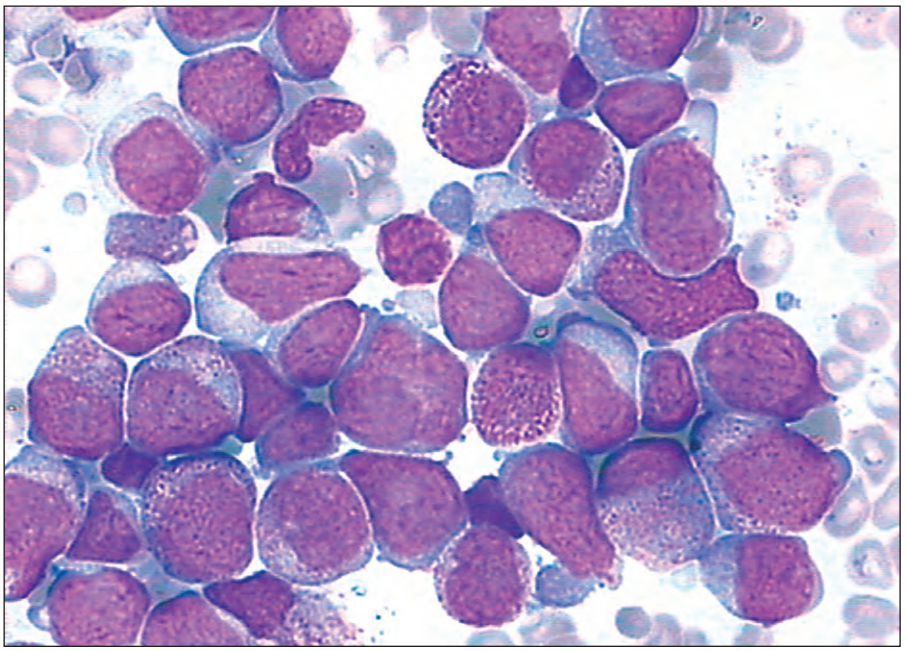


**Fig. 4.84** AMML; bone marrow aspirate from the same patient as Fig. 4.83A and B. The marrow is hypercellular with 90% of cells belonging to a heterogeneous blast population. In comparison with the peripheral blood, it is immediately apparent that a larger proportion of cells show numerous granules; the majority of these cells meet the WHO criteria for granular blasts (e.g. the three in the bottom right corner), while others, with numerous dark granules, eccentric nuclei and compact chromatin (such as that high in the centre) have the morphology of promyelocytes (and are thus excluded from the blast count). Other blasts, in general smaller, appear agranular with scanty or moderately represented cytoplasm that has neither granules nor Auer rods. Yet others, such as the larger cell in the centre, evoke the monocytic lineage because of the tendency of the nucleus to be irregular or convoluted.

shows increased dysmorphism, particularly in cases with the above-specified abnormalities of chromosome 16, which are not assigned to this category.

The immunophenotype often shows heterogeneity, with the co-existence of different blast populations characterised by variable expression of markers of immaturity together with antigens typical of granulocytic and monocytic lineages (such as CD14, CD11b, CD11c, CD64 and, in more differentiated forms, CD4).

### Acute monoblastic and monocytic leukaemia (similar to FAB M5a and M5b AML)

AML, NOS with monocytic or monoblastic predominance represents around 10% of cases of AML. The peripheral blood usually shows monocytosis, sometimes very marked (see Report 4.5). There can be extramedullary masses, cutaneous and gingival infiltration and involvement of the central nervous system. In these cases, 80% or more of the leukaemic cells are monoblasts, promonocytes, monocytes or a mixture of these cell types.

In acute monoblastic leukaemia (and in FAB M5a AML), the dominant population, generally comprising at least 80% of blast cells, is composed of monoblasts. These are large cells with a low or intermediate N:C ratio (Figs 4.89 through to 4.92). The nucleus is round or oval, in the most immature forms without any irregularity, but sometimes slightly indented. The chromatin forms a very loose reticular network: in some cases it has the most delicate interweaving seen in haematological preparations. The nucleoli are usually readily seen, prominent, vesicular and present in variable numbers. The cytoplasm is voluminous, weakly basophilic, pale blue or greyish, rarely a more intense blue; there may be cytoplasmic protrusions or pseudopods of variable appearance; the cytoplasm may contain empty vacuoles or some azurophilic granules, rarely more than four or five, usually close to the nucleus. Monoblasts do not contain Auer rods, although these may be present in myeloblasts, which are present in low enough numbers that they do not meet the criteria for myelomonocytic leukaemia. There is a certain morphological variability in monoblastic leukaemias, ranging from cases in which the cells are similar to myeloblasts with a high N:C ratio to cases in which the cytoplasm is more typically monocytic – ample, dusty appearing and vacuolated. More often than in other types of leukaemia, the cytoplasmic projections detach, by clasmacytosis, giving rise to cytoplasmic fragments and hyaline bodies that can be counted as platelets in automated analysers.[110]

In acute monocytic leukaemia (and also in FAB M5b AML), the bone marrow monocytic component is comprised of a variable but significant number of promonocytes (≧20%),[13] which

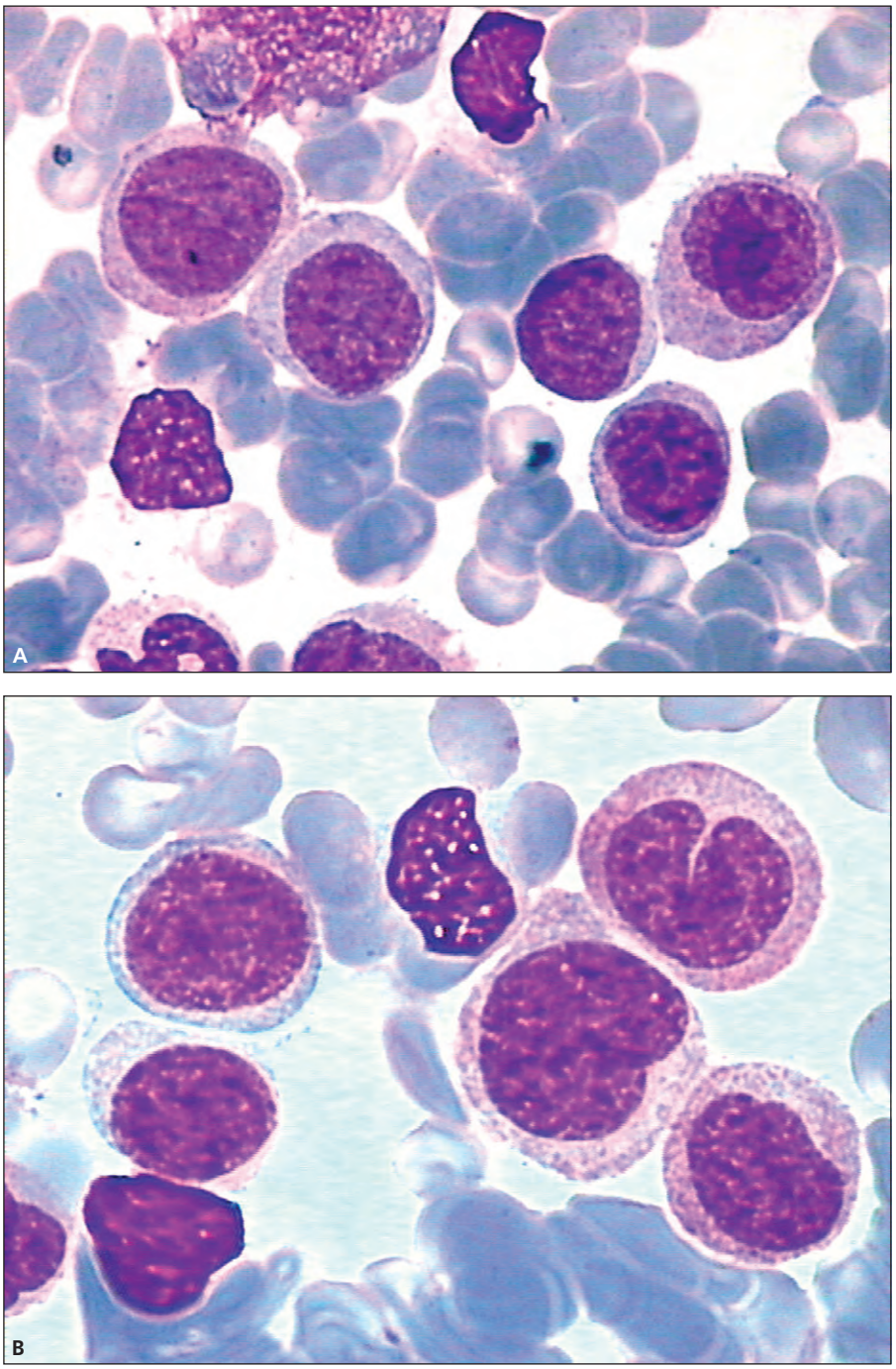



**Fig. 4.85** AMML; bone marrow aspirate from another patient with more obvious monocytic differentiation. (A) The two large cells seen top left are monoblasts, with round nuclei, chromatin showing some small foci of condensation and little evidence of nucleoli; the cytoplasm is plentiful and surrounds the greater part of the nucleus; the background staining is pinker in the cell on the left (which would require a nuclear notch, even if minimal, to be classified as a promonocyte) and weakly basophilic in the cell on the right. Proceeding towards the right there is a small undifferentiated blast with homogeneous chromatin and a high N:C ratio, a similar cell with more cytoplasm and clumped chromatin below and to the right, and a very typical promonocyte top right (one notes the nuclear folding and the granularity of the cytoplasm). (B) From left towards the right: a small atypical monocyte; a monoblast with basophilic cytoplasm; a large promonocyte with a folded nucleus; two immature monocytes. The morphological borderline between promonocytes and immature monocytes is not very clear in this case, which has cells showing considerable, although not homogeneous, chromatin condensation, even in the monoblast.

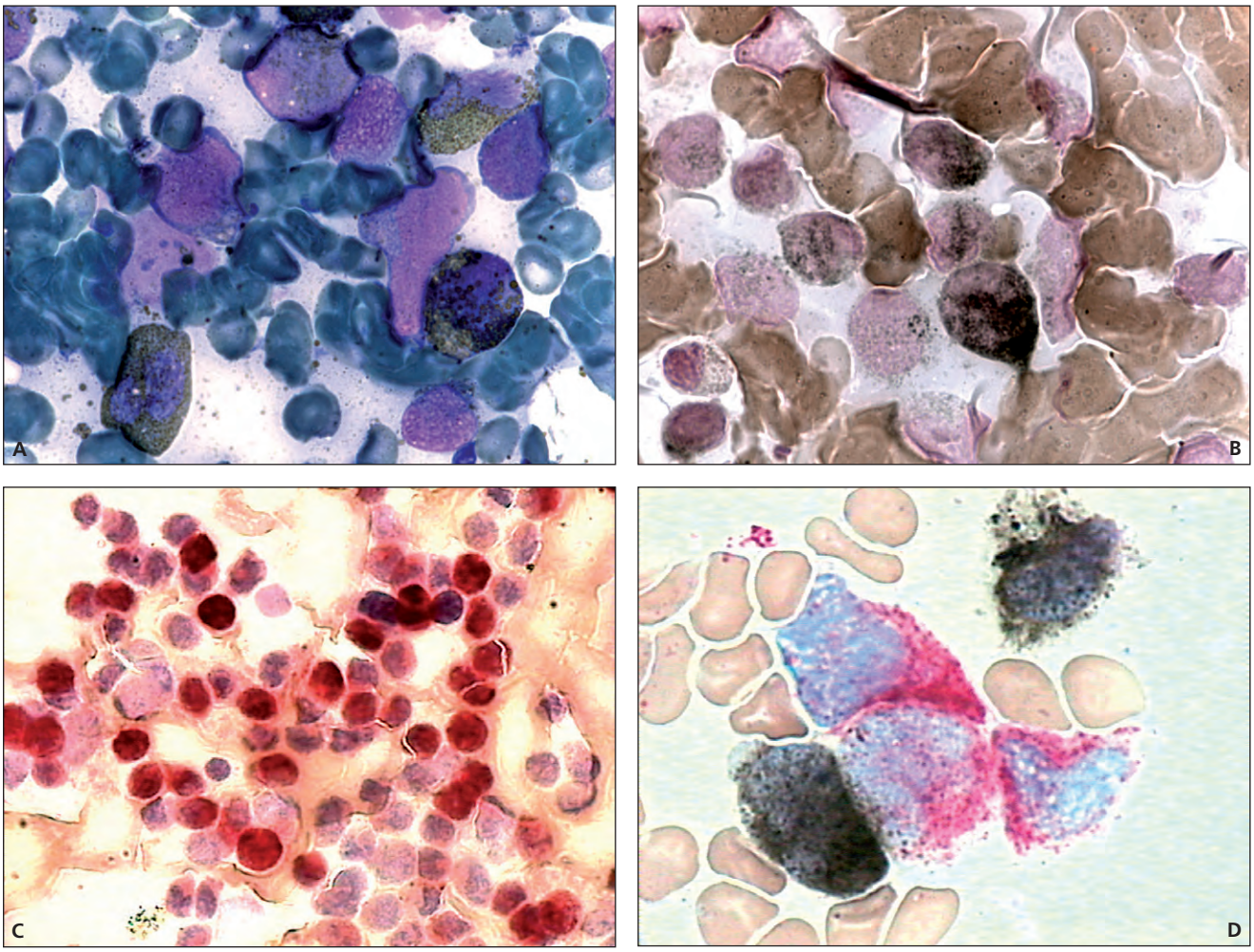



**Fig. 4.86** AMML; bone marrow aspirate. Cytochemical stains are diagnostically very useful in these cases with mixed and heterogeneous differentiation. (A) Peroxidase. The blasts may be negative or show various degrees of positivity, depending on the direction and extent of differentiation, whether granulocytic or monocytic. (B) ANAE. The reaction shows increasing positivity, and thus a greater abundance of the brown precipitate, from monoblasts to promonocytes to monocytes. In monoblasts there is often a granular reaction, sometimes focal, which leaves large areas of the cytoplasm still visible. (C) Double esterase stain: this method combines a cytochemical reaction for naphthol AS-D chloroacetate esterase (CAE) (granulocytic esterase), which produces a reddish precipitate in blast cells showing granulocytic differentiation and in maturing neutrophils, with ANAE (a monocytic esterase), which in this film has given a less dark precipitate. The two directions of differentiation are thus well demonstrated in AMML. (D) Double esterase, at higher power. The two cells with an abundant brown precipitate are identifiable as monocytes, because of the intensity of the reaction and the convoluted shape of the nucleus. The cell in the centre with a moderate amount of granular and diffuse, cherry-red precipitate is a myeloblast; the other two cells are a myelocyte and a metamyelocyte.

according to the 2008 WHO criteria must be considered as equivalent to blast cells,[11] and of more differentiated cells, atypical monocytes or monocytes. The promonocytes are voluminous cells with a low N:C ratio. The nucleus is large and irregular, with the outline appearing folded, cerebriform, convoluted or contorted: it is the nuclear morphology, in fact, that provides the criteria to distinguish this cell from a monoblast (Figs 4.93, 4.94 and 4.95).

The chromatin structure is still reticular, but there may be small areas of condensation into points or lines. Its transparency is such that folding may be seen beneath the nucleus. There are one or two nucleoli, sometimes prominent, more often partly blending with the chromatin network. The cytoplasm is abundant, weakly basophilic, pale or greyish, always less basophilic than that of monoblasts, with a sandy or ground-glass appearance resulting from the presence of very fine salmon-coloured granules; in some cases the granules are more clearly azurophilic, or have an opaque brick-red colour and are relatively large, standing out very obviously from the pale cytoplasm. Vacuoles are often present. Monocytes show a further reduction in the

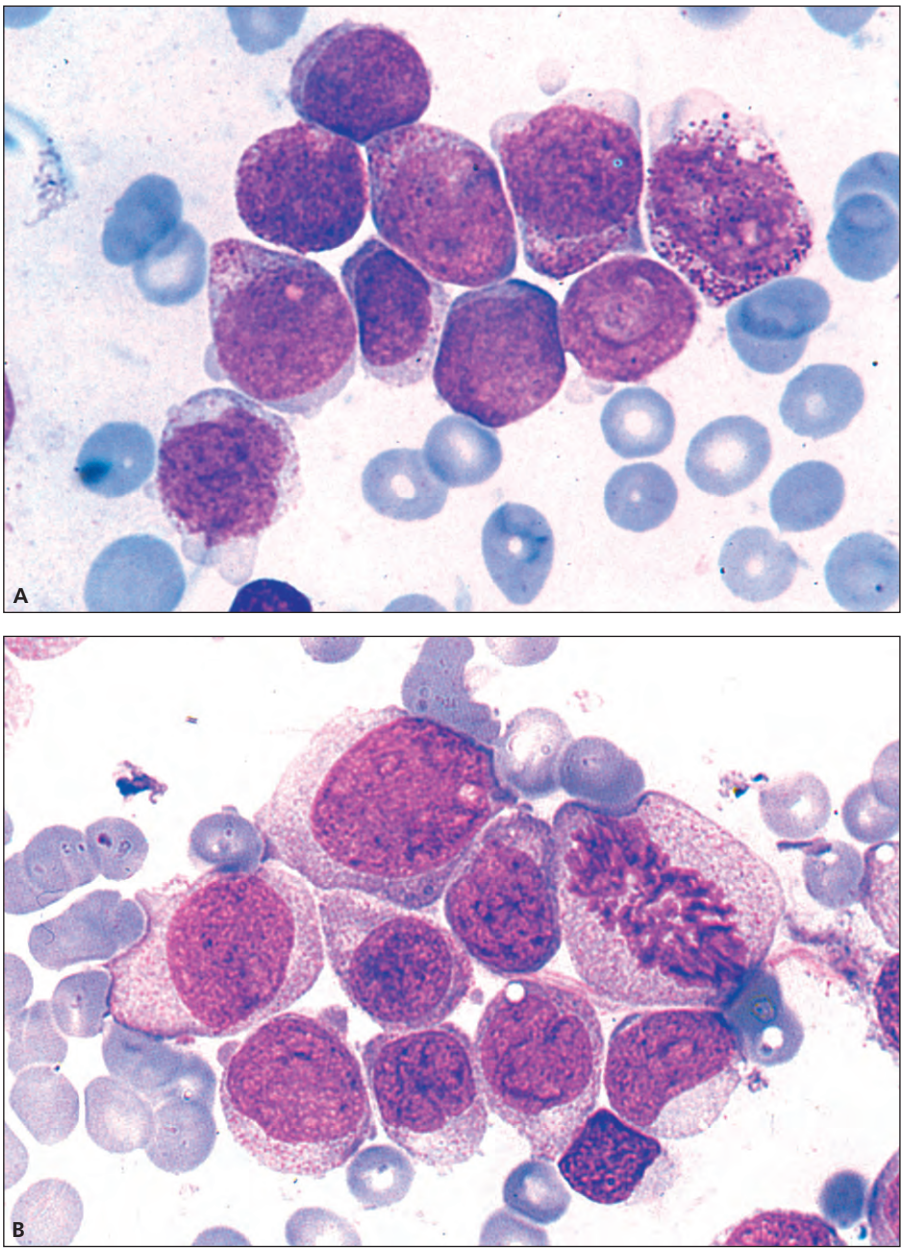



**Fig. 4.87** Another two examples of the leukaemic populations in bone marrow aspirates of patients with AMML. (A) Various blast cells show signs of granulocytic differentiation, in the form of numerous azurophilic granules on a background of weakly basophilic cytoplasm. Two blast cells, one above the other in the centre of the image, are agranular with little sign of differentiation. A cell on the right has larger granules and pale cytoplasm. The chromatin is homogeneous and shows some condensation. The only cell that morphologically suggests the monocyte lineage is the promonocyte bottom left. Cytochemical reactions demonstrated that the morphologically less differentiated blasts were negative for peroxidase and positive for ANAE. (B) In this case, the blastic population has morphological characteristics more suggestive of the monocyte lineage. There are large blast cells with plentiful, finely granular cytoplasm with a peripheral zone of basophilia; the nucleus is oval and has chromatin that is very delicate with numerous nucleoli of varying sizes. Some cells have identical cytoplasm, but with a nucleus that is indented or convoluted and linear chromatin condensation: this appearance is typical of promonocytes. On the right, a mitotic figure in metaphase: the amount of cytoplasm is double that of the other cells.

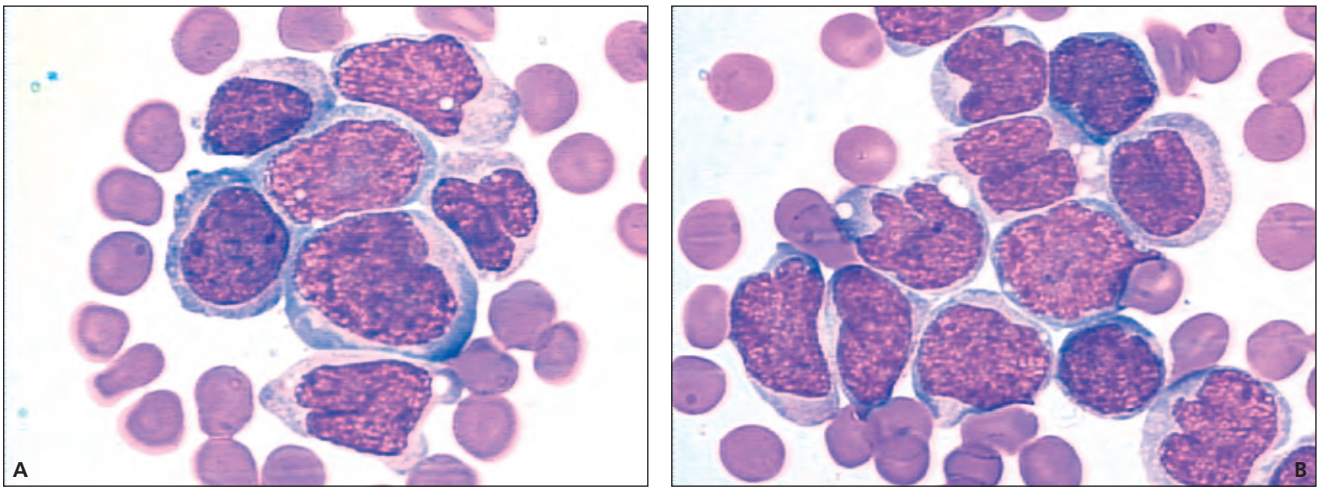



**Fig. 4.88** AMML; bone marrow aspirate in a case in which monocytic differentiation predominates over granulocytic. (A) In the centre, three blast cells with some morphological differences, particularly with respect to the nuclear conformation: the cell on the left has a regular oval nucleus with little chromatin condensation; that on its right is doubled in size with an indented, certainly tetraploid nucleus, the chromatin of which shows a clearer distinction between dark clumps of condensed chromatin and paler areas; the upper cell has a pale nucleolus that is not very prominent. The other cells are not blasts, but more mature cells of monocyte lineage. (B) In this field, the most numerous cells are the promonocytes, with large nuclei with a contorted or indented nuclear outline, with non-homogeneous chromatin composed of alternating pale and dark areas: the abundance of pale parachromatin gives these nuclei a blown-glass, spongy, light appearance. The cytoplasm is consistently well represented with tonal variation from basophilic to sky-blue to greyish.

N:C ratio and are morphologically similar to their normal counterparts, with a complex nuclear form and variable chromatin density. Rare Auer rods belong to occasional myeloblasts associated with the monocytic proliferation (Fig. 4.94).

Peroxidase is usually negative in monoblasts, but there can be weak granular positivity in promonocytes; darkly staining granules are more often demonstrated with a Sudan black B stain. Monocytic esterases, ANBE and ANAE, are typically strongly positive, the latter being inhibited by sodium fluoride. In 10–20% of cases, ANAE is negative; aberrant positivity for CAE has been described. The acid phosphatase reaction is diffusely positive. It is also possible to demonstrate lysozyme activity cytochemically, mixing the leukaemic cells with a suspension of the bacterium *Micrococcus lysodeikticus*, the suspension then being spread on a glass slide and subsequently stained: the bacteria adjacent to lysozyme-secreting blast cells are lysed and thus appear pale on panoptic staining.

The immunophenotypic markers most characteristic of acute monoblastic and monocytic leukaemias are CD14, CD11c, CD11b, CD4, CD64, CD68, CD36 and lysozyme. There is also a variable expression of myeloid surface antigens and, sometimes, of MPO. CD34 is positive in a third of cases, as is CD7.

A morphological features that is not common in AML in general, but which is more frequent when there is monocytic differentiation, is erythrophagocytosis (Fig. 4.96), which is often, but not always, associated with t(8;16)(p11.2;p23.3). This subtype is usually monoblastic or, more rarely, myelomonocytic, with the highest incidence being in children. The blast cells have a monocytoid appearance and have fairly obvious cytoplasmic granules: their principal distinguishing feature is haemophagocytosis, particularly erythrophagocytosis. There is often a haemorrhagic diathesis due to hyperfibrinolysis. There may be central nervous system and other extramedullary localisation. Haemophagocytosis by leukaemic cells (i.e. spontaneous ingestion of red cells, platelets or, more rarely, granulocytes by blast cells), can also be seen in other types of AML, more often, but not only, when there is a monocytic component.[111–113] In some cases, what appears to be haemophagocytosis may actually be the phenomenon of emperipolesis, in which the active role is played by the cells that have apparently been ingested. These appearances must not be confused with haemophagocytosis due to macrophage activation caused by viral infection and associated as a reactive phenomenon with haematological neoplasms including MDS. In these cases, the haemophagocytic cells are not leukaemic blasts, but are well-differentiated macrophages with abundant post-phagocytosis vacuoles and the detritus of digested cells.

With regard to a cytological differential diagnosis, AML with monocytic differentiation can sometimes be difficult to distinguish from the microgranular variant of APL, because of the appearance that cells of the latter condition assume in the peripheral blood. In these cases, in addition to a morphological assessment of well-made and stained films of both the peripheral blood and the bone marrow, which are often very evocative, it is important to employ cytochemistry, which shows strong MPO activity in the promyelocytes and ANAE activity in

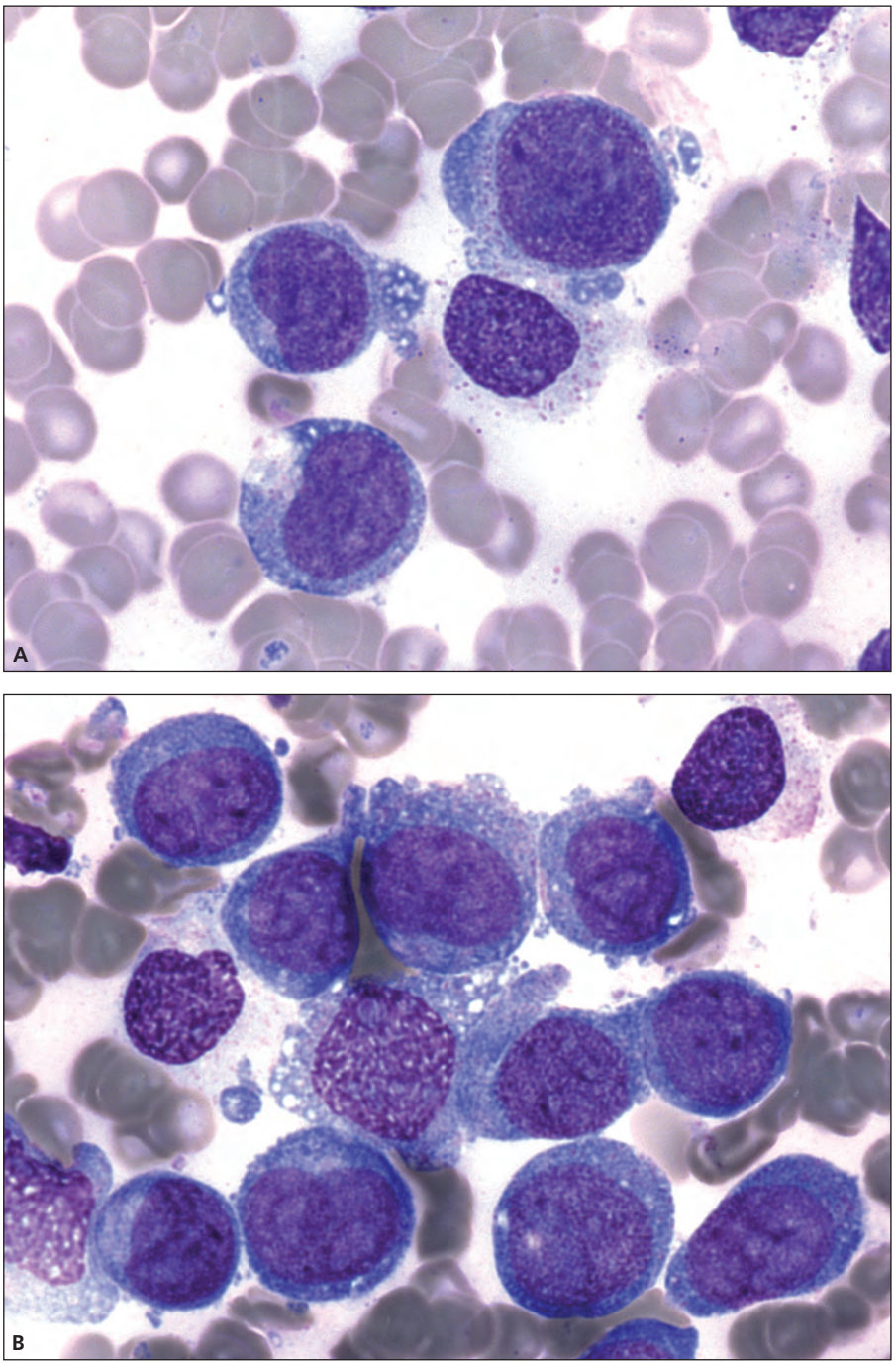



**Fig. 4.89** Acute monoblastic leukaemia. (A) Peripheral blood film. The leucocyte count is high because of the presence of numerous, morphologically typical monoblasts: large size, N:C ratio not high, reticular and homogenous chromatin, nucleoli that are numerous even though they are not prominent and cytoplasm showing a variable degree of basophilia. The largest cell, at the top, has an almost round nucleus with a single straight part on the side that borders the cytoplasm. This matches a large thick basophilic projection on the left; some granules are seen in the elongated, pale paranuclear zone; at bottom right the cytoplasmic membrane is deformed into two large bluish rounded projections containing small vacuoles. On the left, two smaller monoblasts, with similar characteristics but indented nuclei. (B) Bone marrow aspirate. The marrow is heavily infiltrated by monoblasts with analogous morphological characteristics. One notes the extreme delicacy of the chromatin, particularly in the cells with round nuclei, which have large vesicular nucleoli, with perinucleolar chromatin condensation, but which are equally obvious because of their paler colour. The cytoplasm is abundant, basophilic, agranular, less vacuolated than that of the circulating cells and sometimes ragged or with the cytoplasm extending into pseudopodial projections. Even if some nuclei have a suggestion of indentation or folding, none of these cells has characteristics that would lead to it being classified as a promonocyte.

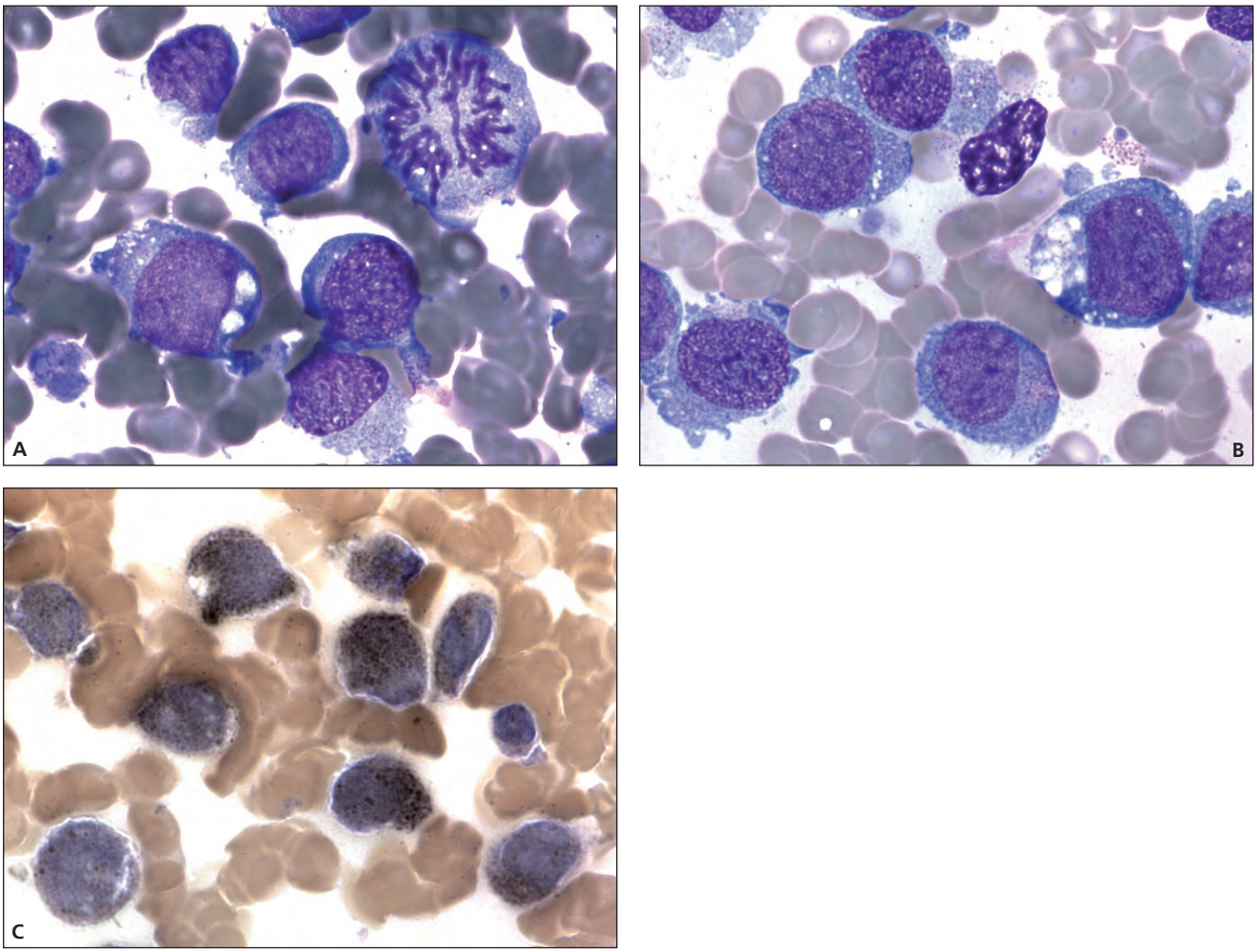



**Fig. 4.90** Acute monoblastic leukaemia, same patient as Fig. 4.89; bone marrow aspirate. (A) Mitotic figure in a blast in anaphase, with long chromatids that have separated and spread out within the cytoplasm prior to the migration to the two poles that precedes cell division. (B) One monoblast has a grape-like cluster of vacuoles in the paranuclear area. Another, at the bottom, has fine azurophilic granules. The chromatin, consistently fine and reticular, forms a network that is somewhat coarser in the cell seen bottom left. The numerous cytoplasmic projections are tending to become detached, which could lead to a factitious increase in the platelet count. (C) An ANAE stain gives a positive reaction, although of variable intensity, in all cells.

promonocytes. In some cases, the bone marrow contains less than 20% of blasts and appears to be completely replaced by promonocytes and monocytes: making a distinction from chronic myelomonocytic leukaemia in these patients can be difficult.

Monocytic leukaemias also need to be distinguished from the very rare cases of malignant histiocytosis in leukaemic phase, a condition that, according to the 2008 WHO classification, represents a systemic presentation of histiocytic sarcoma.[114]

### Acute erythroid leukaemia (similar to FAB M6 AML)

The erythroid leukaemias represent 4–5% of all cases of AML and are characterised by an aggressive clinical course.[115] Since the first description of this condition,[116] cytomorphology has played a critical diagnostic role. The criteria proposed for the diagnosis of AML M6 were one of the major innovations of the FAB group in 1985.[7] They proposed a basic criterion of erythroid hyperplasia: more than 49% of ANC had to be erythroblasts. The second FAB criterion was that blasts had to comprise at least 30% of NEC. The 2008 WHO classification maintained this concept, but as for other types of AML the blast threshold was reduced to 20% of NEC. In summary, the current definition of acute erythroleukaemia is based on the presence in the bone marrow aspirate of at least 50% erythroid cells with blasts comprising at least 20% of NEC. Proerythroblasts are not counted with blast cells (with the exception of the rare cases, described below, of pure erythroid leukaemia). In cases in which blast cells are less that 20% of NEC and erythroid cells are 50% or more of NEC, the

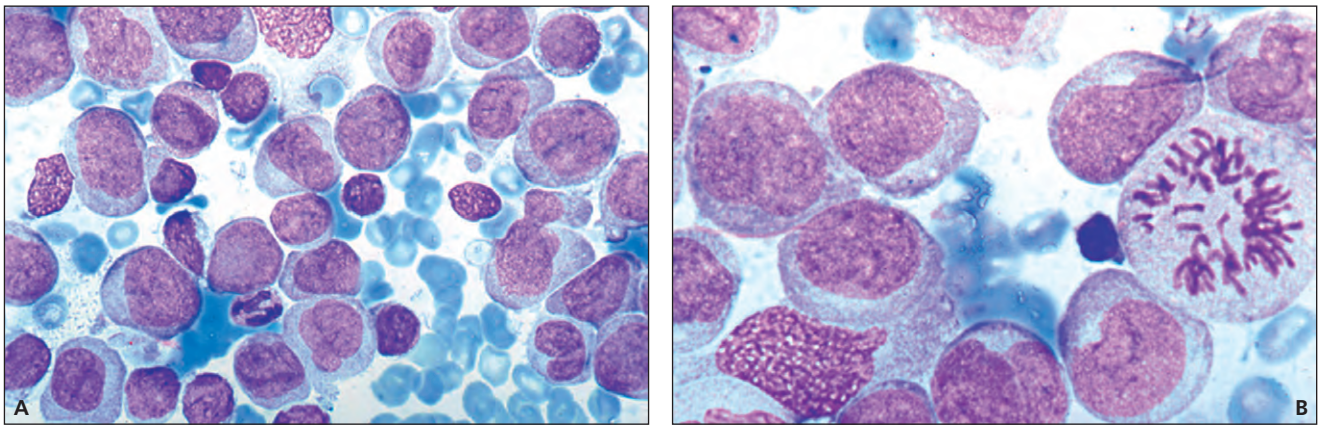



**Fig. 4.91** Acute monoblastic leukaemia; bone marrow aspirate. In this case, the leukaemic infiltrate is composed of monoblasts and promonocytes in similar proportions. (A) The large cells with round nuclei, a loose reticular chromatin pattern with nucleoli and ample basophilic cytoplasm are monoblasts. The promonocytes have a similar chromatin pattern, sometimes with a thicker chromatin network, or appearing swollen and transparent, but can be recognised particularly because of the presence of nuclear notches, folds and lobules. A pair of typical promonocytes, for example, are seen in the centre, close to the lower edge. Top left, a neutrophil promyelocyte. (B) The blasts in this field mainly have the morphological features of monoblasts. Again, one can note the particular delicacy of the chromatin network of these leukaemic cells; this and the tendency of the abundant cytoplasm to surround the nucleus more or less completely represent the most useful and distinctive elements for cytomorphological diagnosis. On the right, a blast cell in mitosis has long thin chromatids, often still joined to the centromere.

diagnosis is MDS and the criteria for diagnosis and classification described in Chapter 3 are applied.

M6 AML was thus the only case in which, applying the FAB criteria, it was possible to make a diagnosis of AML with blast cells, as a proportion of ANC, being below the 30% threshold (being above that level, however, when expressed as a percentage of NEC). Such cases are also diagnosed as AML in the WHO classification, but with the altered threshold of 20% blast cells. In this classification, however, this is also possible for three subtypes of AML with recurrent genetic abnormalities. The WHO classification, like the last FAB classification, follows a strictly numerical approach without there being any particular requirement for specific morphological characteristics (such as erythroblasts with bizarre morphology, multiple nuclei, lobulated or fragmented nuclei, giant forms and megaloblastosis). The requirement for an erythroid component of at least 50% remains, however, controversial. This leads to the exclusion from the diagnosis of erythroleukaemia of the not rare cases with a bone marrow aspirate showing a lower percentage of erythroblasts, but with these being markedly dysplastic and abnormal, and associated with a blastic proliferation.[117] Conversely, a diagnosis of erythroleukaemia may be made inappropriately in cases that present with a very high percentage of erythroblasts, and a blast count below 10% of ANC: in these cases, it is possible for the progress of the disease to be closer to that of MDS, and aggressive treatment may not be indicated.[118]

Under the general heading of erythroleukaemia there are two different types of neoplasm that involve the erythroid lineage.[119,120] The first, which is much more common, is that which better fits the classification of erythroleukaemia and which shows a mixed erythroid and myeloblastic proliferation: the most common picture is that of erythroid hyperplasia with associated dyserythropoiesis and with an increased count of blast cells, some of which are granular and which express granulocytic markers. This corresponds to Di Guglielmo's original description in 1917, to which the eponym 'Di Guglielmo's syndrome' was subsequently applied.[121] In patients with erythroleukaemia, there is always severe anaemia, often in the context of generalised pancytopenia. In the peripheral blood, typical myelodysplastic feature are often apparent: anisopoikilocytosis, anisochromasia, defects of haemoglobinisation, polychromasia, basophilic stippling, Howell-Jolly bodies and erythrocyte fragments resembling schistocytes. In some patients, dysplastic erythroblasts at all stages of maturation are also present. Platelets are large and dysmorphic. In addition to dysplastic neutrophils, one may observe granulocyte precursors and myeloid blast cells, either agranular or granular, sometimes with Auer rods. In the bone marrow, dyserythropoiesis, which is often severe, is a consistent feature: asynchronous maturation, nuclear dyskinesis, karyorrhexis, multinuclearity, gigantism and bizarre nuclei of all types (Figs 4.97 through to 4.101). In the most immature erythroid component, which is generally comprised of proerythroblasts and basophilic erythroblasts with a marked tendency to megaloblastosis, the cytoplasm is often vacuolated; the coalescence of vacuoles can lead to the formation of large apparently empty lacunae. Abnormal erythroid precursors can show erythrophagocytic activity. The non-erythroid leukaemic component is composed of blasts, which can be similar to those observed in any type of AML, with the exception of APL. In the

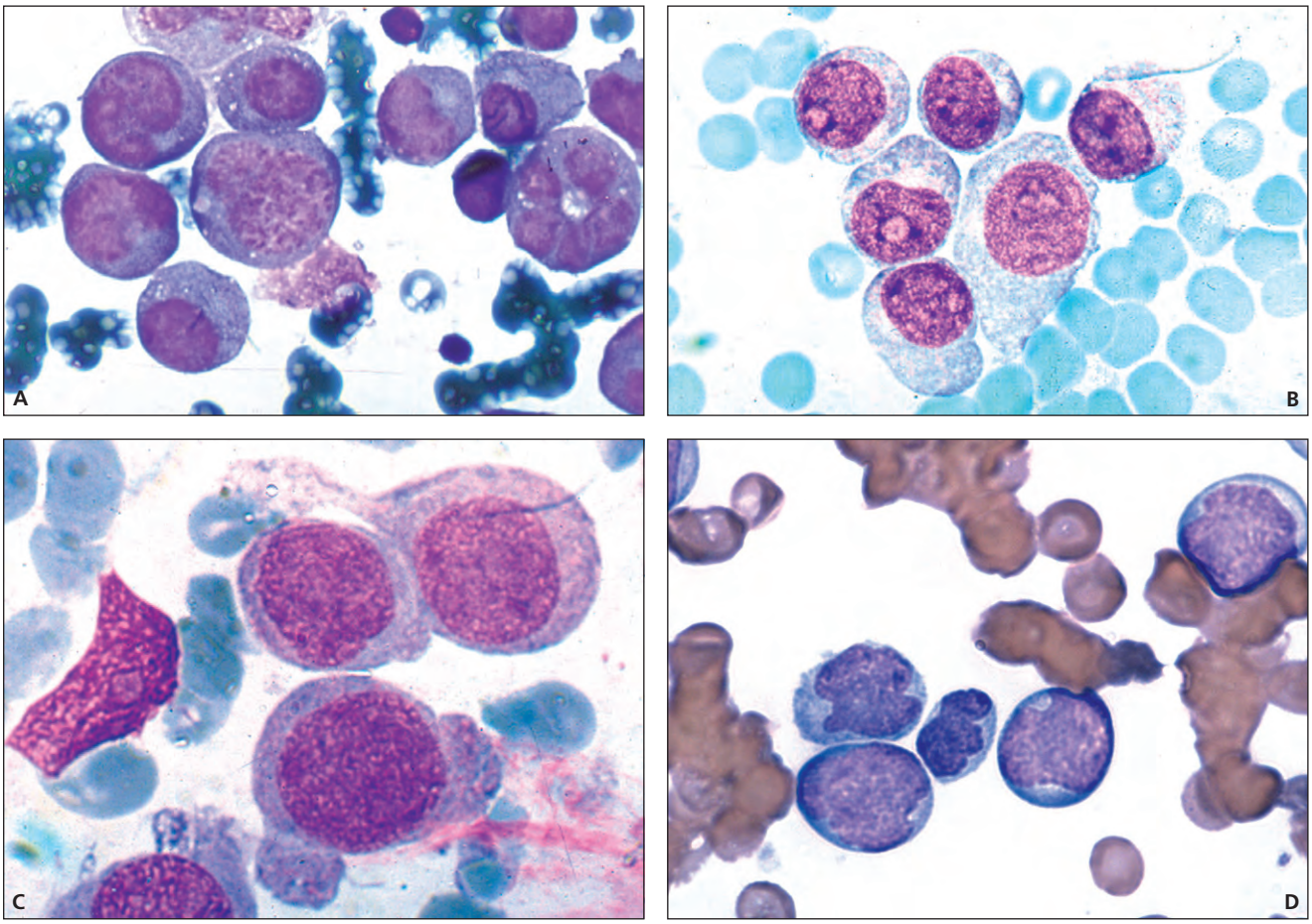



**Fig. 4.92** Morphological heterogeneity of monoblasts in four different cases of acute monoblastic leukaemia. (A) Bone marrow aspirate. In this patient the blasts, attributed to the monocytic lineage with the support of immunophenotyping, are characterised by the abundance and the basophilia of the cytoplasm, the greater density and compactness of the chromatin, the tendency of the nucleus to become deformed with notching and lobulation, or to condense in an eccentric position so that the cell sometimes assumes a rather plasmacytoid appearance. The two cells on the left and the large lobulated cell on the right can be designated promonocytes. (B) Peripheral blood film. Monoblasts with plentiful pale basophilic cytoplasm perforated by microvacuoles. The nuclei are round, having a slight concavity in only one cell. The chromatin forms a reticular network that is denser and less homogeneous than usual, appearing granular in the largest blast. The prominent vesicular nucleoli are particularly clearly seen in this patient. (C) Bone marrow aspirate. Two very typical large monoblasts, with finely stippled chromatin, have very fine pale red granules in the plentiful violaceous cytoplasm that completely surrounds the nucleus. The cytoplasm is extending into large pseudopodial protrusions. The cell at the top in the centre, with identical cytoplasm and a folded nucleus with slightly denser chromatin, is a promonocyte. (D) Peripheral blood film. Three monoblasts that lack any specific morphological features: the irregularity and the angulation of the nucleus, the high N:C ratio and the tendency of the chromatin to clump make these cells morphologically similar to myeloblasts. The two cells with convoluted nuclei, however, can be classified morphologically as an immature monocyte (on the left) and a monocyte (on the right), and provide a useful context that suggests the right diagnosis, which nevertheless needs cytochemical and immunophenotypic analysis.

majority of cases, myeloblasts predominate, these having a small number of peroxidase-positive granules and isolated Auer rods. Granulocytic and megakaryocytic dysplasia is frequent and marked. Sometimes basophils are increased; they may show dysmorphism and immaturity. This form of erythroleukaemia can evolve morphologically, with the expansion of the non-erythroid component, into the picture of AML that is undifferentiated, granulocytic or myelomonocytic.

As already indicated, if myelodysplasia is present in at least 50% of cells of at least two lineages, e.g. erythroid and granulocytic, or erythroid and megakaryocytic or, as occurs not infrequently, in all three lineages, the diagnosis according to the 2008 WHO classification is not that of AML, NOS with erythroid differentiation, but rather is AML with multilineage dysplasia.[122] The same considerations apply to cases of erythroleukaemia that develop from a preceding MDS or that present, as happens fairly often, with cytogenetic alterations specific for MDS.[119] Patients who present with mutations in the *NPM1* gene (about 20% of cases[123]) are assigned also to that specific category. Finally, it is not rare for therapy-related AML to have the morphological

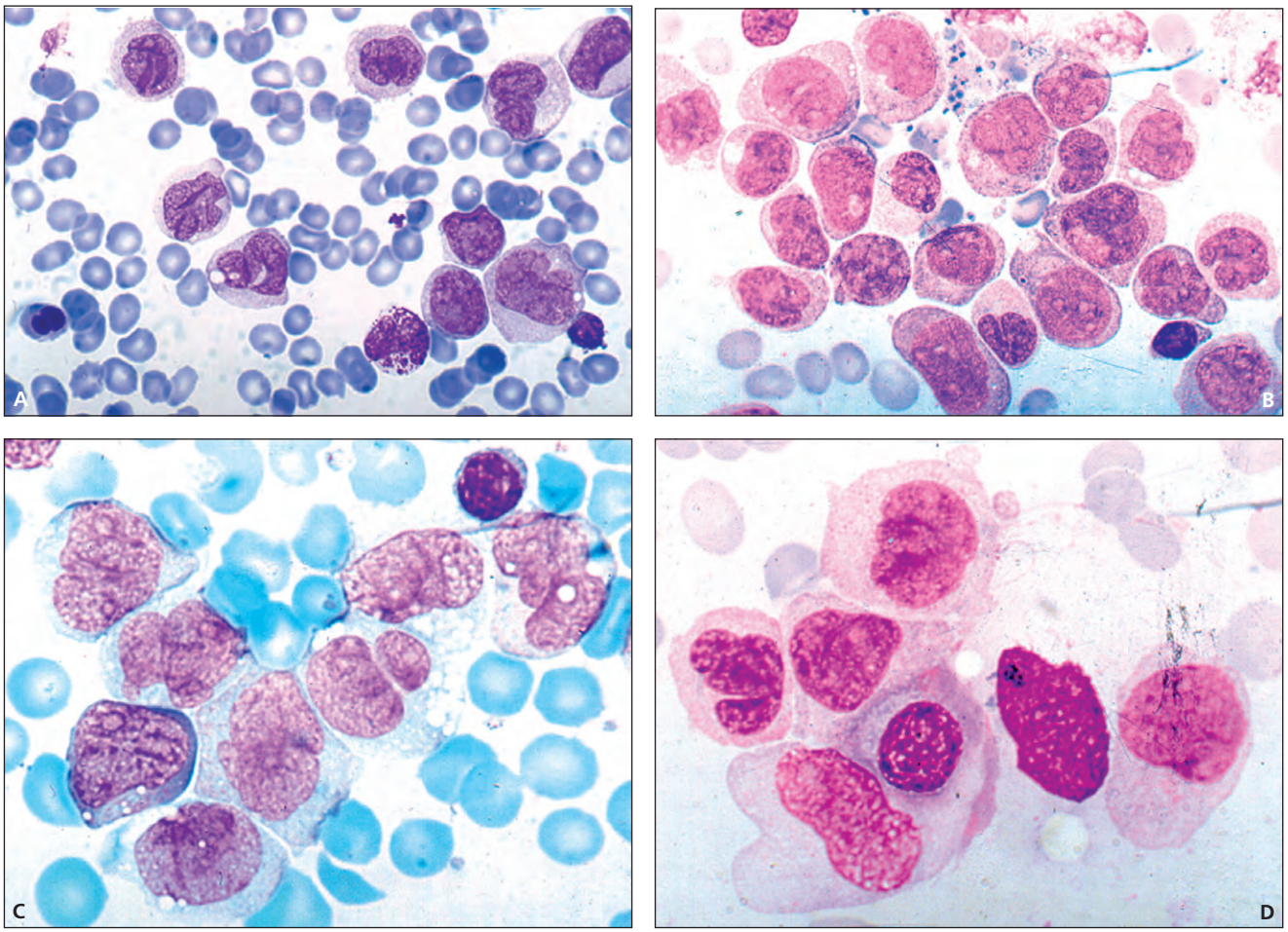



**Fig. 4.93** Acute monocytic leukaemia, NOS. Four cases in which promonocyte predominate over monoblasts. (A) Peripheral blood film. Bottom right, two small undifferentiated blasts and a large very typical promonocyte: folded nucleus, expanded chromatin and plentiful weakly basophilic cytoplasm with a violaceous tinge, a sandy texture and some vacuoles. The majority of the other cells are also categorised as promonocytes, essentially because of the open chromatin pattern and the presence of nucleoli. The features of monocytic differentiation are more apparent in the circulating cells. There is an orthochromatic erythroblast with a lobulated nucleus and a basophil granulocyte. (B) Bone marrow aspirate. In this image also the majority of the leukaemic cells are promonocytes with delicately reticular chromatin and folded or indented nuclei. There are some monoblasts which, however, have a round nucleus. There are multiple clearly seen nucleoli in the majority of these leukaemic cells. (C) Peripheral blood film. On the left, an intensely basophilic monoblast with a prominent nucleolus and clumped chromatin. Five promonocytes with bizarre nuclear configurations and swollen chromatin that to some extent resemble the morphology of anaplastic large cell lymphoma. On the right, two immature monocytes with more condensed chromatin. (D) Bone marrow aspirate. A plasma cell in the centre, four promonocytes and, on the left, a well-differentiated monocyte.

features of leukaemia with erythroid differentiation. For all these reasons, the frequency of cases diagnosed as erythroleukaemia is destined to reduce considerably with the general adoption of the WHO criteria.[124,125]

The second type of erythroleukaemia is that which is designated 'pure': it corresponds to the picture that Di Guglielmo described later, in 1926, under the name of acute erythraemic myelosis or acute erythraemia, which was later named in his honour 'Di Guglielmo's disease' or 'Di Gulglielmo's syndrome'. In these extremely rare and prognostically very adverse cases there is a pure, isolated erythroid proliferation that is not accompanied by a frank myeloblastic component (Fig. 4.102). In the bone marrow, two very different pictures may be seen, one differentiated and one undifferentiated, with the possibility of transitional forms. The more differentiated cases are characterised by extreme erythroid hyperplasia with a clear deviation to the left of the maturation curve and a very striking inversion of the myeloid:erythroid (M:E) ratio. There are always cytological abnormalities of the large proerythroblasts and the basophilic erythroblasts that dominate the morphological picture (designated by Di Guglielmo's 'paraerythroblasts'): giant multinucleated forms, megaloblastic and markedly dysplastic nuclei and fairly abundant cytoplasm, which is basophilic with frequent protrusions, sometimes so vacuolated as to resemble the cells of

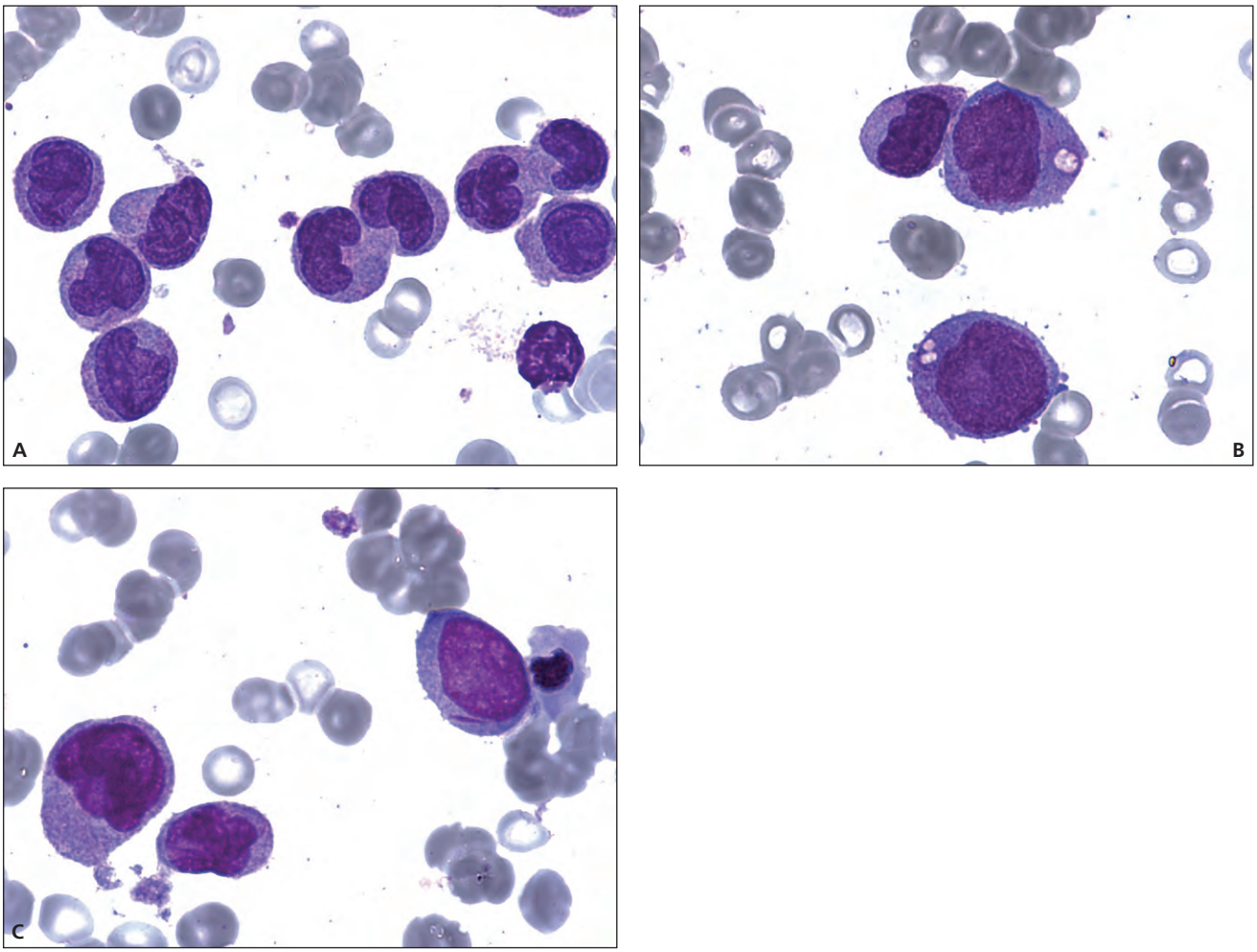



**Fig. 4.94** Acute monocytic leukaemia; bone marrow aspirate. (A) All the cells are promonocytes with convoluted or folded nuclei; nucleoli are not clearly seen; in this patient the cytoplasm is characterised by a particularly intense basophilia, with a violaceous tinge and a ground-glass appearance resulting from the presence of numerous fine azurophilic granules that are not individually identifiable. (B) At the top, an immature monocyte and a promonocyte with a large cytoplasmic inclusion with a paracrystalline structure. At the bottom, a monoblast: the chromatin is finely reticular with large pale areas corresponding to nucleoli; there are two cytoplasmic inclusions and the cytoplasmic membrane has numerous fine projections. (C) Bottom left, a promonocyte and a smaller immature monocyte. Top right, a blast cell with an Auer rod has the morphological characteristics of a myeloblast (nuclear angulation and adherence of the nuclear membrane to the cytoplasmic membrane), showing the participation, even if minimal, of the granulocytic lineage in the leukaemic process.

Burkitt lymphoma: the vacuoles of erythroleukaemia, however, are more variable in size and shape than those of Burkitt cells. At the other extreme, one finds cases of morphologically undifferentiated leukaemia in which the blasts are arrested at a very early stage of erythroid maturation (corresponding to BFU-E or to CFU-E). Their erythroid nature can be shown only by the use of immunophenotypic markers or by ultrastructural examination. The blasts in these immature erythroid leukaemias can be confused with those of other morphologically undifferentiated leukaemias, such as lymphoblastic or monoblastic, but certain features may suggest their true nature: they are often medium sized or large; the nucleus is voluminous with one or two nucleoli; the cytoplasm is intensely basophilic with the exception of a paranuclear zone corresponding to the arcoplasm; close to this area one may observe a few peroxidase-negative azurophilic granules, small vacuoles and clear areas corresponding to hypertrophic mitochondria.[126]

Faced with this heterogeneity, it is important that morphology is assessed together with the results of other, complementary diagnostic methods. Peroxidase is negative in erythroblasts, in proerythroblasts and in the undifferentiated blasts of pure erythroid leukaemia; however, this reaction can show clearly the granulocytic nature of the blast cells in the more common mixed form of erythroleukaemia. The pathological

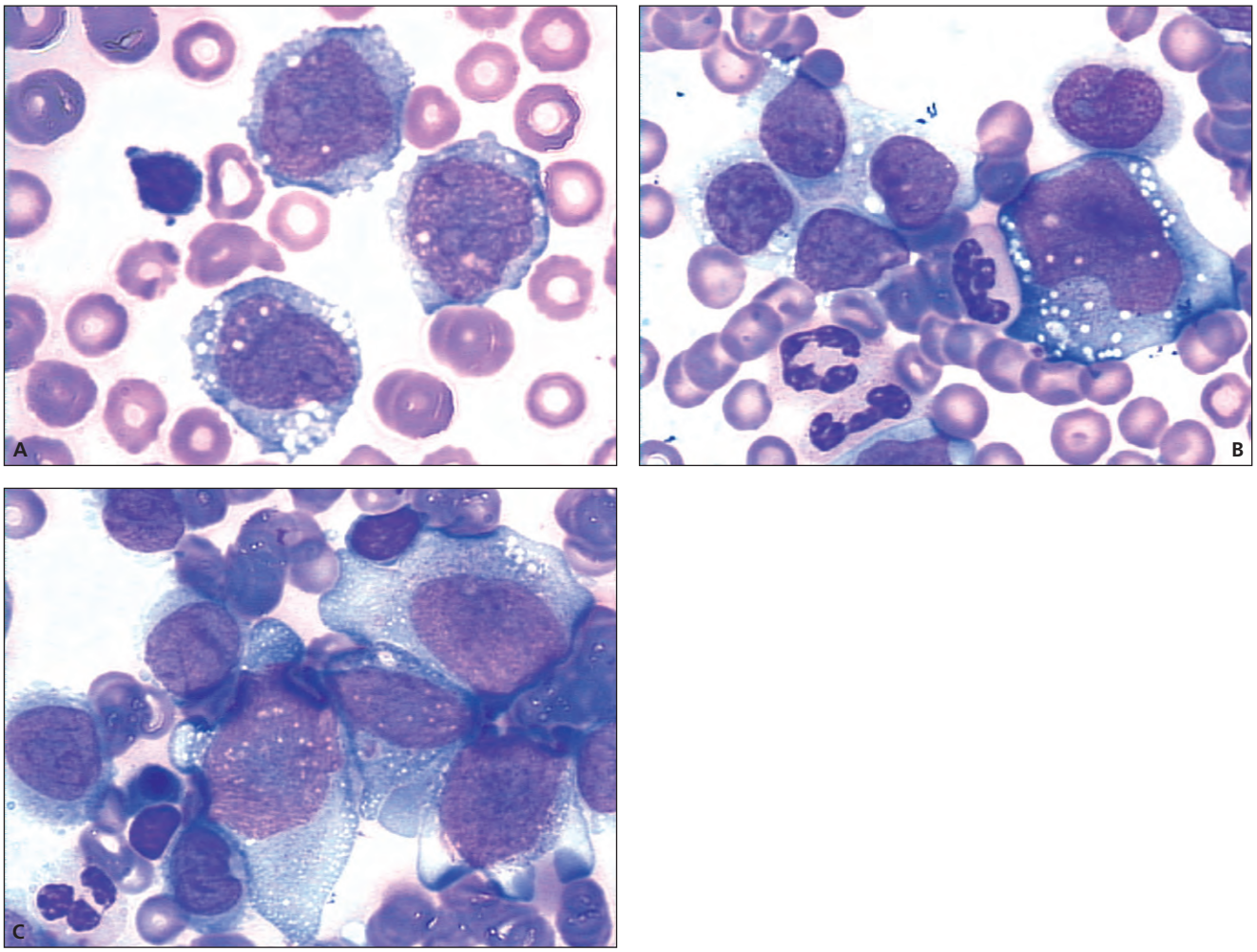



**Fig. 4.95** Acute monocytic leukaemia, with a high grade of anaplasia and hyperdiploidy. (A) Peripheral blood film. Three large leukaemic blasts with plentiful basophilic cytoplasm perforated by vacuoles of various sizes. The nuclear outlines show a variable degree of irregularity. The nucleus is polygonal in the cell at the top, which has very fine chromatin and a large vesicular nucleolus and must be classified as a monoblast. The other two cells have more indented and folded nuclei and chromatin that is slightly more condensed into fine knots; the nucleoli are quite obvious: applying the 2008 WHO criteria, these cells must be classified as promonocytes. (B) Bone marrow aspirate. In addition to a number of monoblasts and three hypogranular neutrophils, there is a giant, almost tetraploid, blast cell with intensely, although not homogeneously, basophilic cytoplasm, containing numerous vacuoles and azurophilic granules that are clustered close to the nuclear indentation. (C) A group of giant monoblasts characterised by the extreme delicacy of the chromatin network, which is forming a fine reticulum. The cytoplasm is tending to expand like a veil. Tiny vacuoles are present in the cytoplasm and, in some cells, also in the nucleus.

erythroblasts are PAS positive, with diffuse cytoplasmic staining in the more differentiated cells and large blocks of intense red in the more immature forms. PAS positivity of erythroblasts is typical of erythroleukaemia, but not pathognomonic since it can also be observed in MDS, in haemolytic anaemia, in thalassaemia major and in megaloblastic anaemia: its diagnostic value is therefore modest. In erythroleukaemia with heavily vacuolated cells, staining with Oil Red O is negative, thus differing from Burkitt lymphoma cells, in which the vacuole contents are strongly Oil Red O positive (they are also PAS positive). Various esterase stains, and in particular ANAE, can give various types of non-specific positivity; an acid phosphatase stain shows focal positivity in the Golgi zone. The frequent abnormality on an iron stain is of interest; staining of leukaemic erythroblasts can be diffuse or there may be large siderotic granules only occasionally arranged as a perinuclear ring.

Immunophenotyping is important, even although it is not always easy to interpret. Myeloid blasts have the same profile as is seen in AML with little differentiation. With regard to erythroblasts, the typical markers are glycophorin and haemoglobin A (revealed with monoclonal antibodies), which however are not particularly early markers, since they appear at the proerythroblast stage. The expression of the transferrin receptor, CD71, is seen from the BFU-E stage to the young reticulocyte,

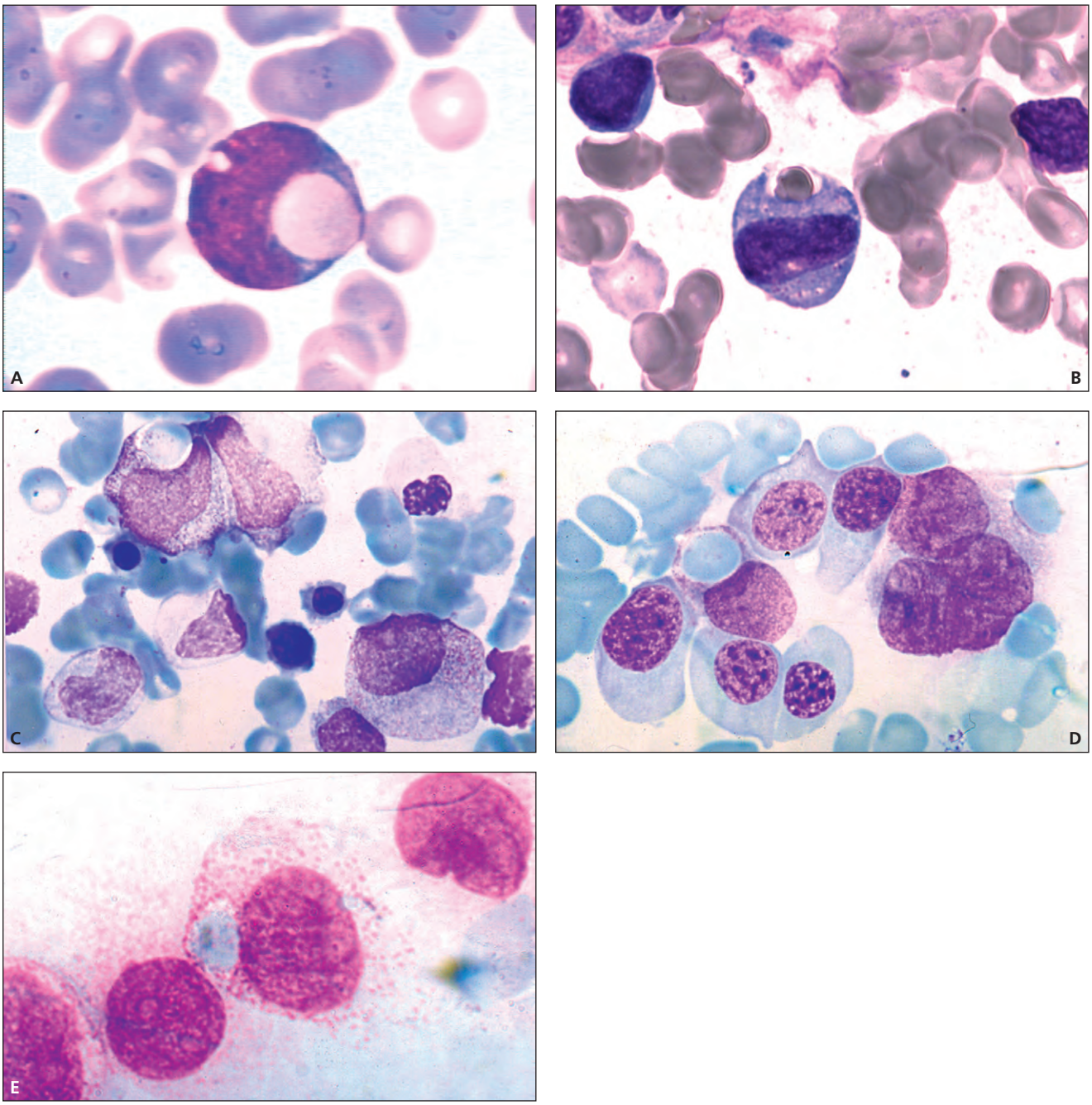



**Fig. 4.96** The variable features of erythrophagocytosis in leukaemic blast cells and dysplastic granulocytes; bone marrow aspirates. (A) A well-preserved erythrocyte occupies almost all the cytoplasm of this blast cell in a case of AML, NOS, without maturation. (B) A leukaemic promonocyte with an erythrocyte within a phagocytic vacuole. (C) A vacuole containing a well-preserved erythrocyte in a dysplastic promyelocyte in a patient with refractory anaemia with excess of blasts (RAEB). On the right, among other cells, a neutrophil at the top and a promyelocyte at the bottom, both with clear signs of dysplasia. (D) A case of acute monoblastic leukaemia with megakaryocytic and erythroid dysplasia: five polychromatic and orthochromatic erythroblasts with clearly megaloblastic features (immature nucleus); a dysmorphic megakaryocte with a lobulated nucleus and immature, transparent chromatin; a blast with a nucleus that has fine, delicate chromatin has extended its cytoplasm to enclose an erythrocyte within a large phagocytic vacuole. (E) An erythrophagocytic monoblast in a case of acute monoblastic leukaemia, NOS. The red cell, still well preserved, appears to be within a vacuole which is surrounded by a concentration of delicate azurophilic granules.

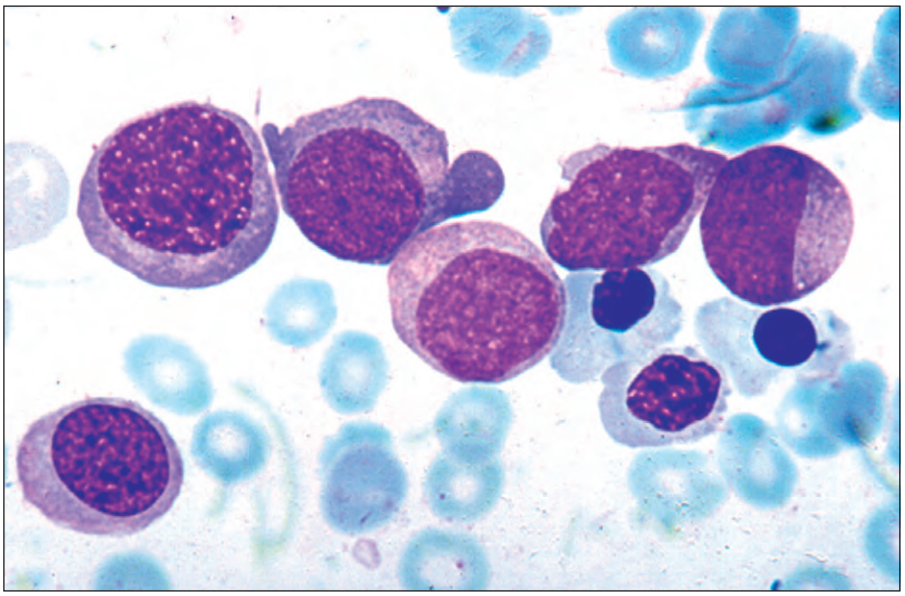


**Fig. 4.97** Acute erythroid leukaemia; bone marrow aspirate. Erythroid cells were more than 49% of bone marrow cells: on the left, two dysplastic erythroblasts with N:C asynchrony since the nuclei have chromatin that is condensed into small clumps, but the cytoplasm is already polychromatic; immediately to the right of the larger cell, a proerythroblast with cytoplasmic blebs; on the right, three orthochromatic erythroblasts with irregular nuclei of various shapes. Next to the erythroblasts are three granular blast cells, indicating that the granulocytic lineage is also part of the leukaemic population.

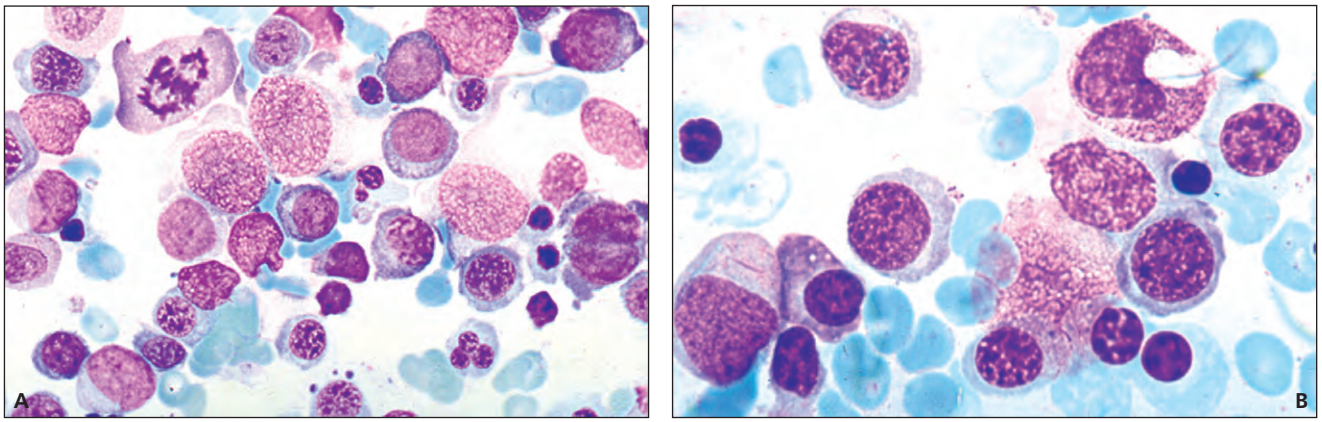



**Fig. 4.98** Acute erythroid leukaemia: examples of the variable degree of participation of the erythroid and granulocytic lineages; bone marrow aspirates. (A) Megaloblastic erythroid cells comprise 90% of cells while myeloblasts are 7% (70% of non-erythroid cells [NEC]). Although blasts are less than 20%, this case satisfies the 2008 WHO criteria for the diagnosis of acute erythroid leukaemia. Erythroid maturation is occurring, even if the maturation curve is left shifted with an excess of proerythroblasts; the degree of megaloblastosis is not consistent, being severe in some cells, such as the largest cells with vermicular chromatin, and moderate in others. Dyserythropoietic forms are sometimes bizarre, such as in the erythroblast with a trilobed nucleus near the bottom; immediately above are a pair of orthochromatic erythroblasts at the end of mitosis and in the top right corner there is an intercellular bridge. To the left of centre and at the bottom, two non-erythroid blast cells with eccentric nuclei, loose reticular chromatin and a moderate quantity of pale cytoplasm. Top left, an abnormal mitosis. Close to the right edge, a promegakaryocyte. In cases such as this, it is prudent to assay vitamin $B_{12}$ and folic acid before starting antileukaemia chemotherapy. (B) The erythroblasts of another patient have less abnormal maturation, with moderate megaloblastoid features (delayed nuclear maturation). Bottom left, a myeloblast with plentiful cytoplasm containing an Auer rod. Adjacent to it is a plasma cell with a reddish tinge to the cytoplasm and a small lymphocyte: these two cells must be excluded from the count of NEC. In the myelogram of this case, the erythroid series was 61%, NEC were 36% and blasts, which were 14% of all nucleated cells (ANC), were 39% of NEC.

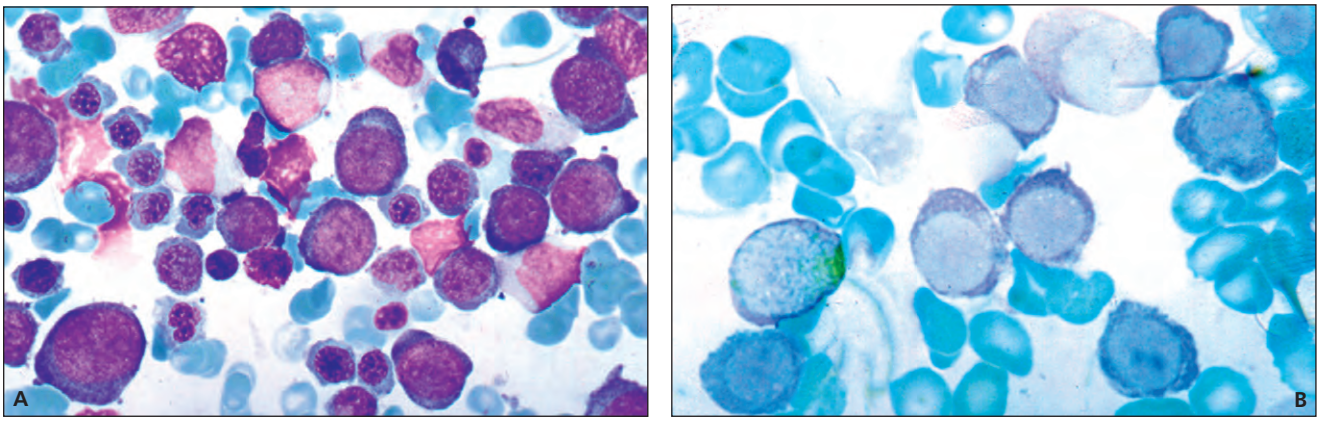



**Fig. 4.99** Another case of acute erythroid leukaemia with very marked erythroid hyperplasia and moderate dyserythropoiesis; bone marrow aspirate. (A) The leukaemic cells are almost all proerythroblasts, recognisable from the intensely basophilic cytoplasm and the round nucleus with immature, homogeneous, granular chromatin, and of erythroblasts in a more advanced stage of maturation, often characterised by nuclear dysmorphism. In this context, one notes three myeloblasts, one high up in the centre, another on its left and the third lower down on the right, characterised by eccentric, polygonal or angulated nuclei with a very fine network of pale chromatin, clearly visible nucleoli and weakly basophilic cytoplasm. (B) A peroxidase stain shows clearly the negativity of the erythroid precursors and the distinct, focal, paranuclear activity of a myeloblast.

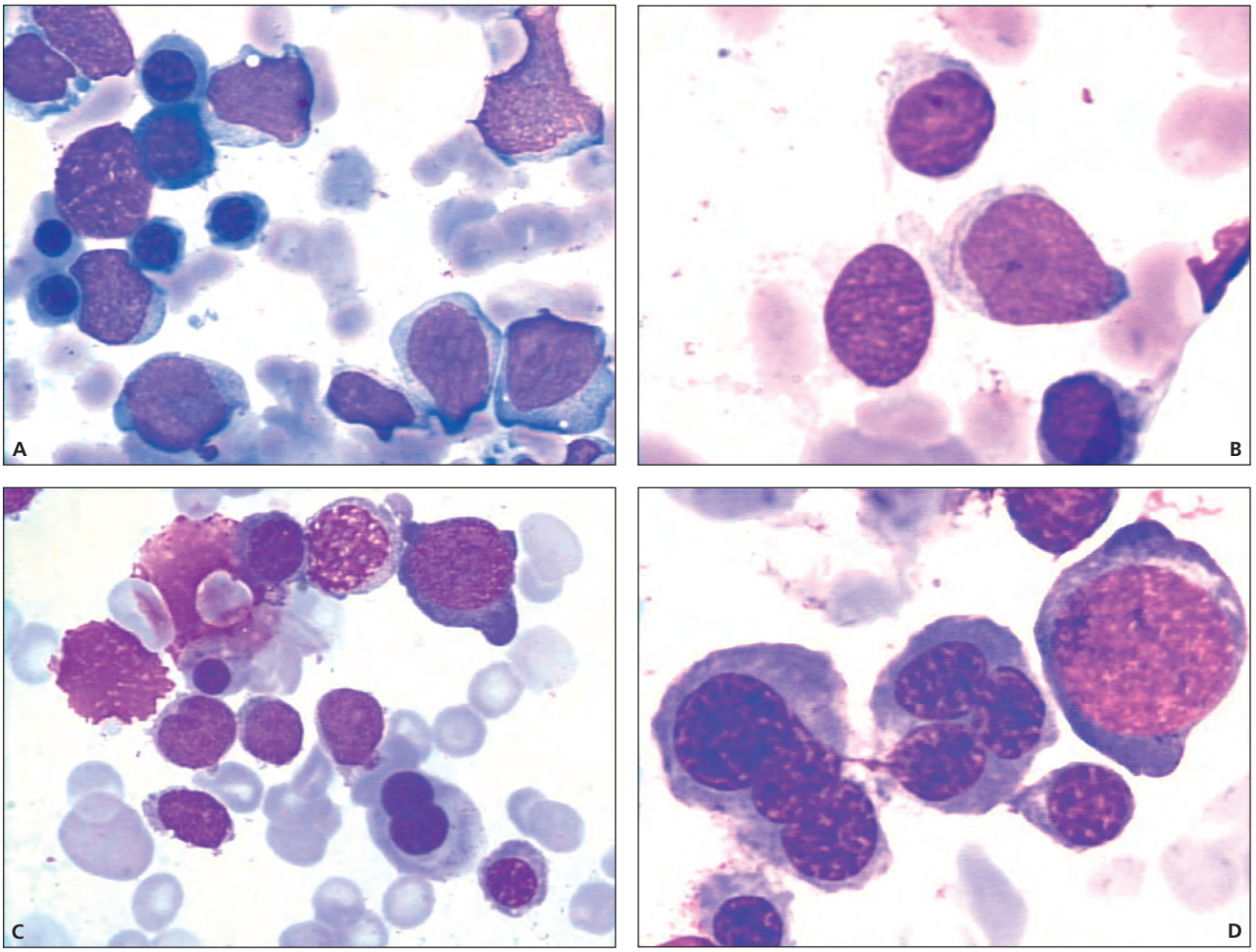



**Fig. 4.100** Acute erythroid leukaemia with dyserythropoiesis and an increased proportion of myeloblasts (38% of NEC); bone marrow aspirate. (A) In this field one observes, considering only the intact cells, six erythroblasts with dyserythropoietic features, a lymphocyte and five myeloid blasts, of which only that in the bottom right corner seems to have cytoplasmic granules. One notes the nuclear morphology of these blast cells, with an angulated outline but some straight sections, very fine homogeneous chromatin and, in some cells, nucleoli that are not prominent: these are characteristics that permit an easy distinction from erythroid precursors. (B) In the same film, a myeloblast with an Auer rod. (C) Four small agranular blast cells, a proerythroblast (top right) and some erythroblasts, one of which is an orthochromatic erythroblast with basophilic stippling and a bilobed nucleus with totally condensed chromatin. (D) A proerythroblast with megaloblastic features and two monstrous erythroblasts, with polychromatic cytoplasm and multiple or multilobated nuclei, two of which appear to be joined by a thick chromatin bridge.

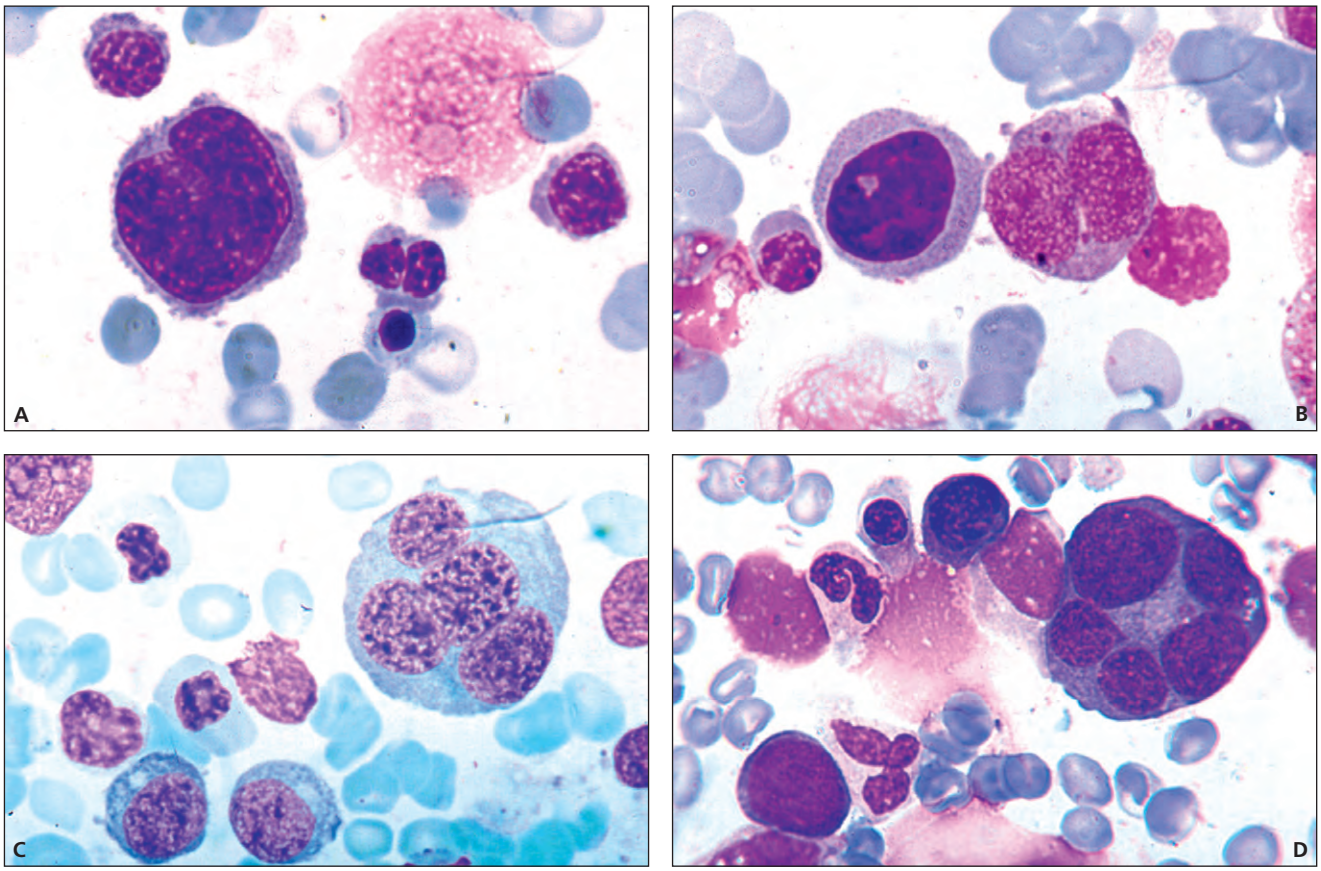



**Fig. 4.101** Bizarre erythroblast morphology in bone marrow films of patients with acute erythroid leukaemia. (A) Giant erythroblast with scanty polychromatic cytoplasm and an enormous hyperdiploid nucleus with a deep incision in the nuclear outline and chromatin strongly condensed into large blackish, coal-like clumps, separated by dark ruby-red spaces. (B) On the right of a small dysmorphic polychromatic erythroblast, two large abnormal erythroblasts, also with polychromatic cytoplasm, which appear granular as the result of apoptosis or degeneration. The nucleus of the erythroblast on the left has dark ruby-red pyknotic chromatin, while that on the right has a pale double nucleus with vermicular chromatin, arrested in early prophase. (C) A giant erythroblast with four nuclei, with chromatin condensed into small clumps and cytoplasm similar to that of the erythrocytes; the more mature erythroblasts have dysplastic nuclear features. (D) Another erythroblast with basophilic cytoplasm and five nuclei, one of which is almost tetraploid. In the bottom left corner, a proerythroblast with finely granular chromatin.

but can be abnormally reduced and also lacking in specificity, since it is expressed in some non-erythroid cells (immature thymocytes, T-ALL, some AML). In the rare pure erythroid leukaemias, useful immunophenotypic features include: CD36, the thrombospondin receptor, which is not very specific since it is sometimes also positive in megakaryoblasts and in monocytes and macrophages; the Gero antibody, directed at the Gerbich antigen; carbonic anhydrase. Haemoglobin A and glycophorin can often be detected, when expressed, in immunohistochemical preparations.

Specific cellular features have been described on ultrastructural examination, such as the presence of numerous polyribosomes, with long chains of rough endoplasmic reticulum and, particularly, aggregates of ferritin molecules localised in mitochondria or in cytoplasmic granules. Rhopheocytotic vesicles are typical, appearing as invaginations in the cell surface membrane associated with ferritin molecules that are still extracellular. Ultrastructural cytochemistry has demonstrated platelet peroxidase (PPO) activity in the perinuclear cisterns and in the endoplasmic reticulum.[126]

From the point of view of practical diagnostic cytomorphology, the differentiation of the various types of erythroleukaemia from MDS is not usually difficult, based on the quantitative criteria of the FAB group, with the modifications of the WHO 2008 classification (Table 4.2): quantification of bone marrow blasts cells is a defining element and must be done with great accuracy, notwithstanding the imperfect reproducibility of this evaluation (e.g. when distinguishing between atypical or dysplastic promyelocytes and granular blast cells).[12] It is also not exceptional for a RAEB to evolve fairly rapidly into a typical acute leukaemia with mainly erythroid differentiation, which is classified as AML with MRC. The diagnosis of

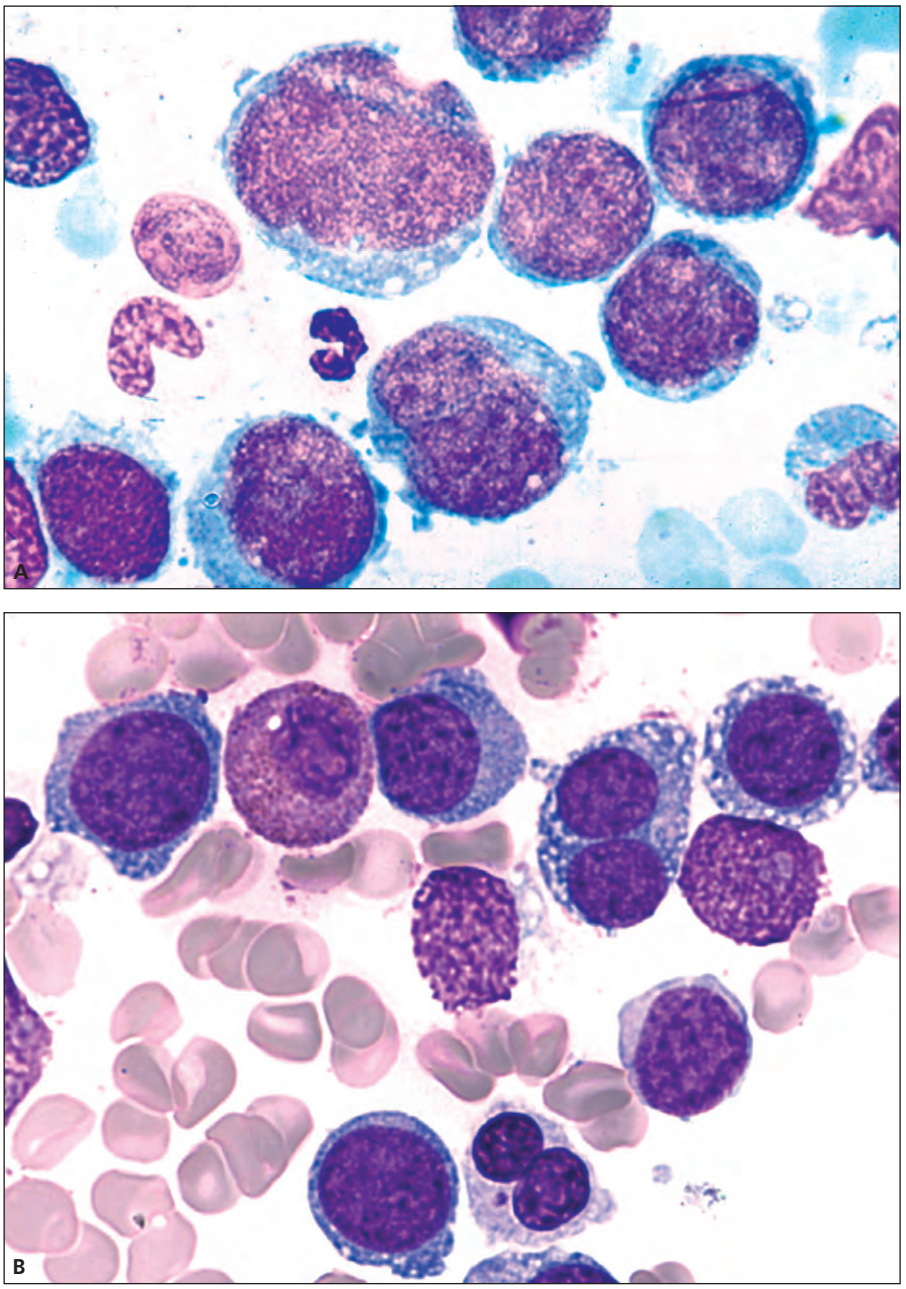



**Fig. 4.102** Two cases of pure acute erythroid leukaemia, confirmed by immunophenotyping; bone marrow aspirates. (A) In this patient the marrow has been invaded by a population of immature cells of variable size with the features of megaloblastic proerythroblasts. The nuclei are large and round with a distinct outline; the larger nuclei show some irregularity and indentation of the nuclear outline. The largest cell is probably tetraploid; small nucleoli can just be seen in this cell. There is a variable amount of deeply basophilic cytoplasm with small heterogeneous vacuoles; in some cells there is a small area of pale perinuclear arcoplasm. A small neutrophil and a metamyelocyte have hypogranular cytoplasm. (B) Erythroid blasts with less differentiated morphology. Their erythroid origin can be suspected because of the presence of two cells with morphology similar to that of proerythroblasts, top left and in the centre at the bottom. In the upper part of the image, from the centre towards the right, there are three blasts with nuclei (double in one cell) with clumped chromatin and a distinct rounded outline, and with well-represented cytoplasm, in two instances with numerous variably sized vacuoles. Lower down, an undifferentiated blast cell with scanty basophilic cytoplasm and a binucleated polychromatic erythroblast with a detached nuclear fragment.

**Table 4.2** Diagnostic algorithm for the differential diagnosis of patients presenting with a percentage of erythroid precursors ≥50% in the myelogram (performed on 500 cells, on at least two different films of a bone marrow aspirate)

| Erythroid series* | Blasts as % of ANC# | Other | Diagnosis |
|---|---|---|---|
| ≥50% | ≥20% | Absence of dysplastic features in 50% or more of at least two series (erythroleukaemia) | AML, NOS with erythroid differentiation |
| ≥50% | ≥20% | Presence of dysplastic features in 50% or more of at least two series | AML with myelodysplasia-related features |
| ≥50% | <20% | Blasts as % of NEC§ ≥20% | AML, NOS, erythroid (erythroleukaemia) |
| ≥50% | <20% | Blasts as % of NEC§ <20% | MDS (for the diagnosis of subtype use blasts as a % of ANC#) |
| ≥80%^ | Rare | Granulocytic series rare or absent | AML, NOS erythroid (pure erythroid leukaemia) |

**Explanatory myelograms for differential diagnosis based on blasts as a percentage of ANC# or NEC§**

| Myelogram | Case 1 | Case 2 | Case 3 |
|---|---|---|---|
| Granulocytic series | 49% | 24% | 14% |
| Erythroid series | 25% | 65% | 76% |
| Blasts (of ANC) | 20% | 5% | 4% |
| Lymphocytes | 4% | 4% | 4% |
| Plasma cells | 1% | 1% | 1% |
| Mast cells | 1% | 1% | 1% |
| M:E ratio | (70/25) = 2.8 | (30/65) = 0.46 | (19/76) = 0.25 |
| Blasts (of NEC) | Not assessed | (5/30) × 100 = 16.6% | (4/19) × 100 = 21.1% |
| Dysplasia by WHO criteria | No | No | No |
| Cytotoxic therapy | No | No | No |
| Recurrent genetic alterations | No | No | No |
| Morphological diagnosis | AML, NOS | MDS of RAEB-1 type° | AML, NOS erythroid† |

*Percentage of morphologically recognisable erythroid precursors.
#Percentage of non-erythroid blasts among all nucleated cells (ANC).
§Blasts as a percentage of non-erythroid cells (NEC), lymphocytes and plasma cells also being excluded.
^Erythroid precursors that are undifferentiated or with very little differentiation (with immunophenotypic confirmation).
°Because blasts are <10% of ANC.
†Because blasts are 19% of NEC.

erythroleukaemia can sometimes be complex in patients with preceding MDS who have been receiving erythropoietin therapy, since the erythroid expansion can be the result of stimulation by the drug.[125] The morphological differential diagnosis, particularly in the case of pure erythroid leukaemia, also includes ALL, lymphoma in leukaemic phase and multiple myeloma: cytoplasmic basophilia is, in fact, a feature that plasma cells and erythroblasts have in common.[127,128]

Various non-neoplastic conditions characterised by bone marrow erythroid expansion with dyserythropoiesis can lead to the suspicion of erythroleukaemia: poisoning by heavy metals such as arsenic, the effect of antineoplastic drugs and of chloramphenicol and congenital dyserythropoietic anaemia.[129] It is sometimes particularly difficult to make a distinction between erythroleukaemia and megaloblastic anaemia, this having obvious therapeutic and prognostic implications. It is not rare for megaloblastosis as a result of a deficiency state to present with trilineage cytopenia and a bone marrow picture of hyperplasia, dyserythropoiesis, dysgranulopoiesis and dysmegakaryopoiesis that is so marked as to raise the suspicion of a high grade MDS or a leukaemic process.[130–132] The distinction is even more difficult in histological sections. In megaloblastosis, chromosomal instability has even been described, which can lead to reporting of cytogenetic defects.[133] The most useful criteria that lead to the correct diagnosis of megaloblastic anaemia (see Chapter 2) in these cases are:

- An accurate verification of the lack of an elevated blastic component, subsequently supported by immunophenotyping (percentage of CD34-positive cells);
- Peripheral blood macrocytosis: MCV values above 120–130 fl are not seen in erythroleukaemia and are characteristic of megaloblastosis; it is not exceptional, however, to find cases

of megaloblastic anaemia in which the erythrocyte size is little increased or indeed normal (e.g. related to co-existing iron deficiency or thalassaemia);
- Gigantism of the intermediate stages of granulocyte maturation (myelocytes and metamyelocytes);
- The presence in the peripheral blood of neutrophils with more than five or six nuclear lobes;
- Clinical data that suggest a possible deficiency state (previous gastrectomy, neoplasia or gastric atrophy, intake of relevant drugs such as antidepressants for long periods of time).

In cases of diagnostic uncertainty, it is always advisable to delay antileukaemic treatment until the results of vitamin $B_{12}$ and folate assays are available and also to carry out a therapeutic trial (*ex adjuvantibus*) with these vitamins, which have no effect on leukaemia but lead to a rapid increase in the reticulocyte count in the deficiency states. Starting intensive combination chemotherapy in an easily treatable megaloblastic anaemia is an error that must never be permitted to happen.

### Acute megakaryoblastic leukaemia (similar to FAB M7 AML)

The first criteria for the recognition of acute megakaryoblastic leukaemia were proposed by the FAB group in an article that recognised the need to overcome the limitations of morphology and cytochemistry[98]: the diagnosis requires confirmation by ultrastructural cytochemistry or demonstration of specific membrane markers. These patients represent less than 5% of cases of AML: the diagnosis must be considered in all cases in which, in the bone marrow or blood, there are observed cells that appear undifferentiated both by morphology and cytochemistry (peroxidase and Sudan black B negative).[134–137] It occurs in adults and in children under the age of five years.

Peripheral blood examination, which is fundamental in those patients in whom bone marrow fibrosis prevents the obtaining of an adequate aspirate, often shows severe pancytopenia. The platelet count is, however, very variable: thrombocytopenia is common, but there can also be thrombocytosis; often the platelets are atypical, large and grey as a result of a lack of granules or the granules are irregularly distributed; there may be circulating micromegakaryocytes, bare nuclei and strands of cytoplasm formed of still cohesive platelets. True undifferentiated blasts are often seen even in cases with leucopenia (Fig. 4.103). All these anomalies are observed with greater ease in buffy coat films.

The marrow picture is variable. Fibrosis is common in all pathological processes involving megakaryocytes, since the blast cells and their differentiated progeny can elaborate cytokines, such as platelet-derived growth factor (PDGF), which stimulate collagen deposition. Attempted aspiration often leads to a 'dry tap' or to only a few drops of medullary blood being obtained; useful information may nevertheless be obtained if an adequate film can be made. When the aspirate is cellular, the cytological picture often resembles that of an ALL, because of the heavy infiltration by small, agranular, rounded blast cells, or there may be blasts with a more heterogeneous size distribution showing one or two nucleoli; in other cases, the atypical cells resemble myeloid blasts because of the presence of

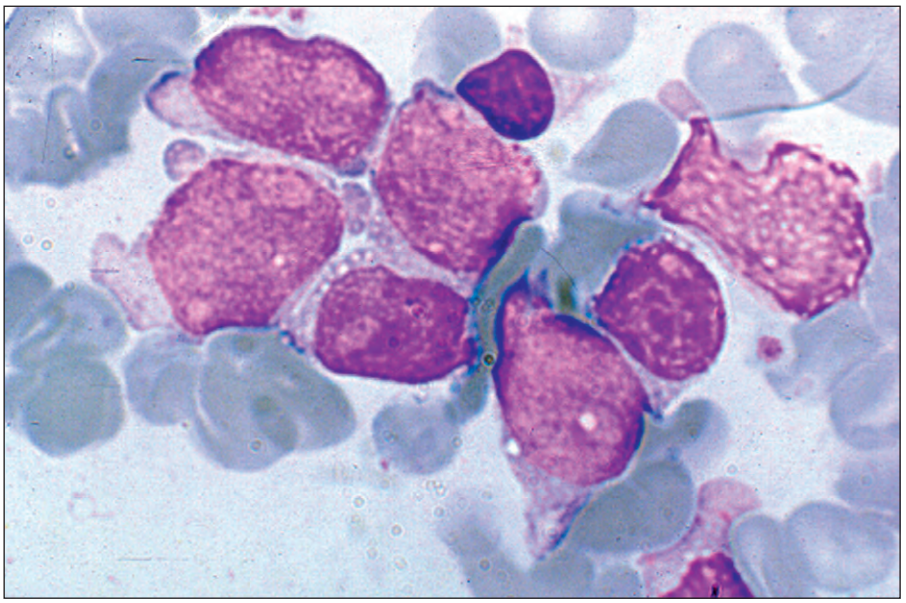


**Fig. 4.103** Acute megakaryoblastic leukaemia, morphologically undifferentiated blast cells, CD41 and CD61 positive; bone marrow aspirate. The bone marrow aspirate was not very cellular and was composed almost entirely of blast cells with no morphological features of differentiation. The cytoplasmic budding and the formation of small cohesive groups of cells suggest a possible megakaryocyte lineage, but these features are not specific. A MPO stain was negative. In cases such as this the value of morphology is limited to indicating the presence of an acute leukaemia that can be classified further only on the basis of investigation of membrane and cytoplasmic antigens.

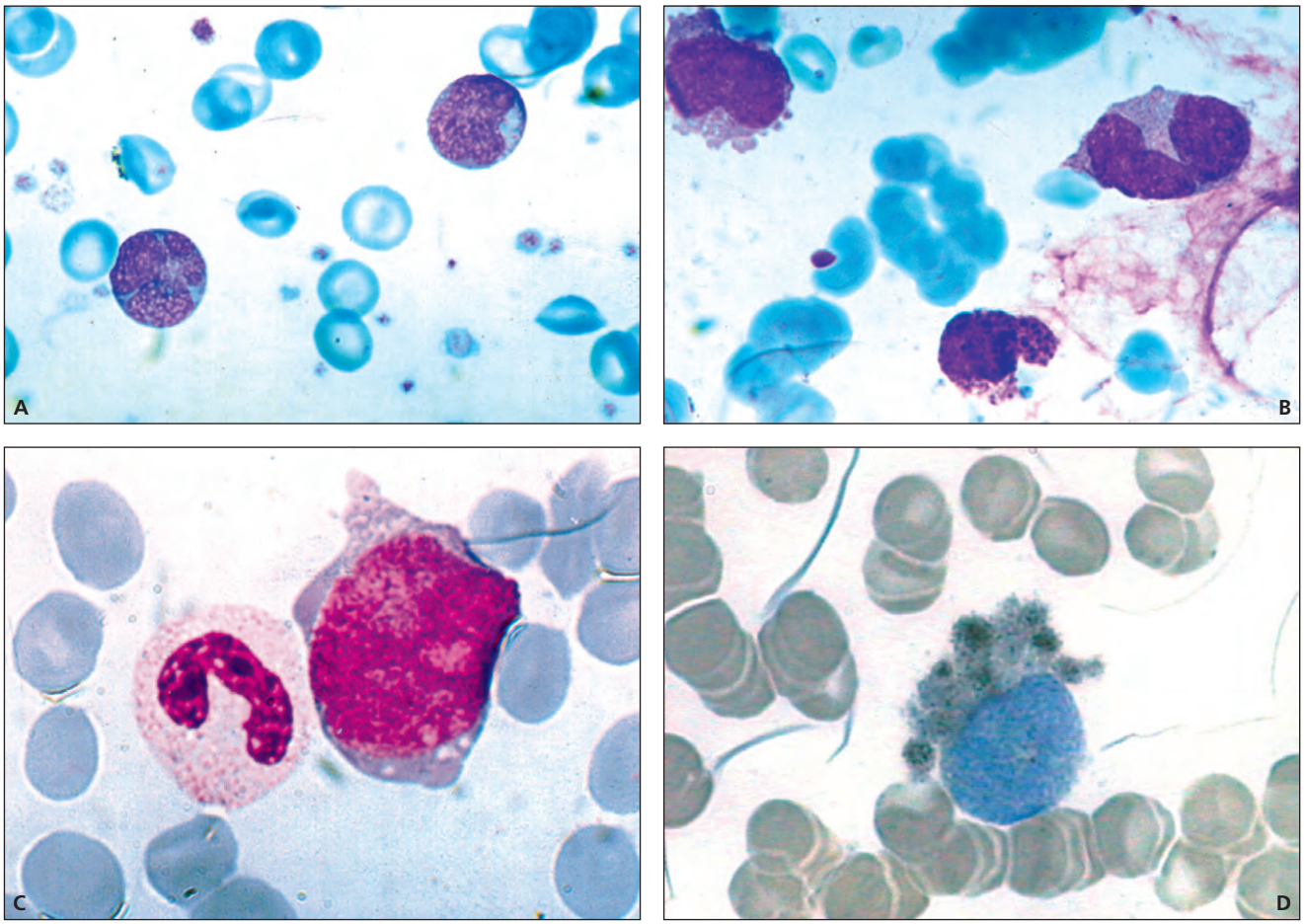



**Fig. 4.104** Morphological features that can suggest the megakaryoblastic nature of an acute leukaemia. (A) Peripheral blood film. On the left, the nucleus of this blast cell, which is less than double the size of an erythrocyte, has chromatin that is condensed into short, worm-shaped forms and is tending to become lobulated without cell division, recalls endomitosis in a megakaryocyte; the cytoplasm is scanty, basophilic and undifferentiated. The cell on the right has similar characteristics, but without forming true nuclear lobes. (B) Bone marrow aspirate. In a hypocellular aspirate, two megakaryoblasts: that on the left has a reniform nucleus and pink cytoplasm that is forming pseudopodia, while the other is hyperdiploid with three nuclear lobes, in part superimposed on each other, and basophilic cytoplasm with fine azurophilic granules. The chromatin is dense and homogeneous in both cells. The cell at the bottom is a basophil granulocyte. (C) Megakaryoblast in a peripheral blood film. In this case, the size and the hyperdiploidy of the nucleus and its dense chromatin with several nucleoli suggest the megakaryocyte lineage. (D) Bone marrow aspirate. Megakaryocyte with differentiated cytoplasm that is forming proplatelets, which are positive on an esterase stain.

occasional azurophilic granules (always, however, peroxidase negative). More often, the leukaemic population is polymorphic and heterogeneous. Certain morphological clues may lead to the suspicion of the megakaryoblastic nature of the leukaemic cells (Figs 4.103 through to 4.108), such as:

- Chromatin characteristics: the chromatin network of megakaryoblasts, though reticular and homogeneous, tends to be denser than in other types of blast cell, recalling that of more mature megakaryocytes and of the micromegakaryocytes that are often also present;
- The shape of the nucleus, in general round and regular, but sometimes in the more typical cells showing an indentation and, particularly, a long groove or longitudinal fissure of variable prominence;
- The presence along the cell surface membrane of protrusions and budding cytoplasm, which sometimes resembles the morphology of large, abnormally staining, dysmorphic platelets;
- The tendency of blasts to form masses or nests, which can resemble metastases;
- The presence of numerous micromegakaryocytes, with mature cytoplasm and one or two small nuclei that have very dense chromatin, and of other cells with clear signs of megakaryocytic differentiation, which is often anomalous; these cells must be excluded from the blast count;
- The co-existence of dysplasia of other haemopoietic lines;
- Peripheral blood anomalies: dysmorphic platelets, micromegakaryocytes and bare nuclei.

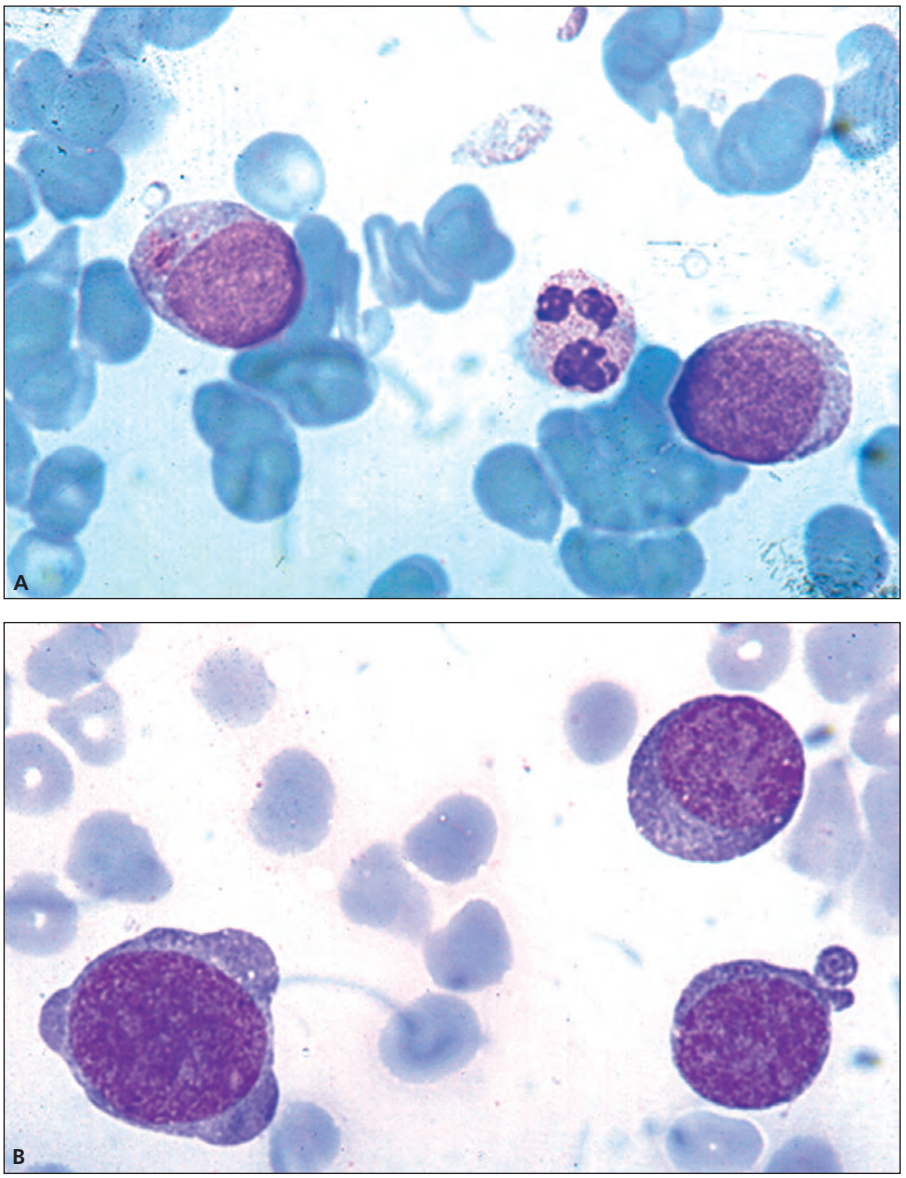



**Fig. 4.105** Acute megakaryoblastic leukaemia; peripheral blood film. (A) In this female patient there is moderate leucocytosis with the presence of a homogeneous population of large blast cells, with a N:C ratio that is not very high. The nuclei are eccentric and oval with a smooth regular outline; their chromatin is granular and homogeneous with stippling created by small dark foci alternating with thin pale spaces: a feature that can also be described as spongy, almost fluffy. There are non-prominent nucleoli. The cytoplasm is well represented and basophilic with numerous small vacuoles and, in the cell on the left, contains a focal accumulation of azurophilic granules that are tending to coalesce. The blasts were peroxidase negative; immunophenotyping showed megakaryoblastic leukaemia. (B) The megakaryoblast on the left has a hyperdiploid oval nucleus with spongy chromatin; its basophilic agranular cytoplasm has formed three large pseudopodial projections. The blast top right has regular nuclear and cellular outlines, while that bottom right has two basophilic cytoplasmic blebs.

The difficulty in bone marrow aspiration makes histological examination of bone marrow sections essential; these can appear hypercellular with normal architecture replaced by a population of blast cells mixed with dysplastic megakaryocytes; sections also confirm the presence of reticulin fibrosis and permit immunophenotyping of the blasts using appropriate markers.

Megakaryocytic leukaemia has a fairly characteristic cytochemical profile, although this is not always easy to determine. The blast cells are negative for granulocytic markers (peroxidase, Sudan black B and CAE). A PAS stain shows diffuse or granular positivity, sometimes more intense in the vesiculated cytoplasm that is detaching from the surface of the more differentiated cells. ANAE often shows focal positivity, localised to

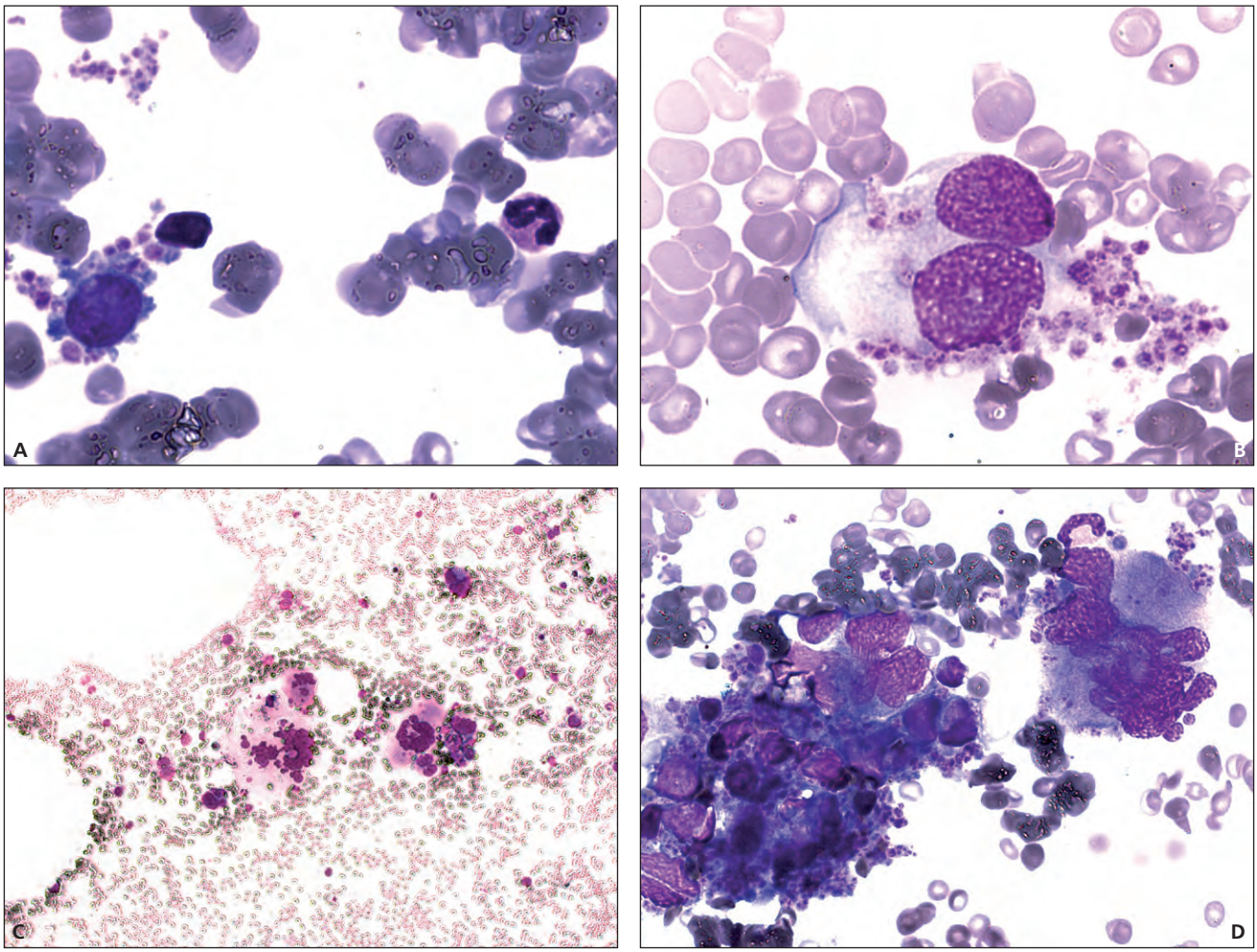



**Fig. 4.106** Acute megakaryoblastic leukaemia in a patient whose blast cells and megakaryocytes had characteristic morphology. The blood count showed severe anaemia, neutropenia and a platelet count of 75 × $10^9$/l. (A) Peripheral blood film. On the left, a megakaryoblast with an oval nucleus with compact chromatin; the cytoplasm is basophilic where it surrounds the nucleus, but is differentiating at the periphery to form dense, hyperchromatic platelets which are separating from the cell in strands (the appearance is similar to that of Fig. 4.104D). (B) Same film as (A). This megakaryoblast appears to have two nuclei, although one cannot exclude continuity at the point of contact. The chromatin is vermicular, like that of cells in early prophase. The left part of the cytoplasm is occupied by a gigantic, optically empty vacuole, while the cytoplasm at the bottom and right of the cell is forming hyperchromatic platelets, which are remaining attached as small clumps. (C) The bone marrow aspirate is hypocellular, but with numerous mature megakaryocytes being conspicuous; some are hyperdiploid and gigantic while others are small and immature. (D) At higher power, on the right, a large basophilic megakaryocyte with a hyperdiploid nucleus composed of an elongated twisted skein of vermicular chromatin. The cytoplasm is intensely basophilic with platelet formation clearly seen at the periphery. On the left, a mass of platelets envelops numerous megakaryoblasts, the individual morphological characteristics of which can no longer be discerned.

the Golgi zone, inhibited by fluoride; ANBE on the other hand is always negative, making a distinction from monocytic leukaemia possible (monocytic leukaemias being positive for both ANAE and ANBE, in a diffuse non-focal manner). An acid phosphatase stain also shows focal positivity that is inhibited by tartaric acid. If megakaryoblasts are very immature, however, all cytochemical reactions may be negative or give rather dubious reactions.

Electron microscopy permits the identification of PPO activity in fresh or fixed cells. In megakaryoblasts the reaction is localised exclusively to the nuclear membrane and the endoplasmic reticulum, and is not found in the cytoplasmic granules or the Golgi apparatus, thus differing from the MPO of cells of granulocytic lineage.[98] By the ultrastructural demonstration of PPO and of platelet-specific antigens, it has been possible to identify a high frequency (19%) of a megakaryoblastic component in various types of AML, particularly in those with granulocytic differentiation.[138]

The main criteria that today permit the recognition of acute megakaryoblastic leukaemia are based on immunophenotyping,

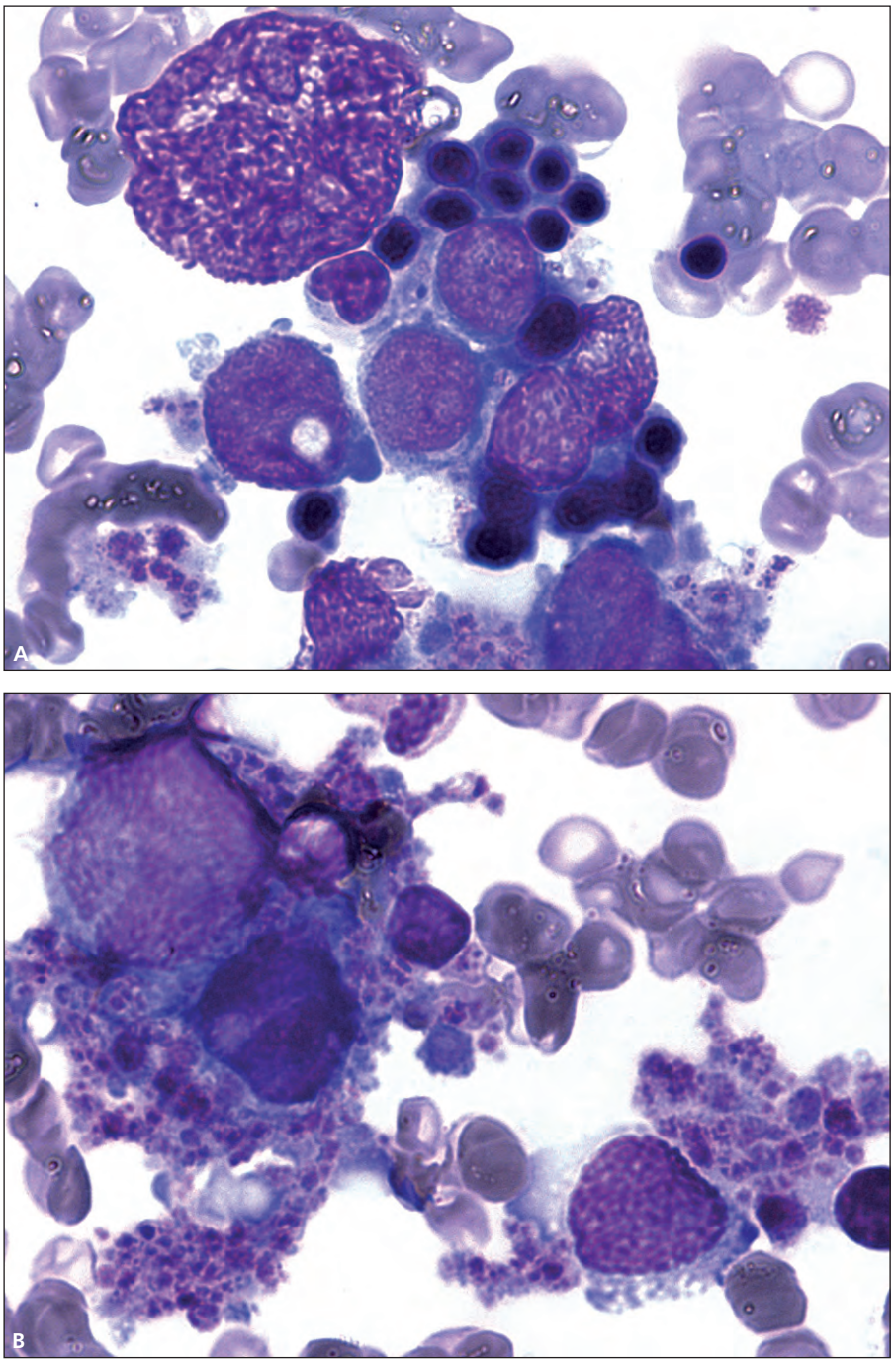



**Fig. 4.107** Acute megakaryoblastic leukaemia, which can be designated as 'with maturation'; same case as Fig. 4.106; bone marrow aspirate. (A) Top left, a large bare megakaryocyte nucleus. Immediately beneath, a lymphocyte and a small accumulation of orthochromatic erythroblasts, which are arranged around a group of large cells. The large cell on the left, with a vacuole, can be recognised as a megakaryoblast on the basis of its large nucleus, the density of its chromatin and the evidence of platelet formation. The two blast cells on the right can be identified as megakaryoblasts because of their spongy chromatin, prominent nucleoli, ragged basophilic cytoplasm and tendency to cluster. (B) Bottom right, a blast surrounded by strips of large hyperchromatic platelets, still attached to each other. On the left, a mass of promegakaryocytes, micromegakaryocytes and festoons of platelets.

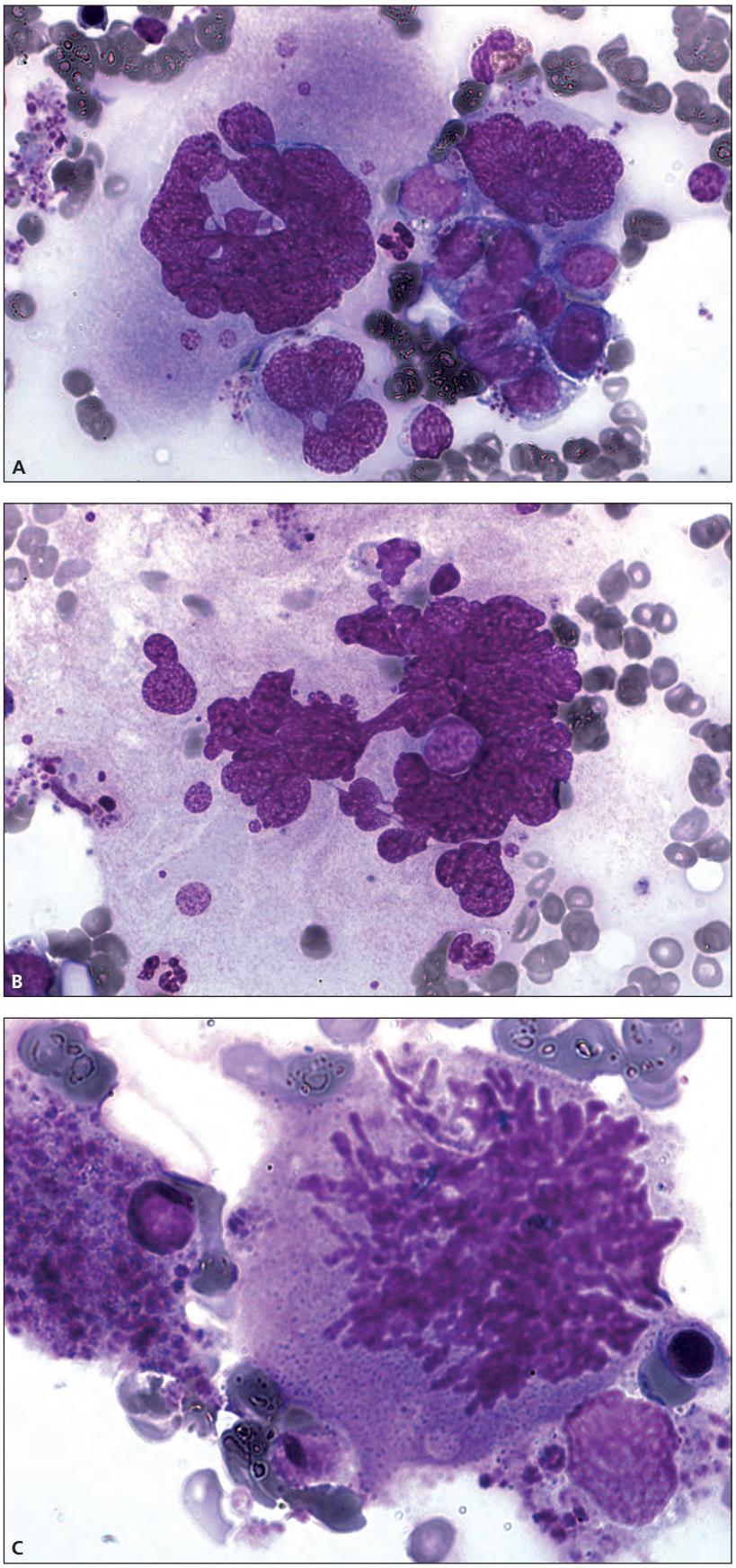



**Fig. 4.108** Acute megakaryoblastic leukaemia with maturation: gigantism and dysmorphism of differentiated megakaryocytes, same case as Fig. 4.106; bone marrow aspirate. (A) A group of dysmorphic megakaryocytes that are heterogeneous with regard to size, morphology and degree of maturation. That on the left is giant and hyperdiploid with some detached nuclear fragments and an immense amount of cytoplasm that is violet because of the admixture of the background basophilia with the colour conveyed by the myriad of fine invisible granules, typical morphology that recalls that of essential thrombocythaemia. On the right, at the top and the bottom respectively, two medium-sized dysplastic megakaryocytes with less cytoplasm and hyperdiploid nuclei of bizarre shapes: between them are a number of blast cells, a promegakaryocyte with a trilobed nucleus and a mature neutrophil. (B) An immense megakaryocyte with a nucleus characterised by extreme hyperdiploidy with the appearance of an exploded cauliflower, with detached lobes. The very extensive cytoplasm shows features of platelet formation at the periphery (or perhaps adhesion of exogenous platelets). The nucleus of a well-preserved blast cell is lodged within a deep indentation in the right part of the nucleus. Two neutrophils, at the bottom, appear to have entered the cytoplasm by emperipolesis. (C) Hyperdiploid mitotic figure in a megakaryocyte precursor with richly granular cytoplasm that is between basophilic and violet.

which gives highly specific results without much difficulty. The most useful markers are the platelet glycoproteins CD61 (glycoprotein IIIa) and CD41 (glycoprotein IIb/IIIa complex), which are more sensitive and expressed earlier than CD42 (glycoprotein Ib). CD36 is also positive. CD45 and HLA-DR are often negative; CD34, CD33 and CD13 are inconsistently positive and only in a proportion of the megakaryoblasts.

According to the 2008 WHO classification, certain types of AML with megakaryoblastic differentiation must be excluded from the category of AML, NOS with megakaryoblastic differentiation, specifically those with recurrent genetic abnormalities. Similarly, the diagnosis of AML with MRC is obligatory for cases of AML with megakaryoblastic differentiation that have the specific cytogenetic abnormalities typical of MDS, or have evolved from a preceding MDS or have cytological dysplasia in at least 50% of cells in at least two haemopoietic lineages. Also excluded are therapy-related AML, acute myelofibrosis with myelofibrosis and acute megakaryoblastic leukaemia associated with Down syndrome, all of which constitute in themselves distinct WHO categories.

The differential diagnosis of megakaryoblastic leukaemia, in addition to the conditions just cited, includes AML, NOS undifferentiated or with minimal differentiation, ALL and pure erythroid leukaemia. Megakaryoblastic/megakaryocytic transformation of MPN, and in particular of CML, can have very similar features to those of acute megakaryoblastic leukaemia, but can be distinguished on the basis of the clinical history of a chronic phase and by the frequent presence of splenomegaly. Bone marrow metastases of solid tumours, such as carcinoma and rhabdomyosarcoma, can be suggestive of megakaryoblastic leukaemia because of the tendency of the neoplastic cells to cluster in small groups.[139]

### Acute myeloid leukaemia with basophilic differentiation

The existence of rare cases of AML with clear evidence of basophilic differentiation has been recognised by numerous authors.[140] From this subtype of AML, NOS the form associated with t(6;9) must be excluded and similarly one must exclude MPN with t(9;22) and a *BCR-ABL1* fusion gene. In a small percentage of patients with AML, NOS with basophilia there are symptoms of hyperhistaminaemia, such as unexplained cough, headache, fever, peptic ulceration and gastrointestinal haemorrhage. The clinical course often shows rapid evolution.

Two variants have been described.[141] The first shows signs of basophilic differentiation on microscopy: in the peripheral blood and bone marrow there are present, together with undifferentiated blast cells, immature cells with large atypical basophil granules (Figs 4.109 throug to 4.113), which stain metachromatically red with toluidine blue, as do a variable proportion of

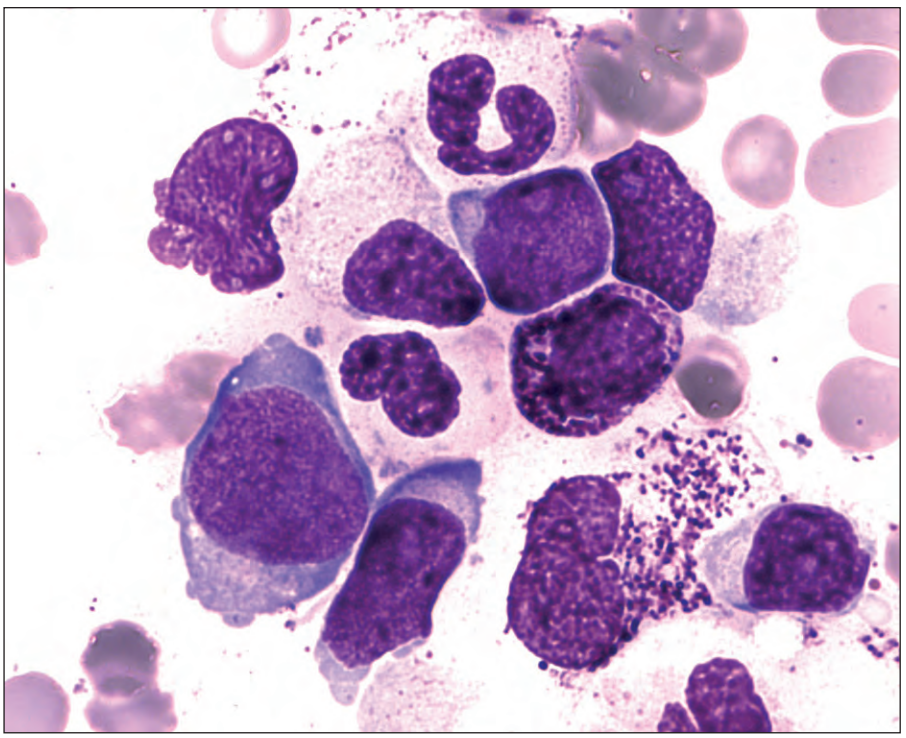


**Fig. 4.109** AML, NOS, with basophilic differentiation; bone marrow aspirate. There are three undifferentiated blast cells, one large with extensive basophilic cytoplasm and two that are smaller; there are neutrophil granulocytes with nuclear dysmorphism and hypogranular cytoplasm; and a basophil granulocyte with fairly plentiful cytoplasm in which the nuclear morphology is more readily appreciated than usual. Another basophil has been damaged during the spreading of the film.

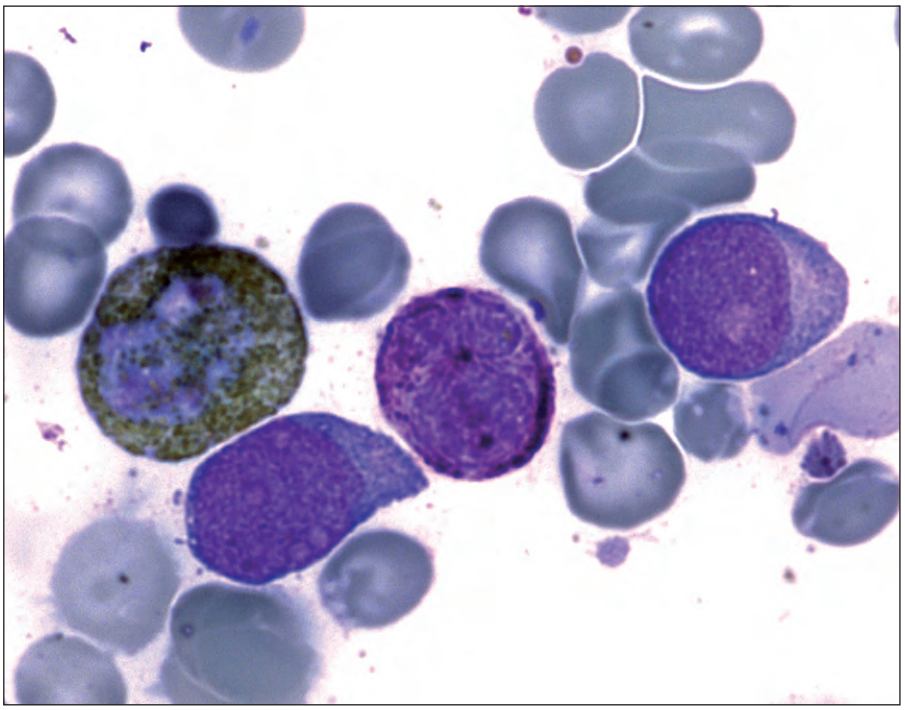


**Fig. 4.110** AML, NOS, with basophilic differentiation, same case as Fig. 4.109; bone marrow aspirate. Peroxidase staining is intensely positive in a maturing progenitor of granulocyte lineage and is completely negative in two agranular blast cells and in the basophil precursor between them; the latter has a multilobated nucleus and violet cytoplasm containing dark granules of various sizes.

dysplastic basophil granulocytes at a more advanced stage of maturation. The nucleus of the blast cells is irregular, covered by granules, with fairly dense chromatin; the cytoplasm is usually moderately abundant and quite weakly basophilic, sometimes colourless; sometimes there are vacuoles; azurophilic granules, usually peroxidase negative, are present together with blackish basophil-type granules. Auer rods are not seen.

In the second variant of basophilic leukaemia, conversely, there are no morphological or cytochemical signs of basophil differentiation. The blast cells are undifferentiated or have scanty cytoplasm containing peroxidase-negative azurophilic granules. PAS staining may show large positive blocks. The diagnosis is made only by demonstrating the characteristic ultrastructural features of basophil-specific granules, designated theta granules because of their characteristic form on electron microscopy[142]; in addition, ultrastructural cytochemistry may demonstrate a distinctive positivity with ruthenium red. The immunophenotype of these cells is characterised by negativity for CD117 and positivity for cytoplasmic CD203, which is very specific for the basophil lineage[143]; expression of CD9 is variable. The immunophenotypic picture is important for the differential diagnosis with mast cell neoplasms, in which the pathological cells express CD117, CD25 and tryptase. In mast cell neoplasms, the mast cells are morphologically fairly typical and mainly mature, without there being an increase in circulating basophils.

### Acute panmyelosis with myelofibrosis

This very rare clinicopathological entity, recognised in the 2008 WHO classification as a form of AML, NOS, is characterised by proliferation of cells of all myeloid lineages, with increased blast cells and associated bone marrow fibrosis. It had been described previously as 'acute myelofibrosis' and 'malignant myelofibrosis'. It is actually a leukaemia derived from a pluripotent stem cell,[144] with fibrosis secondary to the megakaryocytic proliferation.

Clinically it manifests with pancytopenia, fever and bone pain, without splenomegaly. In the peripheral blood film, anisopoikilocytosis is minimal or absent and dacrocytes are not a feature; circulating erythroblasts and micromegakaryocytes may be found.[145,146] The bone marrow aspirate is often inadequate because of marked reticulin fibrosis and histological sections show hypercellularity, focal blastic infiltration, increased megakaryocytes and trilineage dysplasia (Fig. 4.114). The bone marrow biopsy is essential to show the hypercellular bone marrow tissue with diffuse stromal fibrosis and increased proliferation, in variable proportions, of erythroblasts, granulocyte precursors and megakaryocytes. There are numerous megakaryocytes, including small and dysplastic micromegakaryocytes, which are positive for CD61 and CD41. Blasts generally show a focal distribution, occurring in small groups of cells that express myeloid markers (CD13, CD33, CD117),[147] and also megakaryocytic markers.[148]

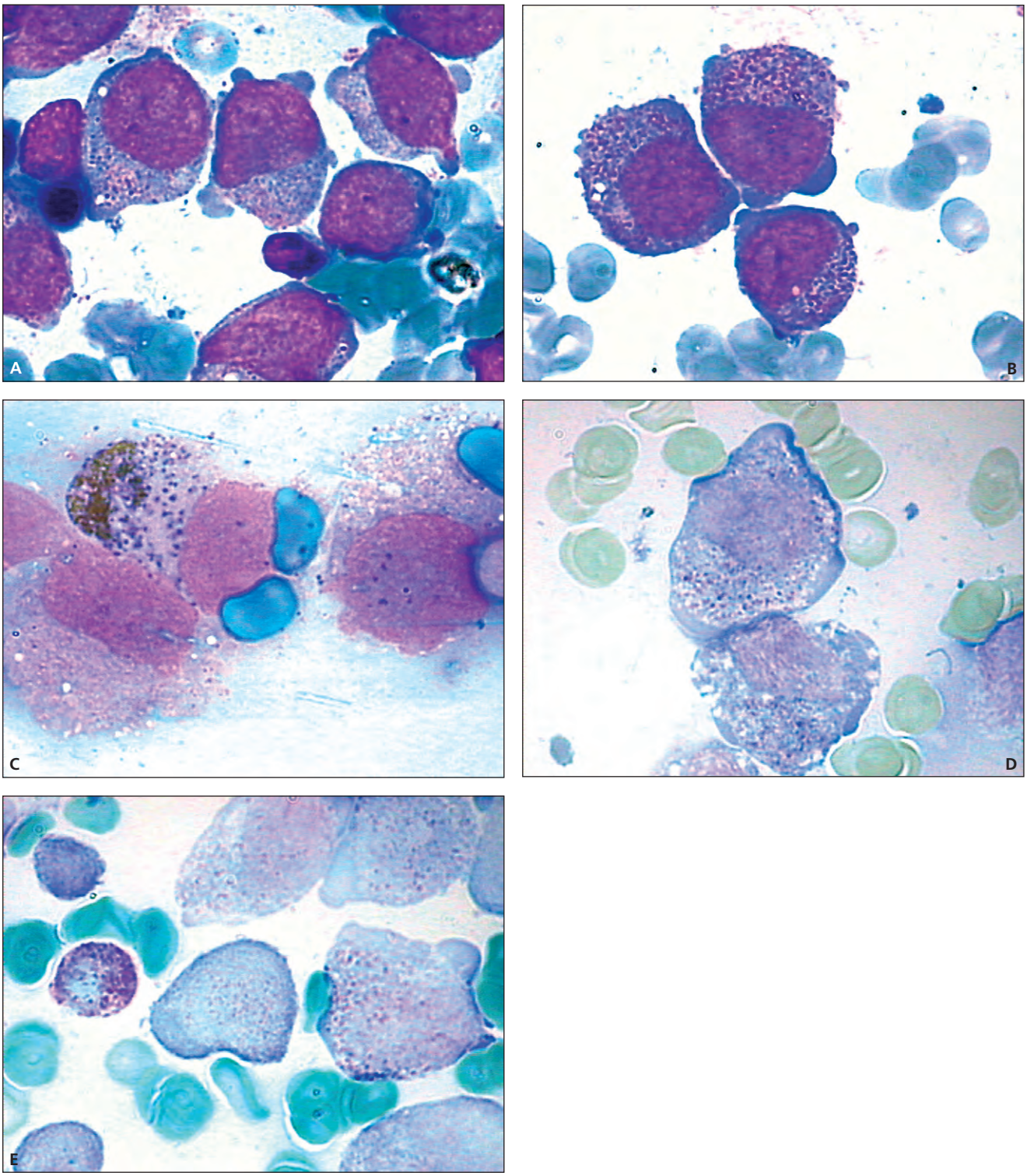



**Fig. 4.111** AML, NOS, with basophilic differentiation; bone marrow aspirates of different patients. (A) The marrow is infiltrated by a monomorphic population of medium-sized blast cells with a N:C ratio that is not increased since there is plentiful cytoplasm; this has the uncommon characteristic of maintaining an intense degree of basophilia despite the presence of numerous dark granules, which are often concentrated in pink patches of cytoplasm. Formation of pseudopodia is common and inside one of these, on the right, part of the nucleus has herniated. (B) These cells show the same features as those just described, with a larger number of large, dark cytoplasmic granules. The nuclei have a rounded or oval outline with some angulation, irregularly condensed chromatin and nucleoli with varying degrees of visibility. (C) A peroxidase reaction shows granular positivity in some blast cells, while in others it is negative. (D) Toluidine blue staining shows that some of the blast cells have basophil granules that stain metachromatically red. (E) The metachromatic staining with toluidine blue is more evident in mature basophil granulocytes, such as that on the left, which is smaller than the blast cells.

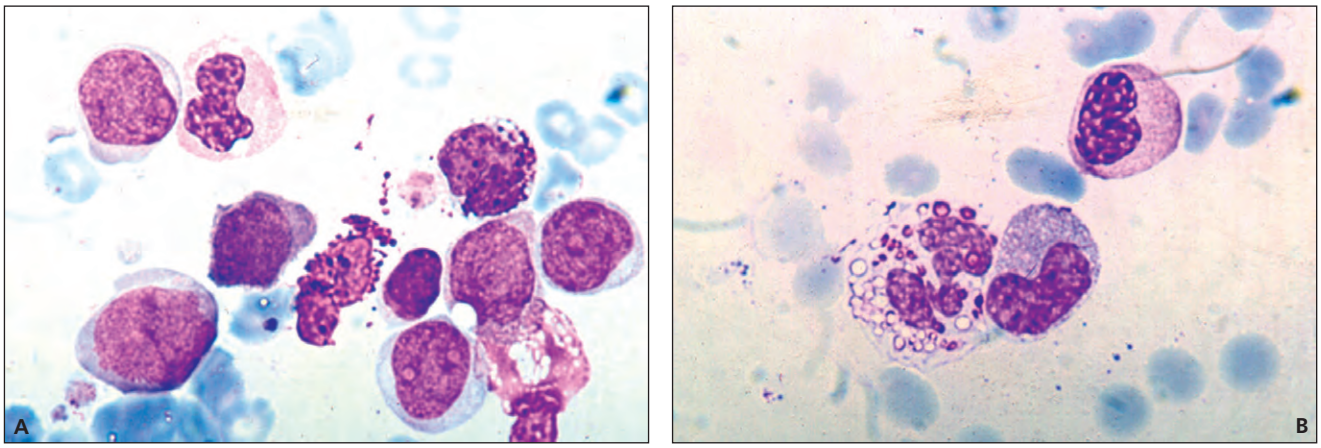



**Fig. 4.112** AML, NOS, with basophilic differentiation; bone marrow aspirate. (A) The blast population is characterised by a lack of morphological signs of differentiation: a high N:C ratio, regular nuclei, condensed chromatin with alternation of pale and dark areas, nucleoli that are often prominent and sky-blue agranular cytoplasm. The recognisable basophils, on the other hand, are differentiated cells with moderate dysmorphism and hypogranularity, as in the two shown here. (B) Other basophils are more frankly dysplastic, such as this cell in which the nucleus is segmented in the same manner as a neutrophil and the cytoplasm is transparent; its granules are large and dysmorphic and often optically empty. On its right, an eosinophil with fine greyish granules and, at the top, a neutrophil, both of which have non-segmented nuclei.

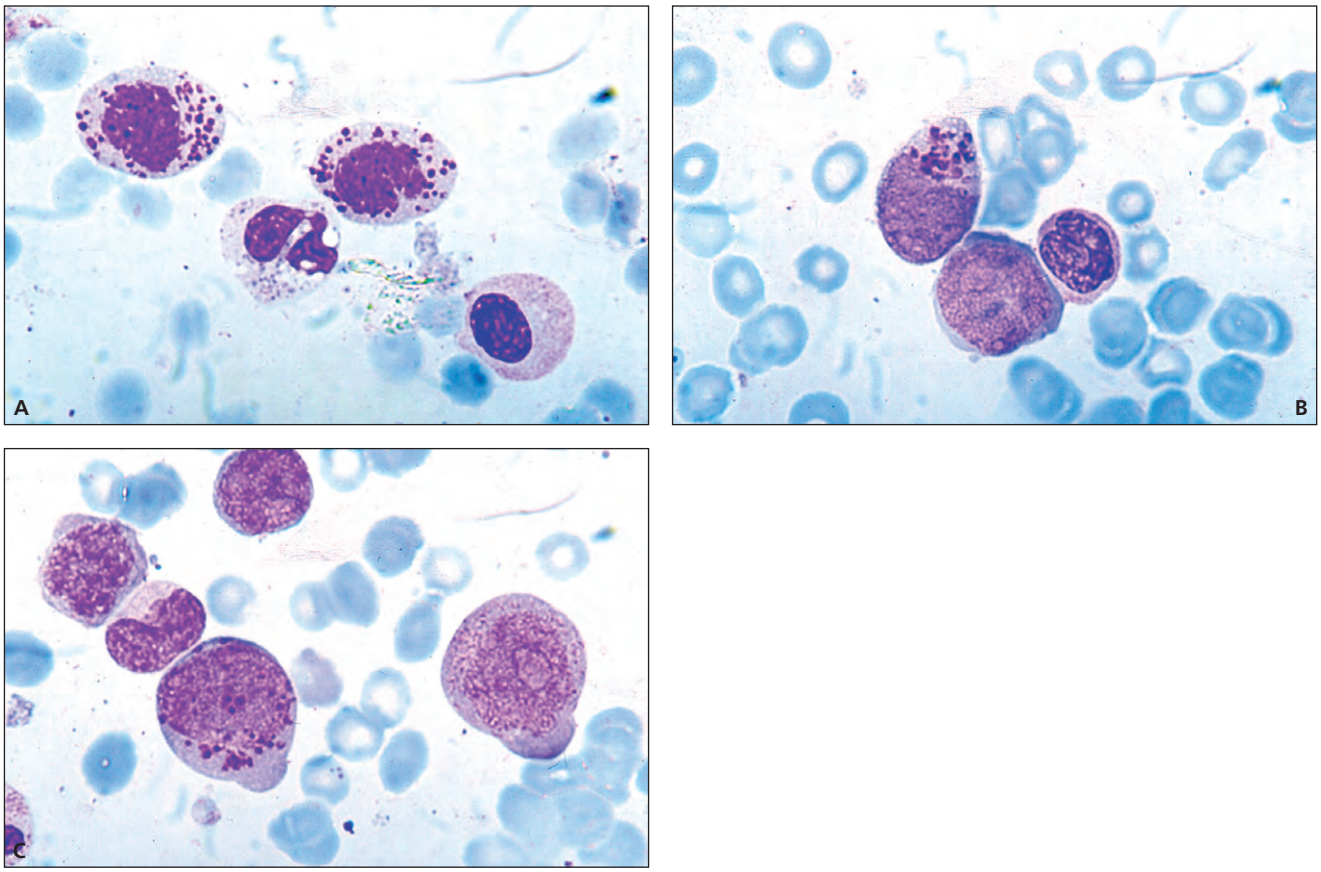



**Fig. 4.113** AML, NOS, with basophilic differentiation and trilineage dysplasia. (A) Peripheral blood film. There are two large basophils in which the plentiful pale cytoplasm contains only a few, characteristic, granules, letting the morphology of the nuclei be clearly seen; a hypogranular vacuolated eosinophil (in the centre); a neutrophil with a non-segmented pseudo-Pelger nucleus and many fine cytoplasmic granules. (B) The same patient as (A); bone marrow aspirate. Two blast cells, one of which has basophil-type granules, and a neutrophil band form. (C) The same patient as (A); bone marrow aspirate. An undifferentiated blast cell, cut off at the upper border, and, at the bottom, a larger blast cell with basophil granules; a third, on the right, has numerous azurophilic granules and a large stamped out nucleolus. In this patient, dysplasia was present in all three haemopoietic lineages, but did not reach the proportion necessary for a classification as AML with MRC; the blast population showed signs of differentiation to all three granulocyte lineages.

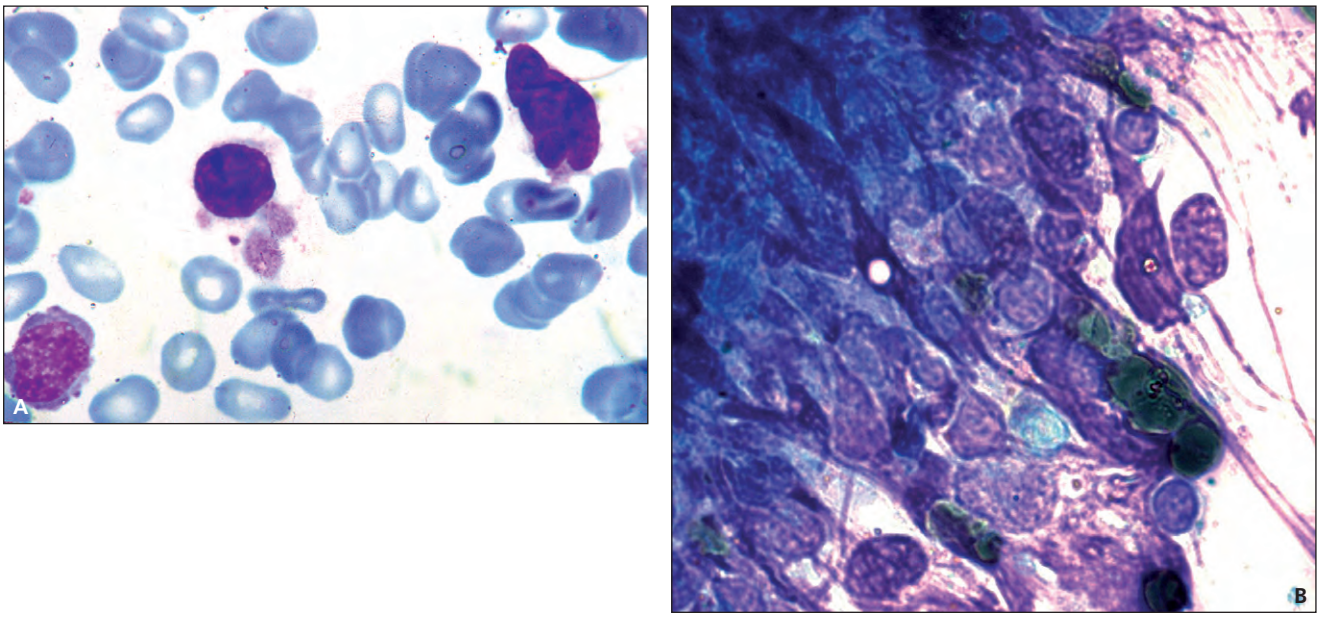



**Fig. 4.114** Acute panmyelosis with myelofibrosis. (A) Peripheral blood film. In the markedly leukopenic blood film there are some very dysmorphic micromegakaryocytes. (B) Bone marrow aspiration did not yield evaluable material. In the context of an apparently necrotic and degenerating fragment, one may observe, as in this case, the presence of blast cells. The diagnosis has to be clinical and histopathological.

This form of AML has a close relationship, so far not totally clarified, with AML, NOS with megakaryoblastic differentiation and with AML with MRC with predominant megakaryocytic differentiation.[149] The differential diagnosis also includes primary myelofibrosis, post-polycythaemia myelofibrosis and post-essential thrombocythaemia myelofibrosis, in which, however, blast cells are not increased; fibrosis associated with bone marrow metastases of non-haematological neoplasms must also be distinguished.

## Therapy-related myeloid neoplasms

Under this heading in the 2008 WHO classification are grouped the myeloid neoplasms that occur as a serious late complication of cytotoxic therapy, administered to patients for treatment of a primary condition that may or may not be neoplastic.[150] The therapy-related myeloid neoplasms include therapy-related MDS (t-MDS), therapy-related myelodysplastic/myeloproliferative neoplasms (t-MDS/MPN) and therapy-related AML (t-AML), comprising 10–20% of cases of such neoplasms.[151–153] This number of such cases is certainly going to increase, in view of the increasing numbers of patients in whom the primary tumour is cured as a result of newly developed efficacious therapy.

These therapy-related conditions are seen in patients who have been treated with ionising radiation, chemotherapy or both, the chemotherapeutic agents implicated being alkylating agents, topoisomerase II-interactive drugs and antimetabolites. The primary condition treated may be either a haematological neoplasm such as Hodgkin lymphoma or non-Hodgkin lymphoma, or a solid tumour, most often of the lung, ovary, breast or prostate. This same group of disorders occurs also in patients treated with immunosuppressive drugs for autoimmune disorders[154,155] or in the context of transplantation.[105,156] In a meta-analysis that included 23 clinical trials with more than 6000 patients,[157] it was underlined that the administration of G-CSF for the treatment of chemotherapy-induced neutropenia permitted the optimal management of infective complications in these patients, but also exposed them to an increased risk of therapy-related haematological neoplasms.[158]

This category includes a very heterogeneous group of haematological neoplasms. The condition is initially identified as MDS, AML or MDS/MPN according to the usual morphological and numerical criteria, such as the percentage of blast cells, the number of monocytes, the number of erythroid cells and the proportion of cells showing dysplasia (Figs 4.115 through to 4.127). On the basis of these findings and the clinical history, cases are then assigned to two groups, t-MDS/AML and t-MDS/MPN. In the 2008 WHO document, it was clearly explained that this group does not include the evolution of MPN, since there are no criteria that permit a distinction to be made between the natural evolution of MPN and a complication of therapy. However, this category does include cases with recurrent cytogenetic abnormalities such as t(8;21) and t(9;11); for such cases the specific cytogenetic aberration must be noted, but bearing in mind that the prognosis is often worse than in *de novo* cases. In other words, cases of t-AML and t-MDS can have

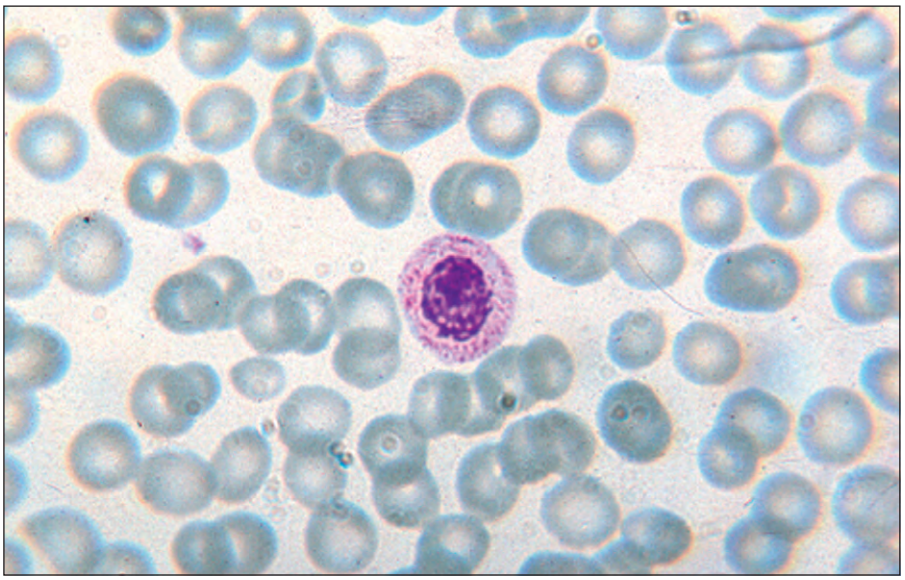


**Fig. 4.115** The onset of a myelodysplastic phase preceding therapy-related AML. A small neutrophil with a pseudo-Pelger nucleus in the peripheral blood film of a patient treated for 22 months, at the beginning of the 1980s, with MOPP combination chemotherapy plus radiotherapy for Hodgkin lymphoma. The presence in the circulation of rare cells of this type indicated the onset of preleukaemic myelodysplasia, which rapidly evolved to AML without maturation and ended with the death of the patient only three months later. The leukaemic population was characterised by the presence of an isochromosome 17.

balanced translocations or inversions that are also present in the first category of the WHO classification, but they are classified as therapy-related neoplasms with the associated cytogenetic abnormality being specified. It should be noted that patients with t-AML associated with t(8;21), inv(16), t(16;16) or t(15;17) do not have the uniformly grim prognosis that is seen in other cases of t-AML. It should be noted that patients with t-AML associated with these specific chromosomal rearrangements have generally been treated for the primary neoplasm with topoisomerase II-interactive drugs, sometimes in association with alkylating agents, irradiation or both; the latent period before development of the therapy-related neoplasm is very short; the sensitivity to antileukaemic chemotherapy appears to be similar to that in patients with *de novo* disease.[159,160]

In fact, the distinction between *de novo* and therapy-related disease is based entirely on the clinical history, since no specific diagnostic markers have been identified. There is considerable biological and clinical similarity between t-AML and t-MDS on the one hand and *de novo* AML and high risk MDS on the other, suggesting a common mechanism of leukaemogenesis in which molecular alterations in genes involved in DNA repair are often identified.

The prognosis of therapy-related myeloid neoplasms is generally much inferior to that of *de novo* equivalents, with less than 10% 5-year survival, survival correlating with the karyotype and co-morbidities as well as with the nature of the treatment received for the primary tumour. Patients with anomalies of chromosome 5, chromosome 7 or both, or with a complex karyotype have worse survivals (median survival being $<12$ months), regardless of whether the morphological diagnosis is MDS or AML and regardless of the blast percentage.[161–163]

Characteristically, a blast count below 5% is reported in a half of patients at diagnosis of MDS. Among the cases presenting as AML, monocytic and granulocytic differentiation are most often seen, although all types of AML have been described, including APL.[161] The majority of cases of therapy-related myeloid neoplasm that present with frank AML are distinguished from *de novo* cases by a tendency to trilineage involvement, difficulty in morphological classification, a higher frequency of cytogenetic abnormalities and generally a poor response to antileukaemic treatment. Cases of therapy-related myeloid neoplasm that present as MDS, regardless of whether or not they evolve to AML, have a poor prognosis and a median survival analogous to that of the cases that present as AML, without any correlation with the blast percentage (this being the reason why, in the 2008 WHO classification, they are assigned to the same category). Bone marrow cellularity is increased in more than half of patients with the picture of trilineage hyperplasia, while bone marrow fibrosis is observed in 15% of cases. A variable degree of dyserythropoiesis is always present and the majority of patients have more than 15% ring sideroblasts. Dysgranulopoiesis is almost always present, but Auer rods are rare; an increased number of basophils, megakaryocyte dysplasia and plasma cell infiltration are often recognised.

In the 2001 WHO classification,[9] two subgroups of therapy-related neoplasms were recognised on the basis of published

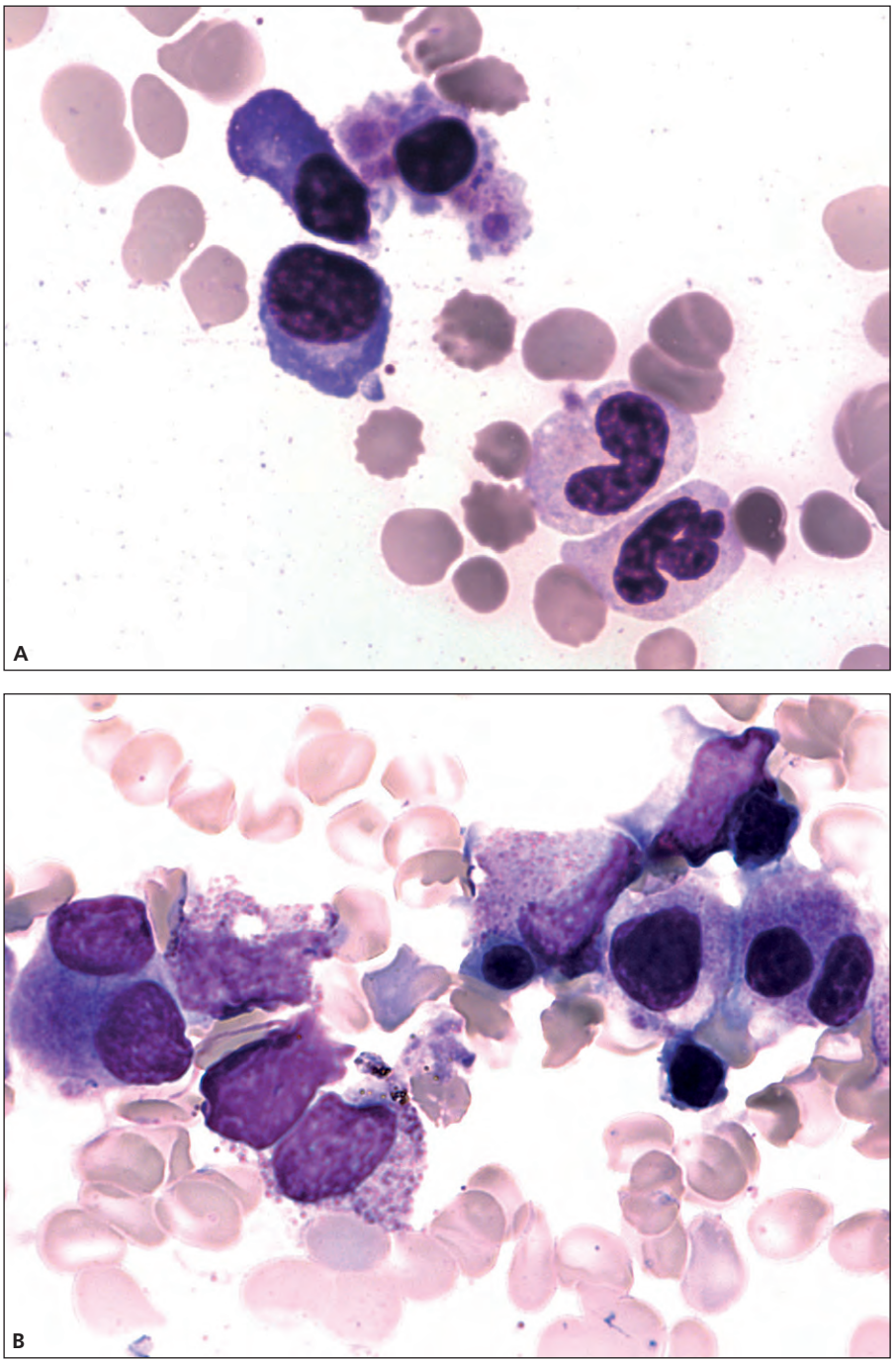



**Fig. 4.116** Therapy-related myelodysplasia in a patient treated four years earlier for carcinoma of the prostate; bone marrow aspirate. (A) From the left: two plasma cells, one mature and one immature; a typical micromegakaryocyte; two hypogranular neutrophils with anomalies of nuclear segmentation. (B) Some neutrophil myelocytes, distorted during the spreading of the film, in which N:C asynchrony can nevertheless be recognised. At each edge of the image, a small binucleated micromegakaryocyte (that on the right has pyknotic nuclei). Adjacent to the latter is a dwarf megakaryocyte with scanty violet cytoplasm and a triangular nucleus. Top right, a small undifferentiated blast cell.

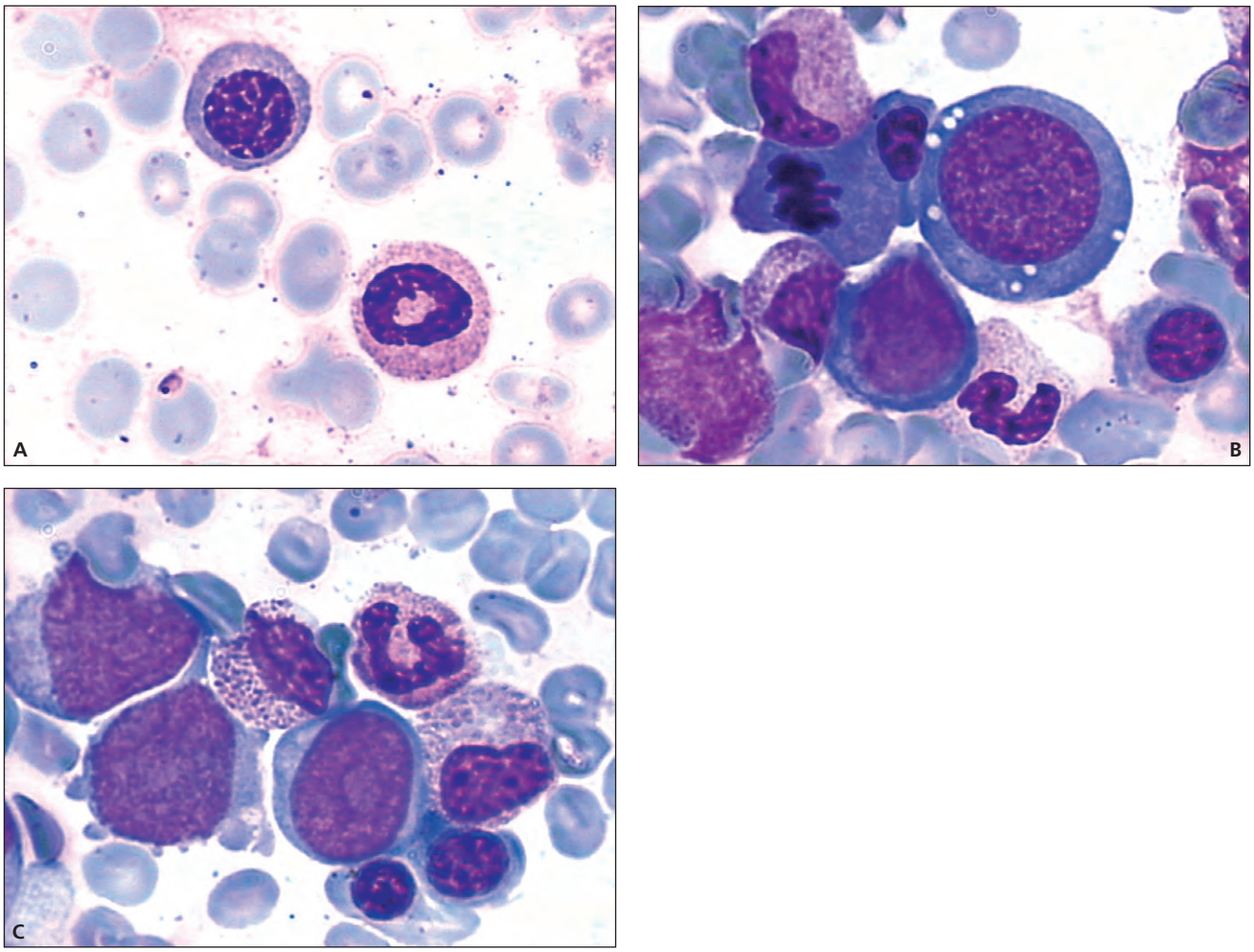



**Fig. 4.117** Therapy-related acute erythroid leukaemia in a patient previously treated with chemotherapy for carcinoma of the breast. (A) Peripheral blood film. Pancytopenia with dysplastic features of neutrophils. Here one observes a ring neutrophil, with several nuclear appendages, and a polychromatic erythroblast with retarded nuclear maturation. (B) Bone marrow aspirate. There are three dysmorphic neutrophil granulocytes and four dyserythopoietic cells at various stages of maturation, showing a tendency to megaloblastosis; one of the latter is in metaphase. (C) On the left, two apparently undifferentiated blast cells with large polygonal nuclei, multiple nucleoli and heterogeneous chromatin; the cytoplasm is moderately basophilic and is extending into a number of pseudopods. The blast cell situated more centrally, at the bottom, has different characteristics, which suggest that it belongs to the erythroid lineage: the nucleus is oval and regular, the chromatin is more homogeneous and granular, there is a large prominent nucleolus in the centre of the nucleus and the cytoplasm is intensely basophilic with arcoplasm.

literature,[89,164,165] these showing differences in clinical and biological features. The 2008 WHO classification did not continue with this subclassification, although this information derived from the clinical history remains useful for considering the nature of the disorder. This earlier classification will be discussed here since it continues to have a valid didactic role and provides a framework that integrates historical and morphological data.

The first group, the commoner and recognised for longer, follows alkylating agents, sometime associated with radiotherapy, with a latent period between five and nine years, but with extremes of less than a year to more than 28 years.[166] The risk is higher and the latent period shorter with certain primary conditions, as in adult patients with Hodgkin lymphoma. This form is usually associated with unbalanced chromosomal abnormalities with loss of genetic material, often involving chromosomes 5 and 7. The appearance of a macrocytic anaemia represents an early sign of MDS; around two thirds of patients develop acute leukaemia after a myelodysplastic phase of about six months. In comparison with *de novo* MDS, t-MDS is more often associated with fibrosis. The AML that develops in these patients is difficult to classify morphologically and rarely shows Auer rods.

The second group, which includes about a third of cases, is associated with treatment with topoisomerase II-interactive drugs, in particular etoposide and teniposide, and in general

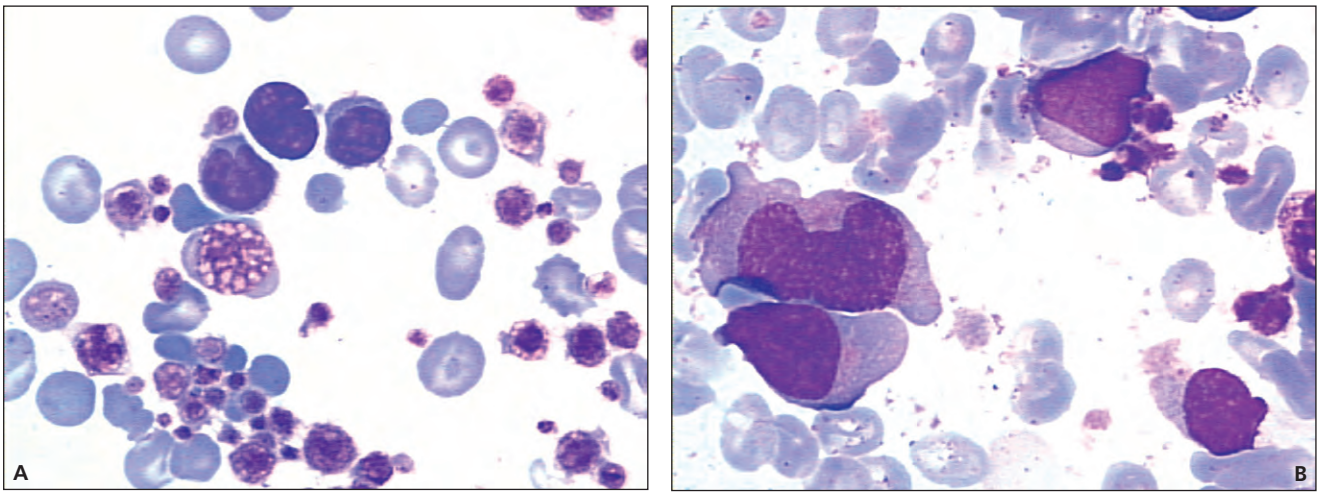



**Fig. 4.118** Therapy-related AML in a patient treated for four years for multiply relapsed, mixed cellularity Hodgkin lymphoma with MOPP, ABVD and MICMA combination chemotherapy and autologous haemopoietic stem cell transplantation. (A) Peripheral blood, buffy coat film. Centre top, a micromegakaryocyte, recognisable from its nucleus with completely condensed chromatin and the basophilic cytoplasm in its nuclear notch. On either side of it, small lymphocytes distinguished by their heterogeneous chromatin condensation and the rim of cytoplasm that totally surrounds the nucleus and has several thin cytoplasmic fringes. Immediately beneath, on the left, a giant platelet with a hypertrophic chromomere forming a pseudonucleus. All the visible platelets, in part joined to form a small carpet, are dysmorphic and very variable with regard to size and the appearance of the chromomere. (B) Bone marrow aspirate. In the scanty aparticulate material there are a number of blast cells, some with features that suggest they are megakaryoblasts, such as the cell with a hyperdiploid lobulated nucleus and azure and violet cytoplasm with a concentration of granules in the nuclear concavity.

presents with balanced chromosomal translocations that often involve chromosome 11 (band 11q23) with rearrangement of the *MLL* gene.[167,168] The latent period is briefer, between six months and five years from the start of treatment; presentation is usually as frank AML without a preleukaemic phase. The marrow is hypercellular, dysplasia is uncommon and morphological classification is usually straightforward: a monocytic component often predominates, and for this reason the morphological diagnosis in the majority of cases is acute myelomonocytic or acute monoblastic/monocytic leukaemia. This form is more sensitive to chemotherapy; survival is better than with disease following alkylating agents, but the frequency of relapse is high.

## Myeloid neoplasms inserted as new categories into the WHO 2008 classification

The 2008 WHO classification has added three newly specified categories to the myeloid neoplasms: myeloid sarcoma, myeloid neoplasms associated with Down syndrome and blastic plasmacytoid dendritic cell neoplasm.

### Myeloid sarcoma

This solid tumour, also designated granulocytic sarcoma or chloroma, is characterised by an extramedullary proliferation of blast cells of one or more of the haemopoietic lineages, which expands and occupies or destroys the architecture of the normal tissue that is involved.[169,170] It can occur at any site, but particularly in the skin, the gastrointestinal tract, the lymph nodes, the testis or the skeleton. It can be diagnosed *de novo*, and is then considered equivalent in all ways to AML; or it can anticipate, follow or accompany AML, or represent the relapse of AML; in these instances it is classified on the basis of morphology, immunophenotype and genetics. It can also represent evolution to AML of MDS or MDS/MPN or be a particular type of blastic transformation of an MPN.

The cytological features can be appreciated from imprints made by pressing material obtained by biopsy or surgery onto a glass slide (Figs 4.128, 4.129 and 4.130). The most frequent appearance is that of a blastic infiltration, with a variable degree of granulocytic, myelomonocytic or monoblastic differentiation; rare examples are trilineage or erythroid. Cytochemistry on the imprint, depending on the lineages involved, shows a variable positivity for MPO and/or various esterases. The final diagnosis is, however, histological. Positive immunohistochemistry for myeloid or monocytic markers or both is fundamental for the differential diagnosis with various types of lymphoma. Genetic investigations give results that are not dissimilar to those of AML in general.

### Myeloid proliferations associated with Down syndrome

Pathological myeloid proliferations associated with Down syndrome are of two types, both of which involve particularly the megakaryocyte lineage[171]: transient abnormal myelopoiesis

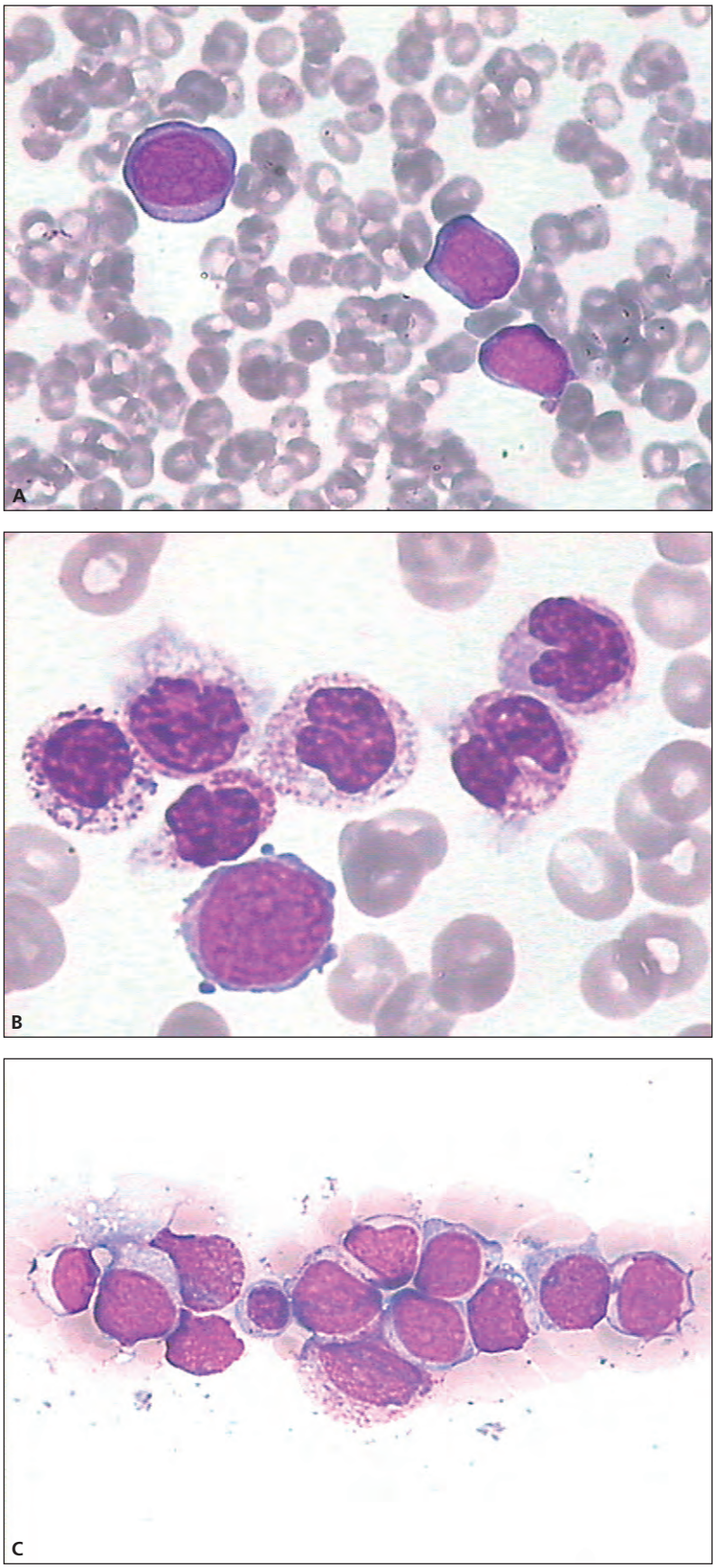



**Fig. 4.119** Therapy-related AML without maturation, in a patient treated with chemotherapy three years earlier for diffuse large B-cell lymphoma (centrocytic-centroblastic). (A) In the peripheral blood film, agranular blast cells with fine chromatin and cytoplasmic basophilia. (B) In the tail of the same film, some dysplastic neutrophils, one of which has a round, non-segmented nucleus; and an agranular blast cell with fine chromatin and a number of nucleoli. (C) The bone marrow aspirate is hypocellular and contains only a small trail of blast cells with the same features as were observed in the peripheral blood. Flow cytometric immunophenotyping showed these cells to be negative for CD34 and TdT, while they expressed MPO weakly and CD13 and CD33 more strongly.

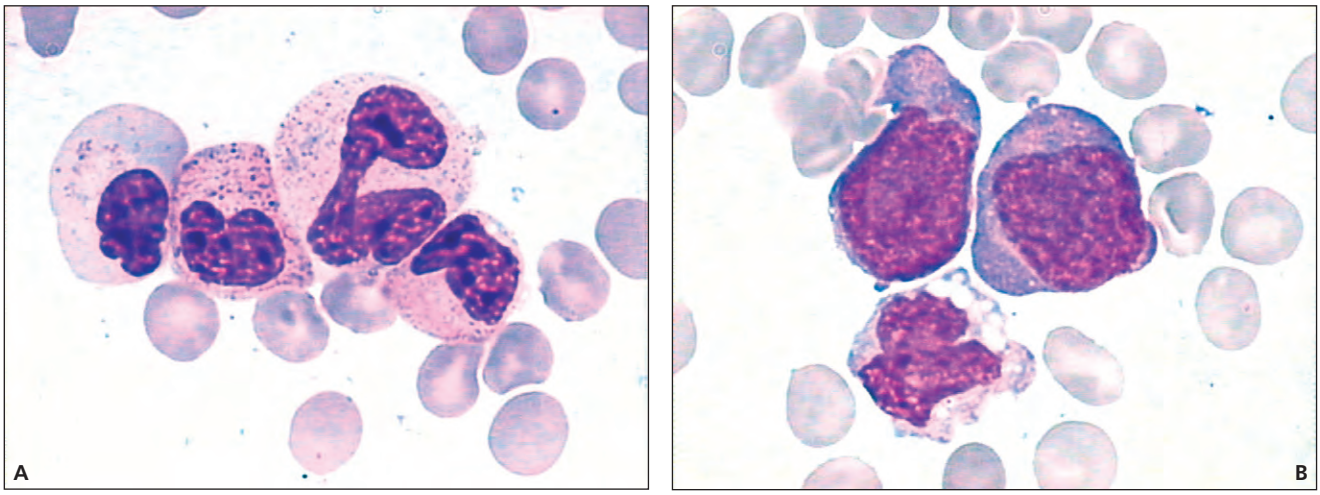



**Fig. 4.120** Therapy-related AML with myelodysplasia in a patient treated with surgery and chemotherapy for adenocarcinoma of the colon; bone marrow aspirate. (A) Three neutrophil granulocytes with nuclear dysplasia and cytoplasmic hypogranularity (moderate in the large cell in the centre and more marked in that on the right). The cell on the left, rather than being a hypogranular neutrophil, appears to be an orthochromatic erythroblast with basophilic stippling and a lobulated nucleus; this is suggested by the clumping of the chromatin and the background colour of the cytoplasm, which is very similar to that of the surrounding erythrocytes. (B) Two large granular blast cells with polygonal nuclei and intensely basophilic cytoplasm.

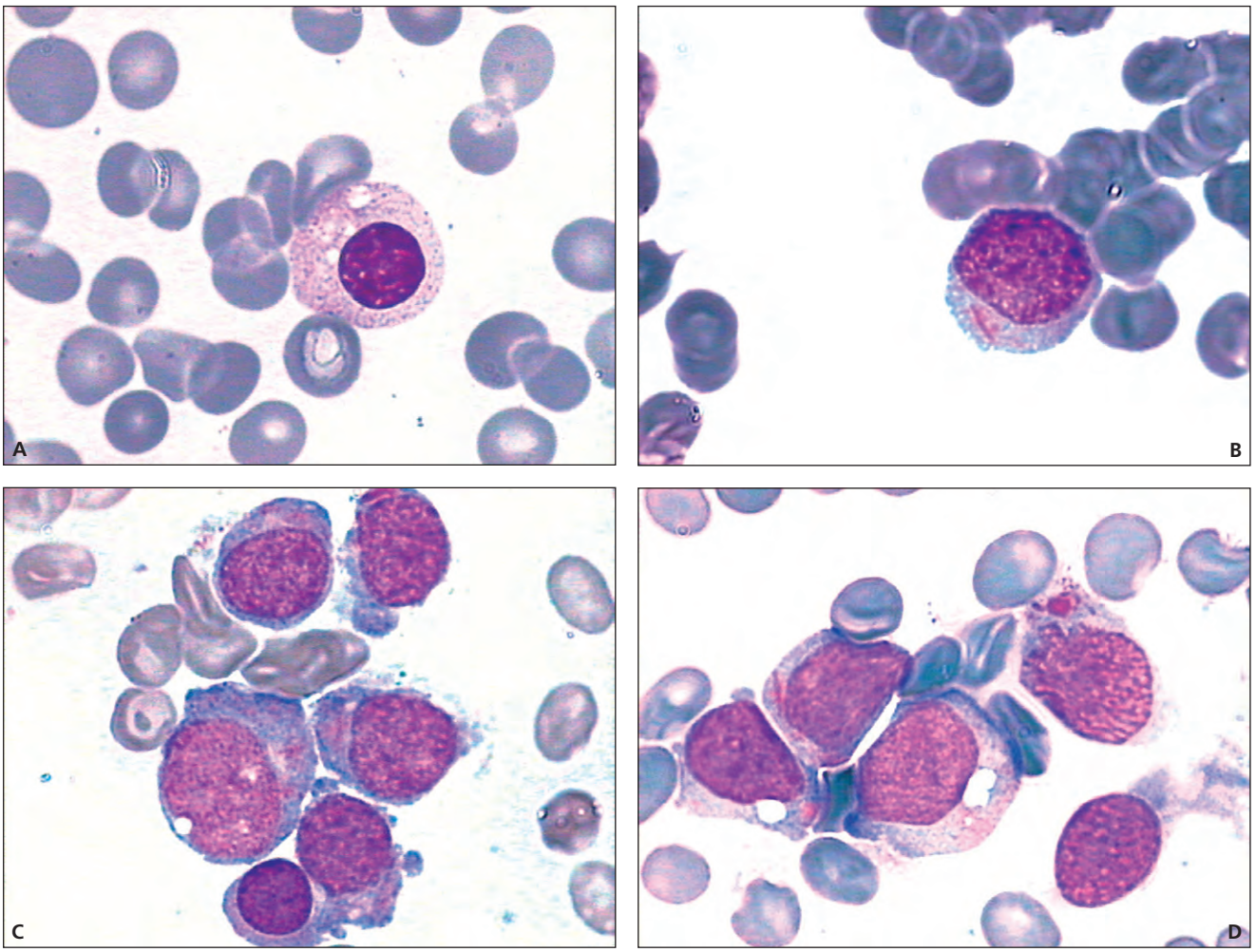



**Fig. 4.121** AML with t(8;21) secondary to protracted treatment with azathioprine for a severe form of psoriasis. The morphology is characteristic and is the same as that in *de novo* AML with t(8;21). (A) Peripheral blood film. Neutrophil with a non-lobated pseudo-Pelger nucleus with chromatin that is not yet pyknotic and vacuolated cytoplasm with quite fine granules on a basophilic background. (B) Peripheral blood film. A typical circulating myeloblast with a single Auer rod. (C) Bone marrow aspirate. The marrow is cellular with 75% myeloblasts, which have ample basophilic cytoplasm containing azurophilic granules and Auer rods. (D) Bone marrow aspirate. In addition to two Auer rods, there are blast cells with coalescent granules.

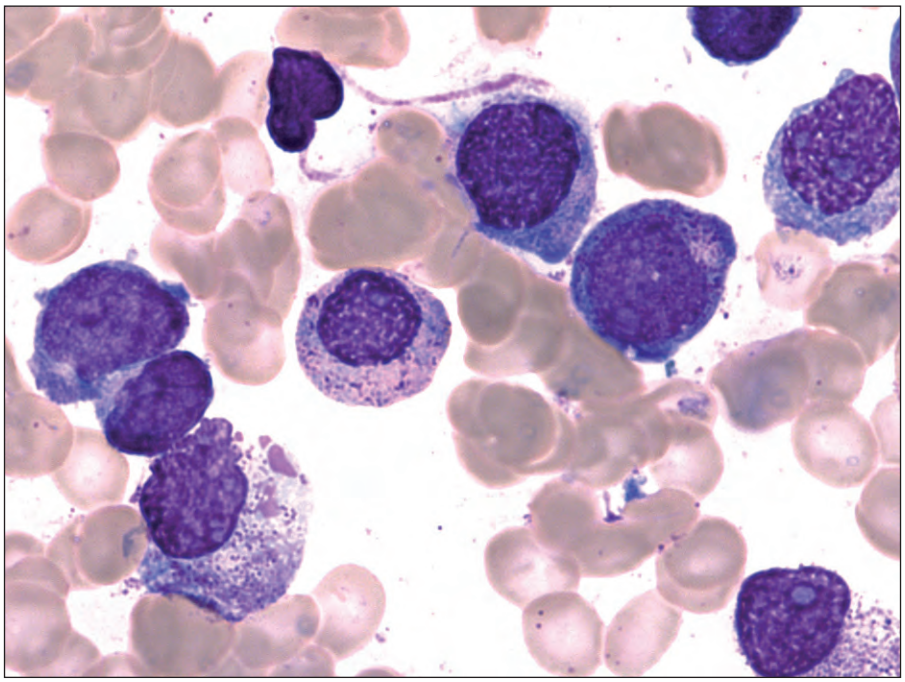


**Fig. 4.122** AML with myelodysplasia secondary to chemotherapy for a tumour of the rectum; bone marrow aspirate. At the top, a micromegakaryocyte with filamentous cytoplasmic projections. From the left, two agranular blast cells; a torn granulocyte precursor in which the coalescent granules form small lakes of hyaline material; another very dysplastic granulocyte. At the top, from the centre towards the right, a promyelocyte with intensely basophilic cytoplasm with a pale Golgi zone and another blast cell adjacent to the edge of the image.

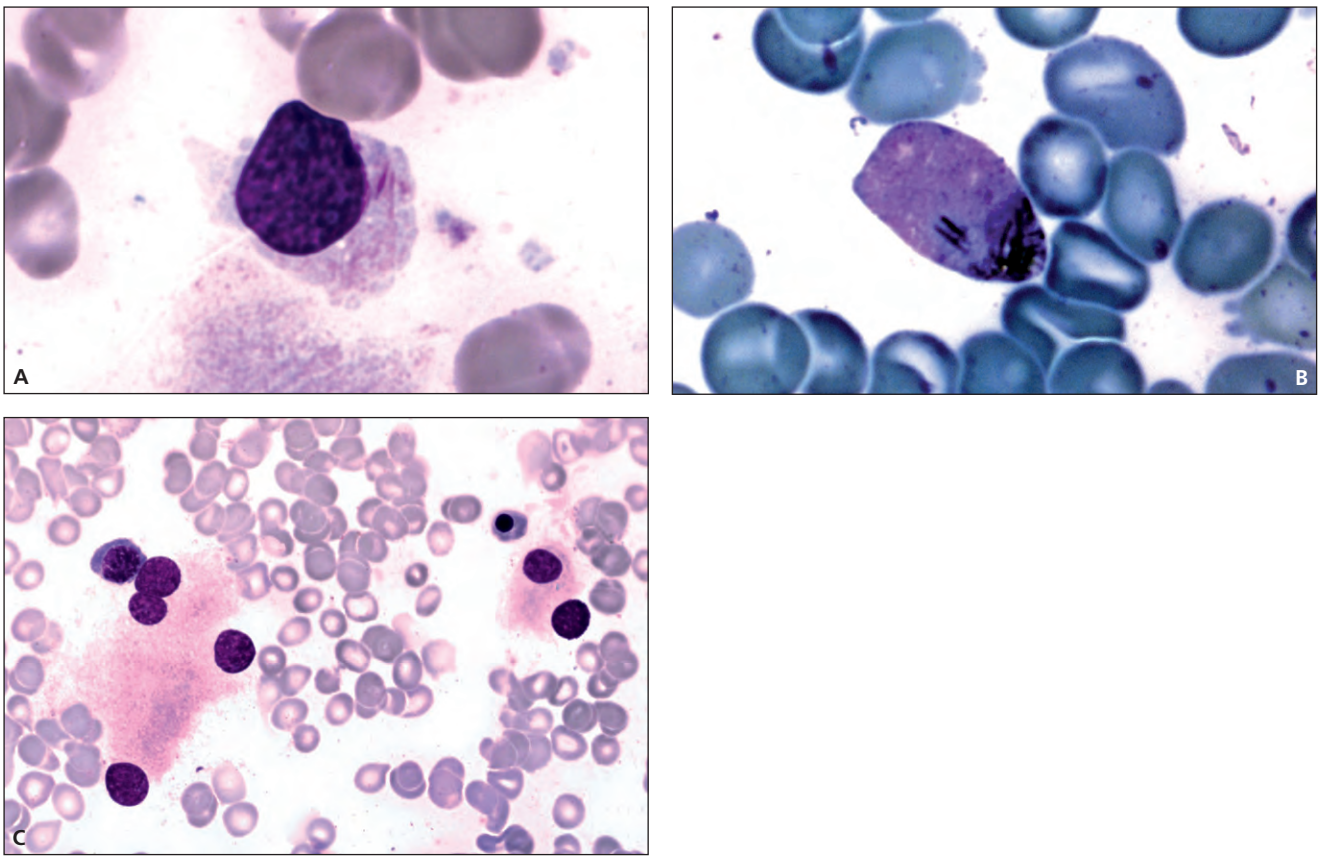



**Fig. 4.123** Therapy-related APL with t(15;17) and features of dysplasia in a patient treated with chemotherapy for carcinoma of the breast. The bone marrow aspirate is very hypocellular. One can recognise abnormal promyelocytes with multiple Auer rods (A) which, as expected, are peroxidase positive (B), and some dysplastic megakaryocytes with multiple separate nuclei (C).

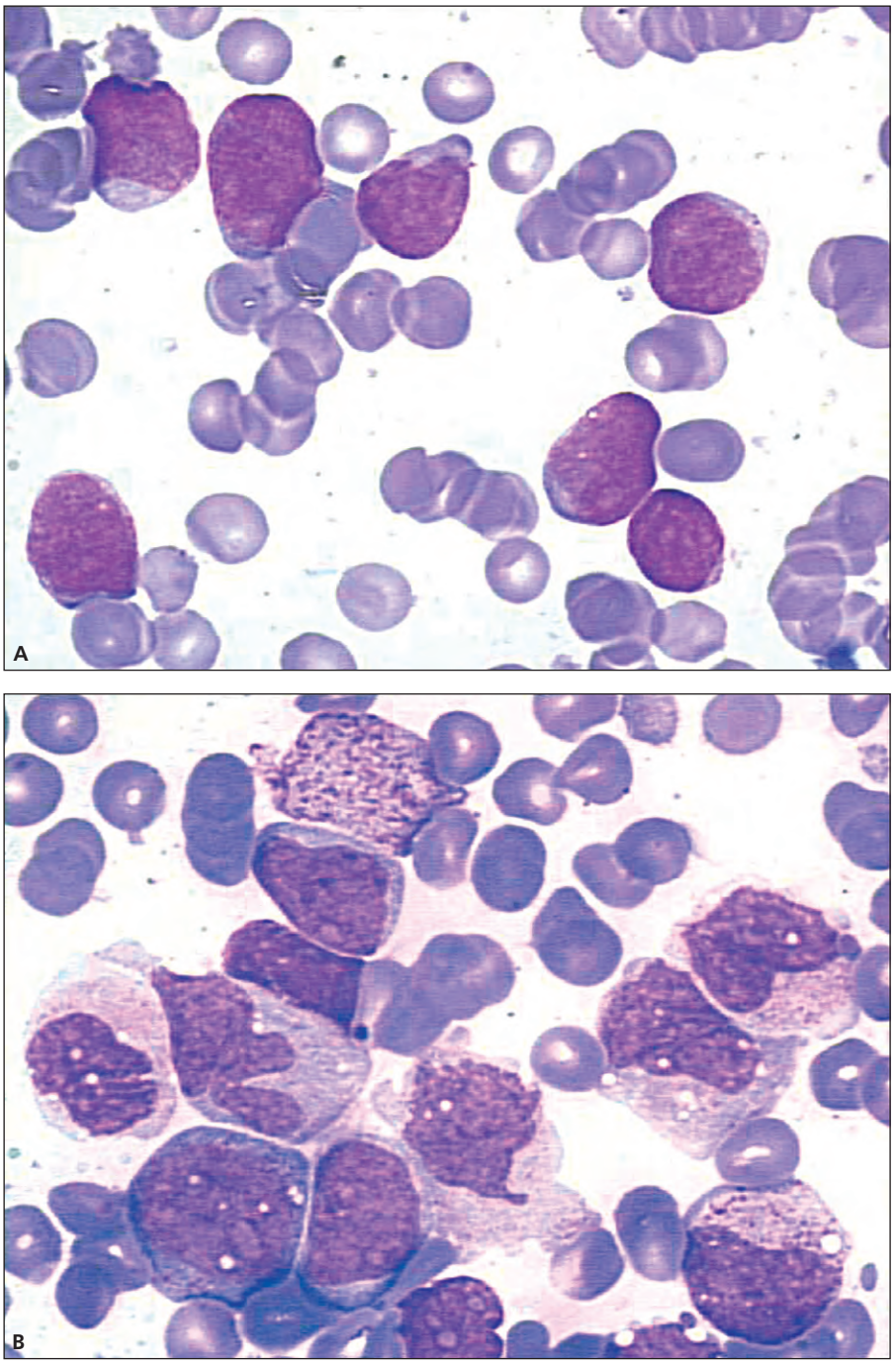



**Fig. 4.124** This patient suffered from acute lymphoblastic leukaemia (ALL), diagnosed in 1991 as FAB L1 ALL, with blast cells positive for CD34, CD19 and CD10. Multiple courses of chemotherapy were given on account of three successive relapses. In 1996, the bone marrow aspirate showed blast cells with rare Auer rods, which were positive for CD34, CD33 and CD15, and negative for lymphoid markers; cytogenetic analysis showed trisomy 8. A diagnosis of chemotherapy-related AMML was made. (A) Bone marrow aspirate on presentation with ALL. The blast cells are small and agranular with a high N:C ratio and homogeneous nuclear chromatin. (B) Bone marrow aspirate five years later, at the time when therapy-related AML was diagnosed. There are heterogeneous blast cells with angulated nuclei and, sometimes, cytoplasmic granules; dysplastic granulocytes; immature monocytes.

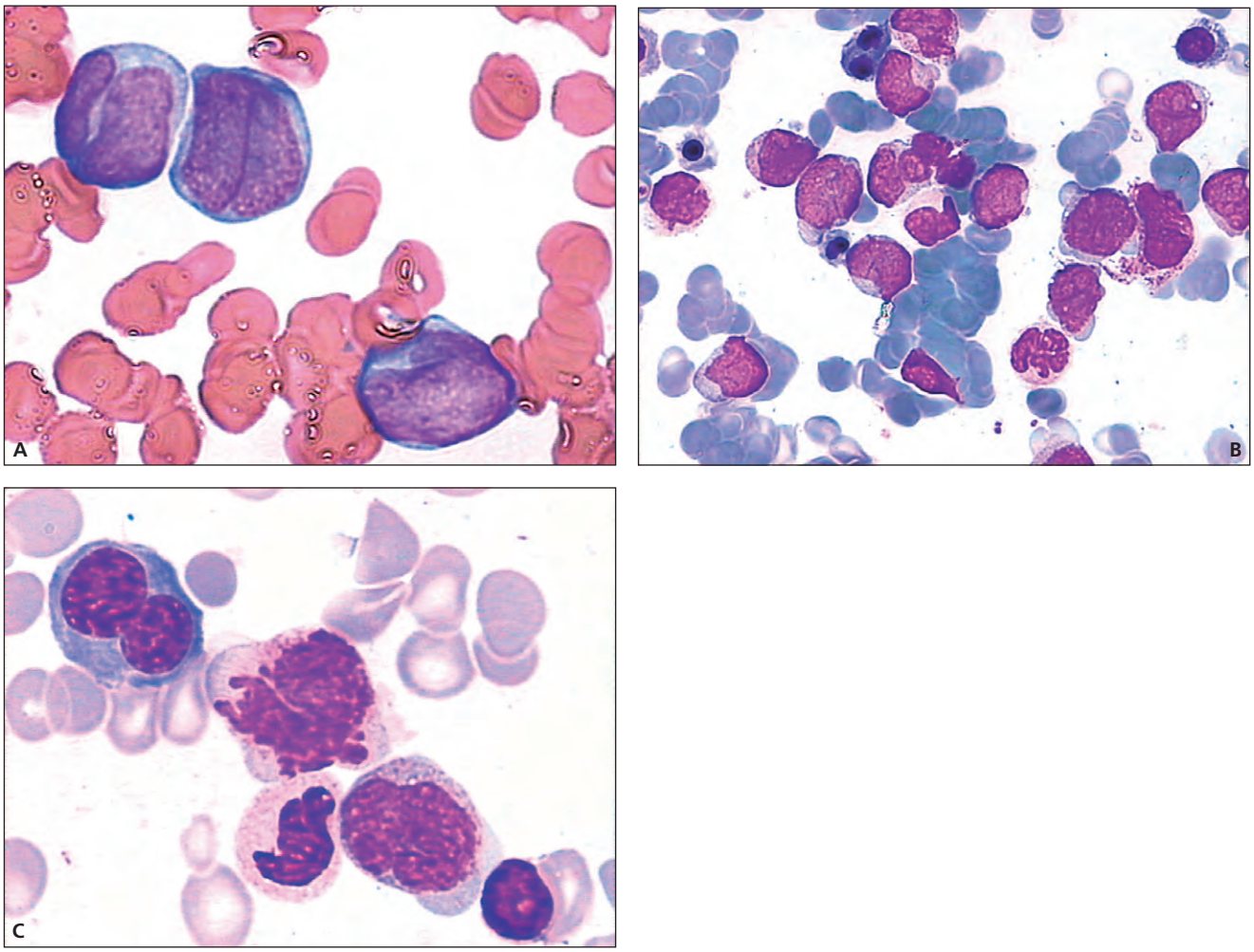



**Fig. 4.125** Another example of AML with myelodysplasia following chemotherapy for carcinoma of the prostate. (A) Agranular blast cells with strange bilobed nuclei in a peripheral blood film. These were positive for granulocytic markers on flow cytometric immunophenotyping. (B) Bone marrow aspirate. Infiltration by blast cells with less bizarre nuclear morphology, only incised or lobated in some cells. Some dysplastic neutrophils and erythroblasts. (C) Bone marrow aspirate. Co-existence of marked dysplasia of the granulocytic series and the erythroid series.

(TAM) and AML, which have been included in a single category, separate from other myeloid neoplasms, in the WHO classification; this classification is based on the sharing of many morphological, immunophenotypic, clinical and molecular characteristics.[172]

Children under the age of five years with Down syndrome have about a 60-fold increased risk of developing AML. The most common form, which includes more than 50% of cases of leukaemia in this age range, is acute megakaryoblastic leukaemia. It is not rare for the leukaemia to be preceded by a preleukaemic myelodysplastic phase, with thrombocytopenia and multilineage dysplasia, similar to refractory cytopenia of childhood: in children with Down syndrome, however, this picture should be recognised as AML even if blast numbers in the blood or marrow do not reach 20%. In the fully developed phase of the disease, the peripheral blood film shows marked anisopoikilocytosis, dacrocytes and giant platelets. The bone marrow aspirate shows frequent hypocellularity and an infiltration by blasts cells with morphological and immunophenotypic feature analogous to those described for AML, NOS, megakaryoblastic. There is multilineage dysplasia. The erythroid series, in particular, is generally definitely megaloblastic. Histology and immunohistochemistry are very important because of the presence of fibrosis. In addition to trisomy 21, the characteristic genetic abnormality in this type of leukaemia, which supports it being assigned to a specific category, is mutation in the *GATA1* gene, which encodes the transcription factor of the same name and has an important pathogenetic role.[173,174] It is necessary to remember, in order not to make diagnostic errors through oversimplification, that children with Down syndrome can also

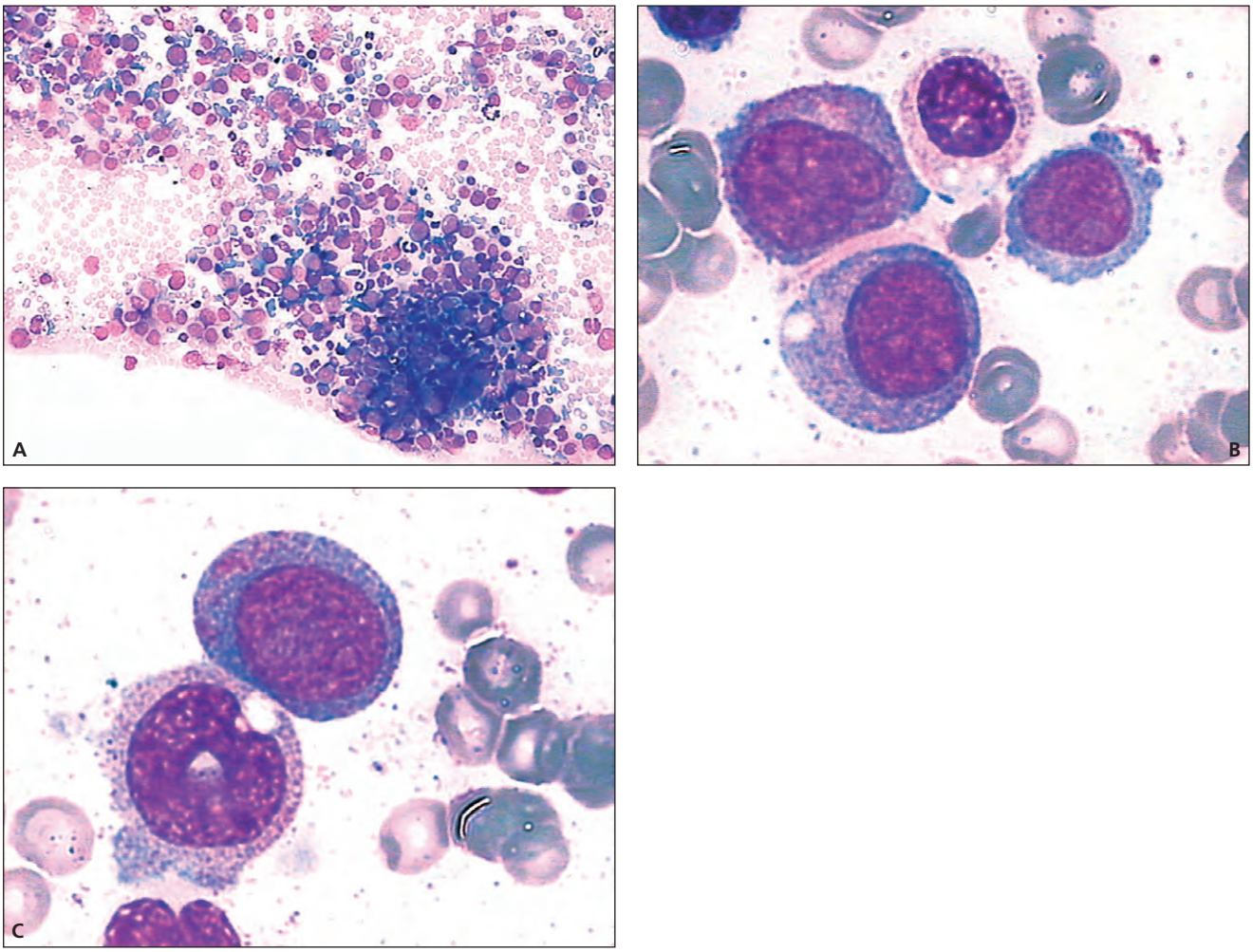



**Fig. 4.126** Therapy-related AML in a patient who had received chemotherapy for non-Hodgkin lymphoma; bone marrow aspirate. (A) The aspirate is cellular with numerous fragments: at low power a certain heterogeneity is seen, with various segmented neutrophils and some erythroblasts. (B) Blasts, mainly granular blasts, are 35% of cells. The neutrophil lineage appears dysplastic. (C) A granular blast cell with an oval nucleus with chromatin condensed into small clumps and multiple nucleoli, one of which is prominent; the cytoplasm is intensely basophilic and contains numerous azurophilic granules; no Golgi zone is apparent. Immediately beneath, a ring granulocyte with a vacuole in the centre of the nucleus and another in the cytoplasm.

develop other types of MDS and AML: a *GATA1* mutation is consistently absent in these cases and the response to therapy is worse.

TAM occurs in around 10% of neonates with Down syndrome and on the basis of the haematological features is not easily distinguishable from AML.[175] It has also been designated 'pseudoleukaemia', but this is inappropriate since it is a clonal neoplastic condition. The full blood count usually shows thrombocytopenia with a marked leucocytosis and a blast percentage that is higher than that in the marrow. The blasts appear undifferentiated, with stippled chromatin, nucleoli and scanty basophilic cytoplasm, which often contains large dark azurophilic granules, similar to those of the basophil lineage and negative for peroxidase and ANAE. The megakaryocytic differentiation of these cells is shown by positivity for PPO and for platelet antigens. Molecular study consistently shows the presence of a *GATA1* mutation. Despite the morphological picture suggesting a serious condition, if the baby survives the potential cardiopulmonary complications, a spontaneous remission occurs within a period of time that varies between two and 14 weeks in the majority of cases.

The existence of this condition means that a diagnosis of AML must be made only with great caution in a neonate with Down syndrome.

### Blastic plasmacytoid dendritic cell neoplasm

This very rare and aggressive neoplasm usually presents either as nodular or plaque-like cutaneous lesions – in which the infiltrate is mainly in the dermis but can extend into the underlying adipose tissue, or as soft tissue or central nervous system

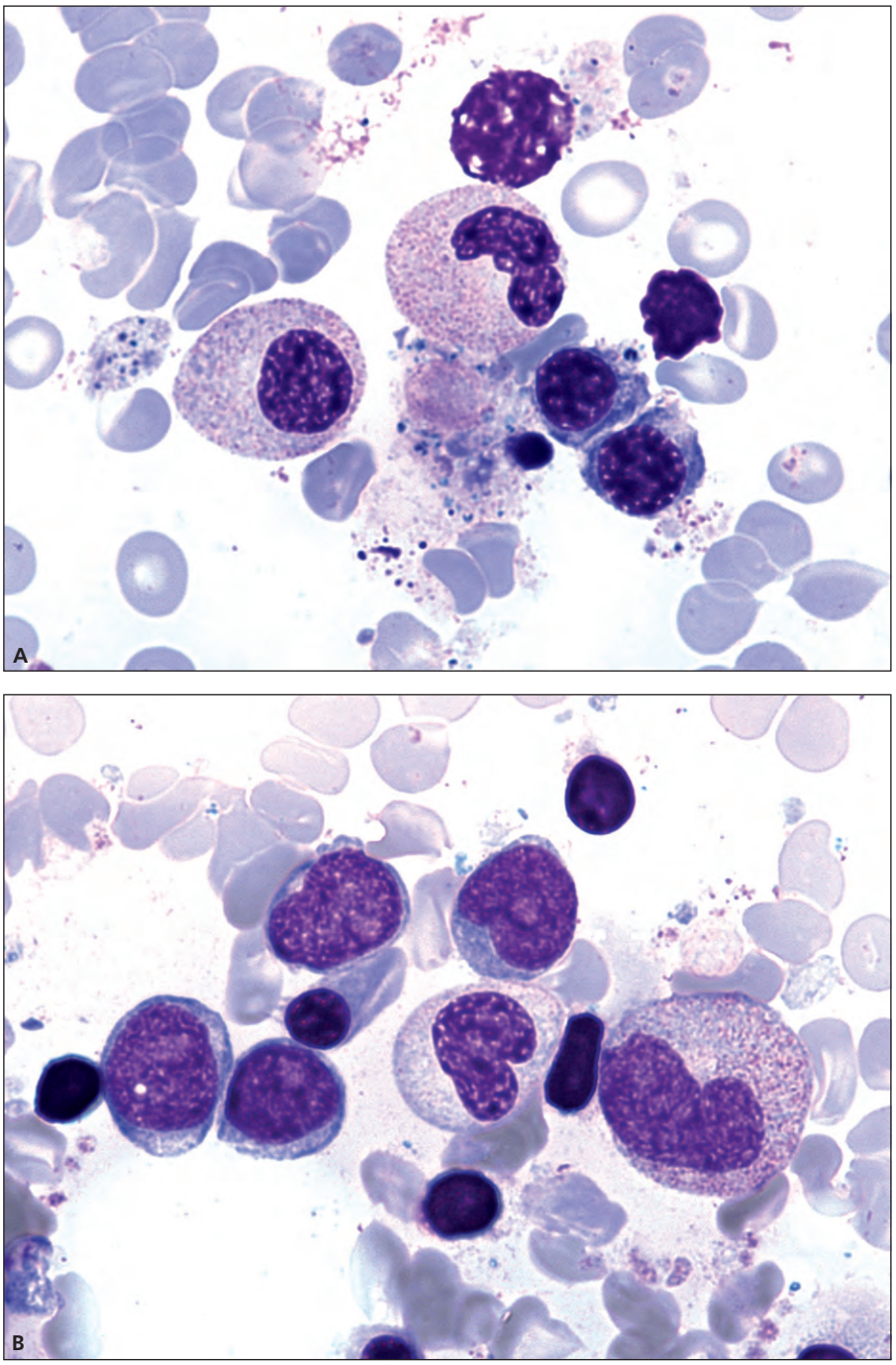



**Fig. 4.127** Therapy-related AML with maturation and multilineage dysplasia in a patient who, six years earlier, had received an autologous haemopoietic stem cell transplant. (A) The granulocytic series is dysplastic. At the top, the nucleus of a macrophage, the lacerated cytoplasm of which is actually seen in the lower part of the image. (B) Four agranular myeloid blast cells, a dysplastic orthochromatic erythroblast, a hypogranular band neutrophil and a metamyelocyte, also dysplastic. At the top, the almost bare nucleus of a micromegakaryocyte.

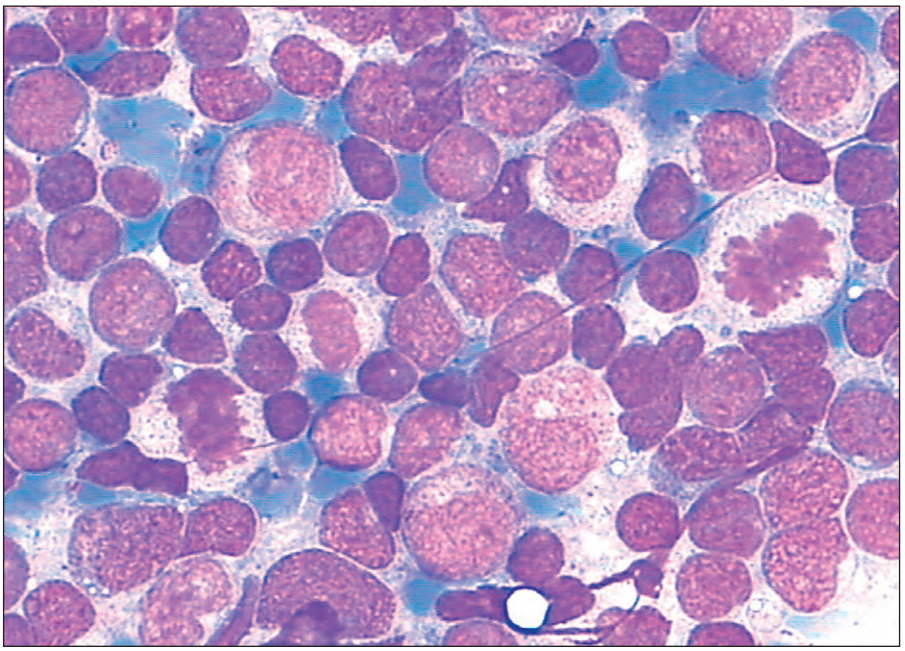
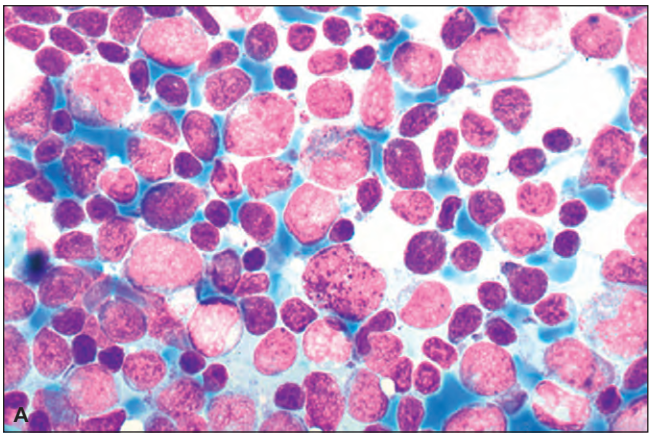
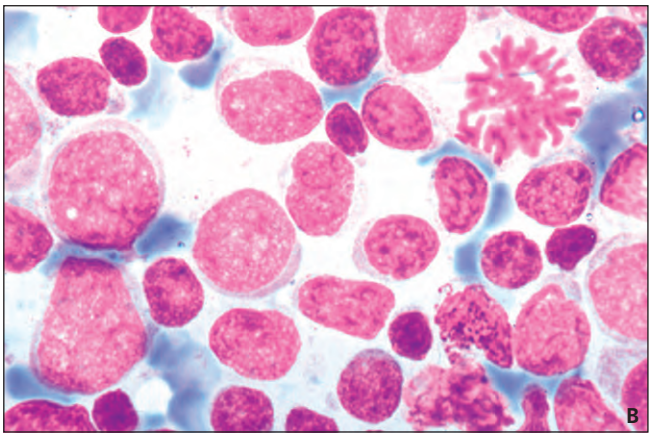


**Fig. 4.128** Myeloid sarcoma localised in a lymph node, observed in accelerated phase of chronic *BCR-ABL1*-positive chronic myeloid leukaemia; imprint preparation after surgical excision. The usual morphology of a lymph node, indicated by the presence of numerous mature lymphocytes, has been altered by the presence of immature granulocytes with plentiful granule-rich cytoplasm and of granular (top right corner) and agranular myeloblasts (top left corner and at the bottom near the right edge). At the bottom in the centre, a promyelocyte with two nucleoli is clearly seen. There are also some mitotic figures.



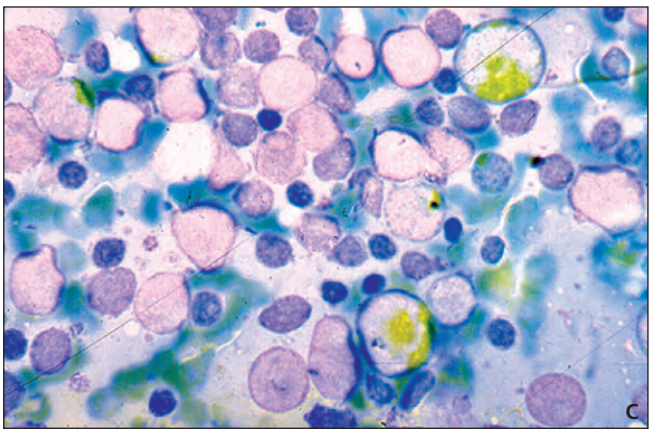




**Fig. 4.129** Myeloid sarcoma localised in a lymph node during the course of AMML with t(16;16); a touch preparation after surgical resection. (A) One observes, admixed with mature lymphocytes, various large blast cells with large, sometimes indented, nuclei with immature chromatin and visible nucleoli. In the centre at the top, an eosinophil myelocyte; a little lower down, a more immature eosinophil progenitor with numerous dark red proeosinophilic granules. (B) At higher power, numerous agranular blast cells, lymphocytes, a small basophil and a mitotic figure. (C) A peroxidase stain shows intense positivity of the granulocytes and focal positivity in some blast cells, e.g. near the top left corner.

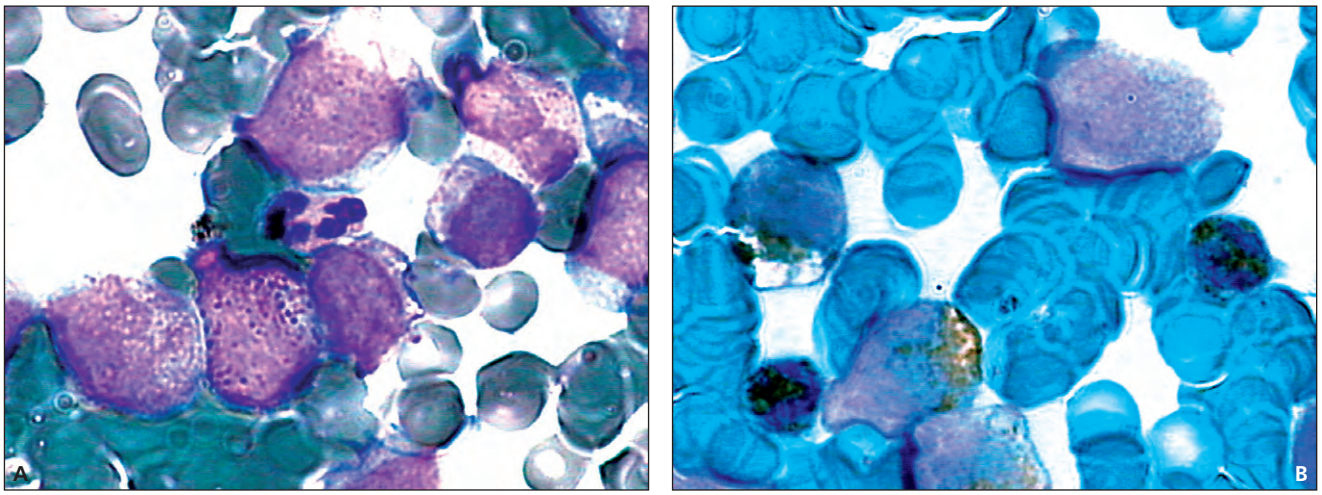



**Fig. 4.130** Cutaneous localisation of myeloid sarcoma with maturation; material obtained by scraping a superficial lesion. (A) There are granular blast cells and immature granulocytes. (B) Peroxidase positivity of the cells that have infiltrated the skin.

disease.[176] Bone marrow invasion is common and can lead to peripheral cytopenia, with circulating blast cells; lymph node infiltration occurs to a lesser extent. Cases have been described in which the disease initially was exclusively in the bone marrow.[177] A bone marrow aspirate usually shows hypercellularity with a relatively monomorphic infiltrate of blast cells, which are sometimes homogeneous with regard to size and sometimes heterogeneous with the presence of small, medium-sized and large cells, with a low or intermediate N:C ratio. The nuclear outline is sometimes regular and sometimes irregular with notches, folding and a tendency to form lobes; the chromatin pattern is blastic, fine and reticular in some cases and more condensed in others, with one or two nucleoli. The cytoplasm is generally ample and basophilic or greyish, with a heterogeneous structure; there are no granules, but sometimes there are numerous vacuoles, corresponding to glycogen deposits, which can form a peripheral corona beneath the surface membrane; it is quite typical for there to be a tendency for the cytoplasm to form long pseudopodial extensions (Figs 4.131 and 4.132).[178,179] Multilineage dysplasia affecting all three haemopoietic series is quite frequent. Similar features can be seen in imprints of bone marrow or lymph node biopsies.

This neoplasm, previously described under the alternative designations of blastic NK cell lymphoma[180] or CD4-positive, CD56-positive haematodermic neoplasm, results from the transformation of a precursor of the plasmacytoid dendritic cells – also known an plasmacytoid monocytes – that are recognised in lymph nodes and activated tonsils, and are capable of producing alpha-interferon; these precursors actually have a multilineage myeloid potential, as is shown by the association of this neoplasm with myeloid neoplasms. The cells are negative with the common cytochemical stains and have a complex immunophenotype in which the co-expression of CD4 and CD56 is typical, variably associated with positivity for CD43, CD45A, CD123, CD68, CD7 and CD33. Non-specific cytogenetic abnormalities are present in a third of cases. Bi-allelic deletion of 9p21.3 has recently been reported as being particularly common.[181]

## Automated analysers and premicroscopic diagnosis of leukaemias

The introduction of sophisticated methods of cellular analysis has improved the approach to blood samples from patients with leukaemia in haematology laboratories that perform automated differential counts. The instruments do not make diagnoses. Their role is to assign cells to the five leucocyte categories normally present in the peripheral blood; in addition, they must indicate, with appropriate, more or less specific, 'flags' or 'alarms', abnormal samples and the type of pathological cell they contain, without necessarily quantifying such cells.

The new technologies explore characteristics of cells different from, but complementary to, those identified by traditional morphology, and sometimes permit original observations, either diagnostic or of research interest. The height of impulses resulting from the passage of cells through analytical chambers of apparatus based on optical or impedance measurements depends mainly on cell size, in addition to a number of secondary variables such as the cell's orientation, shape and refractive index. All the automated instruments that produce a differential count use cell volume as one of the fundamental properties that permit the recognition of cells belonging to various categories, but this is evaluated together with other physical and chemical characteristics of the cell: peroxidase activity, the ability of eosinophil granules to depolarise polarised light, the density, the

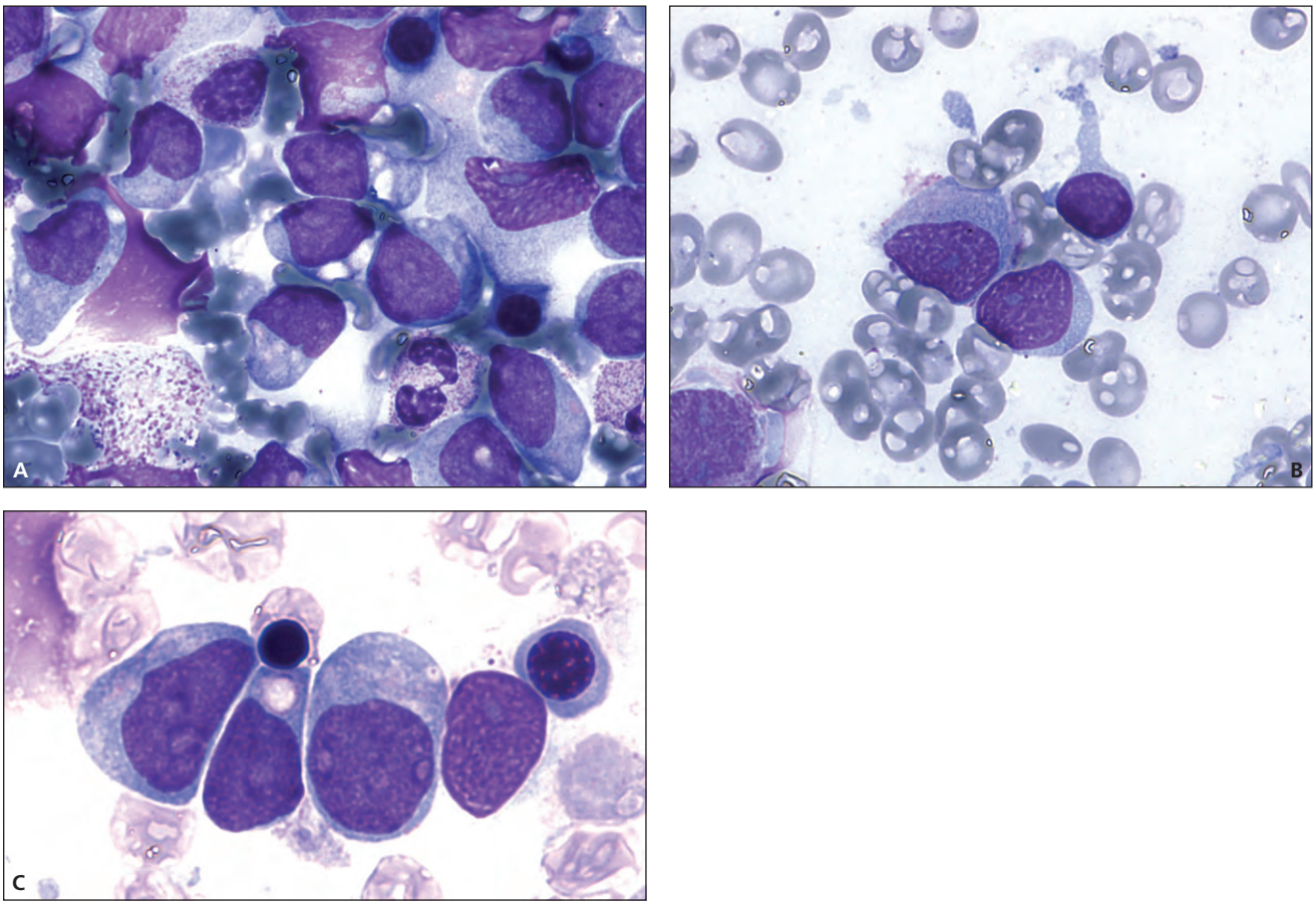



**Fig. 4.131** Blastic plasmacytoid dendritic cell neoplasm; bone marrow aspirate (image courtesy of Dr. Stefano Mancini). (A) The bone marrow is massively infiltrated by medium-sized blast cells with plentiful basophilic cytoplasm, sky-blue in colour, lacking granules but with a reticular pattern, pale circles and, sometimes, large vacuoles. The nuclei are oval or polygonal; the chromatin is compact and reticular, fairly homogeneous (without dark clumps), with clearly seen and sometimes prominent nucleoli. (B) Beside two blast cells with nuclei that have vermicular chromatin and blue, stamped out nucleoli, is a cell with a round nucleus with denser chromatin and cytoplasm that is extending as a long thin pseudopod. (C) Morphology of the blast cells at higher power. Note the delicately woven but compact chromatin with prominent nucleoli, the presence of a large vacuole and the lacy cytoplasm, full of small pale circles.

structural complexity of the cell interior and the content of cytoplasmic granules. These cytological parameters are measured by innovative technology, such as high frequency current, incident laser light, selective cell lysis and fluorochrome staining. Automated instruments are easily able to classify and count lymphocytes, which appear as small, agranular, peroxidase-negative cells with a very simple internal structure, and also neutrophil granulocytes, which are larger with a more complex internal structure resulting from the presence of segmented nuclei and peroxidase-positive granules. Between these two extremes lie a whole range of cells with intermediate properties, such as monocytes and the majority of atypical and immature cells, which each instrument recognises with its own degree of precision and accuracy. Eosinophils are generally well recognised, even though their numbers are low, since they have characteristic properties that lend themselves to measurement by a variety of methods, such as cytochemistry, selective lysis and polarised light. For the basophils, each system applies a specific analytical approach, often very expensive in terms of cost and functionality in relation to a count that is neither very precise nor very accurate.

The analytical accuracy of automated instruments is very high for neutrophils, lymphocytes and eosinophils; there are discrepancies, depending on the analytical method, with regard to the count of basophils and to a lesser extent of monocytes.[182] Based on measurements that are very precise and sufficiently accurate for qualitatively normal samples, automation has on the one hand relieved morphology of the most tedious and least useful of its properties and on the other hand, has increased the clinical utility of the measurements. The great precision of automated methods has found important applications, above all when there is marked leucopenia, with immediate advantages in monitoring haematology patients who have had myeloablative therapy.[183]

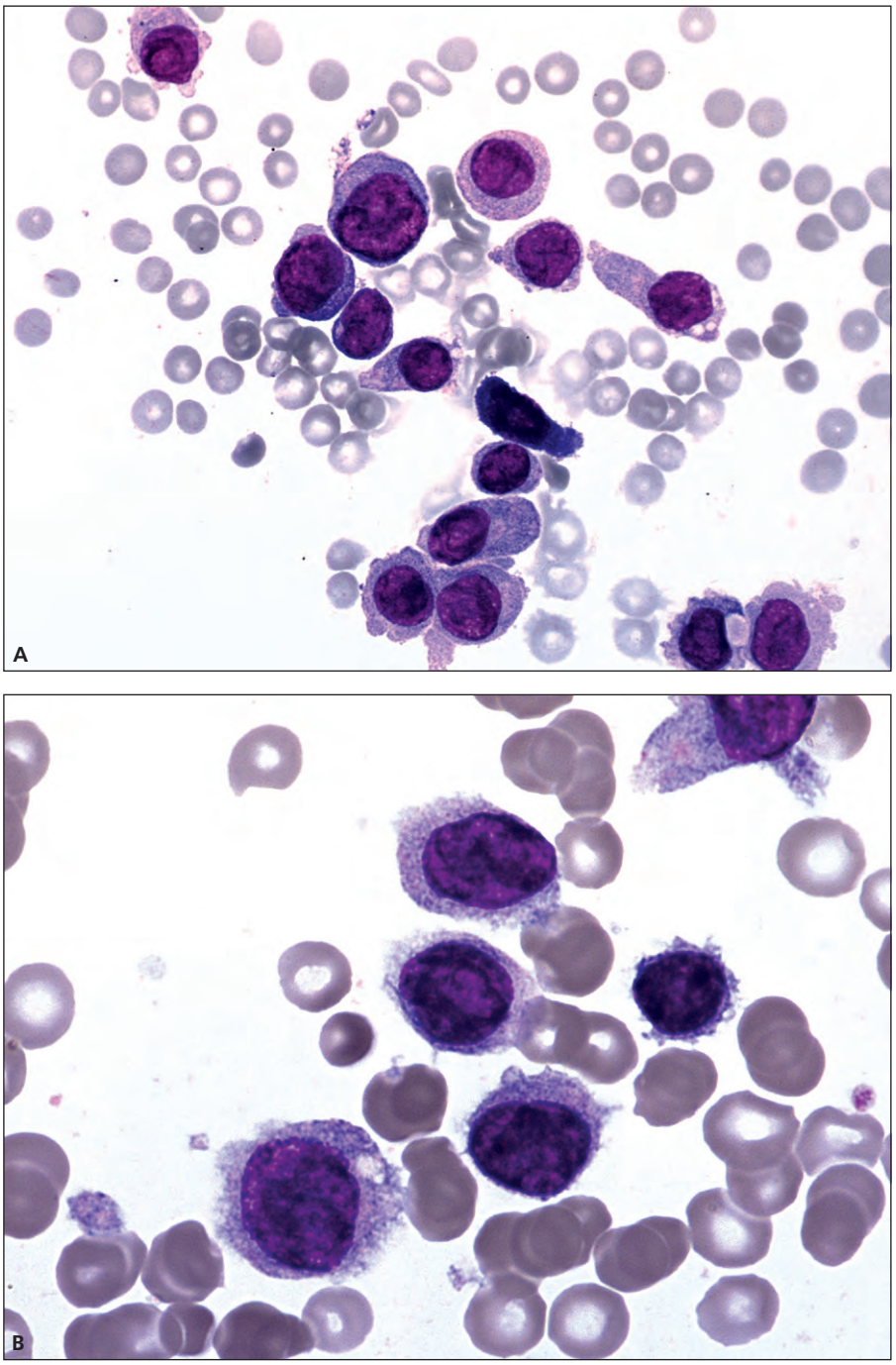



**Fig. 4.132** Blastic plasmacytoid dendritic cell neoplasm; bone marrow aspirate. (A) In a different patient from Fig. 4.131, the population that is infiltrating the marrow is composed of cells with denser chromatin; the cytoplasm sometimes has a violet tinge. There are nucleoli and vacuoles, one of which, in a small cell lower right, is particularly voluminous. Some cells show the cytoplasmic elongation that is typically described in this condition. (B) The nuclei have clumped chromatin and their outlines are sometimes notched. The nucleoli are less visible and not easily distinguished from the pale areas of parachromatin. The cytoplasm is frayed into delicate villous projections and seems to contain very fine granules. (C) In this blast cell, one again sees some of the same morphological features as in Fig. 4.131, such as the abundance of the cytoplasm, its many pale circles, its background colour and the presence of a cell with a round nucleus and cytoplasm deformed into a long projection. Other features are, however, different, such as the tendency of the chromatin to condense into dark clumps, the more convoluted appearance of the nuclei, the fine granules and the numerous short frayed cytoplasmic projections. (D) In this image, a certain cytological heterogeneity stands out: a blast cell with a large nucleus with heterogeneous chromatin has plasmacytoid cytoplasm, which is plentiful and heavily vacuolated; another cell, towards the right, has numerous violet granules; yet another has a quite irregular nucleus and the cytoplasm is bluer than that of the other cells, ultramarine.

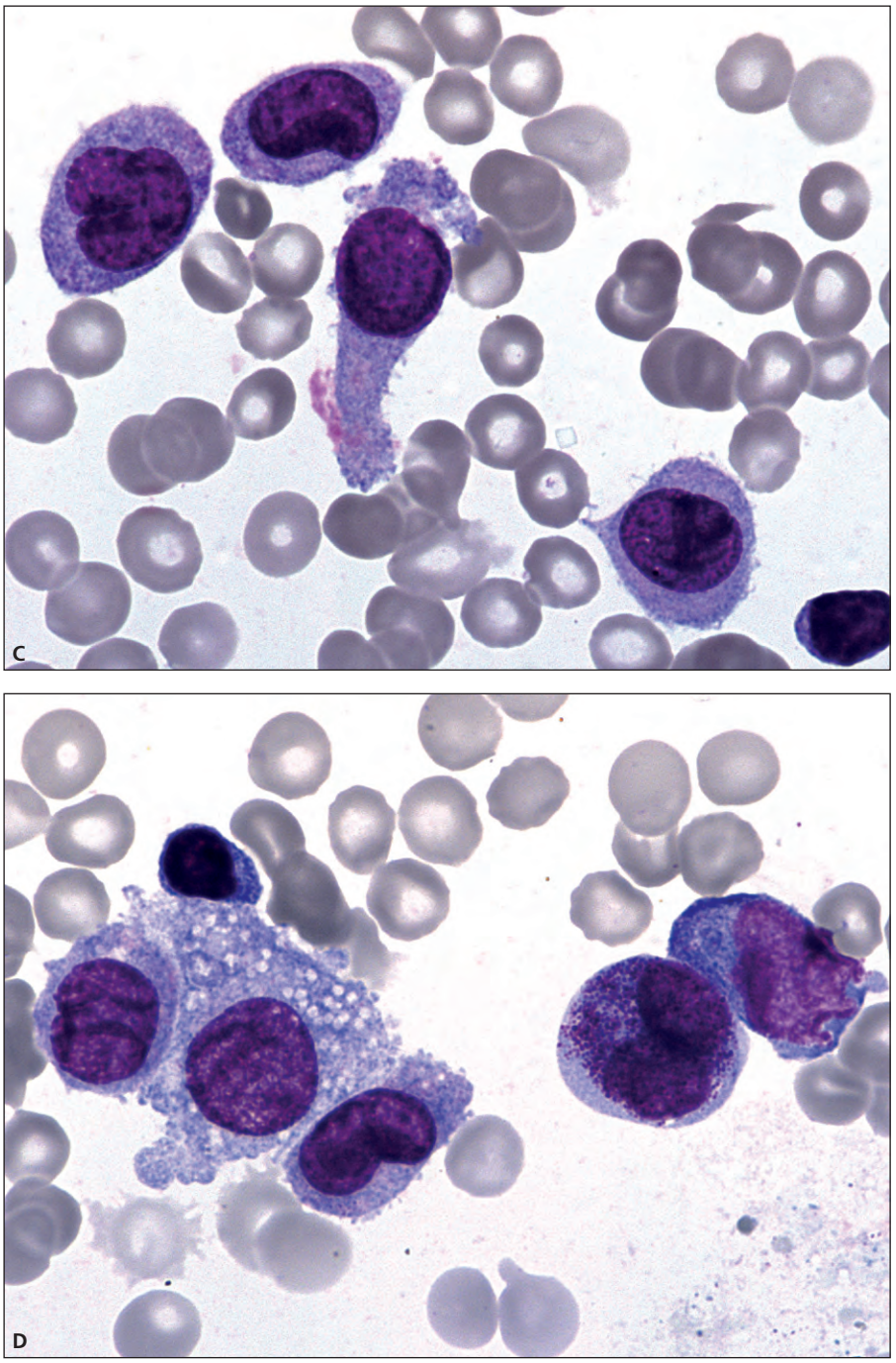



**Fig. 4.132** (*Continued*)

## Recognition of atypical and immature cells

The accuracy of haematological instrumentation cannot yet be considered absolute for the quantitation of cells that do not belong to the five normal leucocyte categories, cells such as blasts, immature granulocytes, atypical lymphocytes and dysplastic cells. Only recently have automated methods for counting erythroblasts been introduced (see Chapter 2). The fundamental role of these systems is to perform an automated differential count and to indicate to the operator those samples that cannot be fully analysed by automated methods and thus require microscopic review.

Automated methods of differential leucocyte counting are not limited to generically flagging samples in which the presence of blast cells, immature granulocytes or other atypical cells

is suspected; they also provide different specific flags indicating the type of abnormality that is suspected. Immature granulocytes, such as promyelocytes, myelocytes and metamyelocytes, lead to a loss of homogeneity of the neutrophil granulocyte population, while blast cells are grouped together in a specific area of the cytogram; these cells, normally not present in the peripheral blood, can be confused, on the basis of their size and the presence of granules, with lymphocytes, monocytes and granulocytes, distorting these populations and being distinguished only on the basis of specific properties, such as their low nuclear density or their resistance to specific lysing agents.[184–186] Various analysers also provide, as an indication to laboratory staff or for research purposes, a numerical evaluation of atypical cells, which should not be included in the report, but is useful for guiding the microscopic evaluation of a blood film and for experimental clinical evaluation, such as the relationship between the automated count of immature granulocytes and the presence of sepsis.[187,188] An evaluation of the sensitivity and specificity of flagging systems of automated analysers, for blast cells in particular, represents one of the more important criteria for judging their clinical utility in patients with any type of leukaemia.[189–197] The distortion of cytological structural parameters analysed by various instruments has recently been used, after transformation into quantitative measurements designated 'positional', for the identification with good sensitivity and efficiency of dysplastic neutrophils with alterations in nuclear morphology, cytoplasmic granularity and peroxidase activity.[198–201]

## Evaluation of atypical cell populations and flags

Leukaemic or dysplastic cell populations tend to produce specific patterns in the cytograms of each instrument that can provide clues for an initial diagnostic approach by microscopy.[202,203] Automated peroxidase cytochemistry permits observations that correlate with leukaemic cells at various stages of differentiation down various pathways.[204–207] The information provided by automated instruments on the characteristics of pathological cell populations in haematological neoplasms also has the potential to be translated, by means of automatic learning applying the principles of neural networks, into diagnostic deductions.[208]

The Siemens ADVIA 120 and 2120 systems represent the most recent improvements in automated flow cytochemistry, initially introduced in 1975. Two of the principal measurements made in their automated channels correspond to classical morphological diagnostic parameters: an evaluation of peroxidase activity, which for more than a century has been used as an additional support to morphology to differentiate various cytological types of acute leukaemia, and that of nuclear chromatin structure, on which the morphologist bases many of his deductions on the maturity or immaturity of the cells being examined. Because of this relationship to classical morphology, the results provided by the analytical channels of the ADVIA systems are particularly easy to compare with the results of microscopy: leukaemic cell populations tend to be distributed in the peroxidase and nuclear density cytograms in a manner that can be interpreted in relationship to our knowledge of cell morphology and function. Consistent images are produced in various types of leukaemia so that they constitute a type of new morphology of cell populations, which can be considered alongside classical morphology of single cells.

The peroxidase channel of the ADVIA system includes a reagent that generates hydrogen peroxide, the substrate of peroxidase, so that the cells that contain the enzyme liberate nascent oxygen which oxidises the chromogen 4-chloro-1-naphthol, forming a dark precipitate that relates to the primary granules. The precipitate is very abundant in eosinophils, well represented in neutrophils, scanty and dispersed in monocytes, and absent in lymphocytes and basophils. The cells stained in this manner are analysed in a flow cell with an optical detection system, which measures scattered light, which is proportional to cell size, and absorbed light, which is proportional to the staining and thus to the peroxidase activity. In this manner each cell generates two items of data relating to the properties analysed: the size and the peroxidase activity. In the graphical representation, the cells fall into compact clusters with homogeneous characteristics (Fig. 4.133A), separated by automatic thresholds. In addition to normal cells, the peroxidase channel also quantifies the percentage of large, peroxidase-negative cells (LUC [large unstained cells]), corresponding, when above the threshold of 4%, to atypical leucocytes, which must be evaluated microscopically and also in relation to the presence or absence of a Blast flag. The MPXI (median peroxidase index) provides information on the median peroxidase activity of the neutrophil population of each sample. Other parameters, not usually reported but useful in the characterisation of anomalous leucocyte populations, are the HPX (high peroxidase) percentage, i.e. cells with high peroxidase activity relative to the median activity of the sample, a generic indication of the heterogeneity of cells of neutrophil lineage, and the percentage of cells 'in saturation', i.e. cells with such strong peroxidase activity that they fall in the final analytical channel on the right of the abscissa.

In the basophil/nuclear density channel, measurements of the scatter of a band of monochromatic light are made by sensors placed at two different angles in relation to the ray of incident light, this providing information on cellular properties: the size, proportional to low angle light scatter (between 0° and 5°), which serves particularly to identify basophils, and the density and conformation of the nuclei of other leucocytes, derived from the quantity of light scattered at a higher angle (between 5° and 15°). The preliminary treatment of the sample in the reaction chamber causes lysis of red cells and of the cell surface membranes of leucocytes, which lose their cytoplasmic contents. Basophils are resistant to the action of the reagent and can be identified by virtue of their preserved size, reflected by measurements high on the Y axis. The nuclei of other leucocytes fall into a continuous group lower down the Y axis, resembling the shape of a tadpole: in the rounded head are found the nuclei of

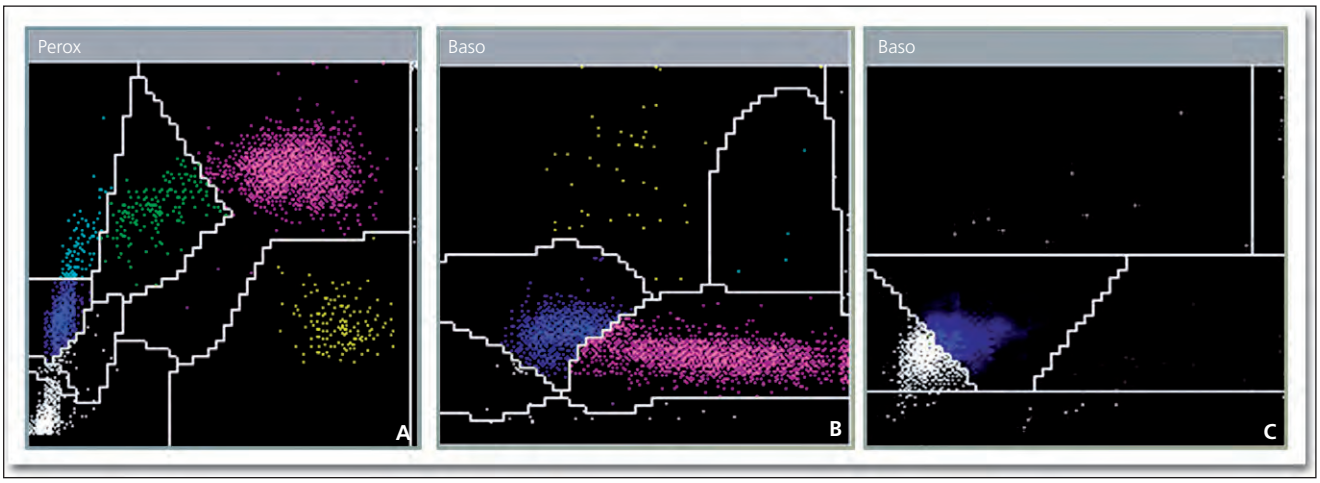



**Fig. 4.133** Morphology of the distribution of cells in the cytograms of the ADVIA system. (A) Peroxidase channel of a normal blood sample. After the cytochemical reaction has occurred, the population of cells is displayed according to their size (Y axis) and their peroxidase activity (X axis), in the following groups, separated by thresholds: background noise (white), lymphocytes (blue), large unstained cells (LUC) (turquoise), monocytes (green), neutrophils (deep pink), eosinophils (yellow). (B) Basophil/nuclear density channel of a normal sample. The nuclei, denuded by the action of the lysing agent, are subdivided along the X axis according to their nuclear density, into a population of so-called mononuclear cells (MN, blue) and polymorphonuclear cells (PMN, deep pink), separated by a valley. The non-lysed basophils are at the top along the Y axis (yellow). This distribution corresponds to the PANDA D0 category. (C) Basophil/nuclear density channel from a patient with AML. The blast cells, because of the low density of their nuclei, are found to the left of the blue MN cluster, and are indicated in white. This distribution corresponds to the PANDA D1 category.

lymphocytes and monocytes, collectively designated mononuclear cells (MN), while in the elongated tail are found the nuclei of neutrophils and eosinophils, collectively designated polymorphonuclears (PMN), which cause increased light scatter at a higher angle because of the marked density of their nuclear chromatin and the irregular conformation of the nuclei (Fig. 4.133B). The nuclei of circulating erythroblasts, with their very compact nuclear chromatin, are found in the right part of the PMN tail. From this analytical channel, the ADVIA system generates various flags. The relationship between the mode of the PMN and MN populations is expressed as the lobularity index (LI), which provides an indication of the degree of nuclear segmentation of neutrophils. The flag for left shift (LSH) of the neutrophil population appears in the presence of a low LI or the loss of the demarcation that normally exists between the head and the tail of the distribution. Blast cells have low density chromatin: this characteristic determines low measurements of high angle light scatter and correlates with the location of blast cells in the left-hand extremity of the head of the MN cluster (Fig. 4.133C). A diagonal threshold, which starts from channel 8 on the X axis, permits a count of low nuclear density signals and thus a quantification of blast cells present in the sample under examination: when the number of signals to the left of this threshold exceeds a predetermined value, the software generates a Blast flag and microscopic review is essential; the percentage value is only for laboratory use.

A comparison of results of analysis in the two leucocyte channels offers very useful information not only for the recognition of possible analytical interference, as for example when there has been inadequate lysis in the peroxidase channel, but also for the recognition of cellular populations that are showing discordant features in the two channels, such as happens particularly with immature granulocytes and erythroblasts or when there is a congenital or acquired peroxidase deficiency. Immature precursors of the granulocyte series – promyelocytes, myelocytes and metamyelocytes – are similar to neutrophils in their cytoplasmic characteristics, including peroxidase activity, and hence are classified as neutrophils in the peroxidase channel, leading only to an increased HPX and, in the case of promyelocytes, to signals in saturation. The nuclei of these cells, however, are not segmented and their chromatin is less dense than that of mature neutrophils: in the basophil/nuclear density channel, therefore, they are classified as MN. For each sample, the system makes a comparison of the sum of neutrophils and eosinophils in the peroxidase channel with the PMN percentage in the basophil/nuclear density channel: a discrepancy of more than 7.5% leads to a flag for immature granulocytes.

## The PANDA 'classification' of cytograms in neoplastic disorders

The ADVIA analytical method for the identification of leucocytes permits a correlation of the manner in which any anomalous leucocyte population is disposed in the cytogram, resulting from their specific properties, with traditional cytomorphological features and with the classification of the disorder. Based on this, and on the consistency of the results in patients with similar disorders, it is possible to trace on the cytograms models of the cellular distribution obtained at the same time as the

quantitative data of the blood count and use this as the basis for a type of classification, designated by the acronym PANDA from the initials of the two methods used (peroxidase activity and nuclear density analysis).[209]

The analysis of the nuclear density in the basophil/nuclear density channel highlights the presence of blast cells with immature, uncondensed chromatin. When these are present in increased numbers, in addition to triggering a blast flag, there is a deformity to the left and downwards of the outline of the usually rounded MN population. This characteristic appearance is seen in the majority of cases of acute leukaemia, regardless of whether they are myeloid or lymphoid, granulocytic or monocytic, more or less differentiated.[189,210] False negatives are more common in ALL in children, as the chromatin density of small lymphoblasts is such that they may fall in the same area as the normal MN population. The morphological abnormality in the cytogram resulting from the reduced nuclear density permits an immediate visual perception of the presence of an anomalous population: this is lacking, however, when the leukaemic cells are differentiated, as in CML and chronic lymphocytic leukaemias (CLL). In the case of CML in particular, the analysis demonstrates the consistent basophilia and the presence of mature granulocytes, while the Blast flag is not triggered in the chronic phase (see Report 5.1); if it becomes positive with deformation of the profile of the MN cluster during the course of disease evolution, it indicates a probable increase in the promyelocyte or blastic population or both, as is observed in the accelerated phase or during blastic transformation. The small lymphocytes of CLL do not deform the MN profile or trigger a Blast flag, which can however occur during prolymphocytic evolution of CLL, in prolymphocytic leukaemia and in hairy cell leukaemia, as a function of the different nuclear characteristics of the nuclei in these conditions. Certain atypical cellular populations are resistant to the action of the lysing agent in the basophil channel, thus causing a pseudobasophilia, with a typical oval cluster being visualised above the horizontal threshold that separates basophils from the nuclei of other leucocytes. This is seen particularly with the atypical cells of non-Hodgkin lymphoma in leukaemic phase and in plasma cell leukaemia (see Report 9.2).

If the measurements made in the basophil/nuclear density channel lead to the suspicion of the blastic nature of a population, it is what happens in the peroxidase channel that provides the basis for a judgement of the type of blast cell and their degree of differentiation. Peroxidase is a faithful marker of the granulocytic nature of a population and provides valuable information on the maturation characteristics. In monocyte populations, on the other hand, peroxidase activity is weak or totally absent in the precursors, monoblasts and promonocytes, and in the mature monocytes is weaker than in all phases of granulocytic differentiation. The distribution of cells that one sees in the peroxidase cytogram is very useful for the initial diagnostic orientation towards an acute or chronic leukaemia. Given that the morphology of anomalous cellular populations in the peroxidase cytogram is determined by cell size and peroxidase activity, it is possible to recognise certain consistent, reproducible, archetypal conformations, designated by the letter P followed by a number between 0 and 6, which express the intensity of the peroxidase reaction as demonstrated in the cytogram (Table 4.3; Fig. 4.134).[211] In the same way, the 'blastic' deformation of the MN population in the basophil/nuclear density channel can be indicated as D1 (when present) and D0 (when absent).

The assembly of all this graphical information constitutes a very informative, rapidly available, diagnostic ensemble, which guides and directs the microscopic morphological analysis, orienting the examination immediately towards the differentiation and degree of maturation of the pathological cells (Table 4.4). The PANDA scheme has been evaluated and scientifically confirmed, with good results for both sensitivity and specificity.[212,213] The PANDA categories do not necessarily indicate a single diagnosis, since diverse leukaemic populations can produce similar graphical representations, and *vice versa*: e.g. the blast cells of AML, NOS with maturation can have variable peroxidase activity, being designated P1, P2, P3 or P4, but they are always categorised as D1 (see Reports 4.1, 4.2, 4.3, 4.4 and 4.5).

It is interesting from both biological and diagnostic points of view that the more frequent subtypes of AML with recurrent genetic alterations identified in the 2008 WHO classification are characterised by a specific and reproducible picture in ADVIA system cytograms: APL, for example, is immediately recognisable from the peroxidase cytogram and is the only type of acute leukaemia that is consistently classified as P6/D1 (see Reports 4.2 and 4.3), while the blast cells of AML with t(8;21) have a sufficiently homogeneous peroxidase activity that they are always classified as P3/D1 (Report 4.1).

**Table 4.3** PANDA classification of haematological disorders: characteristics of the pathological cell populations and morphology of the cytogram. The distribution of the cells in the Perox cytogram defines the P category and the distribution in the Baso cytogram defines the D category

| Peroxidase activity (Perox cytogram) | | | Nuclear density (Baso cytogram) | | |
|---|---|---|---|---|---|
| P0 (a, b) | Absence of peroxidase activity | Large peak or fused with LUC, with a pointed tip, vertical or slightly inclined to the right. The upwards extension reflects cell size (and co-incidence)<br>P0a Pointed peak of variable height, in continuity with lymphocytes<br>P0b Tall fusiform peak, not in continuity with lymphocytes | D0 | Mononuclear cells with normal nuclear density | Regular oval cluster, rounded to the left, not exceeding thresholds |
| P1 | A few cells with weak peroxidase activity<br>Early or partial myeloid differentiation | LUC peak shorter and inclined to the right, with the tip often rounded. The right vertical margin is not distinct because of scattering of signals towards the centre of the cytogram | D1 | Mononuclear cells with reduced nuclear density (blasts) | Deformed cluster, tending to be triangular, with the apex stretched down and to the left. Numerous signals cross the blast threshold and are represented in white |
| P2 | Low or moderate, homogeneous peroxidase activity<br>Intermediate level of myeloid differentiation | A more or less homogeneous cell cluster extends from the LUC area into the monocyte area and partially invades the neutrophil area | (psb) | Pseudobasophilia (add a D1 or D2) | Presence of signals that occupy the basophil area but in an atypical position |
| P3 | Medium to strong homogeneous peroxidase activity<br>Clear and synchronised granulocytic differentiation | Cells form a compact oval group with a fairly clear outline, found on the margin of the lower parts of the monocyte and neutrophil areas | | | |
| P4 | Medium to strong heterogeneous peroxidase activity<br>Advanced heterogeneous granulocytic differentiation | Cells form a large oval or triangular group with the base dispersed to the right, which extends from the monocyte area, invading the neutrophil area | | | |
| P5 | Very strong peroxidase activity, large size<br>Advanced atypical granulocytic differentiation | The cells cluster in the top right corner of the cytogram, usually remaining dispersed, 'in saturation' | | | |
| P6 | Very strong homogeneous peroxidase activity<br>Promyelocytic differentiation | A homogeneous cluster with the right margin 'in saturation'<br>The strong staining causes a reduction in the scatter signal, reducing the apparent size of the cells, which in part occupy the eosinophil area | | | |
| PM | Multiple populations with different levels of peroxidase activity (P0/P1, P0/P2, P0/P3, etc.) | Variable combinations of the above features. The most frequent image is in the form of a V which joins a fusiform LUC cluster to a group of peroxidase-positive cells | | | |

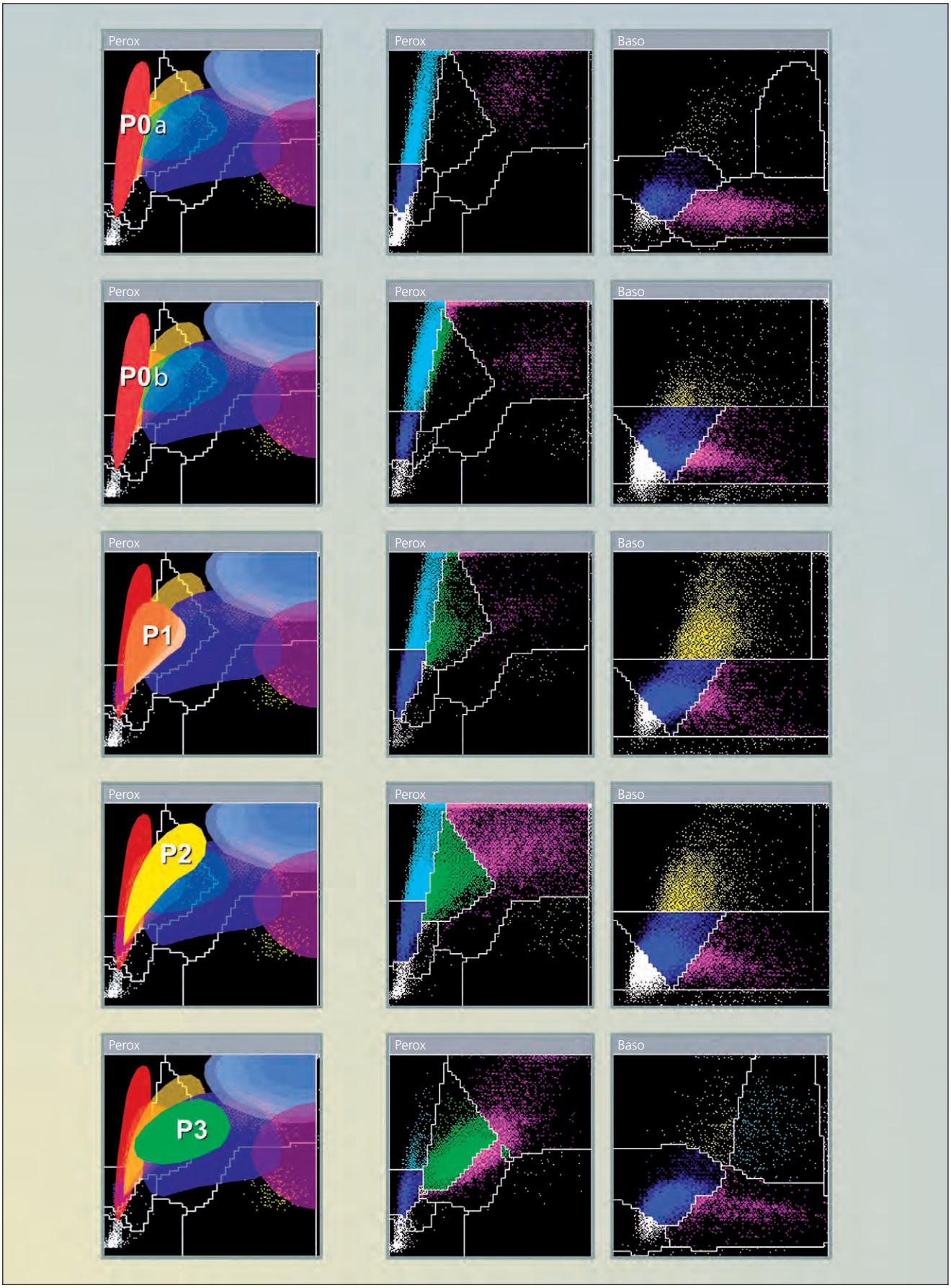



**Fig. 4.134** Morphology of anomalous cellular populations in the peroxidase channel of the ADVIA system: on the left, schematic representation of the P category, according to the PANDA 'classification'; on the right, cytograms of the peroxidase channel and the nuclear density channel of blood samples with the corresponding P distribution.

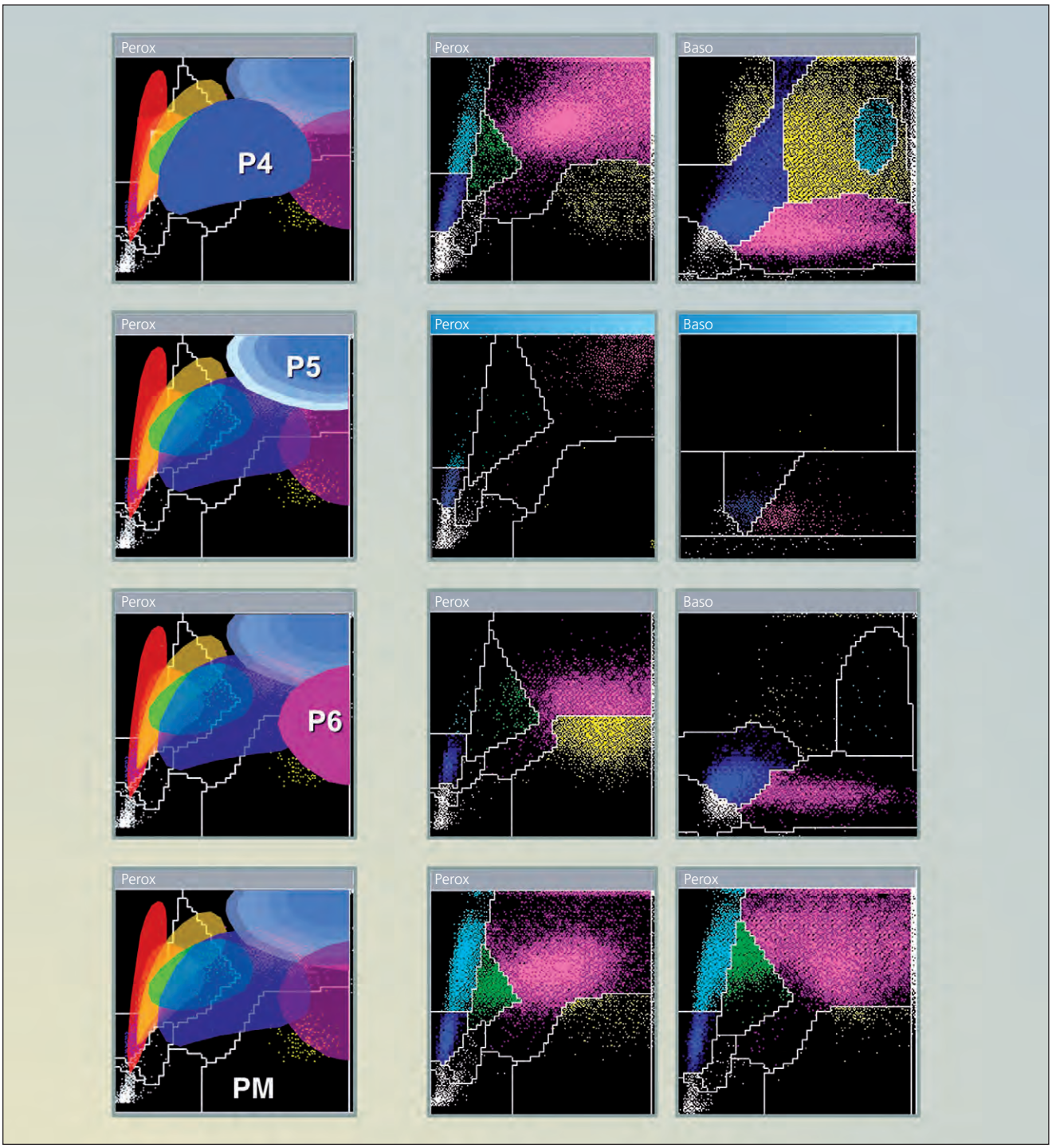
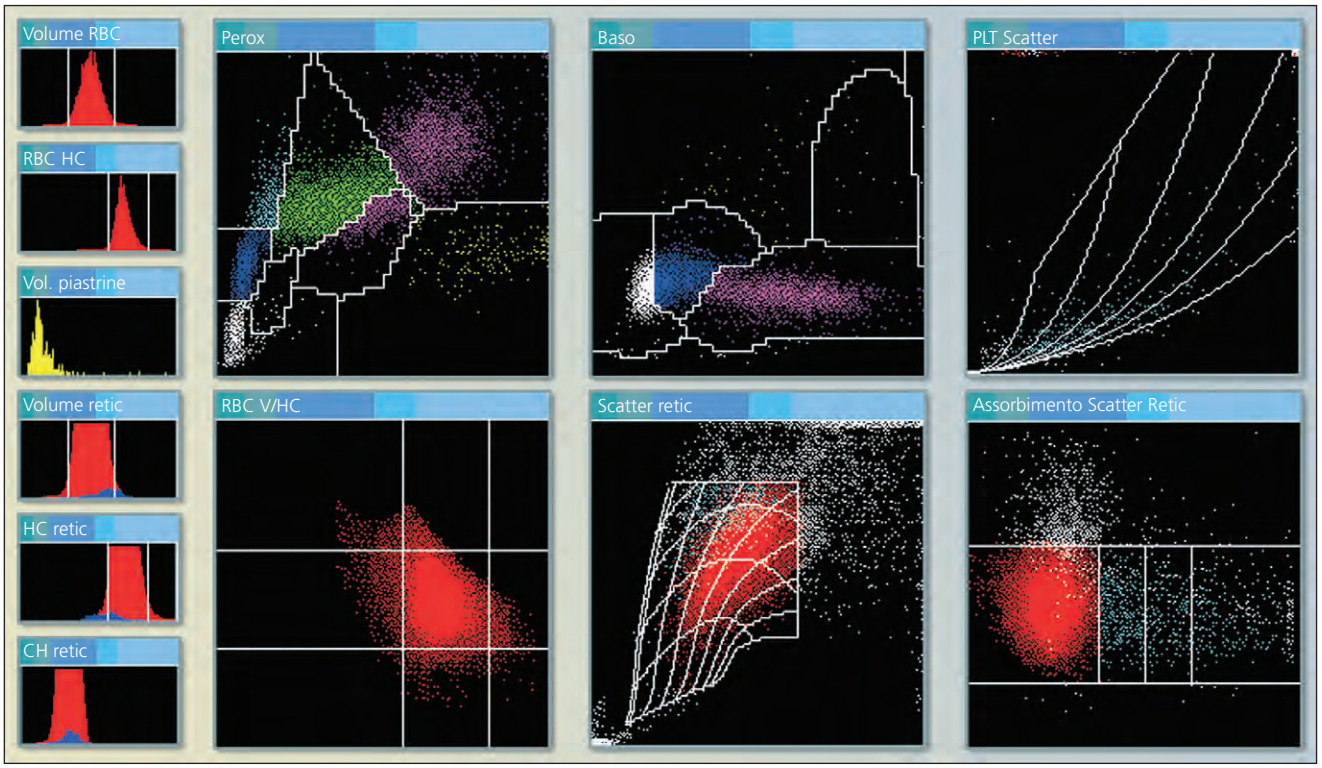



**Fig. 4.134** (*Continued*)

**Table 4.4** PANDA classification of haematological disorders: matrix table. Some conditions can fall into more than one category, depending of the variation in characteristics of the pathological cells

| | D0 | D1 |
|---|---|---|
| P0a | Chronic lymphocytic leukaemia, other lymphoproliferative syndromes, some leukaemia/lymphoma syndromes and rare lymphoblastic leukaemias, infectious mononucleosis | Lymphoblastic leukaemia, leukaemia/lymphoma, acute myeloid leukaemia (AML) with minimal or no differentiation, AML with erythroid or megakaryocytic differentiation |
| P0b | Total peroxidase deficiency | Monoblastic leukaemia, sometimes AML without maturation |
| P1 | Partial peroxidase deficiency | AML with little differentiation, acute monoblastic and monocytic leukaemia, erythroleukaemia |
| P2 | – | AML with granulocytic or monocytic differentiation, some acute monocytic leukaemias, chronic myelomonocytic leukaemia |
| P3 | – | AML with granulocytic differentiation associated with t(8;21) |
| P4 | Chronic myeloid leukaemia (CML) | AML with granulocytic differentiation, CML in blast crisis |
| P5 | AIDS, myelodysplastic syndrome with neutrophil dysplasia, atypical chronic myeloid leukaemia | AML with granulocytic maturation, microgranular variant of acute promyelocytic leukaemia (APL) (sometimes) |
| P6 | – | Hypergranular APL (always), microgranular variant of APL (sometimes) |
| PM | Chronic myelomonocytic leukaemia | Acute myelomonocytic leukaemia, chronic myelomonocytic leukaemia, CML in blast crisis, some AML with granulocytic differentiation |

## REPORT 4.1 Acute myeloid leukaemia with recurrent genetic abnormality t(8;21)



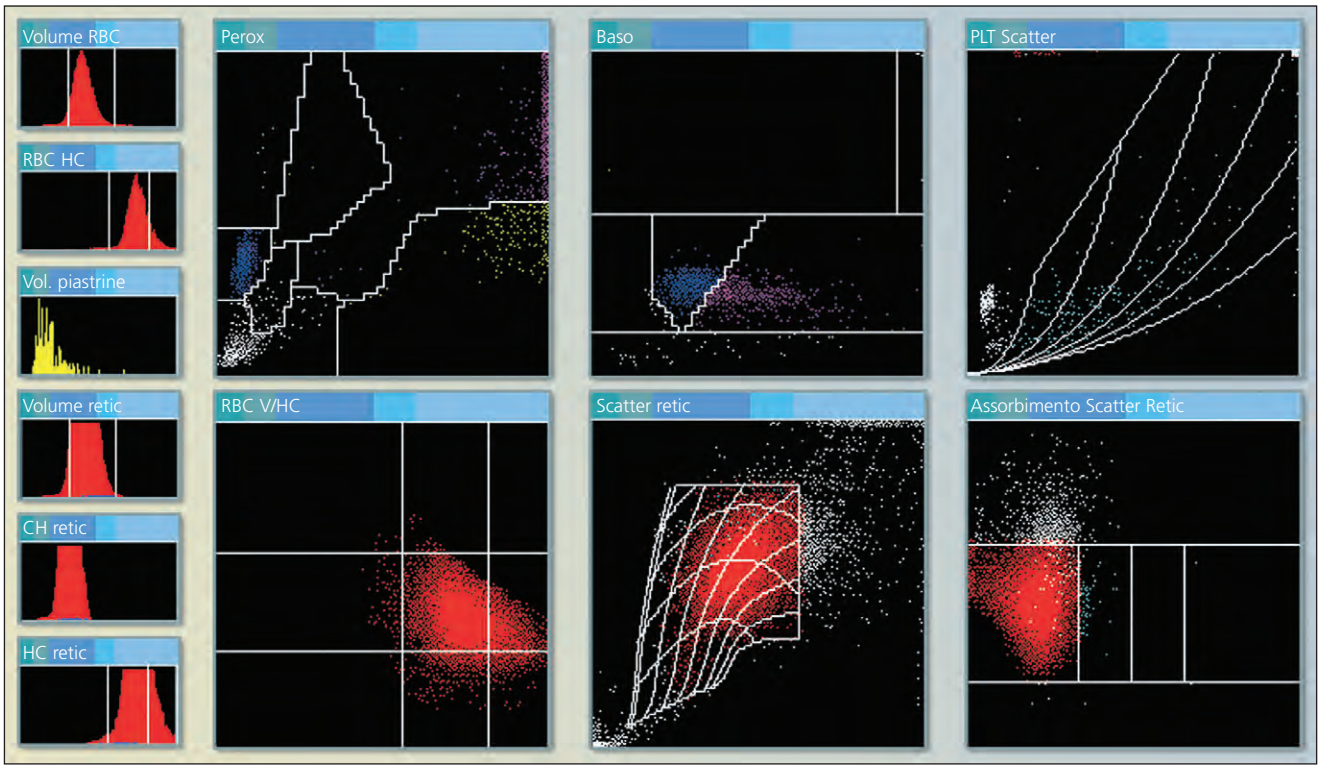

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 148 | g/l | |
| RBC | 4.98 | $\times 10^{12}$/l | |
| HCT | 0.449 | l/l | |
| MCV | 90.2 | fl | |
| MCH | 29.7 | pg | |
| MCHC | 330 | g/l | |
| CHCM | 332 | g/l | |
| RDW | 15.1 | % | |
| HDW | 2.73 | g/dl | |
| HYPO | 1.8 | % | |
| HYPER | 0.8 | % | |
| MICRO | 1.0 | % | |
| MACRO | 1.6 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 1.48 | % | |
| RET | 73.8 | $\times 10^{9}$/l | |
| CHr | 31.0 | pg | |
| IRF (M+H) | 51.9 | % | |
| PLT | 47 | $\times 10^{9}$/l | |
| MPV | 8.6 | fl | |
| PDW | 66.1 | % | |
| Large platelets | 1 | $\times 10^{9}$/l | |
| MPC | 24.9 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 8.74 | | * |
| WBC Perox | 8.17 | | * |
| NEUT | 2.84 | 42.5 | * |
| LYMPH | 1.48 | 17.0 | * |
| MONO | 3.81 | 43.6 | * |
| EOS | 0.23 | 2.6 | * |
| BASO | 0.06 | 0.7 | * |
| LUC | 0.31 | 3.6 | |
| NRBC | 0.45 | 5.1 | * NRBC + |
| LI | 1.88 | | |
| MPXI | −6.3 | | |
| MN | | 76.7 | IG +++ |
| PMN | | 22.3 | |
| BLASTS | | 27.1 | Blasts +++ |
| Perox HPX | | 0.6 | |
| Perox SAT | | 0.0 | |

Incidental detection of thrombocytopenia in an occupational medical check-up. White cell count, haemoglobin concentration, red cell indices and reticulocyte percentage within normal limits; the IRF was increased, an unusual observation in the absence of anaemia. There is relative neutropenia and relative and absolute monocytosis. All values are indicated by asterisks and there are high level flags for Blasts and IG, with NRBC+. The Perox cytogram shows a central compact oval cluster, which is crossed by the line that indicates the lower threshold of the monocyte box. For the major part, the instrument software has classified these as monocytes (green) and to a minor extent as neutrophils. The picture is typical of the PANDA P3 category. In the Baso cytogram, the MN/PMN valley is conserved, but the MN head is deformed downwards and to the left.

**Morphology.** Neutrophils 18.5%, lymphocytes 15.5%, monocytes 4.0%, eosinophils 3.5%, basophils 0.5%, blasts 58%, peroxidase positive, with morphology typical of AML with t(8;21). The IG flag results from the fact that the cells of the blast population have all been classified as MN in the Baso channels, while being classified in part as neutrophils in the Perox channel. Bone marrow cellularity is increased. The morphological picture is shown in Fig. 4.22. The cytogram morphology (PANDA P3,D1) has high predictivity not only for this microscopic picture, but also for this genetic anomaly. The increased IRF is largely due to the very strong signals that are seen in saturation in the Absorption Scatter Retic cytogram.

## REPORT 4.2 Pancytopenia at presentation of acute promyelocytic leukaemia with t(15;17) and FISH positive for *PML-RARA*, in a suspected carrier of hereditary spherocytosis



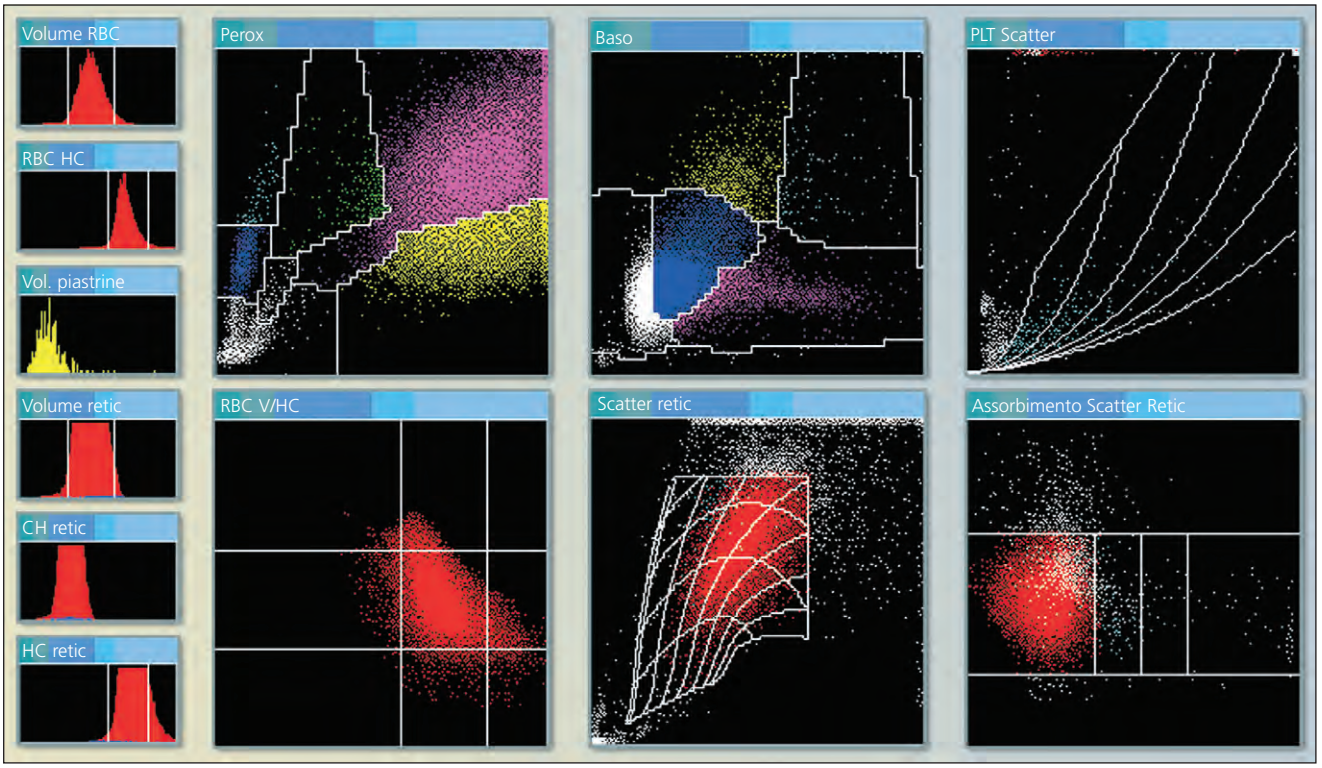

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 81 | g/l | |
| RBC | 2.77 | $\times 10^{12}$/l | |
| HCT | 0.221 | l/l | |
| MCV | 79.8 | fl | |
| MCH | 29.3 | pg | |
| MCHC | 368 | g/l | |
| CHCM | 378 | g/l | |
| RDW | 15.1 | % | |
| HDW | 324 | g/dl | |
| HYPO | 03 | % | |
| HYPER | 123 | % | Hyperchromia +++ |
| MICRO | 38 | % | Microcytosis + |
| MACRO | 01 | % | |
| RBC fragments | 001 | $\times 10^{12}$/l | |
| RBC ghosts | 005 | $\times 10^{12}$/l | |
| RET | 017 | % | |
| RET | 48 | $\times 10^{9}$/l | |
| CHr | 341 | pg | |
| IRF (M+H) | 23 | % | |
| PLT | 27 | $\times 10^{9}$/l | |
| MPV | 113 | fl | |
| PDW | 439 | % | |
| Large platelets | 1 | $\times 10^{9}$/l | |
| MPC | 234 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 1.17 | | |
| WBC Perox | 1.25 | | |
| NEUT | 0.49 | 41.7 | |
| LYMPH | 0.48 | 40.7 | |
| MONO | 0.00 | 0.3 | |
| EOS | 0.19 | 16.6 | |
| BASO | 0.00 | 0.1 | |
| LUC | 0.01 | 0.6 | |
| NRBC | 0.00 | 0.0 | |
| LI | – | | * B-NV |
| MPXI | 44.5 | | |
| MN | | 52.2 | IG +++ |
| PMN | | 41.4 | |
| BLASTS | | 5.3 | Blasts + |
| Perox HPX | | 36.7 | |
| Perox SAT | | 25.5 | |

Severe trilineage cytopenia in a patient with a haemorrhagic syndrome resulting from disseminated intravascular coagulation. The MCV is at the lower limit of normal, while the MCHC, CHCM and proportion of hyperchromic cells are much increased. This picture is suggestive of hereditary spherocytosis, even though the reticulocyte count is low. There is marked leucopenia and, apart from a small population of lymphocytes, all signals in the Perox channel fall into the neutrophil and eosinophil areas and are crowded together in the area of saturation, with very high values for MPXI, HPX and SAT. This is the distribution of PANDA P6, which is specific for APL. The Baso cytogram shows a thin PMN tail. The IG flag results from the numerical discrepancy between the percentage of peroxidase-positive cells and the percentage of neutrophils in the Baso channel. The asterisks result from the absence of a MN/PMN valley (B-NV flag).

**Morphology.** The instrumental lymphocyte count is correct; the remainder of the cells are pathological promyelocytes with distorted pyknotic nuclei, sometime with Auer rods. The recurrent genetic abnormality, t(15;17), already anticipated from the P6 Perox cytogram, is confirmed by cytogenetic analysis and FISH.

## REPORT 4.3 Microgranular variant of acute promyelocytic leukaemia with t(15;17) and FISH positive for *PML-RARA*



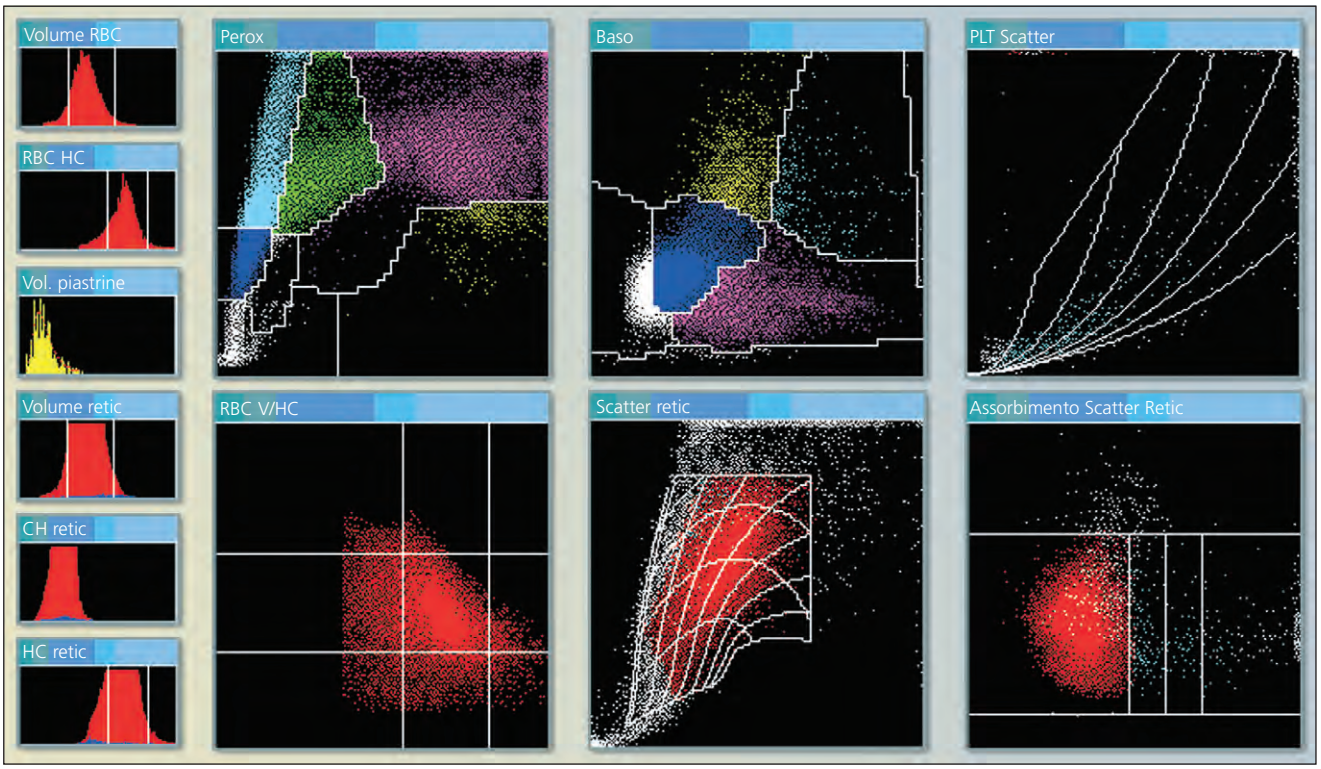

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 113 | g/l | |
| RBC | 3.54 | $\times 10^{12}$/l | |
| HCT | 0.323 | l/l | |
| MCV | 91.4 | fl | |
| MCH | 31.9 | pg | |
| MCHC | 349 | g/l | |
| CHCM | 341 | g/l | |
| RDW | 17.0 | % | Aniso + |
| HDW | 3.16 | g/dl | |
| HYPO | 1.4 | % | |
| HYPER | 2.6 | % | |
| MICRO | 1.4 | % | |
| MACRO | 3.2 | % | Macro + |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 0.29 | % | |
| RET | 10.3 | $\times 10^{9}$/l | |
| CHr | 32.1 | pg | |
| IRF (M+H) | 27.0 | % | |
| PLT | 35 | $\times 10^{9}$/l | |
| MPV | 11.5 | fl | |
| PDW | 47.8 | % | |
| Large platelets | 2 | $\times 10^{9}$/l | |
| MPC | 19.5 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 72.88 | | |
| WBC Perox | 69.56 | | |
| NEUT | 58.60 | 80.4 | |
| LYMPH | 0.49 | 0.7 | |
| MONO | 0.35 | 0. 5 | |
| EOS | 12.35 | 16.9 | |
| BASO | 0.97 | 1.3 | |
| LUC | 0.12 | 0.2 | |
| NRBC | 0.00 | 0.0 | |
| LI | 1.67 | *, B-NV | |
| MPXI | 25.8 | | |
| MN | | 95.0 | IG +++ |
| PMN | | 3.4 | |
| BLASTS | | 22.6 | Blasts +++ |
| Perox HPX | | 0.0 | |
| Perox SAT | | 18,6 | |

Marked leucocytosis: the cells show an atypical distribution and have been classified in the Perox channel as neutrophils and eosinophils. The cytogram shows a large, compact population, strongly peroxidase positive to the extent of saturation, that occupies the neutrophil and eosinophil areas. The reduced chromatin density is shown by the alteration of the head of the MN cluster in the Baso channel. This is a PANDA P6, D1 distribution and is pathognomonic for APL. The flags for Blast and IG underline that the cytological classification of the instrument are not accurate. Through co-incidence, the blastic population invades the basophil area. There is reticulocytopenia. The platelet count is greatly reduced and the platelets are large.

**Morphology.** In the blood film one observes that 94% of white cells are abnormal promyelocytes showing the typical morphology of the microgranular variant of APL, with intense peroxidase activity, which is found also in the bone marrow aspirate. The diagnosis, and the predictivity of such a characteristic instrumental picture, is confirmed by cytogenetic and molecular analysis.

## REPORT 4.4 Acute myeloid leukaemia with myelodysplasia-related changes (evolution from a myelodysplastic syndrome)



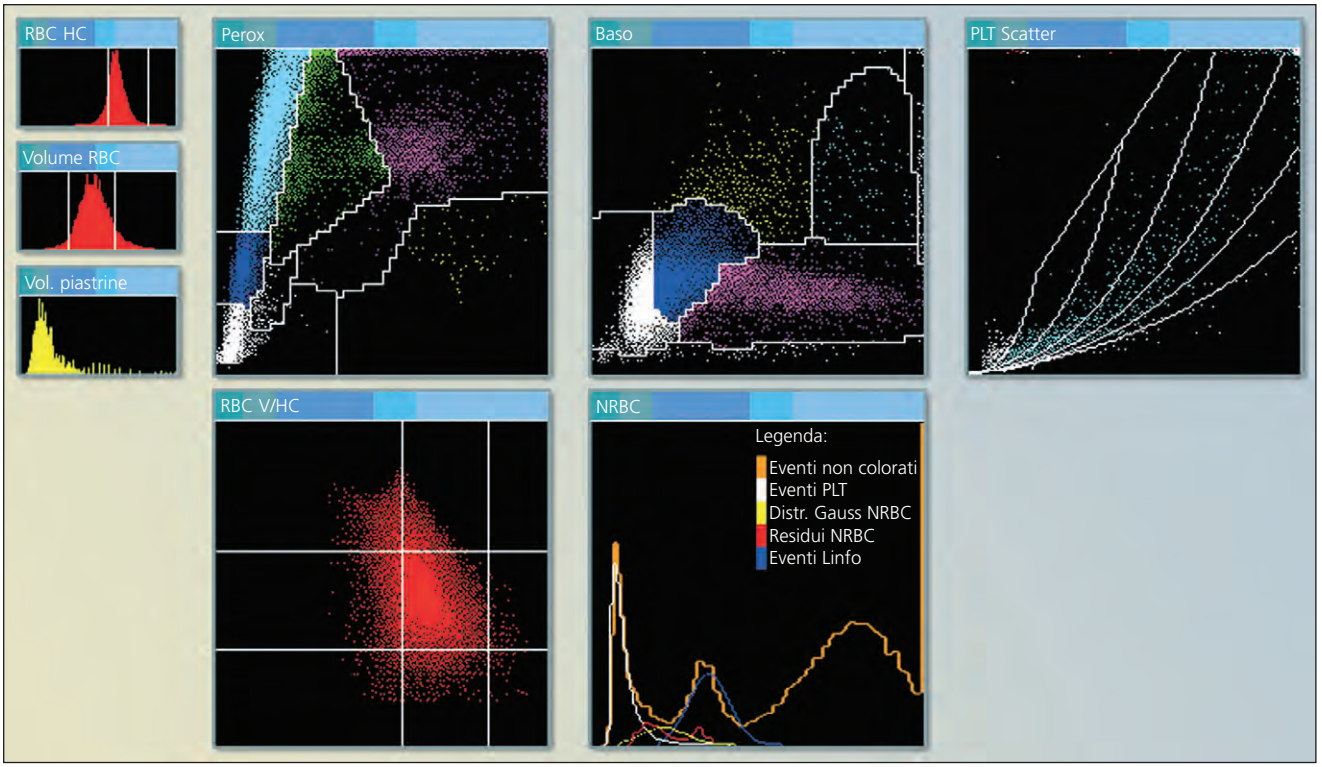

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 83 | g/l | |
| RBC | 3.06 | $\times 10^{12}$/l | |
| HCT | 0.253 | l/l | |
| MCV | 82.6 | fl | |
| MCH | 27.0 | pg | |
| MCHC | 327 | g/l | |
| CHCM | 330 | g/l | |
| RDW | 20.8 | % | Anisocytosis ++ |
| HDW | 4.51 | g/dl | Anisochromasia ++ |
| HYPO | 13.5 | % | Hypochromia +++ |
| HYPER | 2.3 | % | |
| MICRO | 7.9 | % | Microcytosis ++ |
| MACRO | 1.6 | % | |
| RBC fragments | 0.03 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 0.89 | % | RTCADA |
| RET | 27.2 | $\times 10^{9}$/l | |
| CHr | 28.4 | pg | |
| IRF (M+H) | 46.9 | % | |
| PLT | 40 | $\times 10^{9}$/l | |
| MPV | 8.8 | fl | |
| PDW | 17.1 | % | |
| Large platelets | 1 | $\times 10^{9}$/l | |
| MPC | 21.7 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 82.63 | | * |
| WBC Perox | 80.98 | | * |
| NEUT | 12.74 | 15.4 | * |
| LYMPH | 28.88 | 35.0 | * |
| MONO | 8.39 | 10.2 | * |
| EOS | 0.53 | 0.6 | * |
| BASO | 1.51 | 1.8 | * |
| LUC | 30.57 | 37.0 | * Atyp +++ |
| NRBC | 0.86 | 1.0 | * NRBC +, N-RBC |
| LI | 1.64 | | * B-NV |
| MPXI | 13.8 | | |
| MN | | 91.8 | IG +++ |
| PMN | | 5.9 | |
| BLASTS | | 15.5 | Blasts +++ |
| Perox HPX | | 6.4 | |
| Perox SAT | | 18.2 | |

Patient followed for two years with 9% bone marrow blast cells. The automated count shows marked leucocytosis with an excess of lymphocytes and LUC, but also of monocytes. All the values are associated with asterisks, caused by the sample/system flag B-NV (no valley MN/PMN) and N-RBC. The leucocyte distribution in the cytogram, starting from the left, shows a long fusiform shape that starts with the lymphocytes and continues without interruption to a high peak of LUC. From this cluster, a population of cells spreads out across the monocyte area and then expands into a large group of dispersed 'neutrophil' signals. This appearance is designated PM in the PANDA classification. The basophil cytogram is completely deformed by the presence of blast cells. All the morphological white cell flags are activated. The reticulocyte count is within the normal range, but the IRF is excessive; both the asterisk with the sample/system flag RTCADA, and the cytogram with elements in saturation, suggest an invasion of strongly stained particles, perhaps derived from leucocyte fragments.

**Morphology.** Neutrophil granulocytes 12%, with nuclear dysmorphism; eosinophil granulocytes 0.5; lymphocytes 18%; monocytes 2.5%; blasts 38%, in part lacking granules, in part granulated; promonocytes 18%; immature granulocytes 11%, with various dysplastic promyelocytes; orthochromatic erythroblasts 2 per 100 leucocytes. Marked red cell anisocytosis, with mild hypochromia, Howell-Jolly bodies, polychromasia. The bone marrow aspirate shows 54% blast cells and severe trilineage dysplasia.

## REPORT 4.5 Acute myeloid leukaemia, not otherwise specified, monoblastic




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 96 | g/l | |
| RBC | 3.06 | $\times 10^{12}$/l | |
| HCT | 0.282 | % | |
| MCV | 92.1 | fl | |
| MCH | 31.4 | pg | |
| MCHC | 341 | g/l | |
| CHCM | 347 | g/l | |
| RDW | 15.3 | % | Anisocytosis + |
| HDW | 2.62 | g/dl | |
| HYPO | 1.7 | % | |
| HYPER | 0.7 | % | |
| MICRO | 1.7 | % | |
| MACRO | 1.4 | % | |
| RBC fragments | 0.02 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 0.18 | % | |
| RET | 5.4 | $\times 10^{9}$/l | |
| CHr | 30.6 | pg | |
| IRF (M+H) | 34.7 | % | |
| PLT | 100 | $\times 10^{9}$/l | |
| MPV | 11.3 | fl | |
| PDW | 69.0 | % | |
| Large platelets | 9 | $\times 10^{9}$/l | |
| MPC | 23.3 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 33.23 | | * |
| WBC Perox | 32.87 | | |
| NEUT | 2.16 | 6.5 | |
| LYMPH | 4.71 | 14.2 | |
| MONO | 2.59 | 7.8 | |
| EOS | 0.08 | 0.2 | |
| BASO | 0.47 | 1.4 | * |
| LUC | 23.2 | 69.8 | Atyp +++ |
| NRBC | 0.00 | 0.0 | |
| LI | 1.86 | | |
| MPXI | −8.6 | | |
| MN | | 88.1 | |
| PMN | | 9.7 | |
| BLASTS | | 39.1 | Blasts +++ |
| Perox HPX | | 0.42 | |
| Perox SAT | | 0.2 | |

Moderate anaemia and thrombocytopenia, with mild anisocytosis. Marked reticulocytopenia with an increased IRF. Platelet size is increased. The marked leucocytosis results from a marked increase in peroxidase-negative cells classified as LUC, which have given rise to the Atyp flag; in the Perox cytogram they form a large, thick fusiform blue cluster, slightly inclined to the right, cut off at the top because of their large size. A small trail to the right crosses the vertical threshold and is therefore green, indicating the presence of weak peroxidase activity in some cells (P1 appearance). The neutrophil population seems well represented in the appropriate position, but with a tendency to dispersion. The Blast flag results from the distribution of the MN population in the Baso cytogram (D1 appearance). The basophil count is somewhat increased as a result of co-incidence.

**Morphology.** The white cell differential count on the peripheral blood film provides the following results: neutrophils 7%, lymphocytes 9%, blasts 84%. The morphology of the leukaemic population, composed of large blasts cells with ample, often vacuolated cytoplasm, in shown in Fig. 4.94.

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CHAPTER 5

# Myeloproliferative Neoplasms

The myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal disorders arising from a haemopoietic stem cell, characterised by:

- Expansion and proliferation in the bone marrow of one or more myeloid lineages;
- Effective haemopoiesis;
- Presence of organomegaly.

The clonal expansion induces the hyperproduction of cells of one or more lineages; these neoplastic clones have a predisposition to further evolution with loss of the capacity to differentiate resulting in blastic evolution.

The MPN are diseases of adults, with the peak occurrence being between 50 and 70 years; the incidence is about 6–9 new diagnoses per 100 000 persons per annum, without appreciable geographical differences.

The history of our knowledge of this group of disorders includes many milestones of modern oncology, in taxonomic, diagnostic and therapeutic fields. The first descriptions of chronic myeloid leukaemia (CML), published independently by John Hughes Bennett[1] and Rudolph Virchow,[2] date back to 1845: it was the latter who coined the term 'leukaemia' to indicate an illness with a greatly increased number of peripheral blood white cells. More than 100 years later, in 1960, Nowell and Hungerford[3] described a particular cytogenetic alteration in patients with CML, the presence of a small acrocentric chromosome, subsequently known as the Philadelphia (or Ph) chromosome; they indicated that this had a cause and effect relationship with CML. This was the first perception in medicine, viewed at the beginning with scepticism, that a genetic alteration could correlate with the presence of a specific tumour. In 1992, Levitzki proposed the use of an inhibitor of the ABL tyrosine kinase to treat leukaemia that could be considered to be induced by mutation in the *ABL1* oncogene; thus, in 1998 a tyrosine kinase inhibitor was first used in treatment.[4] Between 1879 and 1934 other disorders characterised by proliferation of myeloid cells had been recognised and classified as distinct entities: Gustav Heuck described two patients with bone marrow fibrosis and extramedullary haemopoiesis,[5] Henry Vaquez identified polycythaemia vera (PV) for the first time[6] and Emil Epstein and Alfred Goedel identified a syndrome characterised by thrombocytosis without polycythaemia.[7] In 1951, William Dameshek was the first to hypothesise a relationship between PV, essential thrombocythaemia (ET) and primary myelofibrosis (PMF),[8] grouping all these disorders under the designation 'myeloproliferative disorders'; his intuition was confirmed more than 50 years later when four independent groups of researchers identified, in the majority of *BCR-ABL1*-negative classical myeloproliferative disorders (PV, ET and PMF), a somatic mutation of the gene encoding the JAK2 Janus kinase: the allele *JAK2* V617F, in which valine replaces phenylalanine in exon 14, codon 617, nucleotide 1849, leads to a gain-of-function of the kinase that correlates with the pathogenesis.[9–12]

The 2008 World Health Organization (WHO) classification introduced some relevant modifications to the nosography of these conditions[13,14]:

- The term myeloproliferative neoplasm (MPN) was introduced with the aim of emphasising the neoplastic nature of this condition, which was neither reactive nor idiopathic;
- Mastocytosis was included in the MPN group, since it was recognised that this disease was a haemopoietic stem cell disorder;
- The *BCR-ABL1* rearrangement was specifically identified as a diagnostic marker of CML;
- In the context of *BCR-ABL1*-negative MPN, analysis to detect *JAK2* mutation was introduced into the diagnostic algorithm;
- Examples of primary hypereosinophilia that lacked rearrangement of *PDGFRA*, *PDGFRB* and *FGFR1* were included within the MPN group.

New diagnostic guidelines for the diagnosis of each of the subtypes of MPN were given in specific sections. Table 5.1 lists the entities that are included in the MPN group in the 2008 WHO classification.

## Chronic myeloid leukaemia, *BCR-ABL1* positive

*BCR-ABL1*-positive CML is the most frequent of the MPN and represents about 15% of all leukaemias. It is a myeloproliferative

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**Table 5.1** Categories of myeloproliferative neoplasm included in the 2008 WHO classification(adapted from Swerdlow 2008,[13] with permission of IARC)

- Chronic myeloid leukaemia *BCR-ABL1* positive
- *BCR-ABL1*-negative myeloproliferative neoplasms
  - Chronic neutrophilic leukaemia
  - Polycythaemia vera
  - Primary myelofibrosis
  - Essential thrombocythaemia
  - Chronic eosinophilic leukaemia, not otherwise specified
  - Mastocytosis
  - Myeloproliferative neoplasm, unclassifiable

disorder characterised in the peripheral blood by marked neutrophil leucocytosis with immature granulocytes in the circulation and an increase of basophil granulocytes. It is associated with a specific genetic lesion, the balanced translocation between chromosomes 9 and 22, t(9;22)(q34.12;q11.21): chromosome 22 is shortened, which results in the majority of cases, in the presence of the Ph chromosome. The translocation leads to the formation of a hybrid gene designated *BCR-ABL1*, which in turn leads to the production of an anomalous protein, p210, which has increased tyrosine kinase activity and is implicated in the pathogenesis of the condition by means of constitutive activation of certain transduction signals. Rarely, the breakpoint in the *BCR* gene is in a different position and this leads to formation of a fusion protein of a different molecular weight (p230, p190). From the biological point of view, it is interesting that in acute myeloid leukaemia (AML) associated with t(9;22) the anomalous fusion protein is usually BCR-ABL1 p190, while in the rare neutrophilic variant of CML the protein is usually BCR-ABL1 p230.[15,16] The genetic lesion arises in a multipotent haemopoietic stem cell and has been detected in the granulocytic, erythroid and megakaryocytic lineages, and also in B and T lymphocytes.

The natural history of CML usually shows two or three consecutive stages.[17] The condition is usually diagnosed in chronic phase, sometimes as the result of a routine full blood count, before there are any clinical manifestations. At this early stage of the disease, the expansion of the stem cell compartment with a granulocytic orientation causes granulocytic hyperplasia with differentiation being preserved, associated with basophilia. Further evolution, after a period averaging 30 months, is characterised by subacute or acute clinical and haematological deterioration, designated respectively accelerated phase and blast crisis; these phases result from clonal evolution with loss of the capacity to differentiate, insufficient myelopoiesis and frequent involvement of other lineages.

In the natural history of the illness, the median latent period between the occurrence of the molecular alteration and the clinical evidence of its presence has been estimated at about six years; a further period of about two years has been estimated to be necessary to reach a white cell count of $100 \times 10^9$/l; another four years then pass, on average, before the occurrence of accelerated phase or blast crisis.[18–20] The introduction of tyrosine kinase inhibitor therapy has profoundly altered the clinical course of CML, both with regard to quality of life and survival. The first drug of this type to be introduced into clinical practice was imatinib, initially known as imatinib mesylate: this occupies the ATP pocket of the BCR-ABL1 protein, acting as a competitive inhibitor of ATP by blocking phosphorylation of the tyrosine kinase substrate and interfering with its association with effector molecules. From this process, cell death follows[21] (see Fig. 5.15). Around 70–90% of patients achieve a complete cytogenetic remission with this treatment,[22] with a median disease-free survival of more than eight years.[23] According to the recent European Treatment and Outcome Study (EUTOS) scoring system, the percentage of basophils and the spleen size are the best prognostic indicators in imatinib-treated patients, predicting complete cytogenetic remission.[24]

## Chronic phase

### Blood count

Examination of the peripheral blood film is fundamental and often indicates a strong probability of CML: there is usually a marked increase in white cells, with the white blood cell count (WBC) varying between 12 and $1000 \times 10^9$/l, the median value being around $100 \times 10^9$/l. The WBC is above $100 \times 10^9$/l in 70–90% of patients, and above $350 \times 10^9$/l in 25%.[25] The most extreme increases may exceed the linearity limits of automated haematology analysers: in this case a dilution must be made, preferably using autologous or AB plasma. Various authors have reported the tendency of the WBC in patients with CML to show cyclical oscillations with a marked rise and fall over a period of 30–60 days. There is generally a normocytic normochromic anaemia of variable severity: haemoglobin concentration (Hb) can be very low, e.g. 50 g/l, or conversely can fall within reference limits, but usually it is between 80 and 120 g/l: in this phase of the disease, patients do not usually need red cell transfusions. In general, the Hb shows an inverse relationship with the WBC, falling as the mass of leukaemic cells rises. Exceptional cases have been described with an increased Hb.[26] A marked increase in the WBC can lead to turbidity of the blood specimen within the automated analyser after the addition of a lysing agent, with a resultant increase in the estimates of Hb, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC): more recent instruments flag this phenomenon and, in some cases, can correct the value of Hb according to the WBC. Extreme leucocytosis, particularly if associated with severe anaemia, can also cause a false increase in the red cell count, sufficient to affect measurement of the haematocrit (Hct) and the red cell indices. Otherwise, in the absence of any concomitant pathological process, the red cell indices do not show any particular anomaly in CML at presentation. The reticulocyte count is normal, sometimes slightly increased or reduced. The platelet count is increased in a half of

patients at diagnosis, with average values between 400 and 500 × $10^9$/l and above 700 × $10^9$/l in 20% of cases; in 5–10% of patients there is thrombocytopenia, usually of moderate degree, at the time of diagnosis[26]; the platelet count does not correlate with the WBC or with the severity of anaemia.[27] Report 5.1 shows a typical automated instrument printout in CML.

## Peripheral blood morphology

The morphology of cells present in the peripheral blood film of patients with CML is highly characteristic (Table 5.2) and suggests the diagnosis, but this must be confirmed; diagnosis must be based on recognition of the cytogenetic alteration (presence of the Ph chromosome) and/or the molecular alteration (presence of the *BCR-ABL1* rearrangement). In the peripheral blood one sees a marked increase in mature neutrophil granulocytes, with segmented nuclei, and their precursors (Figs 5.1, 5.2 and 5.3). In contrast to what occurs in AML, at the onset of CML one does not see the *hiatus leukaemicus*: all stages of maturation are generally represented, with a tendency to a larger proportion of the more mature cells. The percentage of myelocytes, however, is usually greater than that of metamyelocytes, so that the differential count has two peaks, a myelocyte peak and a neutrophil peak (comprising band forms and segmented neutrophils). The myeloblasts are usually less that 3% and the promyelocytes less than 5%; their percentages tend to correlate with the degree of leucocytosis. The count of immature granulocytes provided by some automated instruments is a generic indicator

**Table 5.2** Blood count and morphological criteria for diagnosis of *BCR-ABL1*-positive chronic myeloid leukaemia in chronic phase

**Peripheral blood**
- Leucocytosis (>20 × $10^9$/l) with neutrophilia
- Presence of immature granulocytes at all stages of maturation (with a double myelocyte-neutrophil peak)
- Mature neutrophils with normal morphology
- Reduction of neutrophil alkaline phosphatase
- Absolute basophilia (>0.3 × $10^9$/l), sometimes with dysmorphism and hypogranularity
- Frequent absolute eosinophilia, with normal morphology
- Possible presence of eosinophil and basophil promyelocytes, myelocytes and metamyelocytes
- Red cells of normal size and morphology
- Rare circulating erythroblasts (<2 per 100 leucocytes) orthochromatic and polychromatic, without atypia
- Platelet anisocytosis with possible gigantism, hypogranularity or small clumps
- Bare megakaryocyte nuclei; micromegakaryocytes not present

**Bone marrow**
- Hyperplasia of neutrophil granulopoiesis with normal maturation conserved
- Blasts <5%; promyelocytes <15%
- Myeloid : erythroid ratio >10
- Consistent increase in mature and immature basophils
- Frequent increase in eosinophils with normal maturation
- Reduced erythropoiesis, morphologically normal
- Megakaryocytes often numerous, reduced size, frequently in small clusters, not dysplastic; no micromegakaryocytes
- Sea-blue histiocytes or pseudo-Gaucher cells
- Histological evaluation: topography of granulopoiesis, evaluation of megakaryocytes, fibrosis and blast numbers with CD34

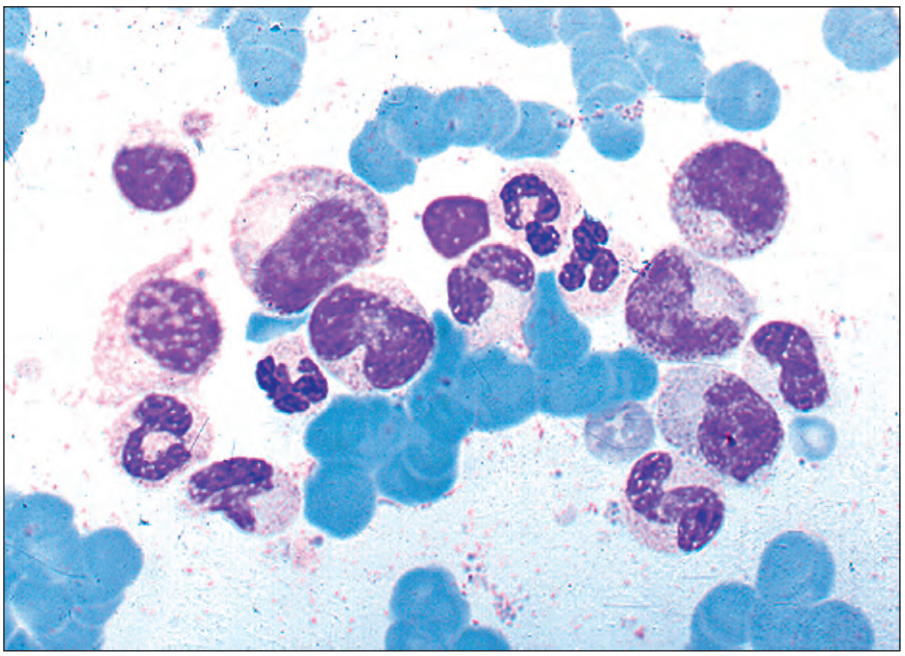


**Fig. 5.1** Chronic myeloid leukaemia (CML), *BCR-ABL1* positive; peripheral blood film. The leucocytosis is a distinctive characteristic. The expansion of the granulocyte component is due mainly to an increase in cells in the intermediate (neutrophil myelocytes and metamyelocytes) and final (segmented and non-segmented neutrophils) stages of maturation. Top left, a small lymphocyte beside a normally formed platelet. The morphology of the neutrophils and their precursors is normal, with pale cytoplasm containing specific granules. There is normal segmentation of mature neutrophils.

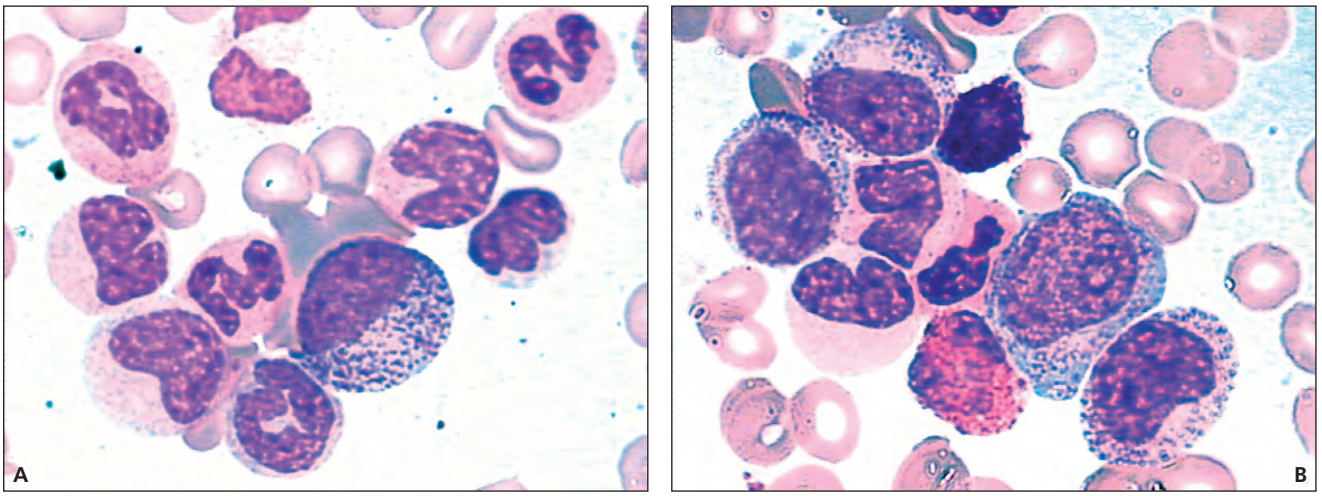



**Fig. 5.2** CML, *BCR-ABL1* positive; peripheral blood film. (A) An early neutrophil myelocyte (or late promyelocyte) with an eccentric nucleus adherent to more than half of the cytoplasmic membrane; the chromatin is heterogeneously condensed into small clumps; there are no prominent nucleoli. The cytoplasm is still basophilic (mild asynchrony with delay of cytoplasmic maturation) and highlights the presence of numerous large dark granules, dispersed in an orderly manner and letting the pale paranuclear Golgi zone be seen. Three metamyelocytes have notched or indented nuclei and chromatin condensed into larger masses; their cytoplasm is changing from sky-blue to pink and the granules are very fine, pale and not individually recognisable. There are three neutrophil band forms with elongated nuclei that are folded in a serpentine manner; the lowest of them appears hypogranular; a fourth, top right, is more mature with almost pyknotic chromatin, well-formed nuclear segments and some nuclear appendages. Centre right, a small monocyte. Such slight asynchrony and dysmorphism is common in CML and should not be considered evidence of dysplasia. (B) On the vertical median of the same film, three mature granulocytes: in the centre is a non-segmented neutrophil with a nucleus that has dense shiny chromatin, at the top is a basophil packed with specific granules that permit only a small central part of the nucleus to be seen and at the bottom, an eosinophil with typical granules. On the left, two metamyelocytes with few granules and, further left, two myelocytes with cytoplasm that is still weakly basophilic. On the right, a large promyelocyte with normal morphology (basophilic cytoplasm, azurophilic granules, an eccentric nucleus, chromatin in small clumps and a nucleolus) and another neutrophil myelocyte. The red cell morphology is normal.

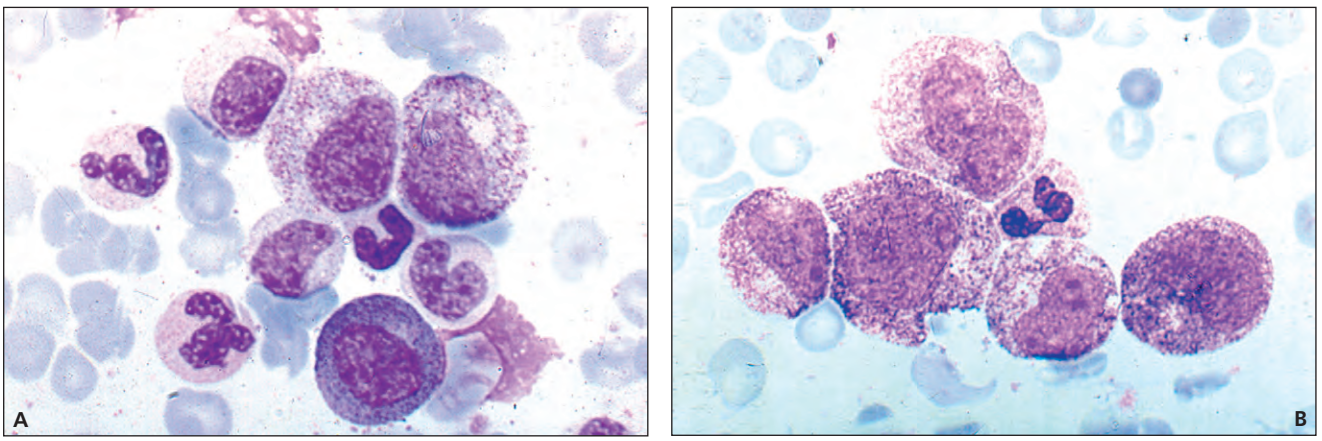



**Fig. 5.3** CML, *BCR-ABL1* positive; peripheral blood film. (A) Three mature neutrophils with segmented nuclei. Three metamyelocytes, recognisable from their small size, even though the nucleus is not yet curved into a horseshoe shape in two of the three, but rather remains rounded. At the top, a large neutrophil myelocyte with an eccentric rounded nucleus and with the cytoplasm full of specific granules of a typical dark brick-red colour; on its right, a promyelocyte, in which the cytoplasm is still weakly basophilic; the small pale paranuclear area is the negative image of the Golgi apparatus; near the lower pole of the nucleus, the chromatin of which is heterogeneous and formed of numerous small dark masses alternating with pale areas, there is a visible trace of a nucleolus. The cell lower down is an eosinophil myelocyte characterised by an oval central nucleus with violaceous clumped chromatin, with a small pale Golgi zone and weakly basophilic cytoplasm packed with large specific eosinophil granules admixed with darker proeosinophilic granules. (B) Adjacent to a small segmented neutrophil with dense chromatin are five immature neutrophil granulocytes distinguished by numerous fine red-violet granules. The largest cell is a promyelocyte with granular cytoplasm and a clearly visible nucleolus with peripheral enhancement on the right of the nucleus, directly opposite the small Golgi zone. The cell further to the right is similarly classified as a promyelocyte, even though no nucleoli are apparent, because of its basophilic cytoplasm that can be seen beneath the very numerous granules. The other three cells can be classified as myelocytes, even if their heterogeneous chromatin lets traces of nucleoli be seen; the larger cell at the top has a nucleus that appears to be tetraploid.

which must always be confirmed by examination of the blood film.[28,29] Both the neutrophils and their precursors have normal morphology: sometimes nuclear segmentation appears anomalous, but not sufficiently so to indicate dysplasia. If dysplasia is present, it raises the suspicion of possible evolution to accelerated phase or blast crisis.

In addition to the leucocytosis and the double myelocyte–neutrophil peak, the third very typical peripheral blood feature in CML is an increase in the percentage and absolute count of basophils, sometimes reaching 30 times their normal level[25] (Fig. 5.4). Sometimes, particularly in the presence of marked leucocytosis, the percentage of basophils does not appear increased, but there is always an absolute basophilia (>0.3–0.4 $\times$ $10^9$/l, often >1.0 $\times$ $10^9$/l). The presence of basophilia is an indispensable criterion for the diagnosis: in cases with basophils less than 1%, particularly when confirmed over time, an accurate assessment of differential diagnoses such as other MPN and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) must be made. Sometimes the basophils of CML show morphological alterations, such as reduction or dysmorphism of granules, sometimes with a characteristic washed-out appearance, even in the early phases of the disease. The presence of

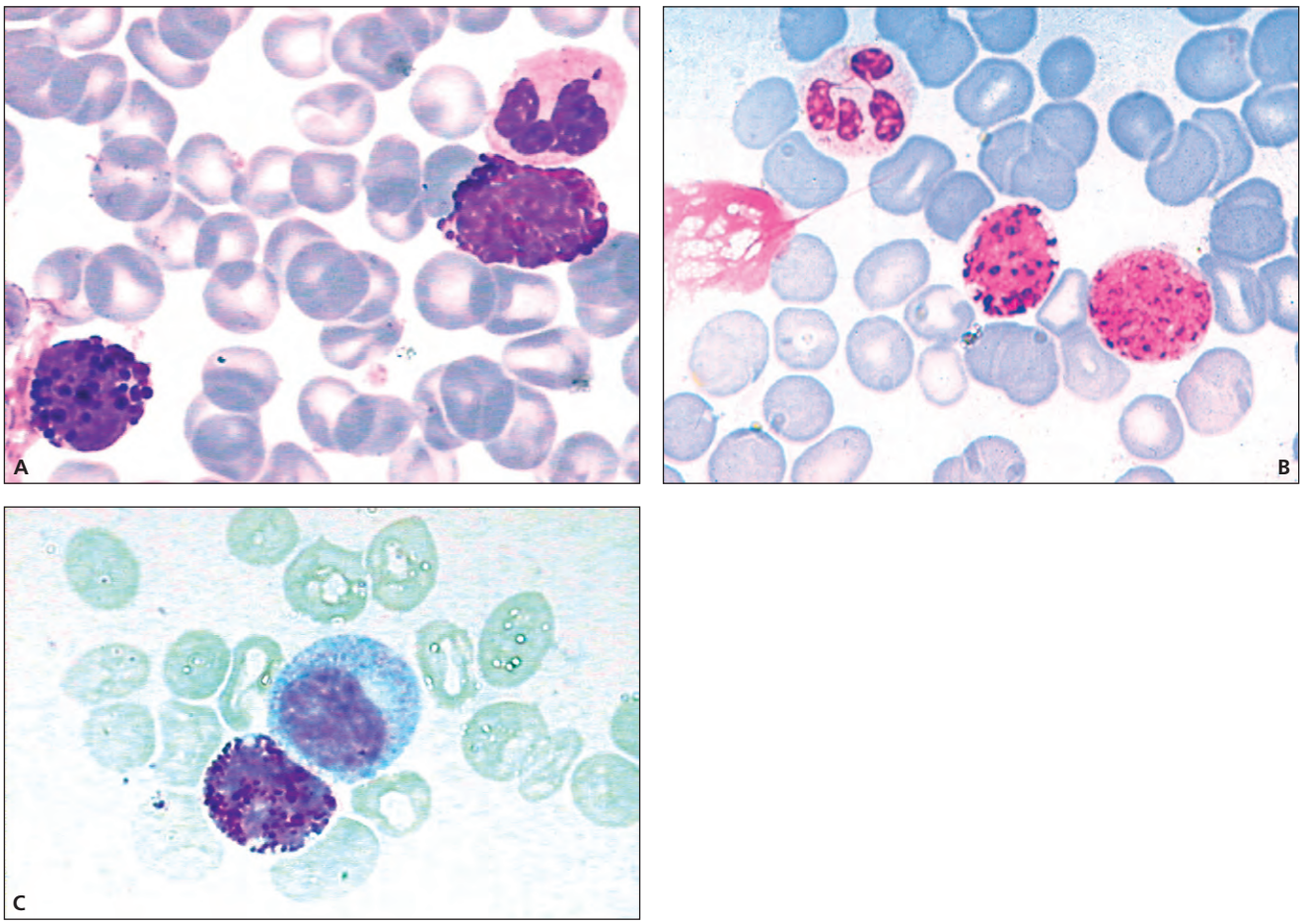



**Fig. 5.4** Basophils in a peripheral blood film in CML, *BCR-ABL1* positive. (A) The increase in basophils is a distinctive feature of this myeloproliferative neoplasm (MPN). In the majority of cases, as in this image, they are morphologically fairly normal. The cell bottom left has scanty cytoplasm occupied by 30 or so large granules that are almost black though with a violet tinge, similar to the colour of a mulberry; they tend to cluster together at the two poles, but also superimpose themselves on the nucleus. The nucleus has a dark prune-red colour and occupies the major part of the cell; it has dense homogeneous chromatin without identifiable clumps and is the shape of a figure of eight. The basophil on the right is similar, but its granules are more numerous and less dark, and tend to blend with each other and with the underlying nucleus. Higher up on the right, a mature neutrophil, with barely visible granules and a nuclear appendage joined to the nucleus by a quite thin filament. (B) In addition to a neutrophil granulocyte with four nuclear lobes and normal cytoplasmic granulation, there are two cells with large purple-black granules. The smaller cell, in the centre, is a mature basophil with very scanty cytoplasm and somewhat fewer specific granules than is usual; top right, it has small cytoplasmic vacuoles. The superimposition of granules on the nucleus is typical of basophils; in this cell the reduced number of granules, which are clustered mainly in the lower part of the cytoplasm, lets the structure of the nucleus be seen. The larger cell on the right is a basophil precursor, classifiable as a basophil myelocyte because of the fairly dense chromatin and the presence of a number of specific granules scattered through the pale cytoplasm that surrounds the nucleus. (C) Positivity of a basophil with toluidine blue. The nucleus is a weak azure colour, like that of the other leucocytes, but the granules have a reddish tinge (metachromatic staining). Top right, a neutrophil myelocyte, the granules of which do not show metachromasia.

these anomalies can make the microscopic recognition of basophils difficult and, more often, the accuracy of their quantification by automated analysers is reduced.[30]

Eosinophil granulocytes are increased, at least in absolute terms, in 90% of patients; in a smaller proportion of cases there are circulating eosinophil myelocytes and metamyelocytes. The monocytes, often increased in absolute terms, are usually reduced as a percentage, less than 3%. An exception is seen in patients with the p190 isoform of the BCR-ABL1 protein, who show a marked monocytosis, which can lead to an erroneous diagnosis of chronic myelomonocytic leukaemia.[31] Half of cases have an absolute lymphocytosis. It is not rare to see some morphologically normal orthochromatic and polychromatic erythroblasts in the circulation: this observation can have an unfavourable prognostic significance, having been identified as an independent adverse prognostic indicator of a shorter chronic phase and earlier acute transformation.[32] The platelets are often morphologically normal, but sometimes they are larger than normal and they can form small clumps in the peripheral blood; megakaryocyte nuclei and strands of megakaryocyte cytoplasm are often found, particularly at the edges of the film.

Neutrophil alkaline phosphatase, demonstrated by either cytochemical or biochemical methods, is strongly positive in 20% of neutrophils in normal subjects, while it is absent or greatly reduced in the majority of patients with uncomplicated chronic phase CML.[33] Reduced neutrophil alkaline phosphatase activity, however, is not specific for CML: it is observed also in other haematological conditions, such as paroxysmal nocturnal haemoglobinuria and some cases of AML or PMF, and also in non-haematological conditions, such as congenital hypophosphatasia.[34] In CML, however, this enzymatic activity has a rather peculiar course: it is absent or reduced in chronic phase, increased in blast crisis and normal in patients in remission. Increased levels of neutrophil alkaline phosphatase can, moreover, be observed transiently during the course of infection, during pregnancy and in splenectomised patients.

The study of patients exposed to atomic radiation at Hiroshima or Nagasaki, in addition to confirming that the development of CML is preceded by a long asymptomatic phase lasting on average about 6 years, showed that the earliest abnormality detected was the appearance of the molecular alteration.[35] The increase of platelets and basophils, together with a reduction of neutrophil alkaline phosphatase, was seen fairly early during the natural history of the disease, when the leucocyte count was still between 10 and 20 $\times$ $10^9$/l; a significant increase in immature granulocytes in the peripheral blood occurred later, as did biochemical abnormalities such as an increase in serum vitamin $B_{12}$ and its transport proteins, and clinical abnormalities, such as splenomegaly.

### Bone marrow morphology

Examination of the bone marrow aspirate is complementary to peripheral blood examination in the diagnosis of CML.[25,36–38] The films are usually rich in fragments (Fig. 5.5). A marked increase in cellularity is a consistent feature with exuberant hyperplasia of the granulocytic series (Figs 5.6 and 5.7). Granulopoiesis usually shows a normal sequence of maturation, even though the ratio between immature and mature forms, as would be predicted, is markedly elevated in the marrow in relation to the blood, signifying that the damage to the marrow-blood barrier is still incomplete. Neutrophil myelocytes are predominant in the marrow aspirate and promyelocytes can be more than 10% (although not >30%); blast cells are rarely more than 5%. The morphology of granulocyte precursors is normal, similar to what is seen in a normal marrow aspirate (Figs 5.8 through to 5.11): this criterion of cytomorphological normality is important in distinguishing the chronic phase of CML from other myeloproliferative conditions that involve the granulocytic lineage. The abundance of granulocyte precursors and the gradual and continuous passage from one maturation phase to another sometimes makes it difficult to assign a cell to a particular phase of maturation rather than to the preceding stage or the stage that follows in the differentiation sequence. Sometimes this difficulty is accentuated by the tendency to asynchrony associated with unstoppable proliferation that leads, for example, to cells with the nucleus of a myelocyte still having cytoplasm that is weakly basophilic with bright red azurophilic granules; or a cell with a nucleus that has chromatin density typical of a myelocyte still containing one or two visible nucleoli. Mitotic figures are numerous, particularly in neutrophil myelocytes, even though the expansion in the total granulocyte mass is not attributable to an accelerated rate of proliferation, but to a massive increase in the pool of progenitors directed towards granulopoiesis and to prolongation of survival in the circulation of mature forms.

Basophilia is also a consistent bone marrow feature and an increase of eosinophils is common (Figs 5.10 and 5.11). Some eosinophil precursors contain, in addition to specific granules, large dark proeosinophilic granules, purplish-red, similar in colour to basophil granules, so that in the past they have sometimes been erroneously considered to be hybrid eosinophil-basophil granules[39]: in fact, they are a well-known morphological expression of early eosinophilopoiesis.

The erythroid series is morphologically normal, but erythroid islands are reduced in number and size,[40] this being related to the increased granulopoiesis: the myeloid:erythroid (M : E) ratio is very high, generally more than 10.

Megakaryocytes are usually increased in CML, sometimes very obviously so, and tend to form small clusters. Their distinctive characteristics are that they are smaller than normal and their nuclei are less lobulated than those of normal megakaryocytes, to the extent that they are sometimes called dwarf megakaryocytes (Figs 5.6A, 5.11A, 5.12 and 5.13). In *BCR-ABL1*-positive CML, the mechanisms by which the majority of megakaryocytes do not reach the normal degree of hyperdiploidy have not yet been clarified: however, in practice these features are consistently present and represent one of the most

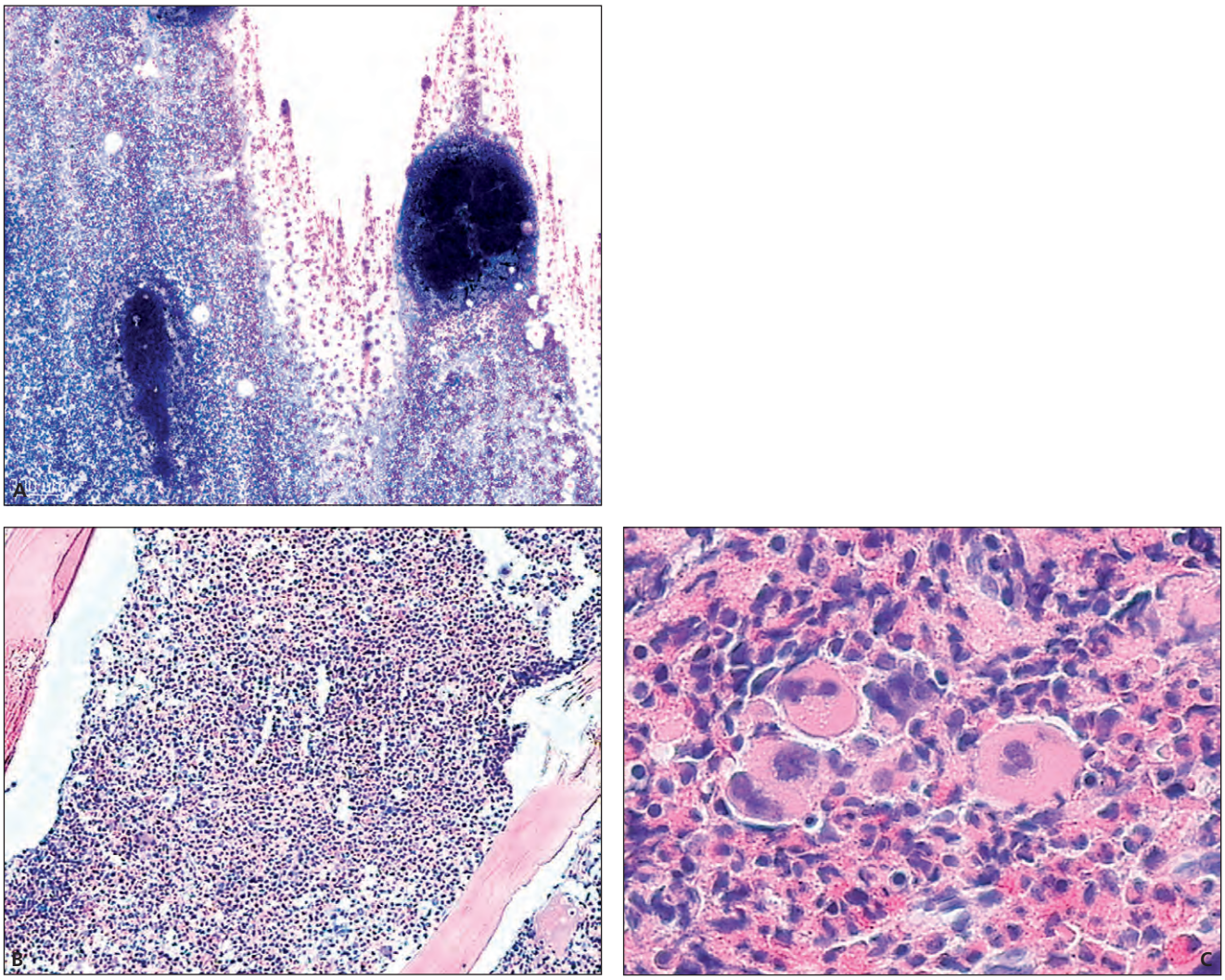



**Fig. 5.5** Hypercellularity of the bone marrow in CML, *BCR-ABL1* positive. (A) Bone marrow aspirate at low power (x10 objective). The film contains large, strongly stained bone marrow particles, which have been dragged into the tail of the film, leaving behind them compact trails of very cellular marrow. The cells in the trails are in a single layer permitting optimal visualisation of cytological details at higher power. (B) Histological section of a trephine biopsy specimen. The cellular marrow is seen to be composed mainly of cells of granulocyte lineage, packing the marrow and totally obliterating fat spaces. There are a number of mainly fairly small megakaryocytes. (C) Trephine biopsy section from a patient with CML in accelerated phase: the marrow is very cellular with numerous dysmorphic megakaryocytes, abundant cells of granulocyte lineage, left shift of the maturation curve and erythroid hypoplasia. Blast cells are below 5%. Some histopathologists consider that cases with granulocytic and megakaryocytic proliferation are a frequent variant associated with a less favourable prognosis.

useful morphological markers of this diagnosis.[41,42] Otherwise, in the chronic phase of CML, other significant dysplastic features are absent (e.g. micromegakaryocytes are never seen). In some patients, megakaryocytes are particularly numerous: this has led some histopathologists to suggest that there is a form of CML characterised by a bilineage granulocytic-megakaryocytic proliferation.[43]

In around a third of patients with CML, one can observe in the marrow histiocytes with cytoplasm laden with cellular debris and lipid inclusions, which accumulate because of saturation of the enzyme system that breaks down the glucocerebrosides that are the product of the increased metabolic turnover of granulocytes[44] (Figs 5.8 and 5.14). These cells can have an appearance analogous to the cells of Gaucher syndrome, being very large (30–60 μm) and oval in shape with a small eccentric nucleus and ample, weakly basophilic cytoplasm containing fine granules; they have the typical appearance of concentric fibrils, giving an onion-skin appearance. In other cases, these storage cells resemble the sea-blue histiocytes of the homonymous syndrome (sea-blue histiocytosis), because of the presence in the cytoplasm of numerous deeply azure granules. The pseudo-Gaucher and sea-blue histiocytes are part of the neoplastic clone

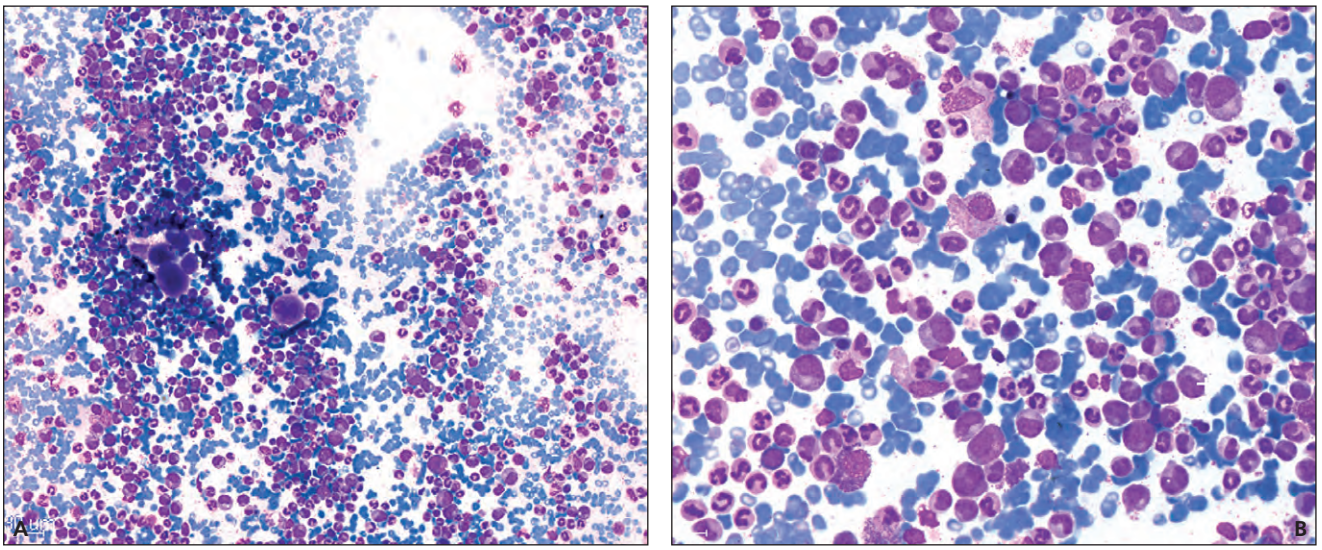



**Fig. 5.6** Granulocytic hyperplasia in CML, *BCR-ABL1* positive; bone marrow aspirate; same film as Fig. 5.5, at a slightly higher power. (A) The trails are hypercellular and heterogeneous in composition, with a prevalence of cells of granulocyte lineage at different stages of maturation. There are a number of segmented and non-segmented neutrophils and numerous myelocytes with plentiful cytoplasm and an eccentric nucleus. In the centre, a small to medium-sized mature megakaryocyte; further to the left, other smaller and darker 'dwarf' megakaryocytes form a cluster. (B) In this field, viewed at a higher power, one can observe the double peak of myelocytes and mature neutrophils, the myelocytes larger with eccentric oval nuclei that are sometimes notched or indented in relation to the Golgi zone and the mature neutrophils sometimes segmented and sometimes band forms. The morphology of these cells is essentially normal. Rare orthochromatic and polychromatic erythroblasts are seen as small cells with a homogeneously black, round nucleus. No blast cells are seen.

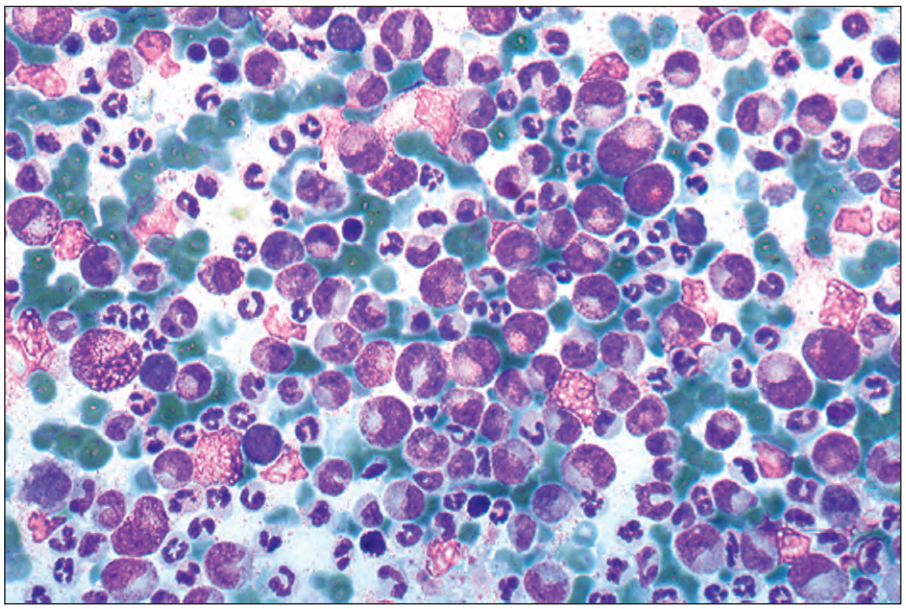


**Fig. 5.7** CML, *BCR-ABL1* positive; bone marrow aspirate. Similarly in this case, at low power, there is a notable increase in cellularity. The leucocytes, more numerous than the red cells, are distributed in a fairly uniform manner through the entire film. One can see, even at this power, the clear prevalence of the two cell types pointed out in the Fig. 5.6: the mature neutrophils in this field constitute more than half of all cells and are recognisable particularly because of their relative smallness and the dark chromatin of their segmented nuclei; the neutrophil myelocytes with a rounded or already slightly indented nucleus, to one side of the cell, show plentiful granular cytoplasm. A little below the centre, a large metamyelocyte with two nuclei. There are two small basophils in the centre, close to the lower edge of the image. There are very few erythroblasts: two, with polychromatic cytoplasm, can be seen near the two top corners of the image. Blast cells are not seen.

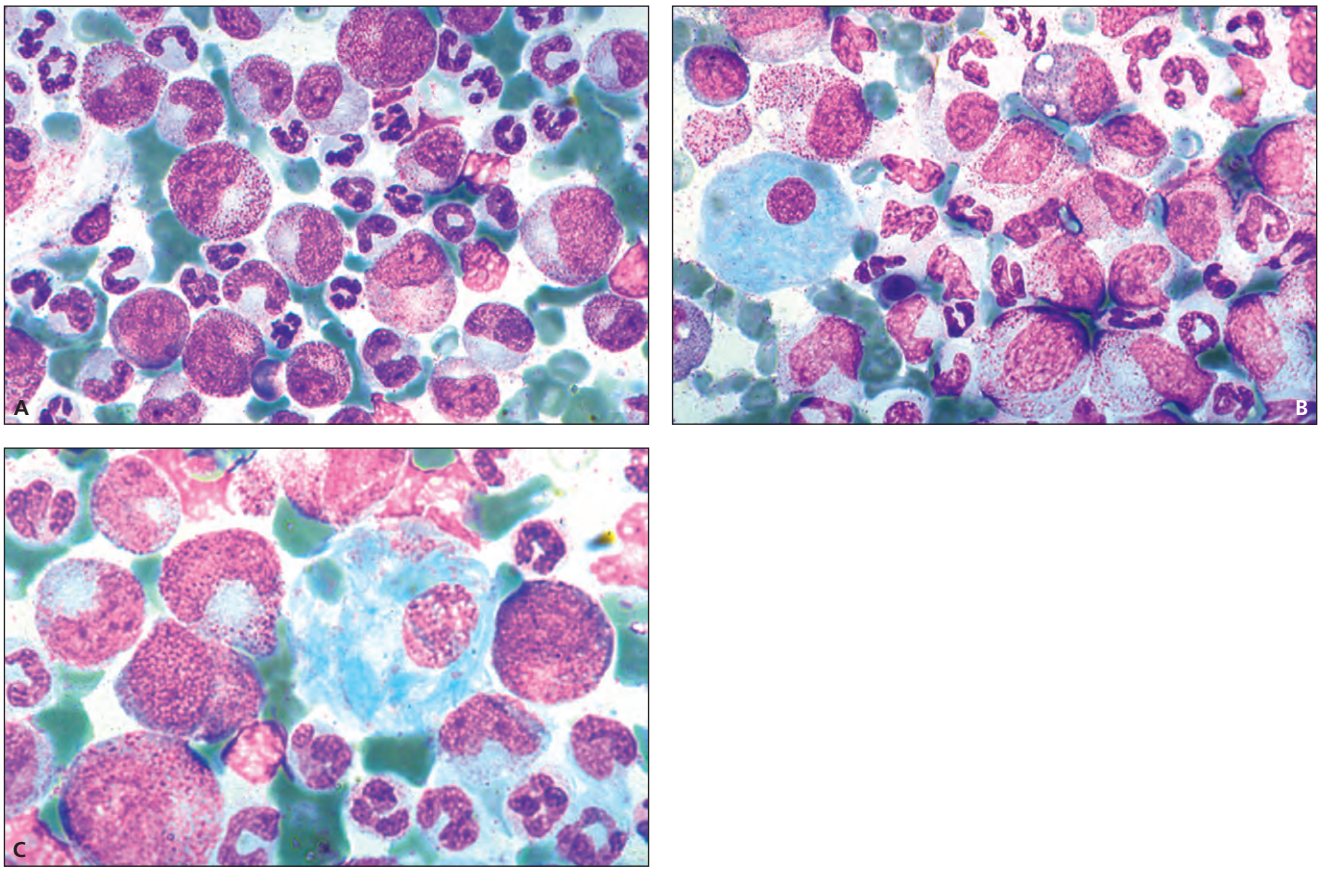



**Fig. 5.8** CML, *BCR-ABL1* positive; bone marrow aspirate from the same patient as Fig. 5.7. (A) At higher power the granulocytic hyperplasia is confirmed, without there being any significant cytological abnormality; this is characteristic of *BCR-ABL1*-positive CML. All the cells in this image belong to the granulocyte lineage; they are mainly mature neutrophils and myelocytes. The nucleus of some of the larger precursors still has a nucleolus: these cells can therefore be classified as promyelocytes, even though the density of the chromatin and the cytoplasmic characteristics (background pale pink or weakly basophilic and numerous specific granules) are in many instances more typical of myelocytes (slight asynchrony with delayed nuclear maturation). There is also hypertrophy of the Golgi apparatus, shown by the size of the pale paranuclear area. Some mature neutrophils have nuclei that are tending to hypersegment. On the left, half way up, is an adventitial reticulum cell with fusiform cytoplasm with indistinct margins. (B) On the left, a typical pseudo-Gaucher cell. The nucleus is perfectly round and eccentric within swollen cytoplasm; the granular, spongy chromatin network is typical of reticulum cells and bone marrow macrophages; the cytoplasm is filled with pale blue concentric lamellae, like onion skins. This cell contains two intact erythrocytes (erythrophagocytosis). Above this macrophage, a fragment of cytoplasm that has been detached from a granulocyte by clasmacytosis and, immediately above, a small blast cell with scanty basophilic cytoplasm. In addition to numerous mature neutrophils and neutrophil myelocytes, there is an eosinophil myelocyte with two cytoplasmic vacuoles close to the top border of the image, just to the right of centre. (C) The cytoplasm of this pseudo-Gaucher macrophage is less compact than that shown in the previous image: it contains plentiful pale blue fibrillar material (glucocerebroside), but also phagocytosed erythrocytes and platelets, and numerous small granules that are probably exogenous in origin. The nucleus has spongy cytoplasm with numerous pale areas. One notes three promyelocytes with plentiful granules that are mainly azurophilic, i.e. violet or pale wine-red, and the prominence of the Golgi zone in the granulocyte precursors top left.

and contain the *BCR-ABL1* transcript[45]; they are always periodic acid-Schiff (PAS) and Sudan black B positive and their cytoplasm is birefringent on examination by polarised light. The presence of numerous storage cells containing glycolipid is more marked in patients in whom a very elevated leucocyte count persists for long periods and is associated with prolonged survival.[46] In contrast to the pseudo-Gaucher cells, the mesenchymal stem cells that differentiate into adipocytes, osteoblasts and fibroblasts are not part of the clonal population.[47] Typically, in more than 80% of patients with CML, macrophages with haemosiderin granules are rare or absent, in the presence of normal values for serum ferritin.[48]

Histological examination of bone marrow biopsy sections is useful for the evaluation of megakaryocyte morphology, any increase in reticulin and the vascularisation of the marrow, which reflects the capacity of clonal stem cells to differentiate into endothelial cells.[49] Study of bone marrow topography in histological sections has confirmed the conservation of qualitatively normal granulopoiesis: granulocyte precursors, in fact, tend to be normally localised, mainly adjacent to the bone

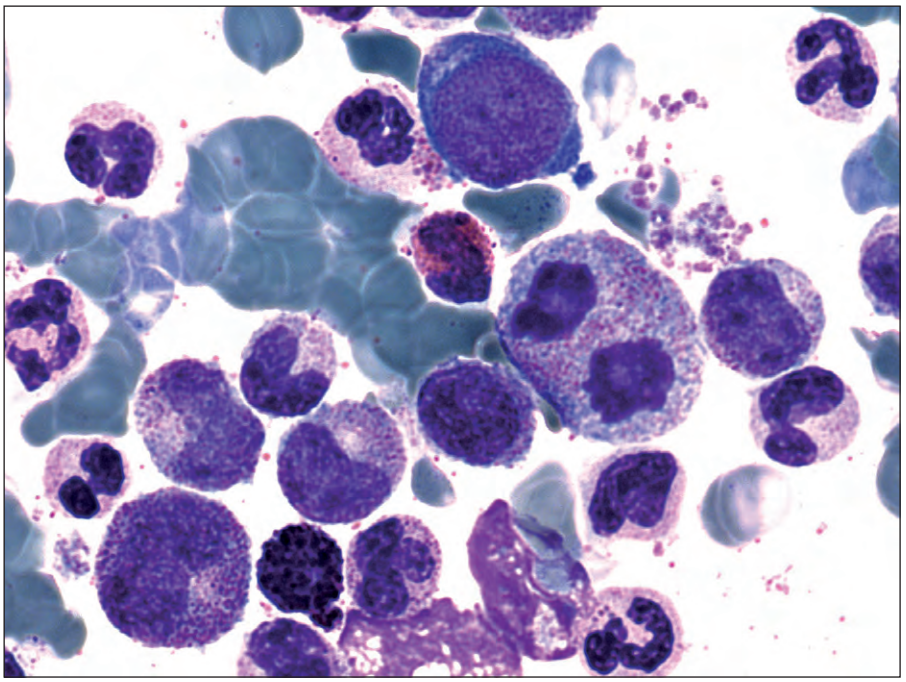


**Fig. 5.9** This bone marrow aspirate from a patient with CML, *BCR-ABL1* positive at presentation is cellular. There are neutrophil granulocytes at various stages of maturation, from myelocytes (such as that in the bottom left corner, which is very granular with a nuclear outline that is typically indented in relation to the pale Golgi zone) to segmented neutrophils with pyknotic chromatin and pink cytoplasm, such as the cell seen immediately to the left of the described myelocyte. The residual cytoplasmic basophilia is made less evident by the appearance of granules. Close to the centre, a granulocyte shows an elegant mitotic figure in a cell in anaphase, with the chromosomes already gathered into two new polar masses, while the granular basophilic cytoplasm has not yet divided. There is also an eosinophil and a mature basophil. The large cell at the top with intensely basophilic cytoplasm and a nucleus with finely granular chromatin and nucleoli is a proerythroblast; it has a cytoplasmic protrusion at its lower edge. Top right, a strand of adherent platelets.

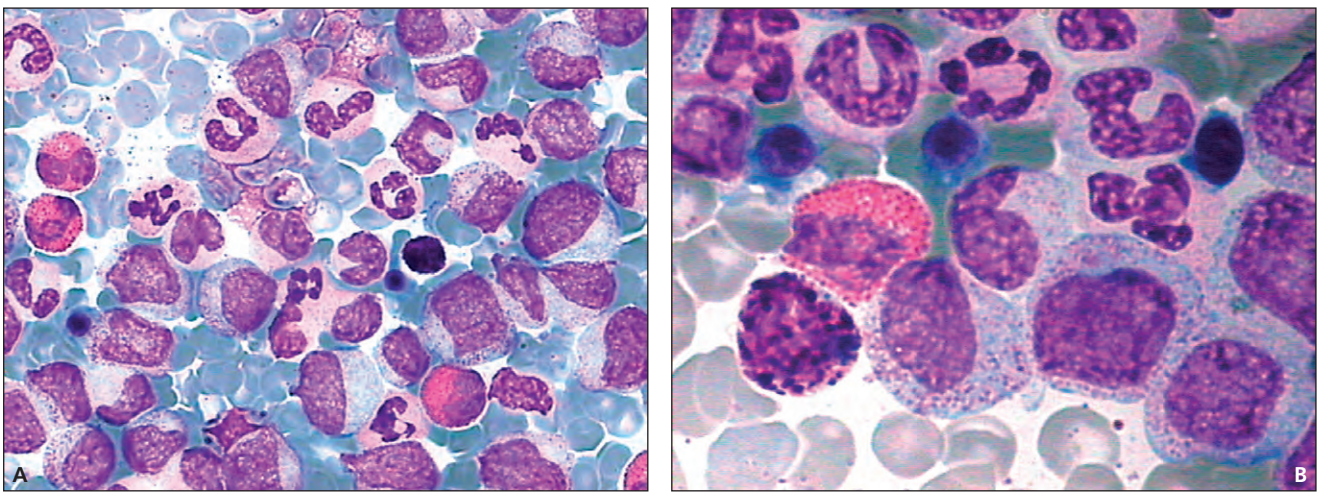
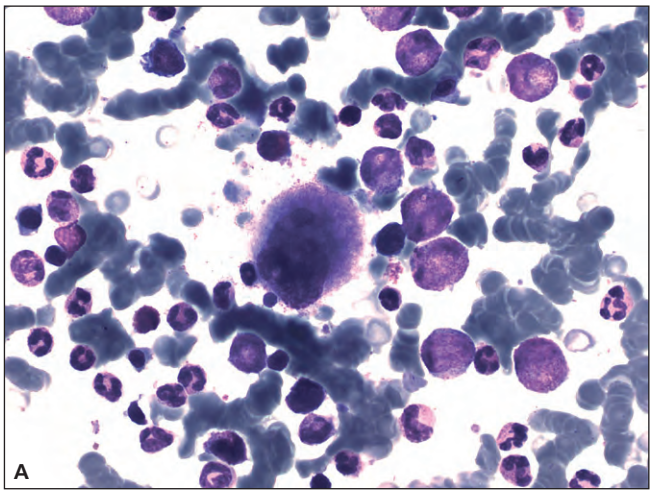



**Fig. 5.10** CML, *BCR-ABL1* positive; bone marrow aspirate. (A) The aspirate is very cellular and is composed mainly of neutrophil myelocytes, with cytoplasm that is more granular than usual and without many cytoplasmic granules, and mature neutrophils, both band forms and segmented: the latter show a tendency to hypersegmentation, perhaps due to a relative lack of folate because of the increased demand resulting from cellular proliferation. Immediately to the left and just below the centre, a promyelocyte; another, with an almost square nucleus, is sited at the same level further to the right, above an eosinophil myelocyte. Slightly higher, a basophil and a small orthochromatic erythroblast. Adjacent to the left edge of the image, two mature eosinophils and, lower down, an orthochromatic erythroblast. (B) The same film at higher power showing cytological details, with representation of all three granulocytic lineages. The band of cells immediately below the midline includes a well-formed basophil, an eosinophil, a myelocyte without many granules, a metamyelocyte and two promyelocytes, recognisable from their chromatin in small discontinuous clumps, their nucleoli and the basophilia of the cytoplasm that does not yet have many granules: their Golgi zones, however, are not visible. Higher up, three erythroblasts and a number of granulocytes in a more advanced stage of maturation.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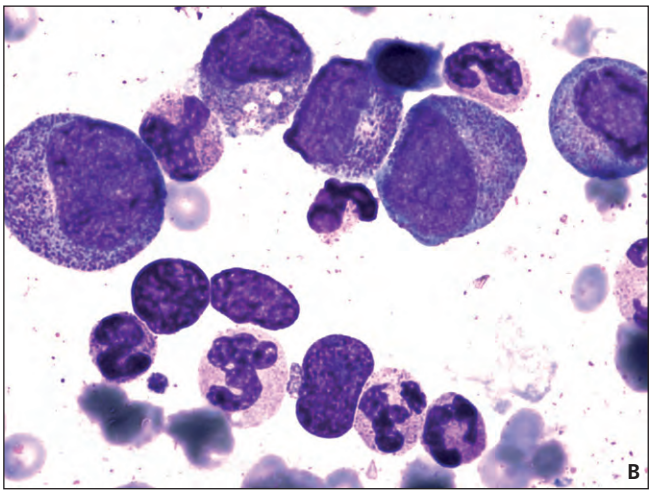
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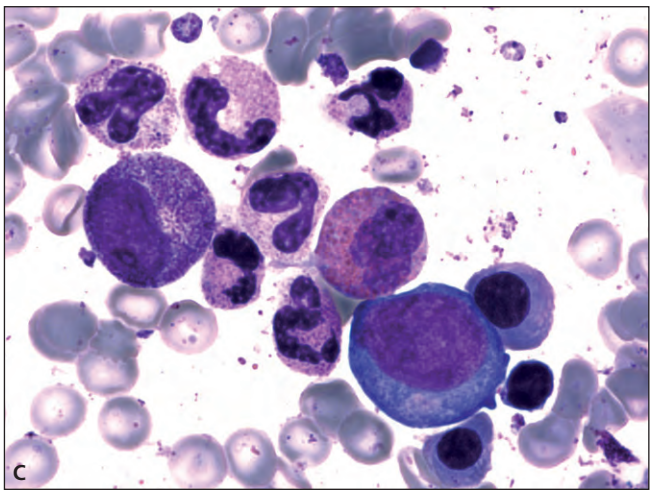
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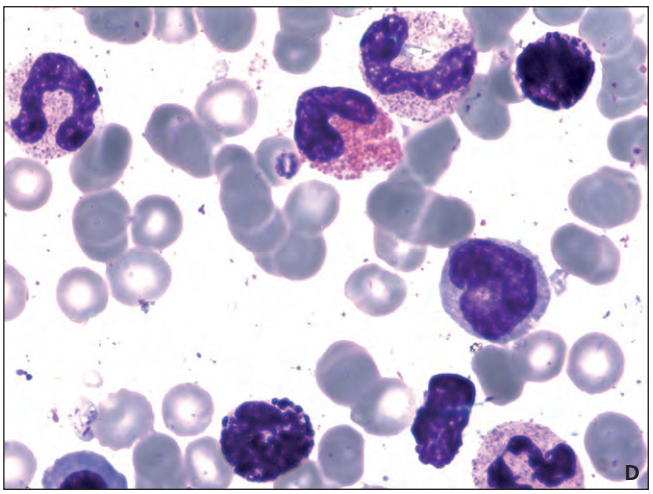
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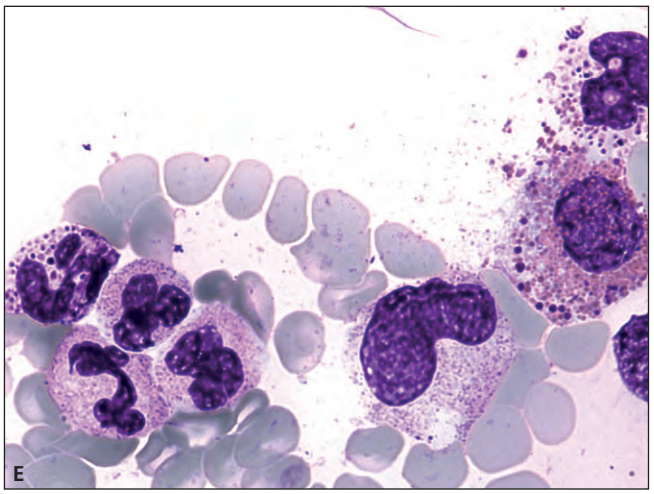
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**Fig. 5.11** CML, *BCR-ABL1* positive, at presentation, two years after treatment of carcinoma of the sigmoid colon by surgical resection, alkylating agents and cisplatin; bone marrow aspirate. The morphological picture, although this is therapy-related leukaemia, is typical, with hypercellularity and granulocytic hyperplasia with slight asynchrony, associated with a moderate increase in basophils and eosinophils. (A) In the centre of the image, a small megakaryocyte, typical of CML, with cytoplasm that is still intensely basophilic in the plentiful central zone that surrounds the nucleus. All around are neutrophils and their precursors, among which one can recognise some promyelocytes on the right. (B) At higher power, among the numerous neutrophils that are tending to hypersegmentation and some bare nuclei of uncertain origin, a large early neutrophil myelocyte on the extreme left (with a Golgi zone that stands out from the background cytoplasmic basophilia), another on the right, similar but smaller and cut off by the right-hand edge of the image, and to the left of the latter a promyelocyte with a prominent Golgi zone, numerous fine granules, heterogeneous chromatin with small foci of condensation and a number of nucleoli. The nuclei of all these progenitors are eccentric, the cytoplasm is plentiful and granular with an intensely basophilic background in comparison with the maturity of the nuclear chromatin (asynchronous maturation with retarded cytoplasmic development and persistence of ribosomes at the myelocyte stage). In the centre at the top, other small neutrophil myelocytes. (C) The largest cell, intensely basophilic, is a proerythroblast, beside which are two polychromatic erythroblasts and a nucleus that has been expelled from an orthochromatic erythroblast. Immediately above, an eosinophil with circular nuclear lobes and cytoplasm that is not fully mature, since there are still some large dark granules and grey cytoplasm indicating persistent basophilia. On the left, a granule-rich neutrophil promyelocyte, with a Golgi zone that creates a 'rising sun' appearance. At the top, a hyperchromic platelet. (D) At the top, a mature eosinophil with a band nucleus. At the bottom in the centre and in the top right corner, two basophils. On the right of the centre, a monocyte. (E) The cell on the left has a segmented nucleus with compact shiny chromatin: its identification as a dysmorphic eosinophil is possible because of the cytoplasmic granules, which are infrequent, dark and immature. The three granulocytes adjacent to this cell are neutrophils. On the right, close to the edge, is a partly damaged eosinophil myelocyte with a very low nucleocytoplasmic (N:C) ratio: the nucleus is eccentric and the cytoplasm contains numerous specific eosinophilic granules and a peripheral ring of dark proeosinophilic granules.

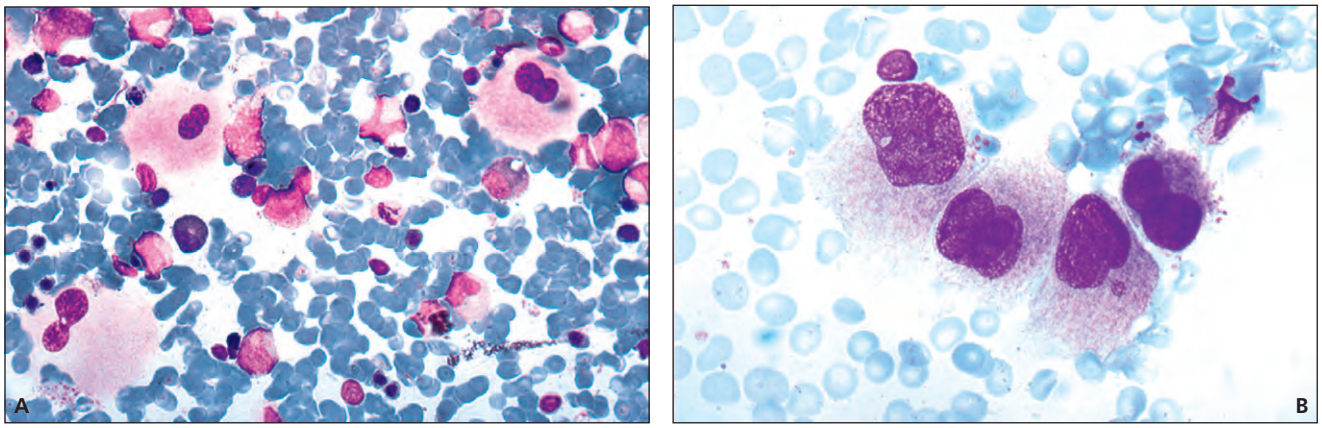



**Fig. 5.12** Dwarf megakaryocytes in the bone marrow aspirate of patients with CML, *BCR-ABL1* positive. (A) The megakaryocytes in CML are often increased in number and of reduced size. This image is of the bone marrow aspirate of a patient who has just received chemotherapy as conditioning for an allogeneic bone marrow transplant. There are three small megakaryocytes with clearly hypolobated nuclei. There is no significant alteration in the cytoplasmic granularity or in the nuclear structure, such as one would observe in a myelodysplastic syndrome (MDS). The reduced size and increased number of megakaryocytes is sometimes a useful criterion for supporting a diagnosis of CML, rather than another MPN in which the megakaryocyte morphology is more heterogeneous and often characterised by hypertrophy and gigantism. (B) In another patient, a group of four small megakaryocytes with scanty, almost mature cytoplasm and nuclei that are moderately hyperdiploid; the cell further to the right is similar to the others, but is smaller with more condensed chromatin and more mature cytoplasm, already starting to form platelets. The morphology of these small megakaryocytes in CML, which have an intermediate N:C ratio, is, however, different from that of the micromegakaryocytes of myelodysplasia, which tend to have pyknotic nuclei, and from that of the megakaryocytes of the 5q- syndrome, which are non-lobated with an eccentric nucleus, plentiful mature cytoplasm and a consistently low N:C ratio.

trabeculae. In biopsy sections, the granulocytic hyperplasia can be confirmed histochemically and immunohistochemically (strong positivity for naphthol AS-D chloroacetate esterase and for antibodies to granulocytic markers), megakaryocytes can be identified (with anti-CD61) and blast cells can be quantified (CD34 positive).[50]

In around 40% of cases at diagnosis there is an increase, ranging from moderate to marked, in reticulin fibrosis, almost always associated with a marked increase in megakaryocytes, significant splenomegaly and lower Hb: these patients are distinguished by a worse prognosis.[40]

Tyrosine kinase inhibitor therapy tends to normalise bone marrow appearances, often with a moderate residual appearance of activation (Fig. 5.15).

## Accelerated phase

The marked expansion of granulopoiesis that characterises the chronic phase of *BCR-ABL1*-positive CML involves, as already

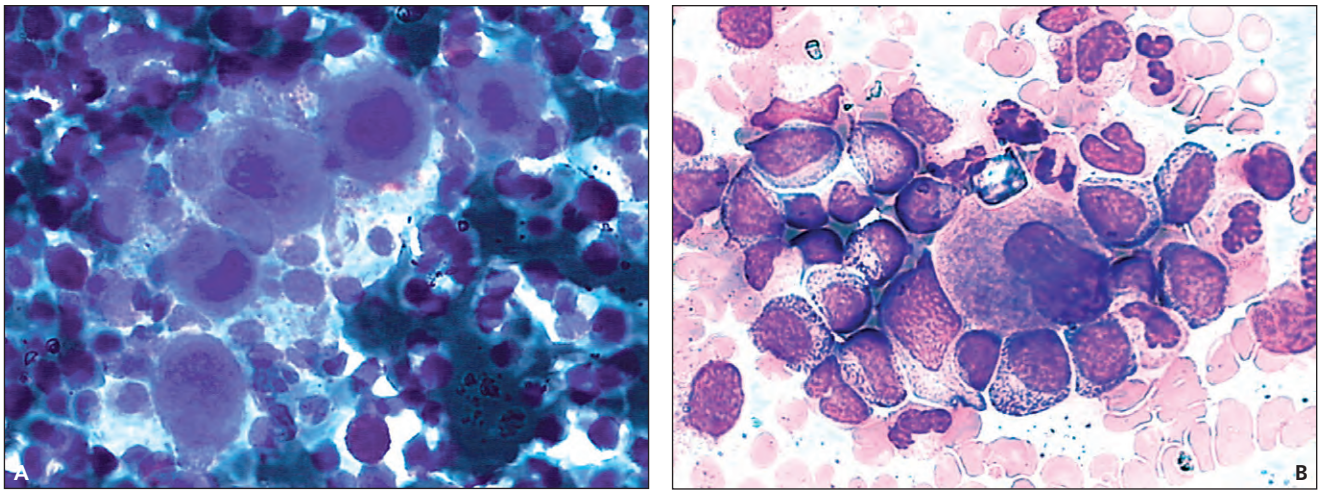



**Fig. 5.13** Other examples of megakaryocyte morphology in bone marrow aspirates in CML, *BCR-ABL1* positive. (A) Five small megakaryocytes, with scanty basophilic cytoplasm, appear lined up in this part of the film, which is too thick to permit an evaluation of morphological details. (B) In another case of CML in chronic phase, with granulocytic hyperplasia and preserved maturation, is a dwarf megakaryocyte, which has morphology that is qualitatively normal, but the dimensions, both of the nucleus and the cytoplasm, are reduced so that the N:C ratio is conserved. Top centre, a hypogranular basophil.

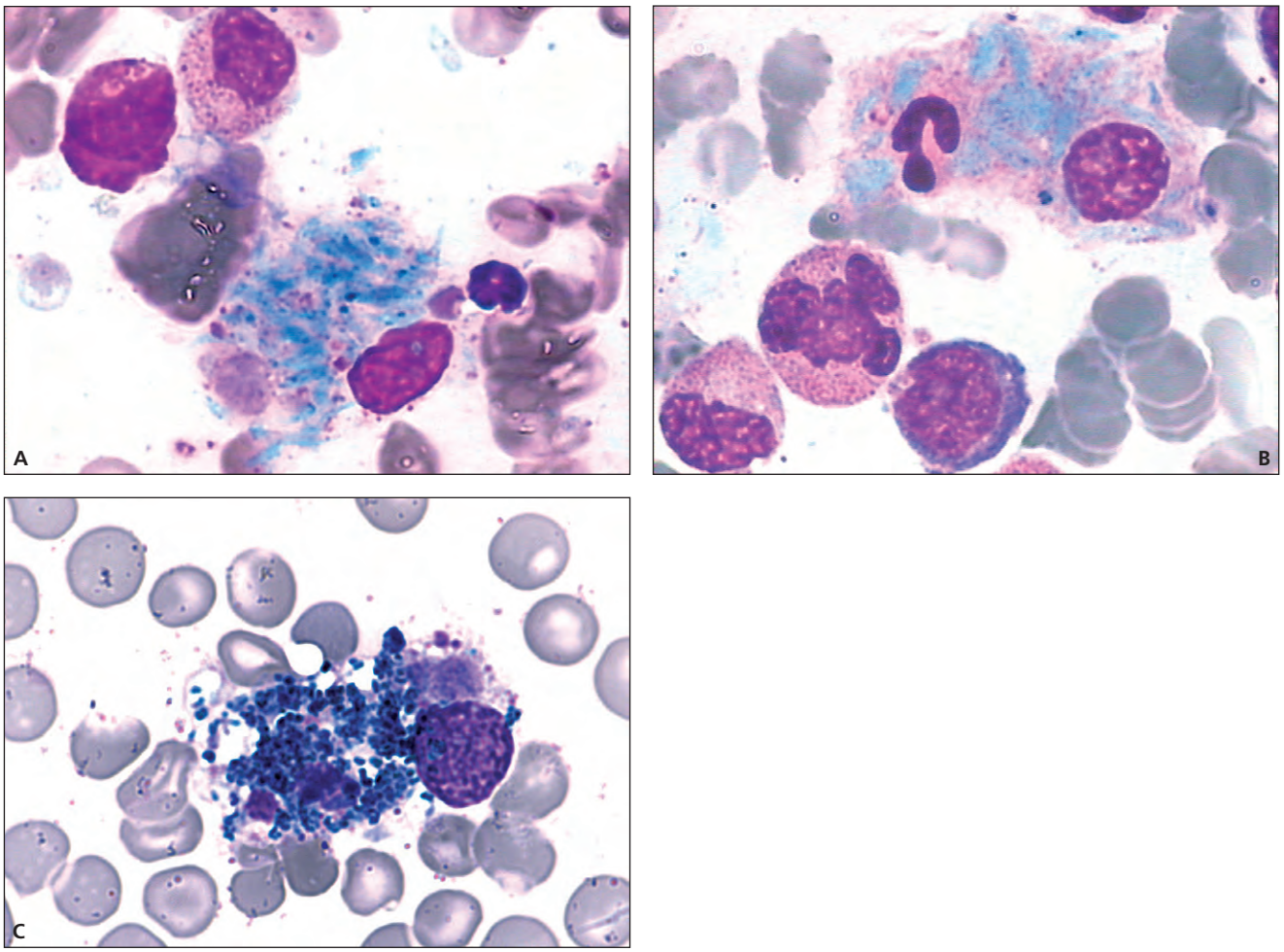



**Fig. 5.14** Macrophages in CML, *BCR-ABL1* positive; bone marrow aspirates of different patients. (A) Typical sea-blue macrophage, conventionally referred to as a 'sea-blue histiocyte': the cytoplasm contains an accumulation of pale blue material (of a pale colour that could be referred to as cerulean). This represents an accumulation of glycolipid, ceroid and lipofuscin, derived from the membranes of cells that have been phagocytosed and broken down. This lipid tends to form elongated, fusiform or rhomboidal structures. Cells of this type can be observed, not only in Niemann-Pick disease, congenital dyslipidaemias and other haematological neoplasms with a high rate of cell proliferation, but also in patients receiving intravenous lipid emulsions in the context or protracted parenteral nutrition (in which the inclusions, however, appear a deeper blue). (B) Another example of a sea-blue histiocyte, caught in the act of phagocytosing a neutrophil granulocyte with pyknotic chromatin; one notes the morphology of the eccentric rounded nucleus with a chromatin structure typical of a macrophage – spongy with small chromatin clumps and pale spaces. This image emphasises the high rate of death of granulocytes in this condition. Bottom left, a dysmorphic tetraploid neutrophil, with a hyperlobated, although not completely segmented nucleus. (C) In other macrophages the inclusions have their usual granular morphology and occupy all the cytoplasm, resembling a bunch of grapes. In this cell, as well as the eccentric spongy nucleus and the dark blue granules, one can observe the remains of degenerating cells.

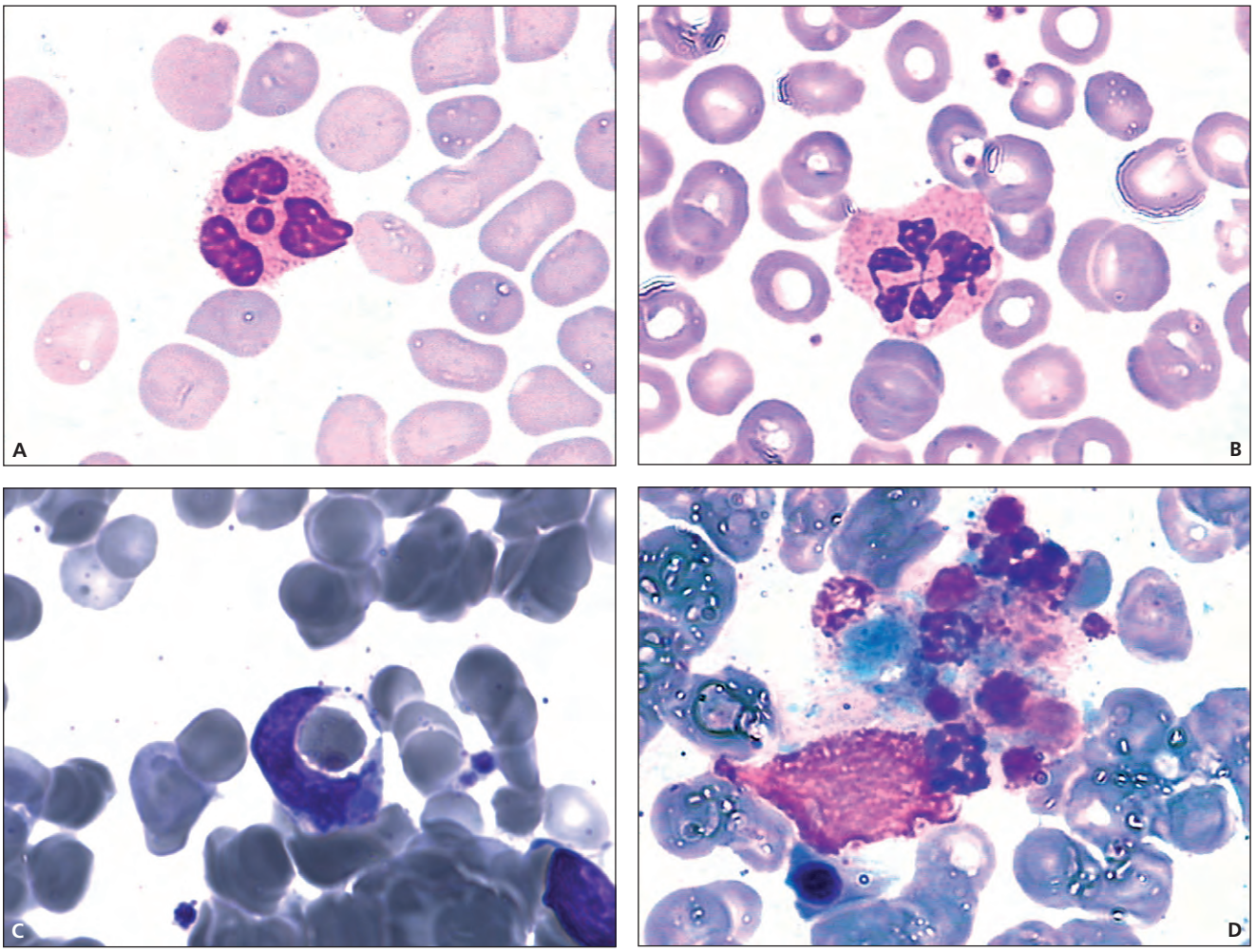



**Fig. 5.15** Cytological modifications in CML, *BCR-ABL1* positive, during treatment with imatinib. (A) Peripheral blood film. Neutrophil with a preaptotic nucleus with detachment of nuclear lobes and shiny chromatin. There is a long drumstick-shaped appendage above the small round central lobe. (B) Peripheral blood film. Hypersegmented neutrophil with the lobes still joined by long thin filaments. (C) Bone marrow aspirate. The film is hypocellular. There is an erythrophagocytic mononuclear cell. (D) Same film as (C). This macrophage with cytoplasm that is partly sea-blue is crowded with nuclear detritus of phagocytosed granulocytes that are being broken down.

described, predominantly the granulocytic lineages and is not initially associated with obvious morphological abnormalities or with significant deviations from the normal sequence of maturation. Qualitative abnormalities of maturation can, however, develop in the peripheral blood or the bone marrow during the course of the illness, mainly in association with a disturbance in the relationship between different blood components and morphological or numerical alterations in blast numbers and/or numbers of immature granulocytes: these indicate, in the majority of cases, evolution towards an accelerated phase or blast crisis. In these advanced stages of the natural history of the disease, in the absence of a response to treatment, the survival of patients is measured in months (on average six to eight months for the accelerated phase and two for blast crisis). Cytogenetic and molecular analysis shows that during acute transformation of CML, new abnormalities are present, the most common of these being duplication of the Ph chromosome, trisomy 8 and the presence of an isochromosome of 17q.

Rarely, patients present already in accelerated phase. More often, this subacute evolution is seen in about 30% of cases of CML diagnosed in chronic phase, in the form of a gradual and progressive clinical and haematological deterioration, which foreshadows a block in myeloid differentiation. Only in a proportion of cases does the transition to accelerated phase indicate that a blast crisis is imminent; rather, in the majority of cases, it is a particular specific form of progression of CML. The haematological signs of acceleration are in part quantitative: a sudden change in the leucocyte count, which sometimes increases very rapidly and sometimes falls to normal or subnormal values; reduced sensitivity of the WBC to therapy;

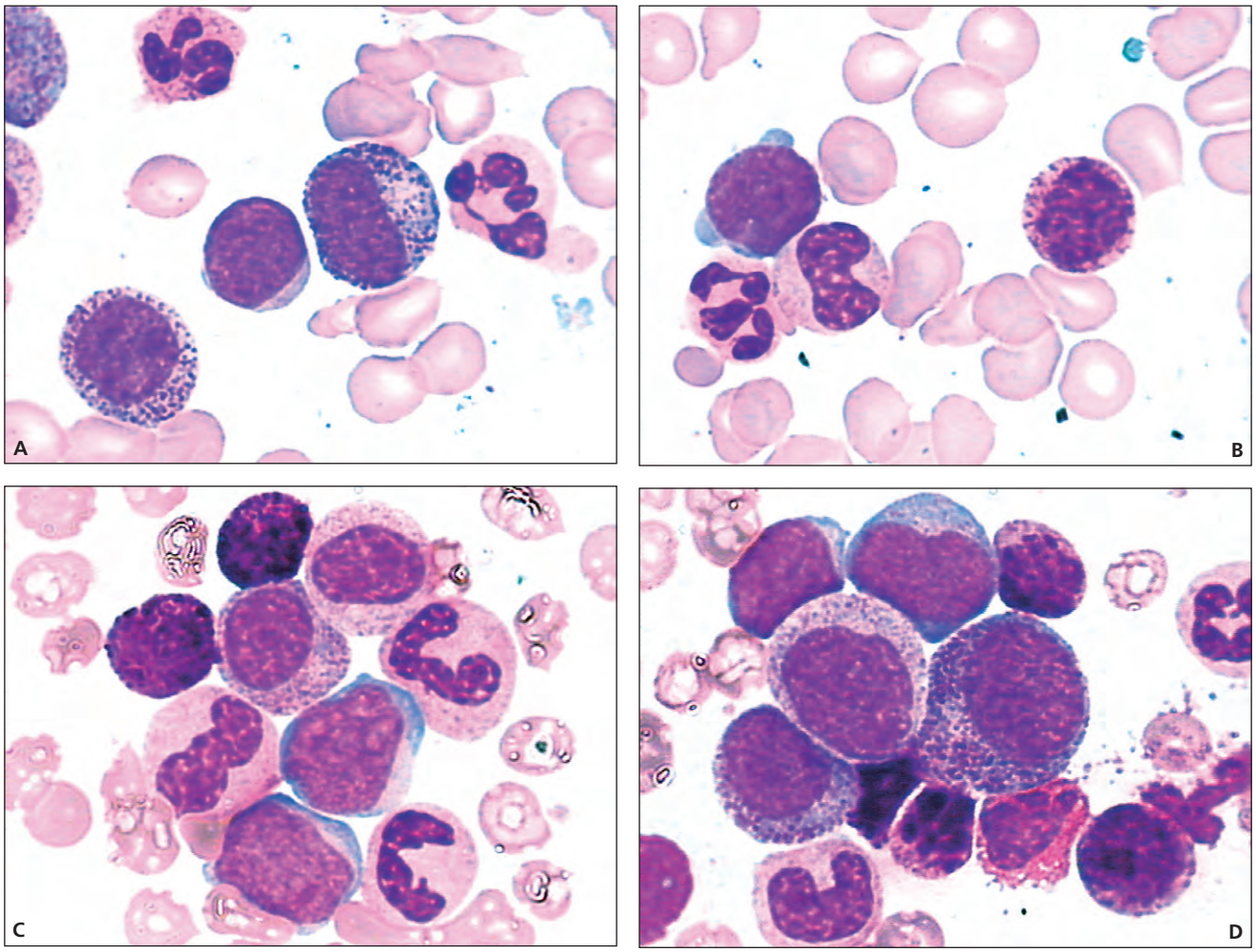



**Fig. 5.16** CML, *BCR-ABL1* positive, onset of accelerated phase identified in a peripheral blood film. The blood count showed mild anaemia, leucocytosis (WBC 24 × $10^9$/l) and marked thrombocytopenia (platelet count 47 × $10^9$/l). (A) There are two segmented neutrophils, one of which has agranular cytoplasm. In the centre, adjacent to an immature granulocyte with asynchronous maturation, which has the nucleus of a myelocyte (clumped chromatin and no nucleoli) and the cytoplasm of a promyelocyte (basophilia), is a small agranular blast cell with homogeneous, fairly compact chromatin and nucleoli that are not very prominent. Bottom left, a dysmorphic myelocyte. (B) From the left: a dysplastic neutrophil (hypogranular with bizarre nuclear segmentation and very dense shiny chromatin); an agranular blast cell, identical to the previous one, with scanty basophilic cytoplasm, with two blebs; a dysplastic neutrophil myelocyte in which granules are reduced to a fine violet dust; a basophil myelocyte with small scattered granules. (C) Near the tail of the film, a small clump of leucocytes comprising two basophils, two myelocytes, three non-segmented neutrophils with atypical condensation of chromatin into clumps (the lower one has agranular cytoplasm and a nuclear appendage) and two blast cells. The blasts are morphologically different from those described in (A) and (B), because of the presence of more plentiful cytoplasm with an elongated perinuclear pale area and of an angulated nucleus with visible nucleoli and more dispersed chromatin. (D) In this further group of cells there are two blast cells, one of which contains ten or so azurophilic granules within plentiful basophilic cytoplasm. There are also four basophils with heterogeneous and atypical morphology (the one on the right is actually a basophil myelocyte), an eosinophil and various cells of neutrophil lineage with all stages of maturation being represented and with there being some dysplastic features.

appearance or worsening of anaemia and thrombocytopenia. Other anomalies are qualitative or morphological in nature (Figs 5.16 through to 5.21): the maturation curve of the granulocyte series tends to deviate in the direction of immaturity, both in the blood and in the marrow, with an increase in the percentage of blasts and promyelocytes; there can be an increase in basophils, monocytes or eosinophils. Signs of unilineage, bilineage or trilineage dysplasia can appear, these being almost always absent in the chronic phase. With regard to mature neutrophils, one can see a reduction or, sometimes, dysmorphism of cytoplasmic granules, and there can also be cytochemical anomalies, such as a partial peroxidase deficiency; the nuclei show abnormalities of segmentation, particularly the appearance of nuclear hypolobation with strongly condensed, almost pyknotic, chromatin, of pseudo-Pelger type. The neutrophil granulocyte precursors are often characterised, in addition to a

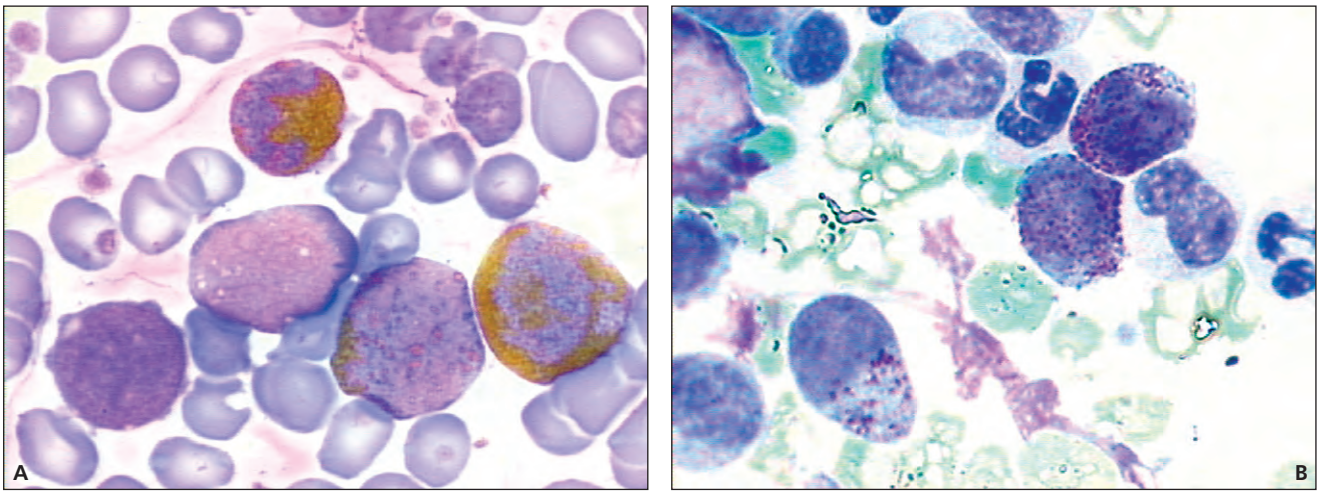



**Fig. 5.17** Cytochemical stains on the same case as Fig. 5.16; bone marrow aspirate. (A) Peroxidase stain. There are three blast cells: the two furthest to the left are peroxidase negative, while the scanty cytoplasm of the third is packed with positively staining granules. The blast bottom left has the typical morphology of a megakaryoblast. The other two intensely positive cells belong to the neutrophil granulocyte lineage. (B) Toluidine blue stains metachromatically (a reddish tinge) only the granules of basophils and their precursors. In this image, there are three, one of which, at the bottom, is particularly immature.

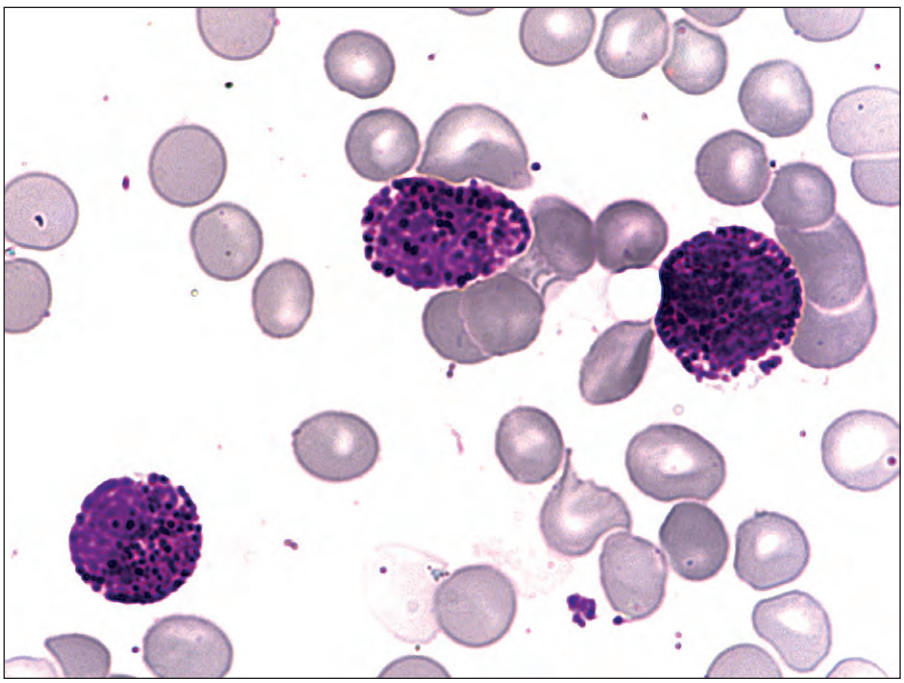


**Fig. 5.18** Marked basophilia in CML; patient in progression to the accelerated phase; peripheral blood film. The three basophil granulocytes that are seen in this image are all morphologically normal. The numerous almost black granules stand out in the wine-red cytoplasm and are clearly visible even when superimposed on the nucleus.

progressive loss of differentiation, by subtle morphological atypia, such as nucleocytoplasmic asynchrony, or by more obvious abnormalities (absence or reduction of granules or the presence of large coalescent granules). Neutrophil alkaline phosphatase activity often increases in this phase of the disease. The number of cells in mitosis increases and sometimes mitotic figures are also seen in the peripheral blood.

In some cases there is a persistent and marked increase in basophils and their precursors in both the marrow and the peripheral blood, with a tendency for these cells to replace the

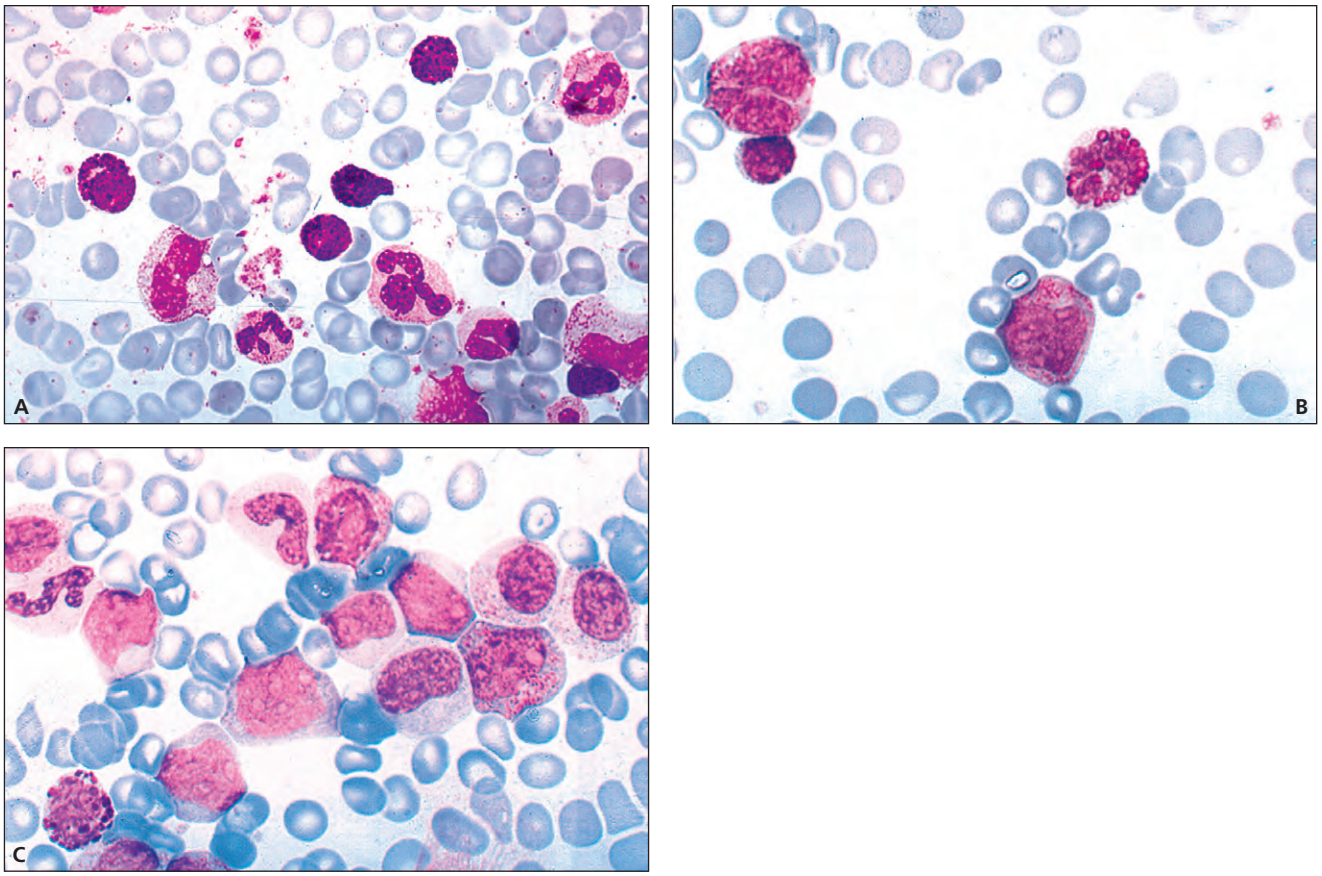



**Fig. 5.19** Accelerated phase of CML, *BCR-ABL1* positive. In this patient, the haematological picture showed a progressive deterioration, characterised by bone marrow insufficiency with anaemia and worsening thrombocytopenia, associated with an increase in peripheral blood basophils (21%) and the appearance of features of trilineage dysplasia. (A) Peripheral blood film. There are four basophil granulocytes with a normal number of granules. There is some neutrophil dysplasia, at least in the large cell with chromatin that is too pale for a mature cell. There are two small masses of platelets. (B) In the same film, at the bottom in the centre, a dysplastic promyelocyte with an angulated nucleus, homogeneous delicate chromatin, numerous prominent nucleoli with peripheral reinforcement, a distorted Golgi zone and numerous dispersed granules. The two basophils have atypical morphology, as is often seen in the basophilia that can characterise the accelerated phase. There are lobulated nuclei with little chromatin condensation: the granules, in particular, are very large but not very numerous, with a washed-out appearance in the centre, and are curiously concentrated above the nucleus and in its immediate vicinity, leaving relatively large parts of the greyish cytoplasm empty. On the left, a small lymphocyte; on the right, a platelet. (C) Bone marrow aspirate. The bone marrow is cellular with the cells being mainly of granulocyte lineage, with a left shift of the maturation curve and an increase of promyelocytes and blasts. In this field, one can observe, in addition to some immature granulocytes with dysplastic features and two band neutrophils, four blast cells with very few granules and reticular dispersed chromatin with prominent nucleoli. On the right, a dysplastic promyelocyte and two myelocytes with hypogranular cytoplasm. In the bottom left corner, a mature, cytologically normal basophil.

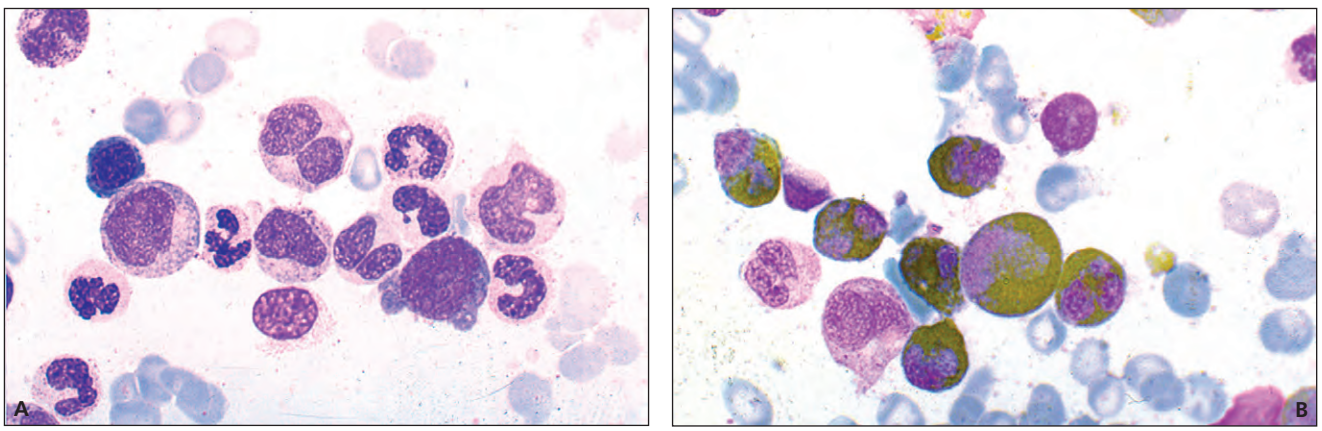



**Fig. 5.20** CML, *BCR-ABL1* positive, in accelerated phase; bone marrow aspirate. The dominant feature in this patient is the severe trilineage dysplasia with a moderate increase in blast cells. (A) Bottom right, a blast cell with granular chromatin, basophil granules and vesicular cytoplasmic protuberances. All the cells of neutrophil lineage have obvious features of dysplasia, both nuclear (reticular chromatin) and cytoplasmic (weak basophilia and hypogranularity). The largest cell, on the left, between a dyserythropoeitc erythroblast at the top and an agranular neutrophil at the bottom, has the nucleus of a blast but the cytoplasm of a promyelocyte. An agranular neutrophil, to the right of centre, has a bilobed, spectacle-shaped nucleus and a drumstick appendage. (B) Peroxidase stain. The negativity of some maturing granulocytes can provide further confirmation of the dysgranulopoiesis that characterises the accelerated phase. In this patient the appearance of a subclone of peroxidase-negative cells of neutrophil lineage was followed, within a few weeks, by progressive anaemia and thrombocytopenia. In this image, the majority of the neutrophils and their precursors are peroxidase positive, as is shown by the greenish-yellow precipitate resulting from the oxidation of benzidine. However, the myelocyte bottom left is negative, as is the neutrophil with the bilobed nucleus immediately above and to the left of it.

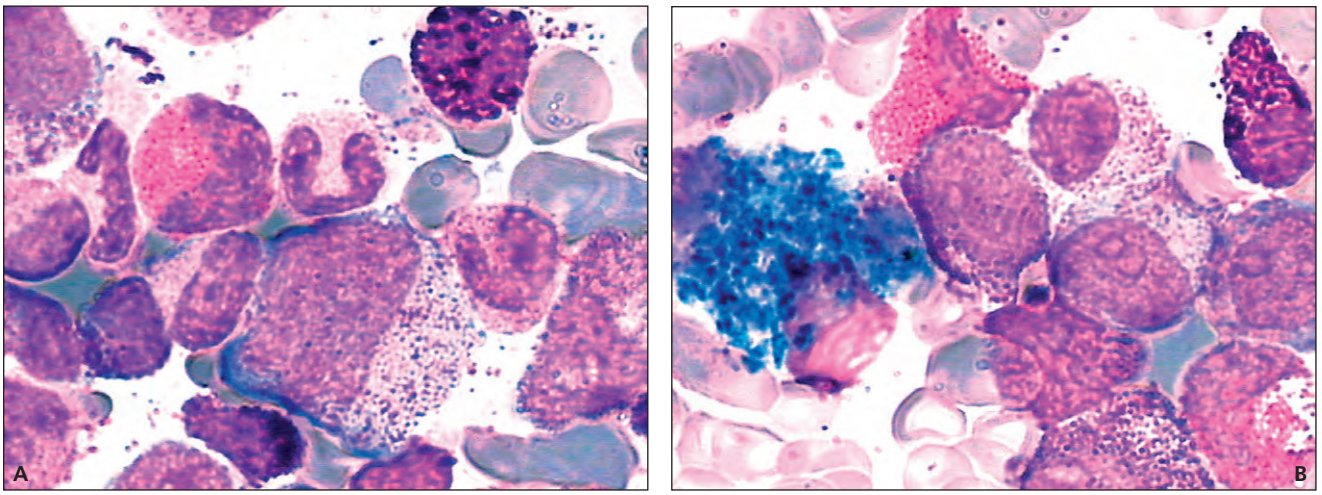



**Fig. 5.21** Bone marrow aspirate of a patient in accelerated phase during imatinib therapy (same case as Fig. 5.15). (A) There are numerous neutrophil granulocyte precursors (in the centre, a large promyelocyte with dysmorphic features), three basophils and an eosinophil. (B) On the left, a sea-blue histiocyte and three very dysplastic promyelocytes. At the top, an eosinophil, a small neutrophil myelocyte and a basophil. In the bottom right corner, an eosinophil metamyelocyte with a large cytoplasmic vacuole.

previously dominant expanded population of neutrophils. Anomalous granulation of basophils is frequent and sometimes this is also seen in eosinophils. The erythroid series may show dyserythropoiesis, with anomalous lobulation of erythroblast nuclei, karyorrhexis, asynchronous nucleocytoplasmic maturation and ring sideroblasts. In the peripheral blood, one may observe dysmorphic erythroblasts, macrocytosis, polychromasia, Howell-Jolly bodies and basophilic stippling; anisopoikilocytosis and the presence of dacrocytes can be indicators of medullary fibrosis.

Platelets are often dysmorphic, with giant forms. Bone marrow megakaryocytes appear dysplastic with a great deal of morphological heterogeneity, from gigantic hyperlobated forms, such as are common in association with thrombocytosis, to smaller elements with small rounded hypolobated nuclei or micromegakaryocytes. Sometimes micromegakaryocytes and bare nuclei are seen in peripheral blood films. In bone marrow biopsy sections, one may see small focal accumulations of blast cells, with atypical megakaryocytes often in clusters and an accentuation of the reticulin network with more or less marked fibrosis.

Report 5.2 shows a typical automated instrument printout in the accelerated phase of CML.

Table 5.3 lists the criteria proposed for the recognition of disease progression in the 2001 WHO classification,[51] which were confirmed in the updated 2008 classification[50]: these are mainly quantitative and are related to an increase in the leucocyte mass (leucocytosis and/or splenomegaly), thrombocytopenia that is not related to treatment, persistent thrombocytosis or an increase in blast cells or basophils. Cytogenetic or molecular evidence of clonal evolution has a similar significance. In the era of tyrosine kinase inhibitor therapy, however, the value of resistance to such therapy as a criterion of progression has not yet been unequivocally shown. The European LeukemiaNet

**Table 5.3** Criteria indicating progression of *BCR-ABL1*-positive chronic myeloid leukaemia* (reproduced from Jaffee 2001,[51] with permission of IARC)

**Diagnosis of accelerated phase**

- *Increased blast cells (10–19%)* (out of total nucleated cells) in peripheral blood and/or in the bone marrow
- *Increased basophils (≥20%)* in the peripheral blood
- *Increasing splenomegaly*, not responsive to treatment
- *Cytogenetic evidence of clonal evolution*
- Abnormalities in the blood count:
  - Worsening anaemia
  - *Thrombocytopenia (<100 × $10^9$/l)* or persistent thrombocytosis (>1000 × $10^9$/l), not responsive to treatment
  - *Increasing leucocytosis*, not responsive to treatment
  - Progressive reduction of the leucocyte count, not related to treatment
- Anomalies, particularly histopathological, in the cytology and histology of the bone marrow:
  - Increase, clustering and dysmorphism of megakaryocytes
  - Granulocytic dysplasia
  - Dyserythropoiesis
  - Deviation to the left of the granulocyte maturation curve, with increased promyelocytes
  - Reticulin or collagen fibrosis
  - Neutrophil dysplasia, with a pseudo-Pelger appearance, hypogranularity or peroxidase deficiency
  - Increase in neutrophil alkaline phosphatase activity
  - Increased circulating erythroblasts with dyserythropoietic features
  - Macrocytosis, not related to treatment; anisopoikilocytosis, Howell-Jolly bodies, basophilic stippling
  - Giant platelets and bizarre forms
  - Increased circulating megakaryocyte nuclei

**Diagnosis of blast crisis**

- *Increased blast cells (≥20%)* (out of all nucleated cells) in the peripheral blood, bone marrow or both
- *Extramedullary blast proliferation*
- Bone marrow trephine biopsy sections show *focal blast accumulations*
- The peripheral blood and bone marrow abnormalities described in accelerated phase can also be present

*In italics, the parameters proposed in the 2001 WHO classification.[51]

group (ELN) diverge from the WHO proposals in adopting, among other criteria, a proposal from the FAB group, that increased promyelocytes should be recognised as evidence of accelerated phase[52]; their proposal is to use only three criteria to define the accelerated phase:

1. The presence in the peripheral blood, bone marrow or both of 15–20% blast cells or more than 30% blast cells plus promyelocytes (even though the blast cells alone are <30%);
2. Peripheral blood basophils more than 20%;
3. A platelet count lower than $100 \times 10^9$/l that cannot be attributed to treatment.[22]

Even though there is not yet complete consensus, in recent clinical studies the proposals of the ELN group have been welcomed.

## Blast crisis

The appearance of more than 19% blast cells (2001 and 2008 WHO criterion, extramedullary blast proliferation (2008 WHO criterion) or more than 30% blast cells in the peripheral blood or bone marrow (2006 ELN criterion) define the blast crisis of CML (Table 5.3): this can represent the natural evolution of an accelerated phase that has lasted for some months or, more often, the sudden and rapid emergence of blast crisis from chronic phase disease. Acute transformation is seen in 60–70% of patients with CML; a small proportion of cases present with an extramedullary localisation, particularly in lymph nodes. As well as worsening of bone marrow insufficiency, with anaemia and thrombocytopenia, and morphological aspects of dysplasia, a blast crisis is distinguished by the rapid expansion of one or more clones of immature blast cells, often showing new cytogenetic alterations, with loss of the capacity to mature and with clinical behaviour essentially identical to that of an acute leukaemia.[53]

From the morphological point of view, transformation of CML is characterised by a notable variability in the cytological picture, related to the derivation of the disease from a multipotent haemopoietic stem cell, from which anomalous clones oriented towards any cellular lineage and varying in their degree of differentiation can be selected and expand. This explains the high frequency of mixed forms and the difficulty that is often encountered in the morphological characterisation of a case. There are thus myeloid blast crises, which can be granulocytic, myelomonocytic or, more rarely, pure monoblastic, erythroid or megakaryocytic; lymphoid blast crises, which represent 30% of the total; and bilineage blast crises. Often, however, the blasts are morphologically undifferentiated and therefore for their identification it is essential to have, in addition to cytochemistry, immunophenotyping and in some cases electron microscopy for ultrastructural morphology and cytochemistry.[54] In myeloid or myelomonocytic blast crisis (Figs 5.22 through to 5.25), which are the most frequent (70%), the blasts in many cases show only minor signs of differentiation: generally, the nuclear chromatin is very delicate, with one or more nucleoli, and there is fairly scanty, often agranular, cytoplasm; Auer rods have been described in rare patients. In other cases, the cytoplasm appears more differentiated, with morphology that recalls that of AML with maturation. Cytochemical investigation shows variable activity for peroxidase, various esterases or both. Even in peroxidase-negative blasts, however, it may be possible to demonstrate the presence of the myeloperoxidase (MPO) enzyme by ultrastructural cytochemistry or by the use of monoclonal anti-MPO antibodies; in the same way, immunophenotyping may demonstrate the presence of myeloid antigens (CD13, CD33) or monocyte antigens (CD14, CD11c). In a minority of cases in blast crisis, there is a population of differentiated cells with the cytological features of hypergranular promyelocytes: in these patients, as in acute promyelocytic leukaemia, there may be a coagulation defect, sometimes fully developed disseminated intravascular coagulation with a fall in the platelet count and the fibrinogen concentration. Positivity for fluoride-sensitive non-specific esterase is important for defining the uncommon pure monocytic crises, which can also be suspected from the presence, more in the peripheral blood than the bone marrow, of an increased percentage of monocytes and promonocytes, not rarely with irregular nuclei and abundant cytoplasmic vacuoles (Fig. 5.26).

The rare erythroid blast crises can sometimes be suspected from the morphology of the blasts: they are often large immature cells with moderately abundant basophilic cytoplasm, sometimes vacuolated, with cytoplasmic protrusions (blebs) which extend in all directions from a large round nucleus with finely granular chromatin, similar to the promegaloblasts of pernicious anaemia; in addition, there is often marked dyserythropoiesis of the maturing erythroid cells in the bone marrow (Figs 5.27 and 5.28). The blasts and the more mature erythroid precursors are PAS positive. It is always necessary to confirm the erythroid nature of a transformation by immunophenotyping (CD71, glycophorin A), which can be supported by ultrastructural morphology (abundant polyribosomes, ferritin molecules in the cytoplasm and rhopheocytotic vesicles with membrane-associated ferritin molecules).

Similarly, megakaryoblasts sometimes have a suggestive appearance (Figs 5.29 and 5.30), with a large oval or round nucleus, compact and homogeneous chromatin that is sometimes deeply cleft and scanty cytoplasm with projections that are similar to primordial platelets or with partial platelet formation; more often, however, their undifferentiated appearance makes necessary confirmation of their nature by immunophenotyping to demonstrate platelet membrane antigens (platelet glycoproteins CD41, CD42 and CD61). Ultrastructural cytochemistry for platelet peroxidase can also be useful; activity is typically localised not in the granules, as in myeloid blasts, but in the perinuclear space and the endoplasmic reticulum. In blast crisis with a significant megakaryoblastic component, bone marrow fibrosis is often marked and there can be evolution towards the picture of myelofibrosis with osteosclerosis.

Around 10% of blast crises involve exclusively or mainly the basophil granulocyte lineage (Fig. 5.31). The blasts in these

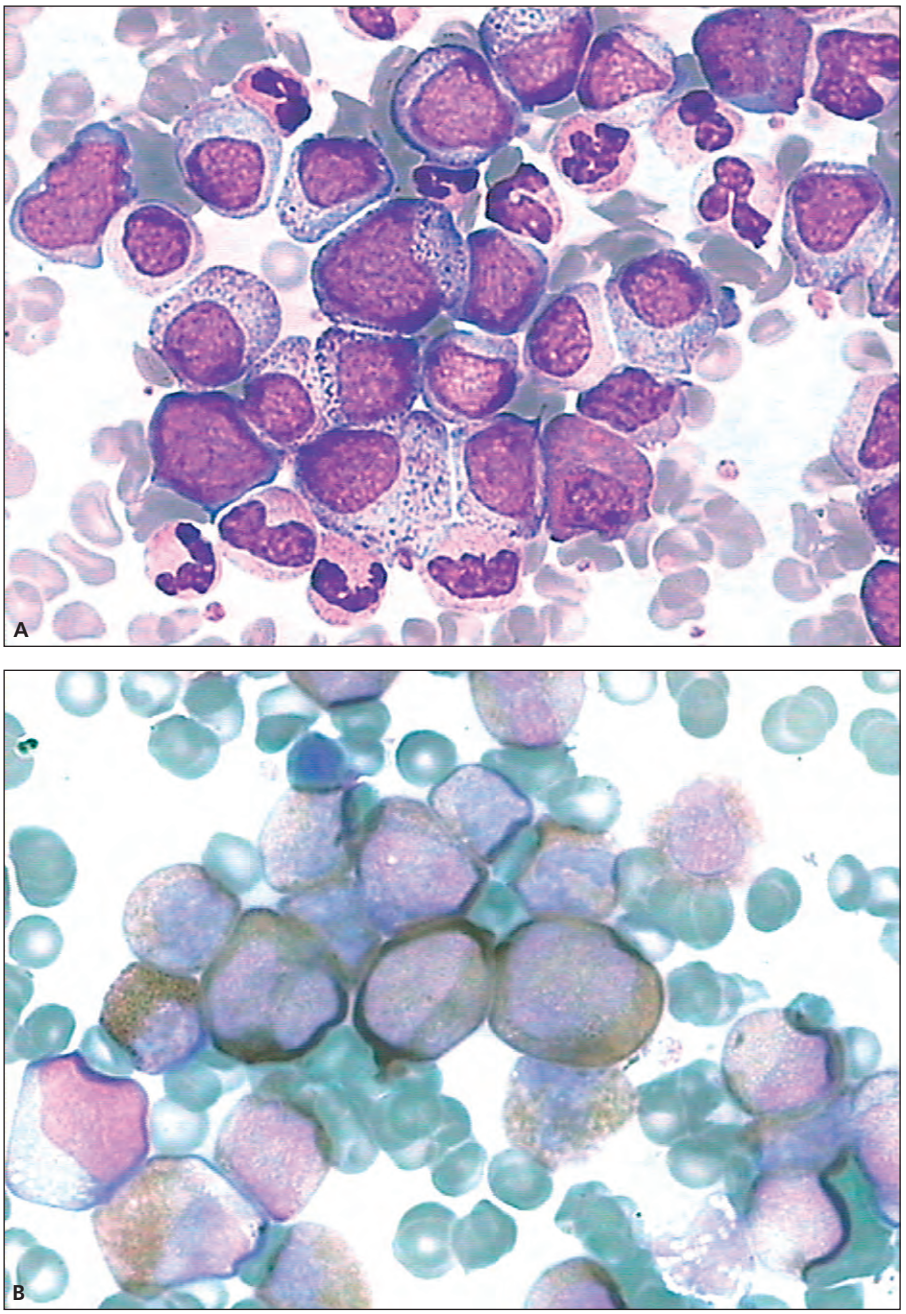



**Fig. 5.22** Blast crisis of CML, *BCR-ABL1* positive; peripheral blood. (A) Marked leucocytosis (WBC 84 × $10^9$/l) with 9% blast cells in the blood and 22% in the bone marrow aspirate. There are two agranular blast cells in the left part of this image and a third small blast, beside a large promyelocyte, a little above the centre. There is marked dysplasia of all cells of granulocyte lineage (cytoplasmic hypogranularity and nuclear dysmorphism). Top right, a cell with an immature nucleus, basophilic cytoplasm and eosinophilic granules crammed in beneath the cytoplasmic membrane, recognised as an eosinophil promyelocyte with asynchronous maturation. An eosinophil myelocyte is visible low down towards the right. (B) Peroxidase stain; peripheral blood film of the same case as (A). All the cells show positivity, of variable degree, with the exception of a blast cell with plentiful pale cytoplasm, bottom left.

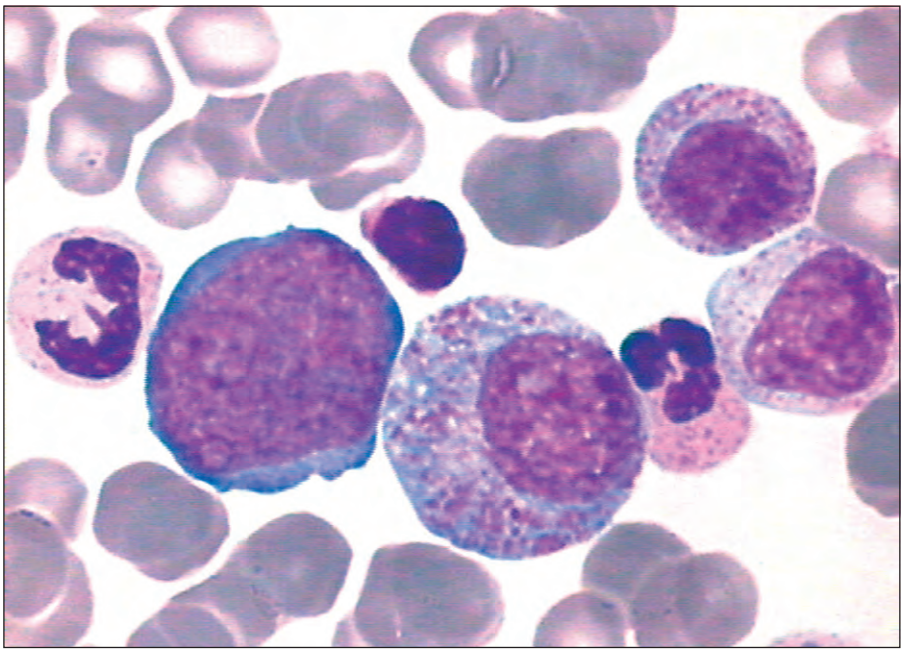


**Fig. 5.23** Myeloid blast crisis of CML, *BCR-ABL1* positive; same case as Fig. 5.22; peripheral blood film. The large blast has a polygonal and angulated, near-tetraploid nucleus with reticular almost homogeneous chromatin with only small areas of condensation, and with nucleoli, one of which, near the lower pole, is prominent. The cell close to it, on the right, is similar in size, but has a different N : C ratio because of the presence of plentiful cytoplasm which is much more weakly basophilic and contains a number of quite voluminous granules, with colour varying from bright purple to brick red, and numerous microvacuoles. The nucleus is eccentric, with chromatin condensed into small clumps with a paler area corresponding to the remains of a nucleolus. These ambiguous characteristics, relating to the dysplasia that accompanies blastic evolution, place this cell on the borderline between a promyelocyte and a myelocyte. The two small myelocytes visible on the right are also dysplastic, as are the two mature neutrophils. The dark nucleus, smaller than an erythrocyte, with a small halo of pink cytoplasm, above the two large cells in the centre, could be an apoptotic neutrophil.

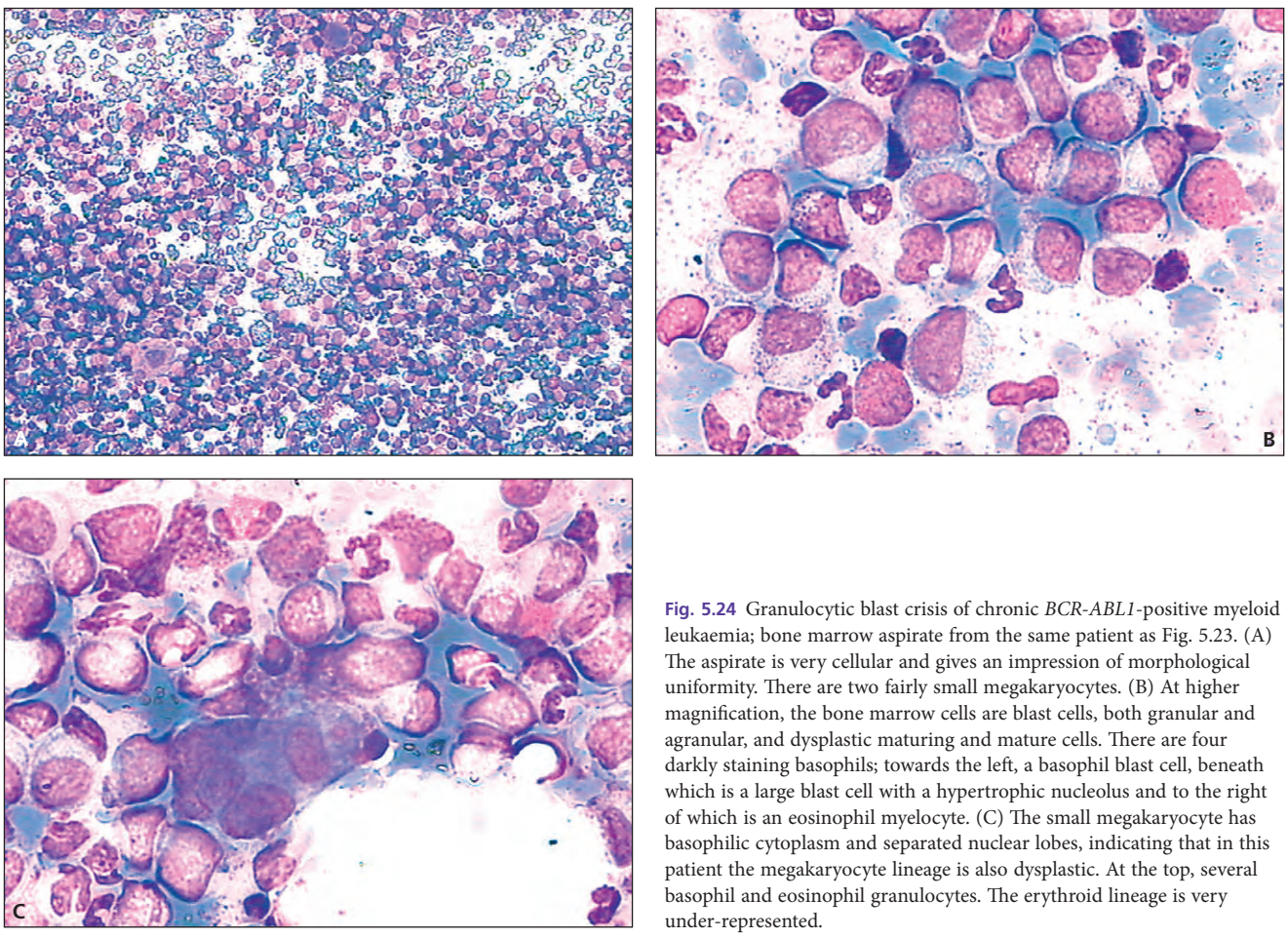



**Fig. 5.24** Granulocytic blast crisis of chronic *BCR-ABL1*-positive myeloid leukaemia; bone marrow aspirate from the same patient as Fig. 5.23. (A) The aspirate is very cellular and gives an impression of morphological uniformity. There are two fairly small megakaryocytes. (B) At higher magnification, the bone marrow cells are blast cells, both granular and agranular, and dysplastic maturing and mature cells. There are four darkly staining basophils; towards the left, a basophil blast cell, beneath which is a large blast cell with a hypertrophic nucleolus and to the right of which is an eosinophil myelocyte. (C) The small megakaryocyte has basophilic cytoplasm and separated nuclear lobes, indicating that in this patient the megakaryocyte lineage is also dysplastic. At the top, several basophil and eosinophil granulocytes. The erythroid lineage is very under-represented.

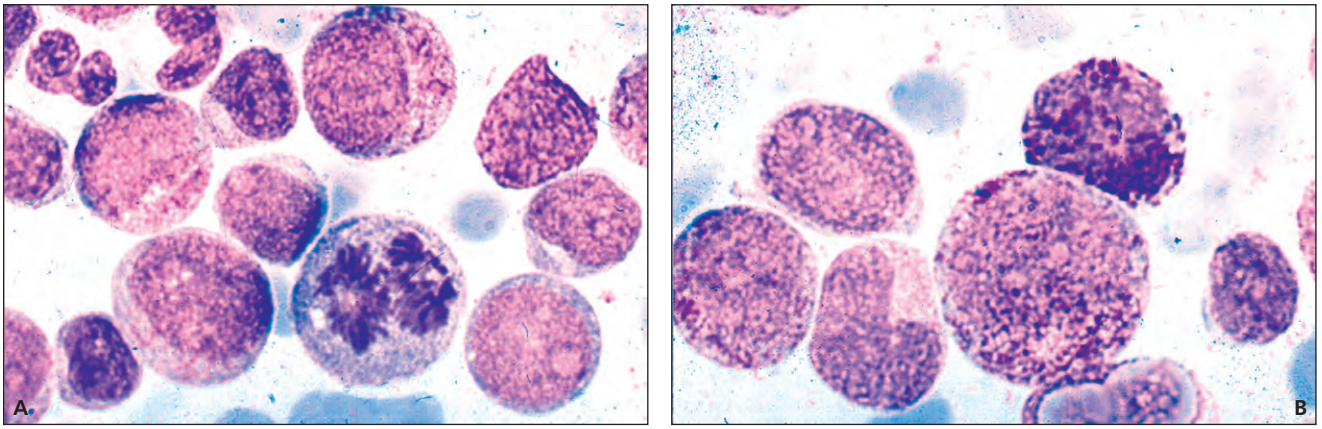



**Fig. 5.25** CML, *BCR-ABL1* positive, myeloid blast crisis; peripheral blood film. (A) At the bottom, a cell in metaphase with very fine cytoplasmic granules; the presence of mitotic figures in circulating cells is rare in the chronic phase of CML, but is seen more often in blast crisis. To the right of the cell in metaphase, a large blast cell with a perfectly round nuclear outline and fine, delicate, homogeneously distributed chromatin, in which stand out two nucleoli, the largest of which has a pale circle of chromatin reinforcement. In the centre on the right, another two smaller blast cells with a less regular, angulated nuclear outline. The three larger cells, these also with immature nuclei with nucleoli of varying prominence, are distinguished by the presence of azurophilic cytoplasmic granules that in some cells are infrequent and in others, such as the larger cell at the top, are more abundant. These cells can be classified, according to the current WHO criteria, as dysplastic promyelocytes. In the top left corner, a neutrophil with an abnormal nucleus and agranular cytoplasm. (B) Top right, a basophil myelocyte with cytoplasm loaded with specific granules. Immediately beneath it, a large atypical promyelocyte with asynchrony between the immature nucleus and the more differentiated cytoplasm: the chromatin is in fact very delicate and contains multiple nucleoli, while the cytoplasm is packed with both pale azurophilic granules and neutrophilic granules (one can see the mixture of small bright red or violet primary granules and other larger, dark red granules); the cytoplasm still has large areas of peripheral basophilia. The cell higher up on the left is a blast cell with scanty cytoplasm: its oval nucleus appears paradoxically more mature than that of the hypergranular promyelocyte, because of the greater density of the chromatin network and the barely visible nucleoli.

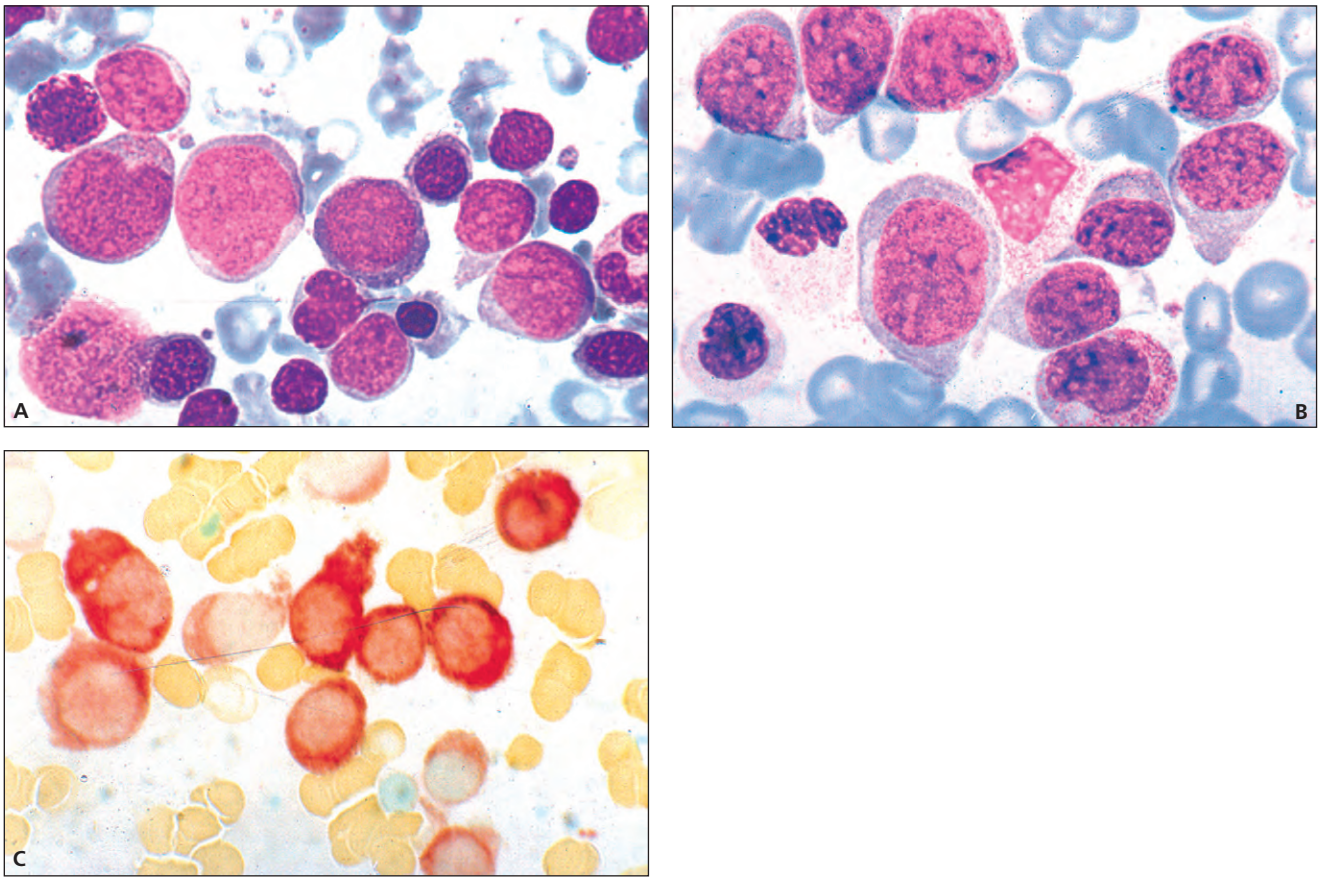



**Fig. 5.26** Blast crisis of CML, *BCR-ABL1* positive; bone marrow aspirate. (A) In this field there are four small, undifferentiated blast cells with scanty cytoplasm and a high N:C ratio; the chromatin is partially condensed and there are nucleoli, sometimes prominent; the nuclear outline has a notch in the cell top left, adjacent to a mature basophil. The two larger cells in the centre are also blast cells: the one furthest to the left has dense granular chromatin with a nucleolus at the lower pole of the nucleus, completely surrounded by condensed chromatin, and a clear nuclear notch; the basophilic cytoplasm contains granules; the more central cell does not have granules and its nucleus, which has a small notch, has looser reticular chromatin, in which are seen at least four nucleoli. On its right, a proerythroblast, below which is a monocyte. (B) In this field, cells of monocytic lineage are more prevalent; one notes both the large blast cell with delicate, open reticular chromatin, similar to that described in (A), and the atypical monocyte in the top right corner. There are three blasts along the top border on the left; lower down are two severely dysplastic mature neutrophils. (C) Numerous blast cells are positive for non-specific esterase (alpha-naphthyl butyrate esterase). Monocytic differentiation of 47% of the blasts was confirmed by demonstration of expression of CD14 on flow cytometry.

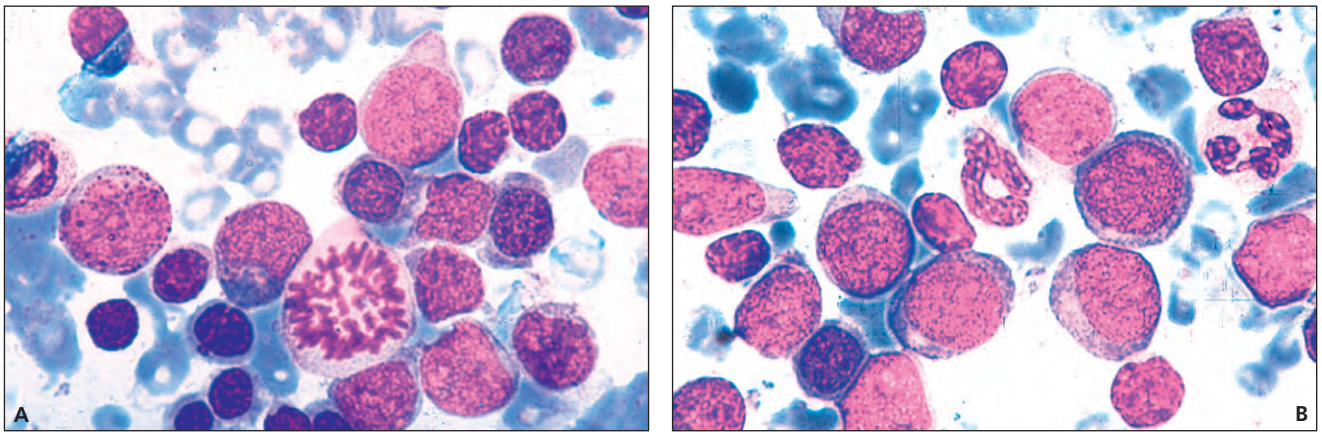



**Fig. 5.27** CML, *BCR-ABL1* positive, blast crisis with erythroid differentiation; bone marrow aspirate. (A) Around what appears to be a granulocyte precursor containing a mitotic figure, there are a number of blast cells with a high N:C ratio; the nucleus is large, round or, often, angulated, with fine delicate chromatin and prominent nucleoli; in the scanty, weakly basophilic cytoplasm one can see very fine azurophilic granules. To the left of the cell in mitosis, a cell with the nucleus of a promyelocyte and the cytoplasm of an eosinophil myelocyte; to the left of this, a large blast cell with a nucleus that has a nucleolus and an irregular, notched outline, and cytoplasm that contains granules similar to the specific granules of basophils. In addition, there are a number of basophilic and polychromatic erythroblasts with clear signs of dyserythropoiesis. In this patient, 20% of the blast cells were peroxidase positive. The dysplastic erythroid series constituted 57% of cells, while blasts were 32% (77% non-erythroid cells [NEC]): had this been a case of *de novo* acute leukaemia, the diagnosis would have been AML, NOS, erythroleukaemia. (B) In the same case as (A): in addition to myeloid blast cells, similar to those described in (A) (e.g. the two cells cut off at the right and left edges respectively), there are at least two large proerythroblasts with voluminous rounded nuclei in the centre of intensely basophilic, agranular cytoplasm. The chromatin network is megaloblastoid, more delicate than is found in normal proerythroblasts. For some cells, such as that found bottom right, beneath one erythroblast and on the right of another – this one also similar to a promegaloblast, with a nucleus mainly adherent to the cytoplasmic membrane – it is difficult to make a secure identification solely on the basis of morphology. Also of note is the large neutrophil, top right, which seems to have two well-formed nuclei, as if there has been nuclear duplication not followed by division of the cytoplasm (incomplete cytodieresis).

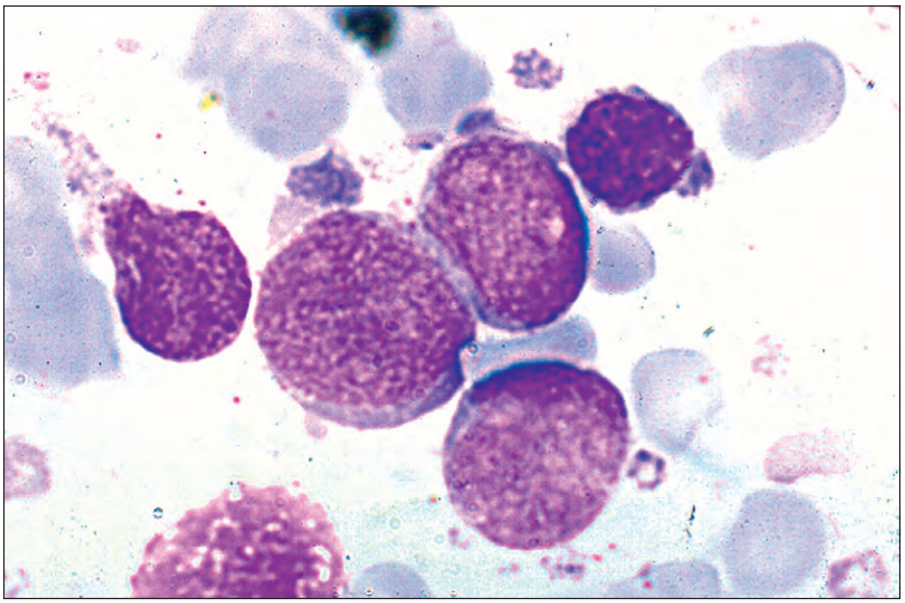


**Fig. 5.28** CML, *BCR-ABL1* positive, immature erythroid blast crisis; bone marrow aspirate. The three blast cells in the centre of the image have large nuclei with a regular, rounded nuclear outline, with finely granular chromatin which resembles, particularly in the largest cell on the left, that of the promegaloblasts of pernicious anaemia. The cytoplasm is rather intensely basophilic and has vesicular peripheral projections or blebs, particularly evident in the two blast cells at the top. The cell top right has the nuclear morphology of a basophilic erythroblast, with a cytoplasmic bleb similar to that in the more immature cells. The elongated nucleus on the left, with immature chromatin and a thin cytoplasmic tail, resembles a hand-mirror lymphoblast and cannot be identified by morphology alone. The blasts in this patient were positive with antiglycophorin A antibodies.

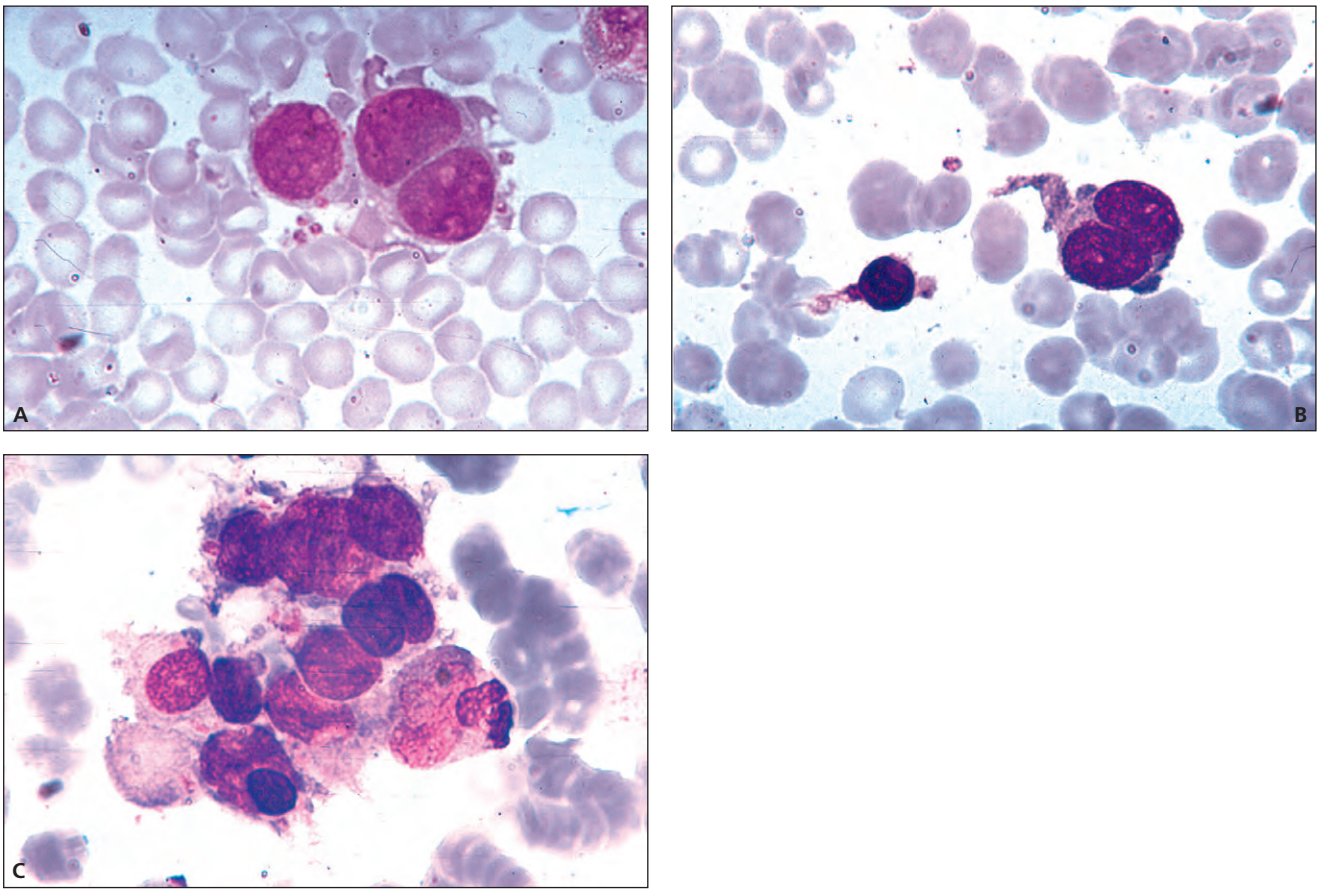



**Fig. 5.29** CML, *BCR-ABL1* positive, megakaryocytic blast crisis. (A) Peripheral blood film. Two circulating megakaryoblasts, one of which is binucleated. The cell on the left has a high N:C ratio; its nucleus is very voluminous with a rounded outline and a dense, homogeneous, reticular chromatin network, without any pale spaces; there are numerous nucleoli, some indistinct, others (at least two) showing perinucleolar chromatin reinforcement; the cytoplasm is basophilic, tending to greyish, and agranular, with cytoplasmic blebs that appear vesicular, but with ragged ill-defined limits, as on the left side. A little further to the left is a pair of small anuclear fragments, resembling agranular platelets, that appear to be detached fragments of megakaryoblast cytoplasm. Lower down are some normally formed platelets, one of which is superimposed on the cytoplasm of the megakaryoblast. The larger, binucleated cell is differentiated from the cell already described only in that endoreduplication has occurred; the nuclei differ from what is expected in the megakaryocyte lineage in that they are clearly separated from each other; otherwise their structure is very similar to what has already been described; the nuclei are closely related, each deforming the profile of the other, with the nuclear outline being straight where they are in contact; the cytoplasm is very irregular with some areas that appear delimited and ready to detach, such as the large cytoplasmic fragment, bottom left. (B) Same film as (A). A binucleated micromegakaryocyte and a circulating micromegakaryocyte. This patient was observed a number of years ago when immunological markers of megakaryocytes were not available. The morphology of these cells, present in the peripheral blood in the context of severe trilineage cytopenia and in association with blast cells similar to those illustrated in (A), does not leave any doubt as to their nature. The atypical binucleate micromegakaryocyte is very similar to that shown in (A), but the nuclear chromatin is denser and each nucleus has two small pale areas that are probably the vestiges of nucleoli; one cannot exclude that the chromatin of the two nuclei is in continuity; the cytoplasm appears more intensely basophilic. The cell on the left is a micromegakaryocyte of the type that is seen in MDS (and which is never present in the chronic phase of CML): it has a small rounded nucleus with completely condensed chromatin, with two cytoplasmic projections; the entire circumference of the cell is composed of ragged cytoplasm. (C) Same case as (A) and (B), bone marrow aspirate. The material is very hypocellular, almost like a desert. However, standing out on this background are numerous masses of these large anomalous cells, often immature and hyperdiploid, which seem quite similar, at least at low power, to metastases of a solid tumour. The cytological characteristics that permit one to assign them to the megakaryocyte lineage, other than the simultaneous presence in the peripheral blood film of cells such as those described in (A) and (B), is the tendency to binuclearity, with nuclei in close contact with each other and the jagged, highly irregular cytoplasmic margins, which are tending to detach, forming rudimentary giant hypogranular platelets. One of these can be seen between the rouleaux of red cells close to the right side of the image. One can see how atypical megakaryocyte precursors show the full range of nuclear maturity, from the most immature, at the top, with chromatin still reticular, to those with compact dense chromatin, such as the micromegakaryocyte with a small, almost pyknotic, nucleus at the lower edge of the mass of cells, to the left of which one notes a strand of greyish cytoplasm that is now detaching itself from a nucleated cell.

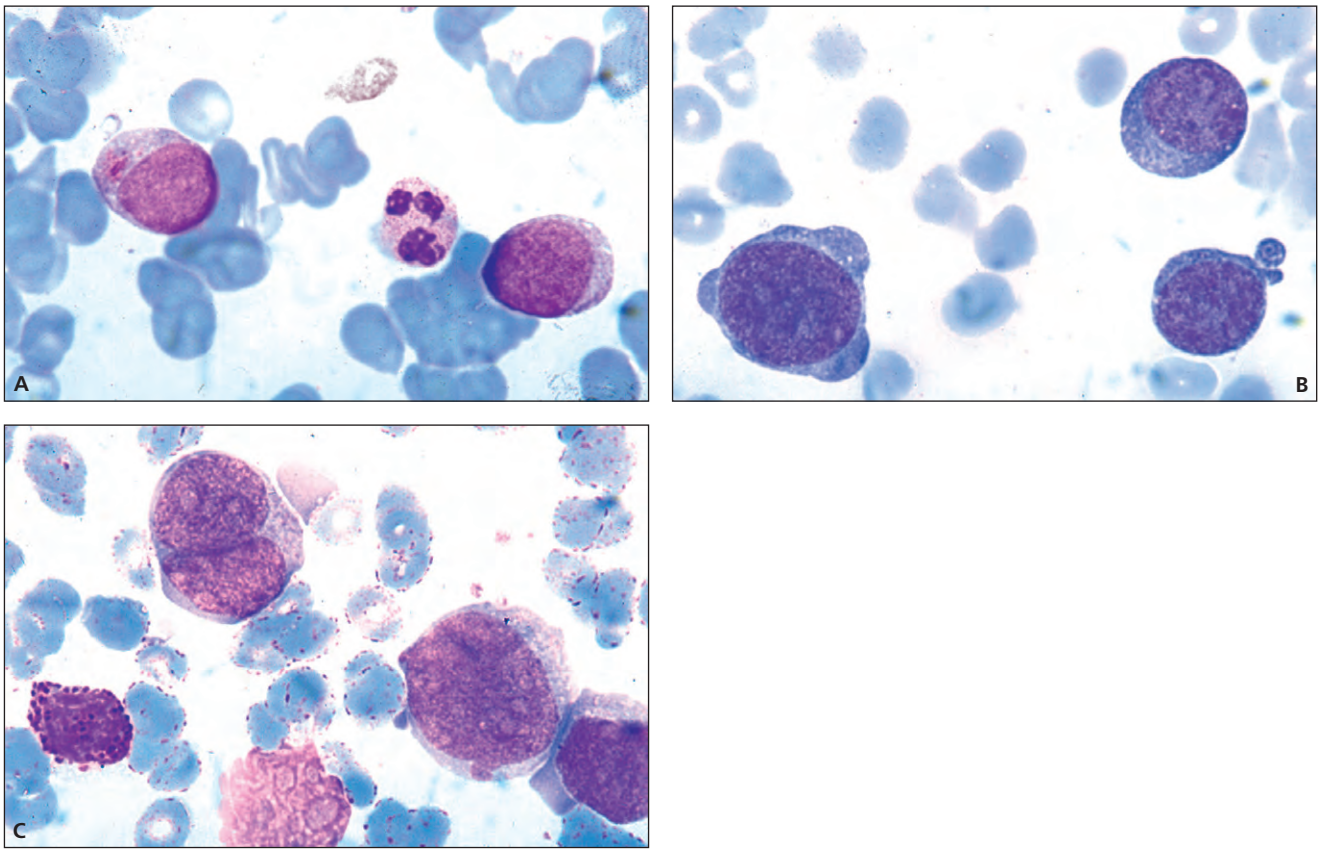



**Fig. 5.30** Another case of megakaryocytic blast crisis of CML, *BCR-ABL1* positive, with the presence of less differentiated blast cells; bone marrow aspirate of a female patient aged 31 years who had suffered from CML for four years. The haematological picture had changed with a marked increase in the platelet count, which had reached values above $1000 \times 10^9$/l, then returning after three months to $170 \times 10^9$/l after an adjustment of her chemotherapy. (A) Attempted aspiration yielded several drops of not very cellular marrow without fragments, composed mainly of mature granulocytes and blast cells with strange morphological feature. They are large and variable in size. The nucleus is very large and always eccentric, round or oval without notches or angulation. The chromatin network is particularly granular and homogeneous with the juxtaposition of uniform and regular fine dots of condensed chromatin. The nucleoli are visible, even if there is no perinucleolar reinforcement. The cytoplasm is plentiful and moderately basophilic, lacking in homogeneity and with a number of small vacuoles. In the cell on the left, a focal accumulation of large azurophilic granules in the centre of the cytoplasm. The peroxidase reaction, not illustrated, was totally negative in the blastic population. In the centre of the image, at the top, a large greyish oval platelet, with granules scattered in an irregular manner. (B) The granular chromatin network, the regularity of the nuclear outline and the cytoplasmic basophilia could suggest the possibility of an erythroid origin of these immature cells. The cytoplasm, always well represented and basophilic, often forms ample pseudopodial projections that tend to detach from the main body of the cell as small spheroidal structures, which sometimes contain small granules or vacuoles. (C) The appearance of the large binucleated blast cell on the left has clear analogies with dysplastic cells of megakaryocyte lineage: the two nuclei are oval and lined up with the major axis of the cell, with nuclear outlines that are mirror images with facing flat surfaces but are otherwise curved. The chromatin is granular, with small areas of condensation and pale spaces forming a loose-knit network; there are multiple prominent nucleoli. Immunophenotyping demonstrated that all the immature cells expressed CD42 and CD61, confirming that this transformation of CML was megakaryoblastic. The presence of numerous basophil granulocytes, bottom left, provides evidence, as often happens in blast crisis, of the preceding chronic phase disease.

cases are heterogeneous with regard to size, with nuclei that have fine dispersed chromatin and prominent nucleoli. The cytoplasm is weakly basophilic and can either be agranular or, sometimes, can contain very large peroxidase-negative azurophilic granules or, again, there may be scanty, more easily recognisable metachromatic granules. Almost always there is a marked increase in mature basophils, often atypical, and of precursors at an intermediate stage of maturation. Even if not always present, the characteristic metachromasia with toluidine blue or alcian blue offers a very useful diagnostic clue in these cases. Often, only ultrastructural examination permits the recognition in the cytoplasm of apparently undifferentiated or lymphoid-appearing blast cells and the presence of basophil granules with their very particular morphology.

It is not rare for there to be mixed bi- or tri-lineage blast populations, with features of a true panmyelosis (Fig. 5.32).

Thirty per cent of blast crises are lymphoid in nature (Fig. 5.33): the blast cells are typical lymphoblasts with scanty

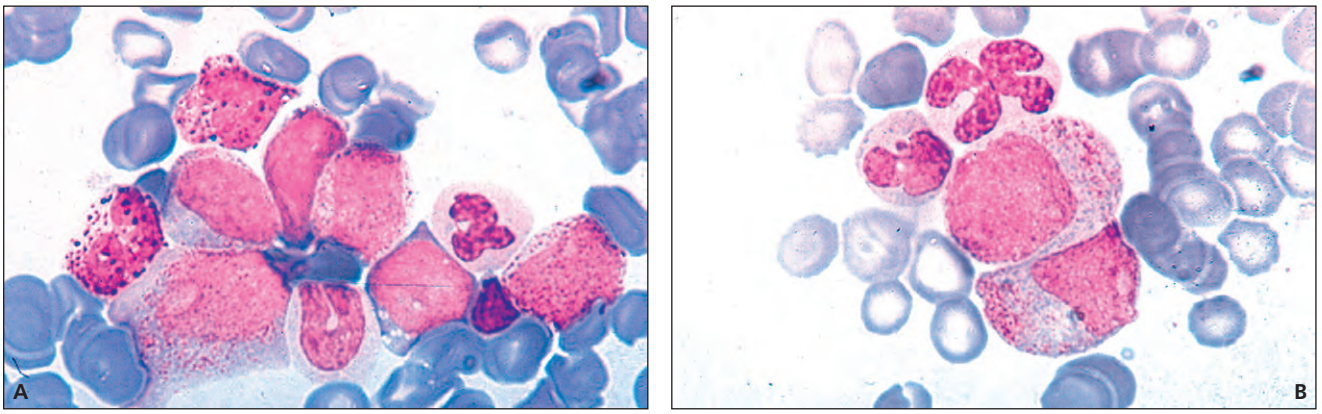



**Fig. 5.31** CML, *BCR-ABL1* positive, basophil blast crisis; bone marrow aspirate. This is from the same patient as Fig. 5.19. This aspirate was obtained ten months later at a time of worsening anaemia and the appearance of immature cells in the peripheral blood. (A) In addition to two mature basophils, with a slight reduction of granules, there are two undifferentiated blast cells, one elongated as a result of compression between adjacent cells, the other with heavily vacuolated intensely basophilic cytoplasm. Another four immature cells have fairly large, bright red cytoplasmic granules, which stand out clearly in a moderate amount of weakly basophilic cytoplasm. These cells were peroxidase negative but, like the mature basophils, stained metachromatically with toluidine blue and thus can be considered atypical basophil promyelocytes. (B) The nuclei of these basophil promyelocytes have reticular loosely-knit chromatin, with pale internuclear spaces and two or three nucleoli. The cytoplasm is plentiful and pale blue with little evidence of a Golgi zone. Granulation appears atypical, both because the granules are tending to be peripheral and because usually the bright red, azurophilic granules of promyelocytes are smaller. Peroxidase activity was lacking. The cell at the top is a dysplastic neutrophil with two band-shaped nuclei.

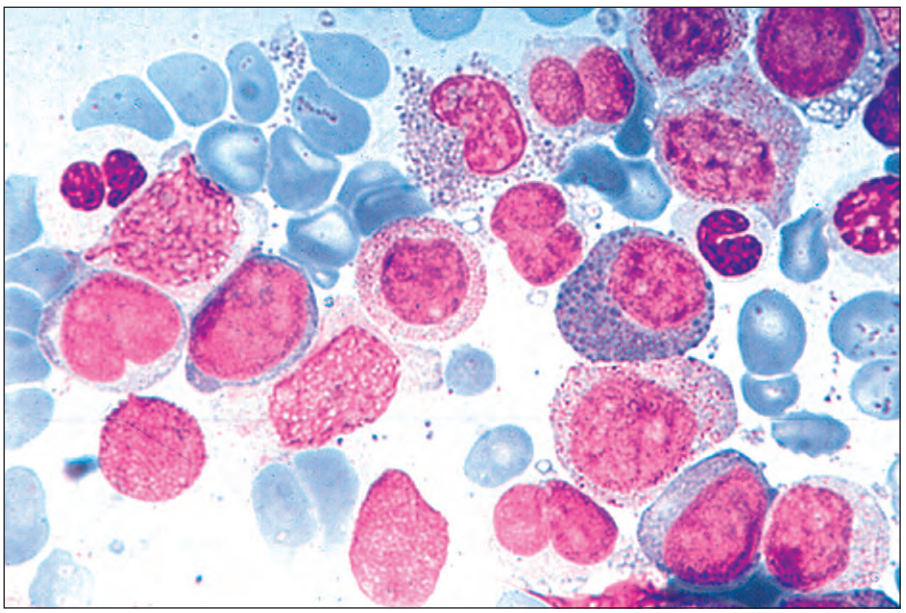


**Fig. 5.32** CML, *BCR-ABL1* positive, myeloid blast crisis; peripheral blood film. In this patient acute transformation is characterised by the involvement of all myeloid lineages, with an increase of undifferentiated blast cells, monocytoid cells and proerythroblasts, together with marked dysplasia of all lineages. There are two very dysplastic mature neutrophils, with pale cytoplasm with no granules and with hyposegmented pelgeroid nuclei with very dark chromatin; two monocytoid cells, one on the left below the dysplastic neutrophil and the other close to the centre; in the top right corner: a proerythroblast with vacuolated basophilic cytoplasm; two undifferentiated blast cells with basophilic cytoplasm, with that on the left showing a resemblance to cells of the erythroid lineage; a promyelocyte and three neutrophil myelocytes; an eosinophil myelocyte and an eosinophil metamyelocyte; a dysplastic erythroblast cut off at the right edge and two cells that are either binucleated or have bilobed nuclei, at the top and at the bottom respectively, both unclassifiable.

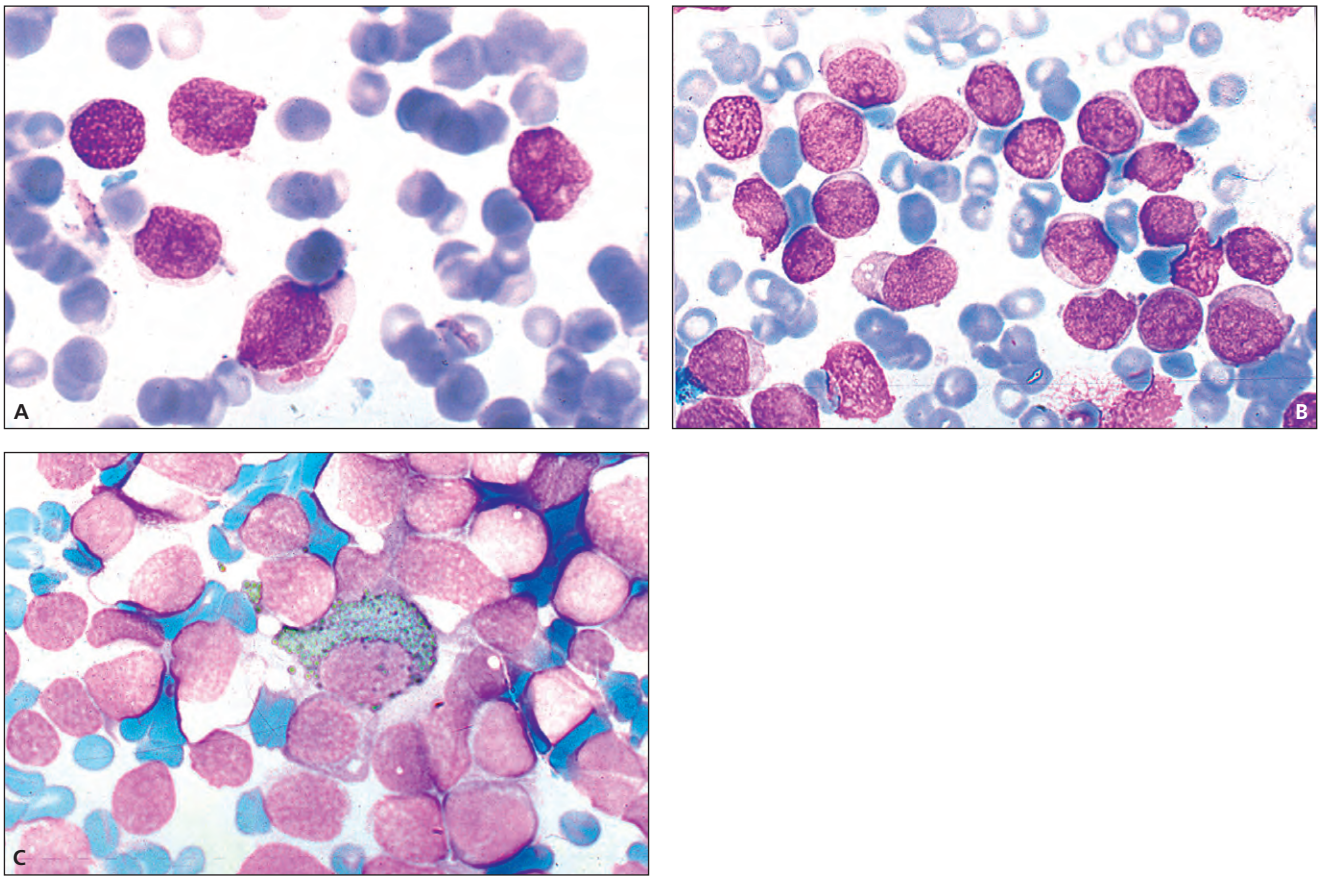



**Fig. 5.33** CML, *BCR-ABL1* positive, B-lymphoid blast crisis. (A) Peripheral blood film. Five blast cells, four of which have the uniform morphology of small lymphoblasts: the cytoplasm is very scanty or almost invisible, weakly basophilic and agranular; the nuclei are round or oval with a homogeneous chromatin network, slightly denser than that of myeloid blasts, with no more than one nucleolus. The larger cell, at the bottom, has more plentiful cytoplasm in which one can observe a nuclear fragment, probably resulting from the persistence of one or more isolated chromosomes lost during a disorderly mitotic division. (B) Bone marrow aspirate. The granulocytic hyperplasia of the chronic phase is no longer present and has been replaced by proliferation of undifferentiated blast cells of variable size. The larger blast cells have a voluminous nucleus with an oval or angulated outline; the chromatin is delicate and homogeneous with one or two nucleoli with an irregular outline, sometimes with a circle of chromatin reinforcement; the moderately abundant cytoplasm is agranular with basophilia that is weak and irregular with peripheral enhancement. The smaller blasts have a quite high N:C ratio, denser chromatin with one nucleolus or none, and very scanty cytoplasm. (C) Bone marrow aspirate, peroxidase reaction. All the blast cells are uniformly negative; the only cell that is packed with peroxidase-positive granules, near the centre, is an eosinophil myelocyte. Immunophenotyping in this patient showed expression of CD19, CD20 and CD10 (common B immunophenotype): cytogenetic analysis showed clonal evolution with duplication of the Ph chromosome in all metaphases and trisomy 8 in about half.

cytoplasm, more condensed chromatin in blocks, with absent or barely visible nucleoli. Peroxidase is always negative, while PAS is often positive. The morphology can be either that of acute lymphoblastic leukaemia (ALL) of childhood with small homogeneous blast cells with regular nuclei or that of the form more common in adults, with blasts of variable size and more abundant cytoplasm, an irregular or notched nuclear outline and one or two easily seen nucleoli. In lymphoid blast crisis, dysplastic features in myeloid lineages are usually absent. The diagnosis is necessarily dependent on immunophenotyping which shows, in the majority of cases, the phenotype of common B-ALL (CD10, CD19, CD20) or, rarely, an immature T phenotype (Fig. 5.34). Finally, the frequency of mixed or biphenotypic leukaemic populations appears relatively more common in blast crisis of CML than in AML at diagnosis (Fig. 5.35), occurring in around 20–30% of patients.[55] The phenotype most often associated with lymphoblastic differentiation in bilineage cases is myeloblastic or megakaryoblastic.

## Morphological effects of tyrosine kinase inhibitors

In patients treated with tyrosine kinase inhibitors, the natural history of the disease is dramatically altered. The response to

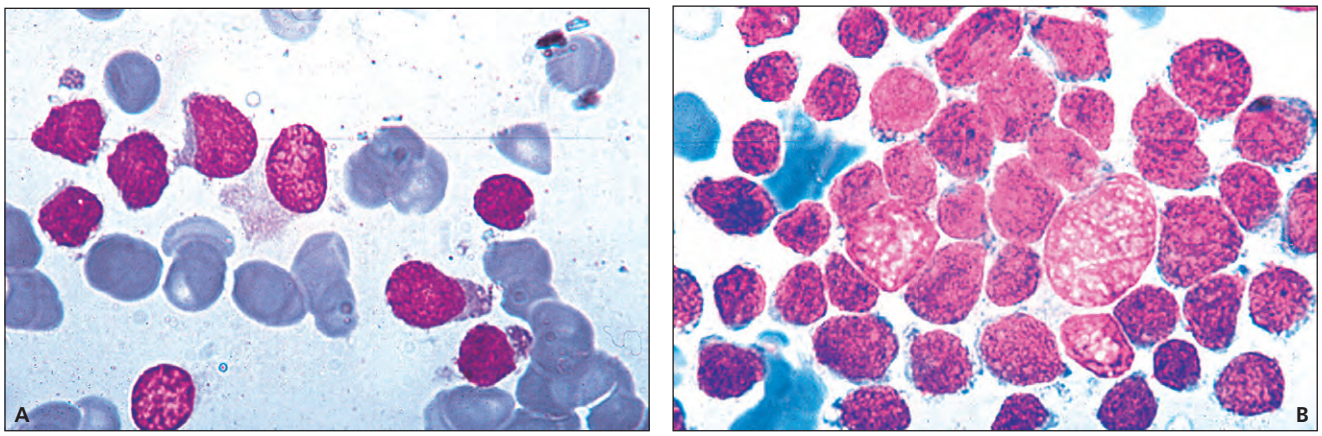



**Fig. 5.34** CML, *BCR-ABL1* positive, T-lymphoid blast crisis. (A) Peripheral blood film. Small blast cells with very scanty basophilic cytoplasm and an irregular, often angulated nuclear outline. The chromatin is a condensed network in the two more spread out cells, while it appears more compact in the smaller cells. The nucleoli are practically invisible. These cells expressed CD3 and CD7, but not CD4 or CD8. (B) Bone marrow aspirate. The blast cells, which are diffusely infiltrating the marrow, appear more immature than those in the peripheral blood: the chromatin network appears better preserved, even though it is dense; in the larger cells there is a small, indistinct nucleolus.

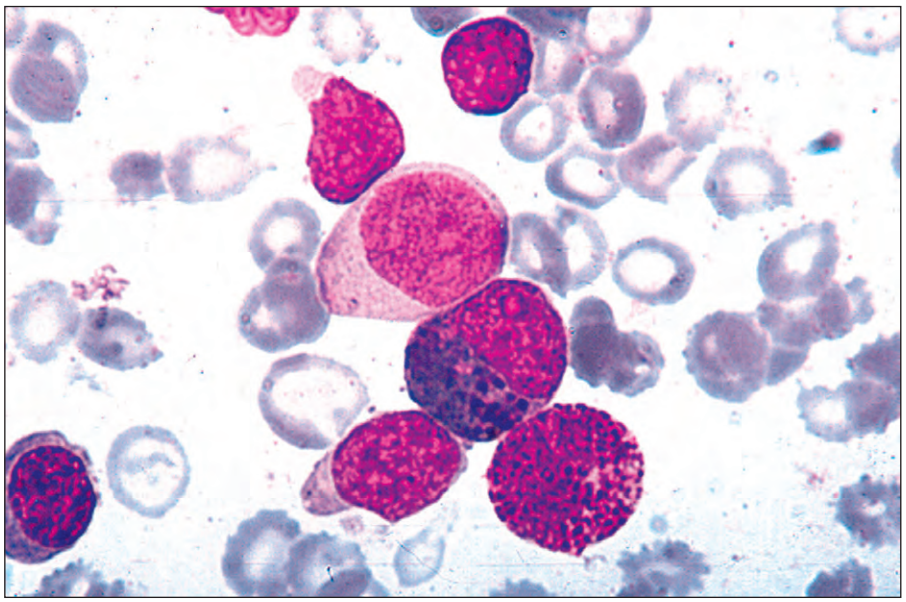


**Fig. 5.35** CML, *BCR-ABL1* positive, mixed (bilineage) blast crisis; bone marrow aspirate. There are two small undifferentiated blast cells, similar to lymphoblasts, with scanty cytoplasm that is elongated at one pole to give a hand-mirror appearance, and a larger blast with a round nucleus that has a much more delicate chromatin network, more obvious nucleoli and plentiful weakly basophilic cytoplasm with fine azurophilic granules. Beneath this cell is a cell with the nucleus of a promyelocytes and cytoplasm packed with blackish granules admixed with eosinophil granules that are darker than those seen in mature eosinophils (an eosinophil promyelocyte). Further down still, a cell of basophilic lineage, which, judging from its size, is likely to be a basophil myelocyte or metamyelocyte. Cut off at the left edge, an erythroblast with the nucleus of a basophilic erythroblast and cytoplasm that is already polychromatic (N:C asynchrony). Immunophenotyping demonstrated the co-existence of blast cells of B lineage (CD19 and CD10 positive) with myeloid blasts (CD13 and CD33 positive).

treatment is actually the best indicator of prognosis and is defined in the following terms[22]:

- Complete haematological remission: platelets less than $450 \times 10^9$/l, white cells less than $10 \times 10^9$/l, basophils less than 5%, absence of circulating immature cells, spleen not palpable;
- Cytogenetic remission (based on the percentage of Ph-positive cells): complete (0%), partial (1–35%), minor (66–95%) or minimal (>95%);
- Molecular remission (based on identification of the *BCR-ABL1* transcript): complete (no evidence of the transcript), major (three log reduction of the transcript, equivalent to a level 1000-fold lower than the initial value).

A complete cytogenetic remission is obtained in around 70–90% of patients.

The tyrosine kinase inhibitors currently available (imatinib, nilotinib and dasatinib) are promiscuous drugs, able to inhibit not only BCR-ABL1 but also other tyrosine kinases. Imatinib and dasatinib inhibit the proliferation of mesenchymal cells in a dose-dependent manner. In addition, it has been shown that imatinib has a biphasic effect on osteoblast differentiation, inhibitory at low concentrations and stimulatory at high concentrations[56]; furthermore, it has an effect on osteoclast-mediated bone resorption through direct inhibition of FMS, KIT, carbonic anhydrase II and the platelet-derived growth factor (PDGF) receptor.[57] Nilotinib inhibits osteoclastogenesis by a mechanism that involves stromal cells[58]: patients being treated with tyrosine kinase inhibitors therefore have an increased risk of developing osteoporosis and osteomalacia.

The use of these drugs in patients with CML causes significant morphological changes (Figs 5.15 and 5.21), which are independent of the stage of the disease and whether or not cytogenetic and molecular remissions are achieved.[59] These changes are apparent by three months after initiation of therapy and involve both clonal and non-clonal cells. For this reason, the 'normalisation' of bone marrow morphology does not necessarily indicate the absence of an abnormal clone, since it is not possible to distinguish, solely on the basis of microscopy, a bone marrow that no longer harbours the Ph-positive clone from another in which it persists. Treatment leads to a reduction of bone marrow cellularity, with a reduction of cells of the granulocytic series and an increase in erythropoiesis, so that the M : E ratio tends to normalise. Sometimes there is a relative erythroid hyperplasia or an increase in stromal cells. The appearance of peripheral blood thrombocytopenia and/or neutropenia, which is seen in some patients, results from excessive myelosuppression and is an indication to immediately suspend treatment. In patients who at the initiation of treatment have an increase in bone marrow blast cells (accelerated phase or blast crisis), there is a rapid fall in the number of blasts that precedes other morphological alterations of the bone marrow. The number of megakaryocytes falls in those patients in whom there was initially an increase; in about half of patients, the megakaryocytes become normal in size and morphology, as also occurs during treatment with interferon alpha.[40]

In bone marrow biopsies, megakaryocyte clusters, defined as comprising at least three cells, tend to reduce in number and disappear; bone marrow fibrosis, when present at diagnosis, reduces significantly, to the point that its presence, which prior to tyrosine kinase inhibitor therapy had a negative prognostic impact, no longer has such an impact[60]; areas of bone marrow necrosis, as seen in patients treated with interferon alpha, are not seen.

At present, there is no evidence that the use of these pharmacological agents increases the risk of a second neoplasm.[61] In some patients, after around three months of treatment with dasatinib, there can be a monoclonal/oligoclonal proliferation of large granular T lymphocytes and NK cells (phenotype of the latter CD3 negative, CD16 positive, CD56 positive) both in peripheral blood and bone marrow; very high levels may be reached ($4–20 \times 10^9$/l); this expansion appears to correlate with a prolonged remission, particularly in patients with an otherwise poor prognosis.[62]

## Chronic *BCR-ABL1*-negative myeloproliferative neoplasms

### Chronic neutrophilic leukaemia

Chronic neutrophilic leukaemia (CNL) is a very rare neoplasm, characterised by neutrophil leucocytosis (WBC $\geq 25 \times 10^9$/l), granulocytic hyperplasia in the bone marrow and hepatosplenomegaly. Until now, little more than 100 cases have been described, mainly in adults.[63] The cell of origin is certainly a myeloid progenitor with a limited differentiation potential. In around 20% of patient, an association with multiple myeloma has been described: thus, at the time of diagnosis it is essential to investigate for, and if possible exclude, the presence of lymphoid clonality and also to evaluate accurately the number of plasma cells in the bone marrow. Furthermore, it is obviously essential to exclude a reactive neutrophilia.

The diagnosis of CNL is largely one of exclusion: it is based on the absence of the Ph chromosome, the absence of the *BCR-ABL1* fusion gene, the absence of rearrangement of *PDGFRA*, *PDGFRB* and *FGFR1*, and on the failure to meet the diagnostic criteria for other MPN and MDS/MPN.

The characteristic morphological features of CNL can be defined as follows (Figs 5.36 and 5.37). In the peripheral blood:

- The percentage of mature neutrophils, segmented and band forms, is greater than 80%;
- The neutrophils are not dysplastic (thus differing from atypical chronic myeloid leukaemia [aCML] CML); toxic granulation is sometimes present;
- The granulocyte precursors (metamyelocytes, myelocytes and promyelocytes) are less than 10%;
- Myeloblasts are less than 1%;

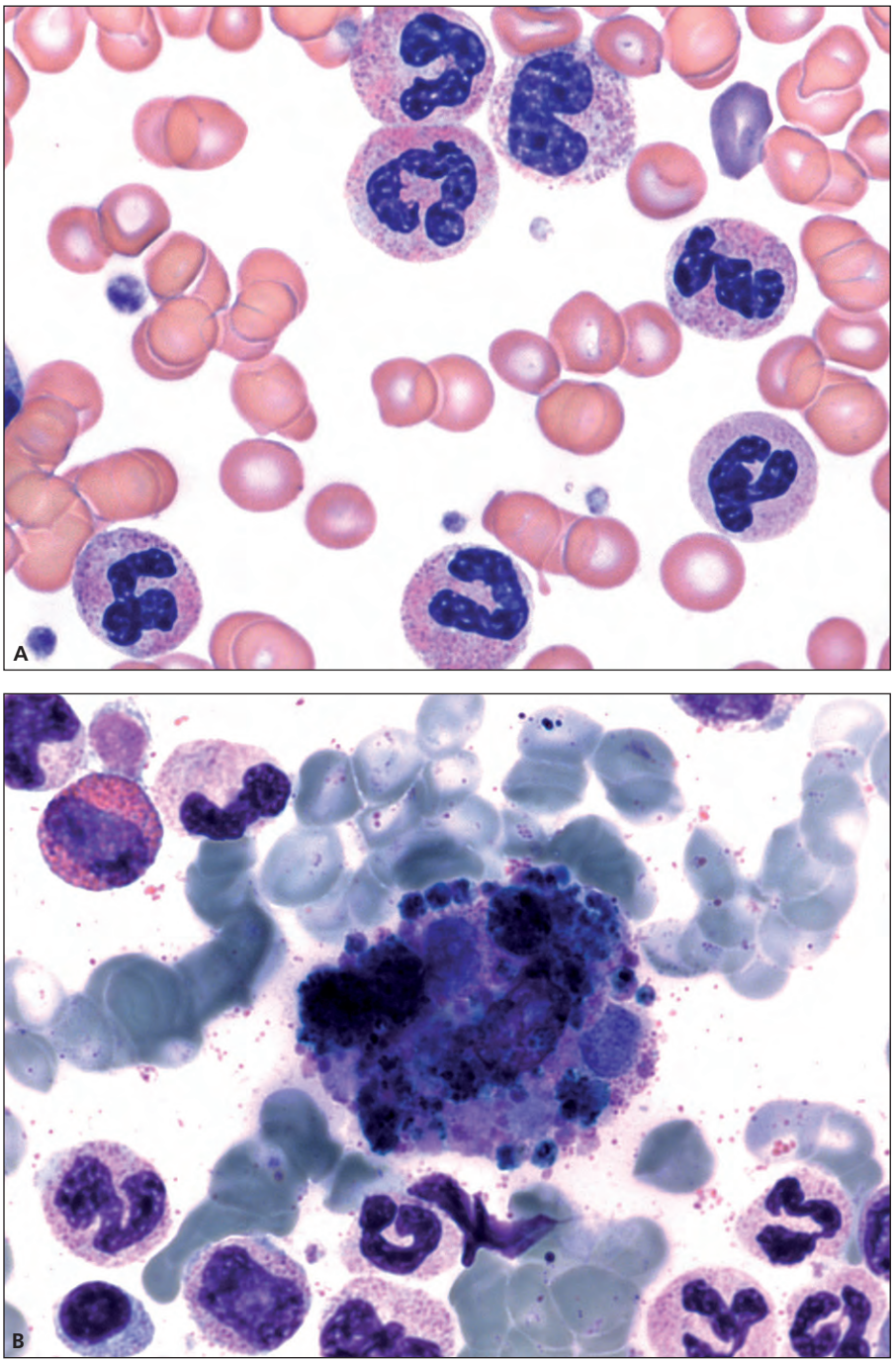



**Fig. 5.36** Chronic neutrophilic leukaemia (CNL). The leucocyte count in this patient was 57 × $10^9$/l, with 91% segmented and band neutrophils and 4% neutrophil myelocytes. There was neither anaemia nor thrombocytopenia. Careful clinical and radiological investigations did not disclose any underlying disease; cytogenetic and molecular analysis did not find any abnormality. (A) The peripheral blood neutrophils do not show any dysplastic features. There is a prevalence of band forms in which the nuclear lobes are still joined by thick bridges rather than thin filaments. The number and appearance of the granules shows some variability: less mature cells, such as the myelocyte at the top with an indented nucleus, have larger, individually recognisable granules on a background of weak basophilia; the more mature cells have either fine dust-like granules conveying a pink-orange colour to the cytoplasm or appear hypogranular, such as the neutrophil bottom right. The red cells and platelets appear normal. (B) In the bone marrow aspirate, in addition to granulocytic hyperplasia with a preserved maturation curve and 3% blast cells, there are numerous macrophages with cytoplasm packed with nuclear detritus.

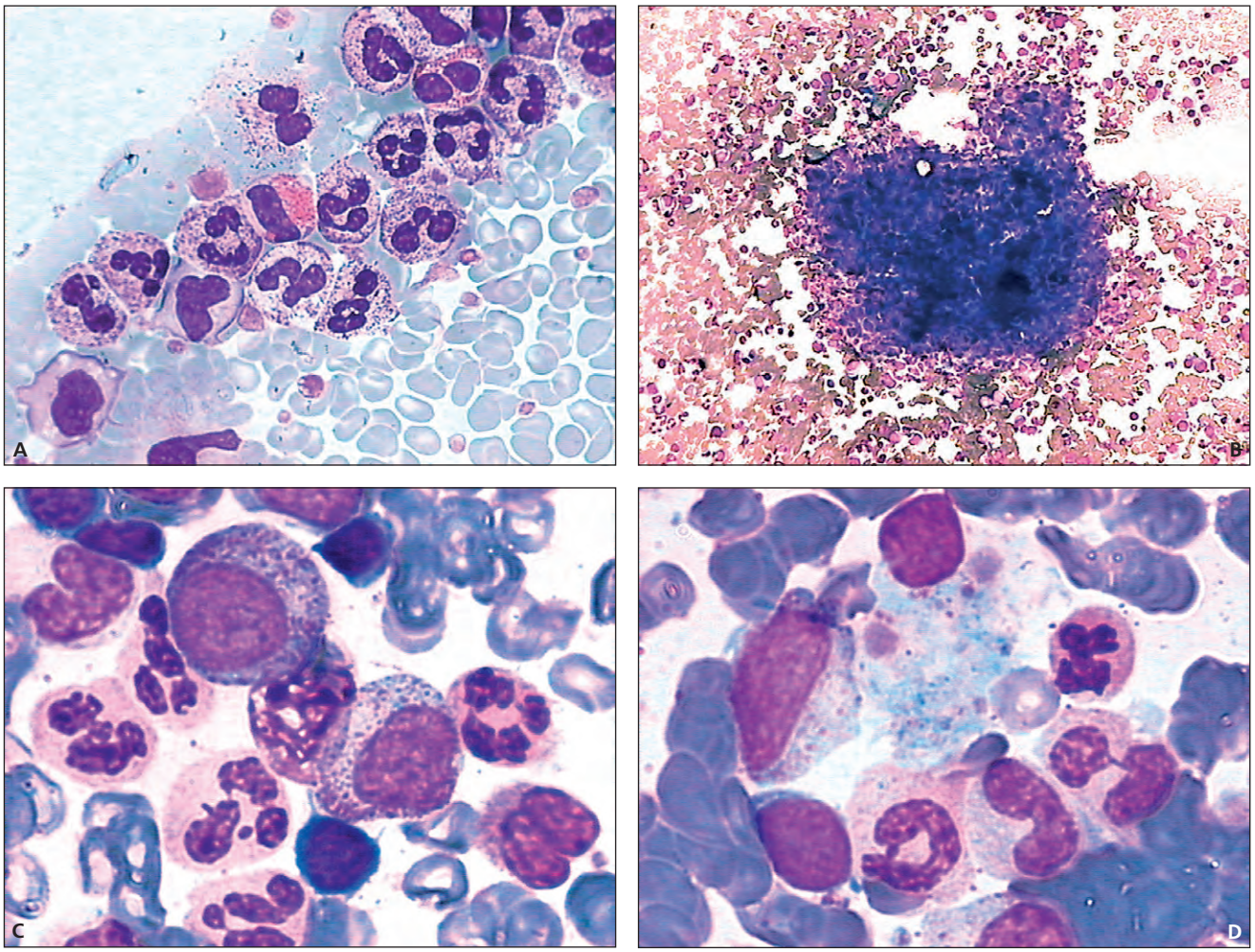



**Fig. 5.37** CNL in a female patient. (A) Peripheral blood film. The numerous neutrophils have normal neutrophil segmentation and a moderate increase in cytoplasmic granules, which appear hyperchromatic. There are two monocytes, a mature eosinophil with a normal, bilobed nucleus (at the top) and an eosinophil metamyelocyte. There are no dysplastic features. (B) Bone marrow aspirate. The marrow is very cellular, with numerous fragments and masses of granulocytes similar to those already seen. (C) Bone marrow aspirate. The maturation curve appears shifted to the right because of an excess of mature forms. The morphological features of the neutrophil granulocyte lineage appear well preserved. At the top, a promyelocyte, a myelocyte and various neutrophils characterised by a tendency to hypersegmentation. One of these has two nuclear appendages, one filiform, the other with the characteristic shape of a drumstick (a small chromatin nodule joined to one of the nuclear lobes by a thin filament). (D) Bone marrow aspirate. In this field there are two blast cells, one of which is granular, three neutrophils with signs of dysmorphism and a small sea-blue histiocyte with the remains of cells in its cytoplasm.

- There is no basophilia (thus differing from *BCR-ABL1*-positive CML);
- There is no monocytosis (thus differing from chronic myelomonocytic leukaemia).

In the bone marrow:

- The M:E ratio is 20 or greater; erythroblast morphology is normal;
- One can observe, inconsistently, a deviation to the right of the granulocyte maturation curve (an excess of mature neutrophils);
- Myeloblasts are less than 5%;
- There is no dysplasia (thus differing from aCML);
- The megakaryocytes are morphologically normal and are not small and hypolobated (thus differing from *BCR-ABL1*-positive CML).

Neutrophil alkaline phosphatase activity is normal or increased. The clinical course is variable, often slow, with gradually increasing leucocytosis and worsening of bone marrow insufficiency. Dysplastic evolution and acute leukaemia have rarely been described.

## Polycythaemia vera

In healthy adults, the bone marrow produces $200 \times 10^9$ red cells per day. In PV the presence in haemopoietic stem cells of a *JAK2*

**Table 5.4** Diagnostic criteria for polycythaemia vera according to the 2008 WHO classification* (adapted from Swerdlow 2008,[13] with permission of IARC)

**Major criteria**

1. Haemoglobin concentration
   >165 g/l in a woman or >185 g/l in a man, or >150 g/l in a woman or >170 g/l in a man (or above the 99th percentile for healthy subjects matched for age, gender and altitude), associated with a documented and sustained increase of 20 g/l (or of 25% in the red cell mass) in comparison with known basal concentration (which is not attributable to correction of iron deficiency)
2. Presence of the *JAK2* V617F mutation or a functionally analogous mutation

**Minor criteria**

1. Trilineage bone marrow hypercellularity (panmyelosis) shown on bone marrow biopsy
2. Serum erythropoietin concentration below the reference range
3. Endogenous erythroid colonies *in vitro*

*Diagnosis requires meeting the two major criteria, or one major criterion and two of the minor criteria.

V617F mutation (or another functionally analogous mutation) causes trilineage proliferation or panmyelosis, which is initially manifest particularly in erythropoiesis, with increased production of morphologically normal red cells. In the prodromal prepolycythaemic phase, erythrocytosis is moderate (around the upper limit of normal) and the diagnosis, if suspected, can only be made on a molecular basis. The *JAK2* V617F mutation is found in more than 95% of cases of PV; this abnormality is not specific, being observed also in other MPN, although at a lower frequency. In 50–80% of patients with PV without *JAK2* V617F, other mutations of *JAK2*, in exon 12, have been recognised.

The criteria for the diagnosis of PV, defined many years ago by the PV Study Group,[64] have been very important both for research and clinical practice, e.g. with regard to the use of aspirin and hydroxycarbamide (previously hydroxyurea). However, in the light of recent molecular discoveries, a new approach to diagnosis has become necessary. Table 5.4 gives the diagnostic criteria for PV according to the 2008 WHO classification.[65]

An early diagnosis of PV, as for all the clinically occult stages of Ph-negative MPN, is very important for the prevention of vascular complications, both haemorrhagic and thrombotic, which may otherwise be the initial clinical feature. In an attempt to achieve earlier diagnosis, the concept of diagnosis of a prodromal phase of the disease has been introduced[66] for *BCR-ABL1*-negative MPN, based mainly on molecular analysis and assessment of the histopathological features of the bone marrow. With particular reference to PV, this approach to the prodromal phase is applied in patients with moderate but persistent erythrocytosis. Screening of such patients is appropriate, based on an initial evaluation for the *JAK2* V617F mutation: patients positive for this mutation are found to develop frank PV in a period varying from six months to two years.

An important early role is also recognised for morphological evaluation of trephine biopsy sections of the bone marrow: the histopathological features characteristic of the prodromal phase of PV are a mild or moderate increase in cellularity with increased proliferation of the erythroid component and, very importantly, typical abnormalities of megakaryocytopoiesis. The megakaryocytes of PV are characterised by extreme variability of size, with small and giant forms and with marked pleomorphism, without significant abnormalities of maturation (Fig. 5.38): this is a picture quite different from that of essential thrombocythaemia (ET), in which giant megakaryocytes predominate. This is particularly useful for the occasional case of early PV that presents with thrombocytosis, thus simulating ET. The morphological features of bone marrow sections are also useful for distinguishing PV in a prepolycythaemic phase from both spurious and secondary erythrocytosis in which megakaryocyte anomalies are not observed; similarly, there is a lack of stromal reactions such as perivascular plasmacytosis, increased detritus-laden macrophages and increased eosinophils, which are all more frequent in PV.[67] In addition to molecular screening and histology, several less specific tests have found a complementary role in the diagnosis of prodromal PV: the determination of serum erythropoietin concentration (which is typically diminished) and evaluation of the growth of spontaneous erythroid colonies.

In the polycythaemic phase, there is always an increase in the total red cell mass. Both in the peripheral blood and the bone marrow, there are lineage-specific stem cells capable of forming endogenous erythroid colonies, i.e. colonies that develop in the absence of exogenous erythropoietin. Serum erythropoietin is almost always below the lower limit of the reference interval.[68] The blood count at the onset of the illness always shows an increase in the red cell count to $7 \times 10^{12}$/l, or even higher. The red cells are generally normal in size and shape (Fig. 5.39), even if already at the outset it is frequent for the mean cell volume (MCV) to be around the lower limit of normal, between 75 and 85 fl, with a moderate reduction of the MCH and MCHC. The Hb at disease onset varies between 180 and 240 g/l and the Hct is correspondingly increased. For monitoring treatment, the Hct is sometimes still measured by centrifugation, more out of tradition than for scientific or technical reasons: the value that is significant for diagnosis if above 0.55–0.60, while during venesection therapy the aim is for a value below 0.50. The reticulocyte count in patients with PV at diagnosis is normal or slightly increased[69]: 44% of patients assigned to therapeutic protocols by the PV Study Group had a reticulocyte count above 1.5%. The immature reticulocyte fraction is increased, corresponding to

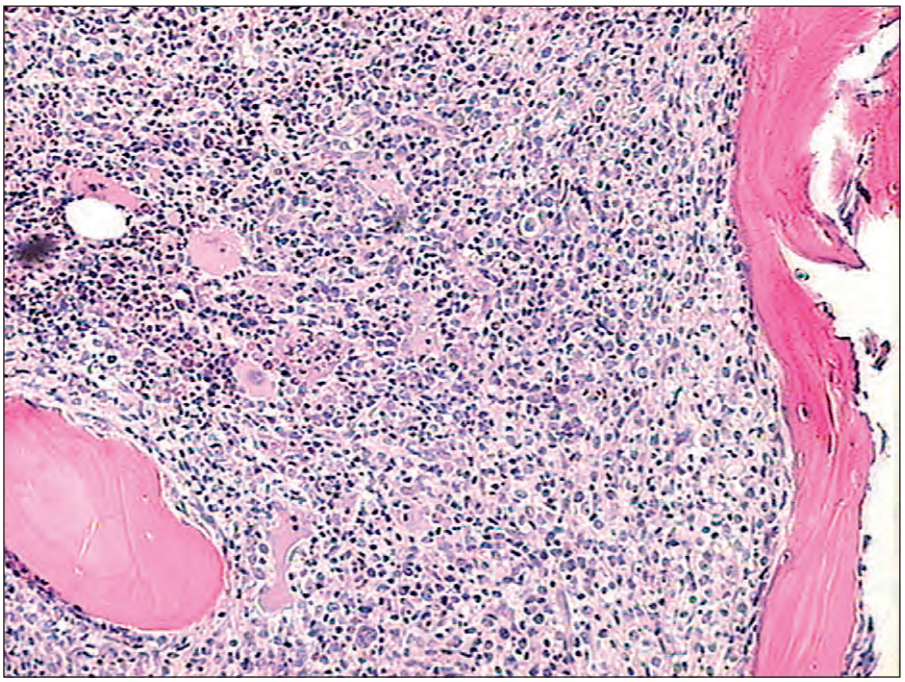


**Fig. 5.38** Polycythaemia vera (PV); section of a trephine biopsy specimen taken at presentation. The bone marrow is very cellular because of trilineage hyperplasia, particularly evident in the erythroid lineage, with an absence of fat cells. The megakaryocytes, in particular, appear numerous and are mainly mature, but polymorphic and heterogeneous with regard to size.

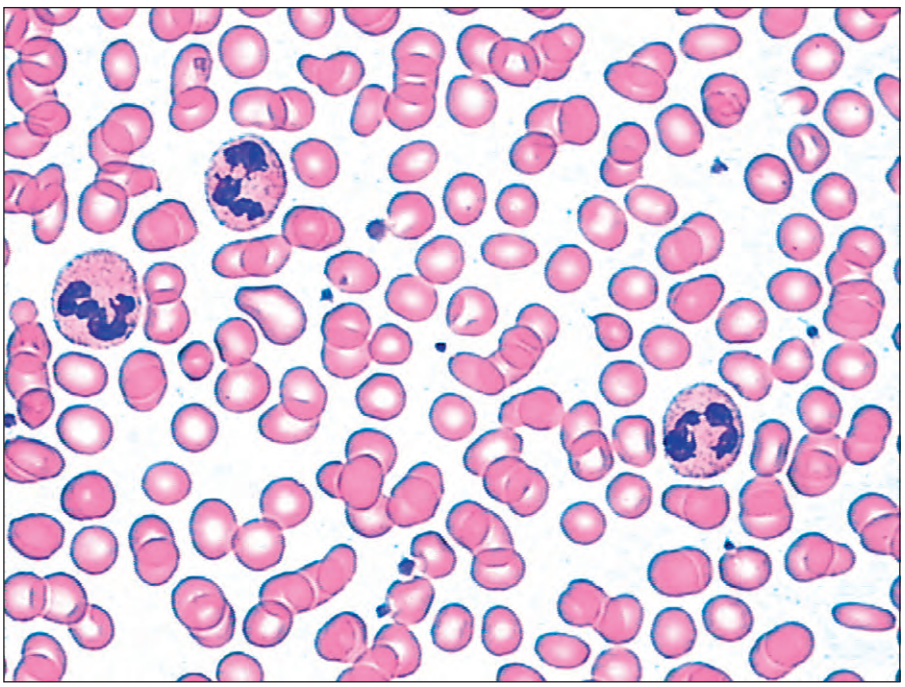


**Fig. 5.39** PV at diagnosis; peripheral blood film. The morphological picture does not have any characteristic features. There are three apparently normal neutrophils and a number of platelets. The red cells appear normal, with a zone of central pallor, very little variation in cell size and no significant tendency to rouleaux formation. There is slight poikilocytosis as evidenced by a small dacrocyte, to the right of centre, some ovalocytes and an erythrocyte fragment in the top right corner.

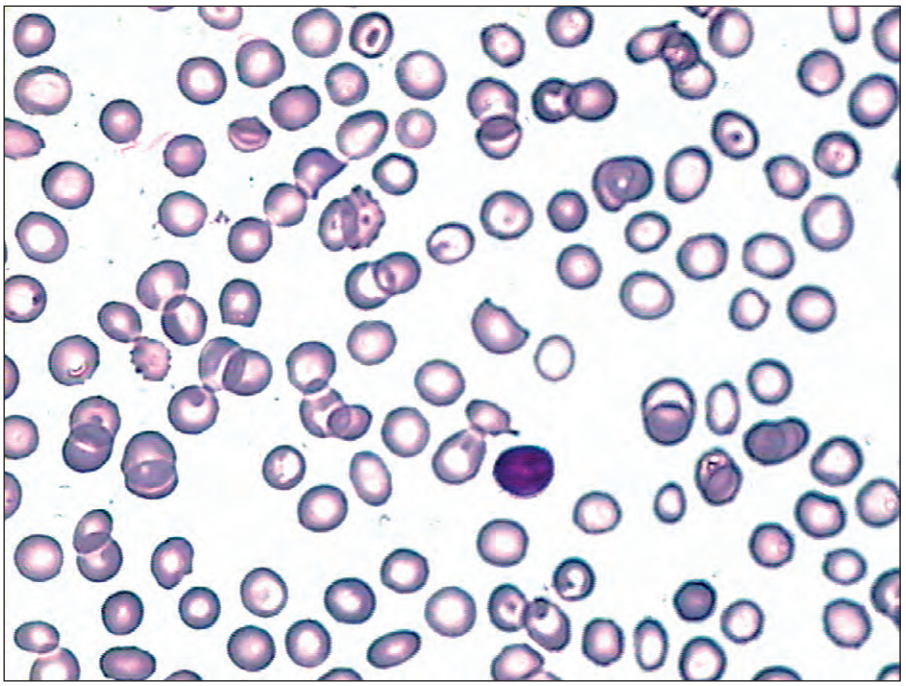


**Fig. 5.40** PV in a patient being treated by periodic phlebotomy; peripheral blood film. The red cells appear microcytic (in comparison with the nuclei of the small lymphocytes) and very hypochromic, a result of iron deficiency secondary to the loss of iron through phlebotomy. There are some echinocytes and two small dacrocytes. To the right of centre, a typical helmet cell, not usually seen in the absence of extramedullary haemopoiesis or thrombotic microangiopathy: the recognition of an occasional red cell fragment of this type raises the suspicion of an increase in bone marrow reticulin fibres, which could be evaluated by histological examination.

the expansion of the bone marrow erythroid component.[70] Occasionally, orthochromatic erythroblasts are seen in the peripheral blood.

One of the effects of venesection therapy is the induction of a state of iatrogenic iron deficiency, which can also be the result of haemorrhagic episodes or occult blood loss: iron deficiency is manifest by the development of microcytosis and hypochromia that become gradually more marked (Fig. 5.40), and that are an almost constant feature of PV being treated by phlebotomy. One is almost always dealing with microcytosis with little anisocytosis and not accompanied by major morphological abnormalities of red cells: the appearance in a patient with PV of severe anisocytosis with poikilocytosis, ovalocytosis, elliptocytosis, target cells, basophilic stippling, Howell-Jolly bodies and, particularly, dacrocytes and circulating erythroblasts always suggests the development of bone marrow fibrosis with imminent transition to the spent phase of the disease or post-polycythaemic myelofibrosis, characterised by ineffective haemopoiesis, myelofibrosis and extramedullary haemopoiesis, particularly in the spleen.[71]

Neutrophil leucocytosis is present in two thirds of patients at onset. The WBC is usually between 10 and 25 $\times$ $10^9$/l, with even higher counts in some patients. The increase in neutrophil granulocytes is both relative (80–90%) and absolute, and is not associated with morphological abnormalities, except sometimes a left shift; some myelocytes and metamyelocytes can be present in the circulation, particularly in cases with more marked leucocytosis; granulocyte precursors tend to increase, together with erythroblasts in the spent phase of the disease (a leucoerythroblastic picture). Myeloblasts are not seen. Neutrophil alkaline phosphatase is often increased in PV as is serum vitamin $B_{12}$ concentration and $B_{12}$-binding capacity. An absolute increase in the basophil count is characteristic, basophils sometimes being 3% of leucocytes; less constant, but frequent, is eosinophilia and monocytosis. A moderate thrombocytosis with a platelet count of 400 to 800 $\times$ $10^9$/l is seen in half of cases and sometimes the value exceeds 1000 $\times$ $10^9$/l. Platelets can be in part atypical, with increased variation in size and the presence of a small proportion of large platelets, sometimes with a reduced granule content.

The bone marrow in PV is hypercellular by virtue of a marked trilineage hyperplasia, this being one of the three minor diagnostic criteria of the 2008 WHO classification (Fig. 5.41). The hyperplasia does not usually affect the M:E ratio or the morphology of these lineages. Often there is a marked reduction in bone marrow fat cells, while around 80% of patients have a normal reticulin network. In around 10% of cases, bone marrow cellularity is within normal limits.[72] Fairly rarely, hypercellularity is bilineage or affects only the erythroid series, with erythroid hyperplasia that is similar to that seen in haemolytic anaemia.

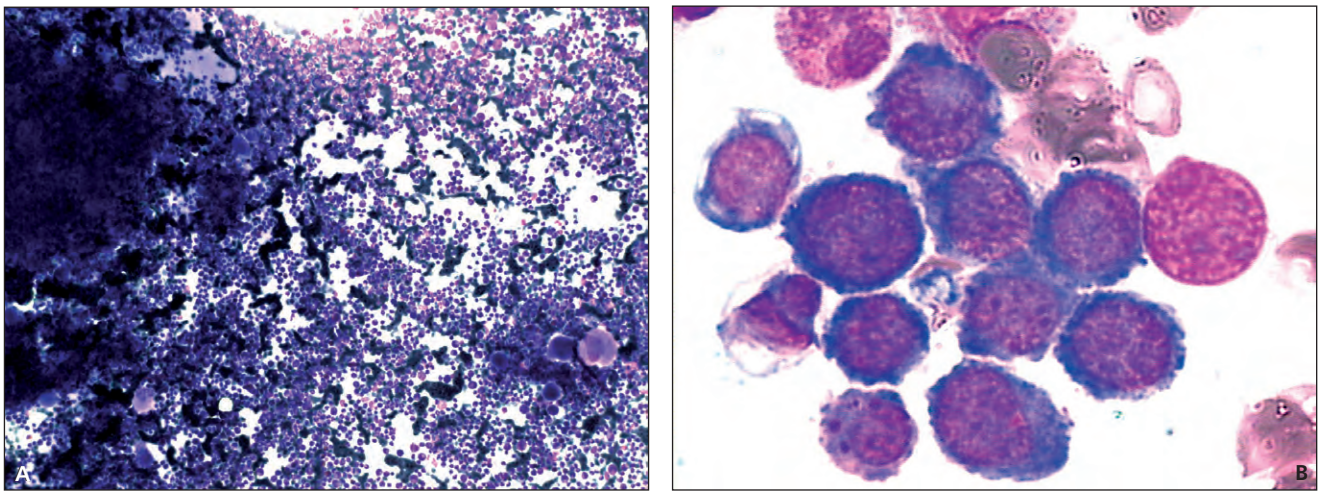



**Fig. 5.41** PV; bone marrow aspirate. (A) In this preparation, with numerous fragments, bone marrow cellularity appears quite increased, with hyperplasia of all haemopoietic lineages and maintenance of a normal M : E ratio. At low power one can see numerous megakaryocytes, which are quite variable with regard to size, maturity and nuclear morphology. (B) The morphology of erythroid precursors in PV is usually normal, as in this small group of mainly basophilic erythroblasts.

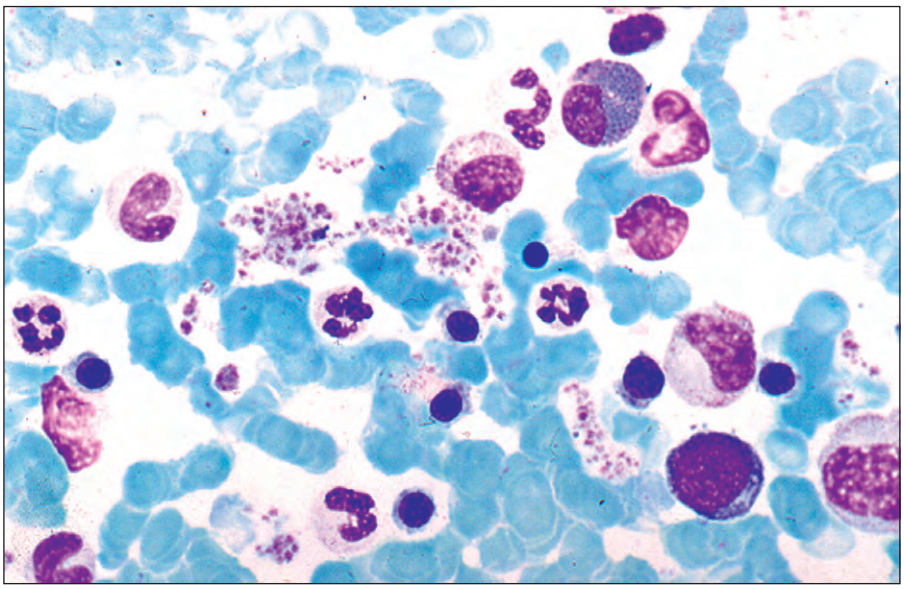


**Fig. 5.42** PV; bone marrow aspirate. The trilineage hyperplasia in the bone marrow in PV is not always reflected in hypercellularity apparent on a bone marrow film. This microscopic field demonstrates the paucity of information that may be provided by cytology in a proportion of patients. There are no morphological abnormalities in any lineage. The presence of a number of mature segmented neutrophils, more numerous than the myelocytes and metamyelocytes, is indicative of considerable contamination of the aspirate by peripheral blood. At the top, on the right, an eosinophil myelocyte. The erythroblasts are small with the normal morphological features of maturation; bottom right, a proerythroblast with intensely basophilic cytoplasm in which there is pale paranuclear sarcoplasm. The presence of numerous masses of platelets is another feature often observed in these preparations, which certainly are not representative of the cells of the bone marrow.

A bone marrow aspirate does not always yield material with adequate cellularity and particles for assessment (Figs 5.42 and 5.43), particularly in cases with increased reticulin deposition. Overall, bone marrow cytology in PV would be barely distinguishable from normal if it were not for the alteration in megakaryocytes, which are often increased in number, either dispersed or clustering to a variable degree and characterised by three types of variability:

- In size, with giant, normal and dwarf forms;
- In nuclear conformation, with cells showing hyperlobation and hyperdiploidy admixed with smaller forms with small pyknotic nuclei;

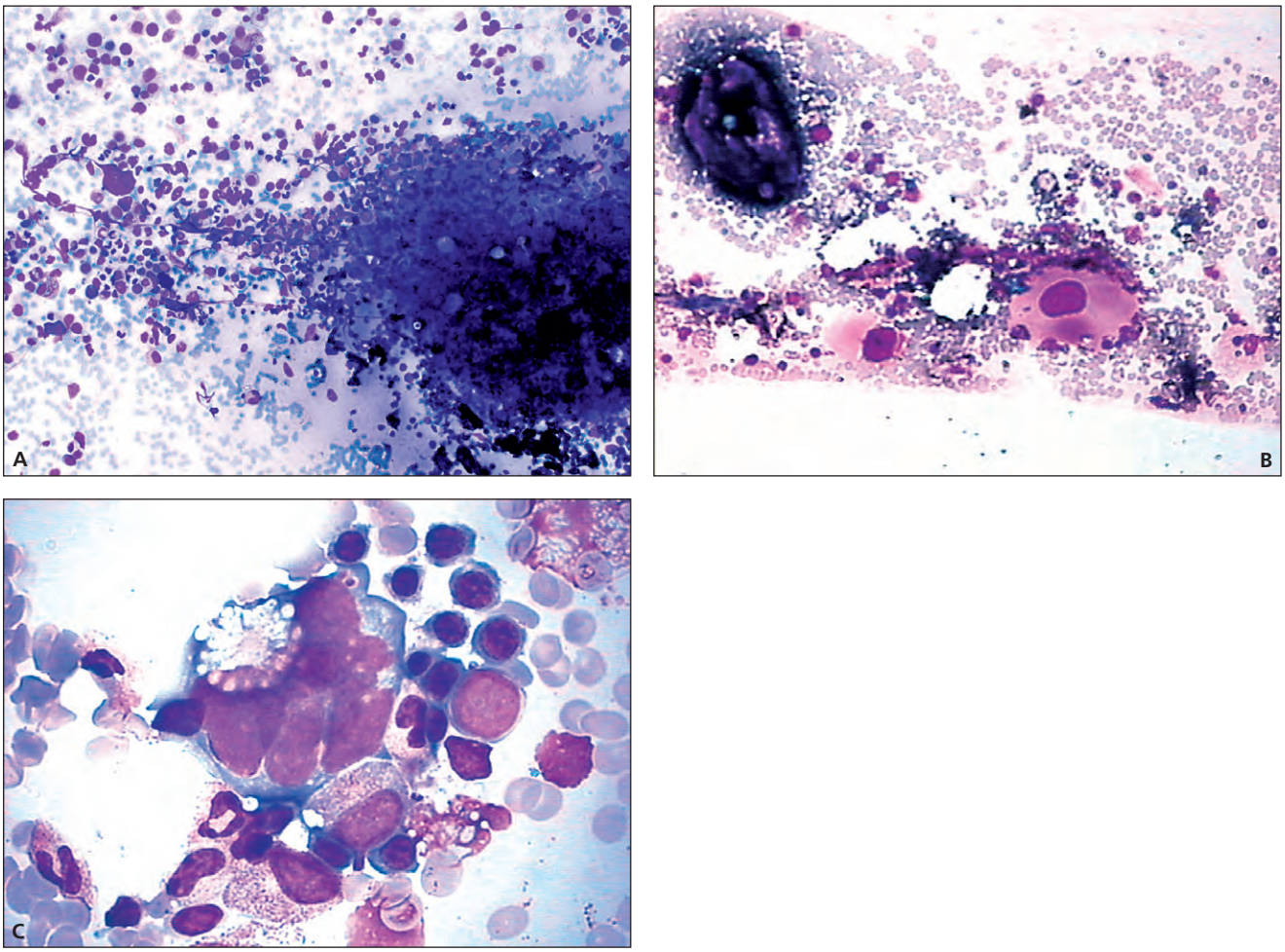



**Fig. 5.43** Bone marrow aspirates of patients with PV. (A) Hypercellular bone marrow fragment, composed of an accumulation of granulocytic and erythroid precursors, packed together. Morphological analysis can be done optimally by examining a trail left behind a fragment during spreading of the film. This permits, even at low power, an appreciation of the cytological heterogeneity, the different stages of maturation, and the absence of monomorphism or marked atypia. The long bluish filaments correspond to large nuclei that have been torn apart. (B) In the tail of a film from another patient, there is a small fibrous fragment, which has left behind only a few erythroid and granulocytic precursors. Megakaryocytes, however, appear numerous; they are very variable in size and yet appear morphologically uniform and indicative of preserved maturation. (C) In another field, in the same film as (B), there is a promegakaryocyte with a hyperdiploid multisegmented nucleus, with moderately condensed homogeneous chromatin, without dark clumps or prominent nucleoli. The cytoplasm is intensely basophilic, particularly at the periphery of the cell. What was a hypertrophic Golgi apparatus has degenerated and formed a giant, multiloculated vacuole, which has in its centre a concentration of pale pink microgranules. At the bottom, from the centre towards the left, a promyelocyte, a neutrophil myelocyte and a metamyelocyte, a pair of band neutrophils and two lymphocytes. The erythroblasts are gathered particularly in the top right quadrant, where there is also a medium sized blast cell with a high N:C ratio and a thin rim of almost transparent cytoplasm.

- Of the cytoplasm, which can be hypertrophic with abundant granules and with platelet demarcation indicative of increased thrombopoiesis or, on the contrary, almost totally lacking cytoplasm ('bare nuclei').

The loss of storage iron, demonstrable with a Perls stain, is a very common finding in the bone marrow in PV, relating to the increased utilisation of iron in erythropoiesis, often aggravated by overt or occult loss of blood and by venesection therapy.

Investigation of haematological features does not always permit PV to be distinguished from secondary erythrocytosis. The distinction may only be made by considering clinical and molecular characteristics together with certain cytological differences shown by secondary cases:

- In the peripheral blood, the usual absence of neutrophil leucocytosis and thrombocytosis;
- In the bone marrow, less marked hypercellularity, which affects exclusively the erythroid lineage, with a reduction in the M:E ratio;
- Normal megakaryocyte morphology (in our experience, the most informative distinguishing feature[73]);

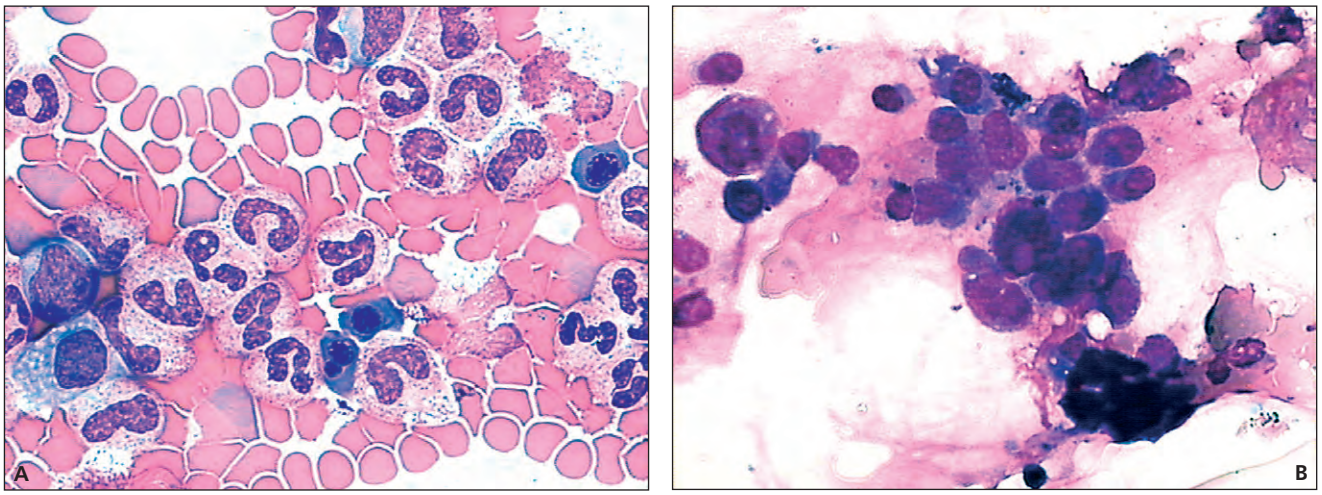



**Fig. 5.44** PV in a patient in transition towards the spent phase. (A) Peripheral blood film. The white cell count is increased because of the presence of numerous neutrophils, which are mainly band forms. There are also numerous erythroblasts, which have polychromatic cytoplasm with delayed nuclear maturation and some dysmorphism, such as the nuclear lobulation visible in the erythroblast towards the bottom. Various erythrocytes, although not evaluable morphologically because the crowding of cells in this part of the film has deformed them, can be seen to have polychromatic cytoplasm. (B) The bone marrow aspirate is hypocellular and not really evaluable, a result of the development of fibrosis. The cells included in this fibrinoid mass are mainly erythroblasts, with some reactive cells, such as the two plasma cells at the top and the mast cell recognisable with difficulty towards the centre of the image.

- Good preservation of iron stores.

Over a period of years, PV often evolves into the so-called spent or post-polycythaemic phase (Figs 5.44 and 5.45), in which one sees a progressive reduction in erythropoiesis as a result of increasing myelofibrosis, with the appearance of extramedullary haematopoiesis in the liver and spleen.[71] In the peripheral blood, the Hb tends to fall and erythroblasts appear, together with the characteristic dacrocytes and myeloid precursors. The appearance of dysplastic features is not common but, when it occurs, is indicative of progression towards an accelerated phase. Table 5.5 shows the 2008 WHO classification criteria for the diagnosis of post-polycythaemic myelofibrosis.[65] In around a third of patients, evolution to AML occurs, with an increase of bone marrow or peripheral blood blast cells to 20% or more.

Reports 5.3 and 5.4 show typical automated instrument printouts in PV.

## Essential thrombocythaemia

In the healthy adult, the bone marrow produces $100 \times 10^9$ platelets per day, by means of fragmentation of long cytoplasmic extensions from megakaryocytes, known as proplatelets. ET is a clonal haemopoietic disorder in which there is a sustained increase in this process. It has many biological characteristics in common with PV,[74,75] such as the formation of growth factor-independent megakaryocyte colonies *in vitro*, the concomitant expansion of the neutrophil granulocyte compartment and the presence of the somatic mutation *JAK2* V617F (or a functionally analogous mutation) in around 40–50% of patients. A characteristic exclusive to ET and PMF, however, is that in some cases a mutation of *MPL* (encoding the thrombopoietin receptor) is observed, the most frequent of these being W515L and W515K and in about a third of cases there is a mutation in *CALR*.

The platelet count threshold indicated for the diagnosis of ET in the 2008 WHO classification is $450 \times 10^9$/l, but often the count is higher than $1000 \times 10^9$/l. The other criteria are based on bone marrow morphology observed in histological sections and on exclusion of other MPN and of reactive conditions (Table 5.6).

In ET the peripheral blood film often shows numerous platelets, mainly morphologically normal, sometimes with subpopulations both larger and smaller than normal and with atypical morphology: sometimes they appear darker than normal, while in other cases they are hypogranular; often there are giant platelets of a bizarre shape with pseudopodia, masses of elongated platelets and genuine fragments of megakaryocyte cytoplasm within which platelets are already forming but have not yet separated (Figs 5.46 through to 5.49). Sometimes there is neutrophil leucocytosis with occasional immature granulocytes or the presence of atypical megakaryocytes and megakaryocyte nuclei in the circulation. The basophil count is normal or, rarely, slightly increased. A mild or moderate anaemia is common; red cell morphology is usually normal, but it is not rare for there to be microcytosis or hypochromia attributable to iron deficiency, which can often be related to a clinical history of repeated haemorrhage or to occult blood loss, particularly involving the

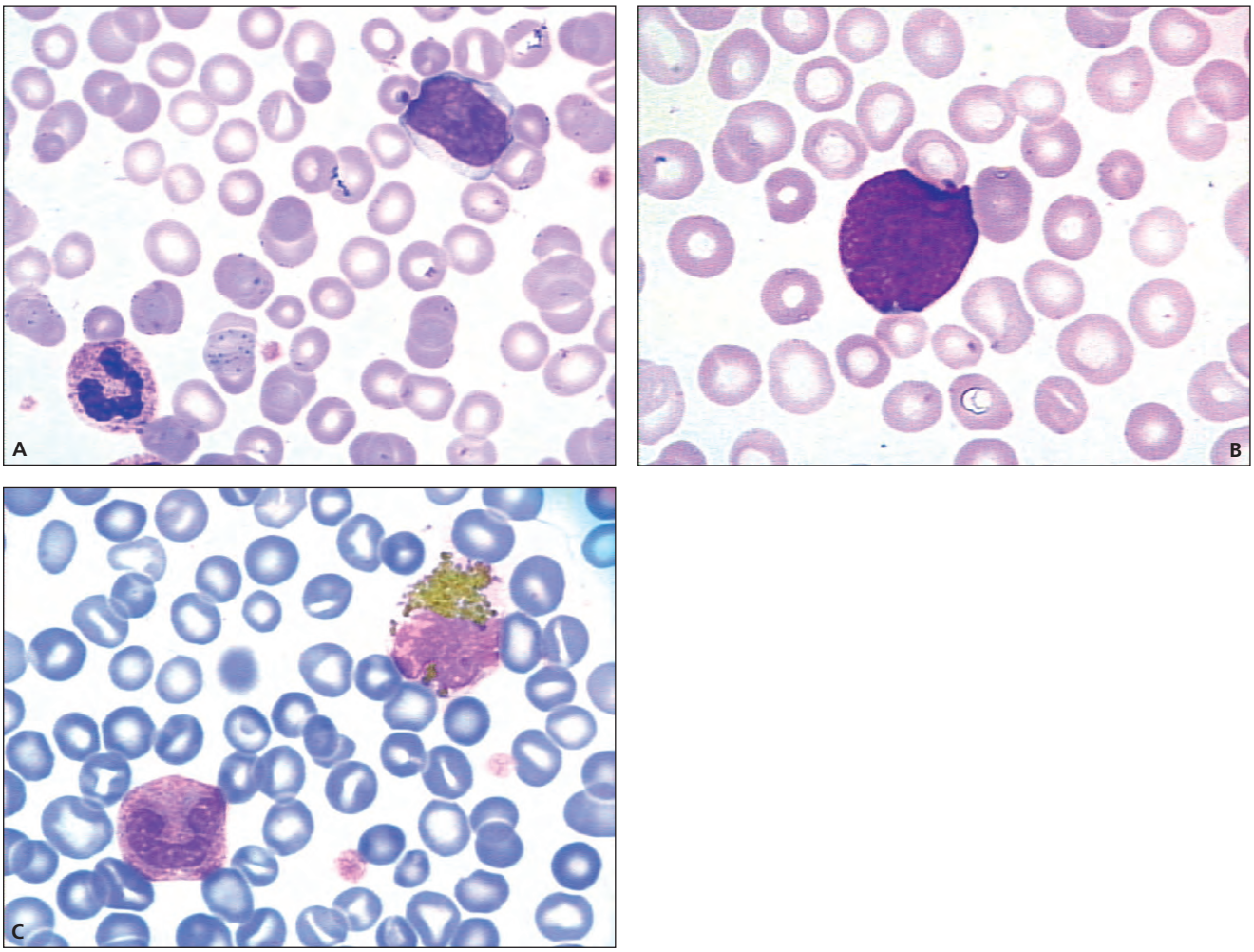



**Fig. 5.45** Peripheral blood film of a patient with advanced stage PV with developing myelofibrosis. (A) A band neutrophil with hyperchromatic granules, an erythrocyte with basophilic stippling and, top right, a medium-sized, agranular blast cell. The red cells show anisocytosis and hypochromia. (B) Circulating megakaryocyte with very scanty cytoplasm that is visible in the notches that are cutting into the lower margin of the nucleus; the chromatin is compact and fairly heterogeneous, with small areas of greater condensation and the remains of nucleoli in the upper part of the nucleus. (C) Peroxidase stain. Top right, an eosinophil with a non-lobed nucleus shows normal positivity with large greenish-yellow granules. The band neutrophil at bottom left, however, is peroxidase negative and has some areas of cytoplasmic basophilia, suggesting dysgranulopoiesis.

**Table 5.5** Diagnostic criteria for post-polycythaemia myelofibrosis according to the 2008 WHO classification (adapted from Swerdlow 2008,[13] with permission of IARC)

**Essential criteria** (both)

1. Previous documented diagnosis of polycythaemia vera according to WHO criteria
2. Bone marrow fibrosis shown on bone marrow biopsy, grade 2–3 (on a scale of 0–3) or 3–4 (on a scale of 0–4)

**Additional criteria** (at least two are necessary for the diagnosis)

1. Anaemia (haemoglobin concentration below the reference range); or sustained reduction in the frequency of therapeutic phlebotomy, the need for cytoreductive therapy or both
2. Leucoerythroblastic blood film
3. Significant increase in splenomegaly
4. Development of at least two systemic symptoms (weight loss, nocturnal sweating, fever)

**Table 5.6** Diagnostic criteria for essential thrombocythaemia according to the 2008 WHO classification (adapted from Swerdlow 2008,[13] with permission of IARC)

1. Platelet count $\geq 450 \times 10^9$/l
2. Increased number of large megakaryocytes on bone marrow biopsy in the absence of erythroid or granulocytic expansion
3. Absence of WHO criteria necessary for a diagnosis of PV, PMF, *BCR-ABL1*-positive CML; detection of the *JAK2* V617F mutation or other marker of clonality; in the absence of *JAK2* V617F, exclusion of reactive thrombocytosis (iron deficiency, splenectomy, recent surgery, infection, inflammation, collagen disease, metastatic tumour, lymphoproliferative neoplasm)*

*The presence of one of these conditions does not exclude the diagnosis of essential thrombocythaemia if the first three criteria are met.

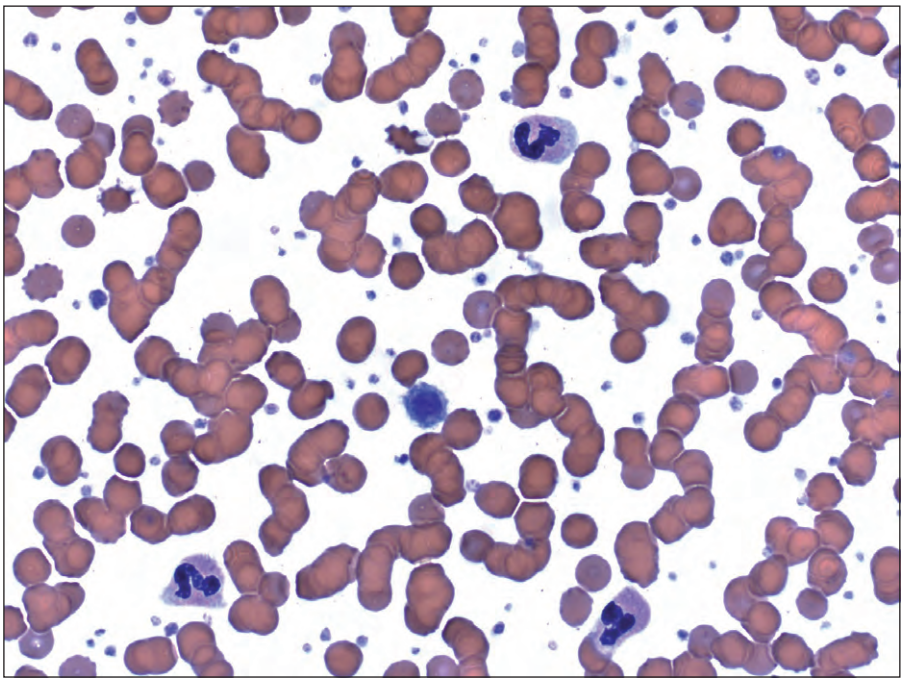


**Fig. 5.46** Essential thrombocythaemia (ET); peripheral blood film. The platelets, well separated from each other, appear very numerous and generally are morphologically normal. There is heterogeneity, not so much because of variation in size (some variation being a physiological feature), but because of variation in the intensity of staining: staining is in fact very variable, from an almost colourless grey in the smaller platelets (e.g. bottom right) to a particularly intense blue, indicating the difference in granule content and the development of the chromomere. A little below the centre of the image, a giant platelet, similar in size to a lymphocyte, with a central hyperchromic pseudonucleus, surrounded by a basophilic hyalomere, which at the periphery is not very distinct and has short villiform projections. The three neutrophils do not show any morphological abnormalities. The red cells have formed rouleaux: close to the top left corner are two echinocytes and, in the centre of a line that joins them, a small hyperchromic acanthocyte.

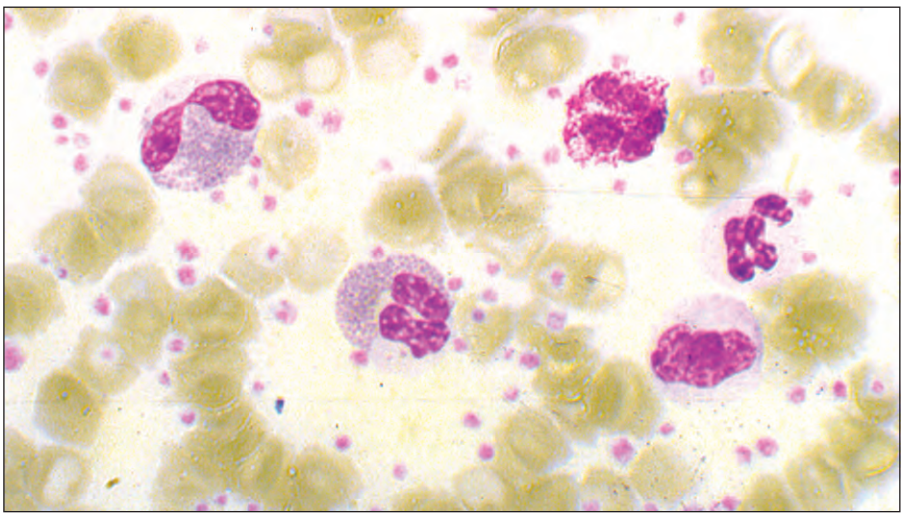


**Fig. 5.47** Essential thrombocythaemia; peripheral blood film. In this patient the platelet abnormality is exclusively quantitative; both the morphology and the size of the platelets appear normal and there is no aggregation. The red cells are forming long rouleaux. Both the basophil and the eosinophil appear morphologically normal, but the neutrophil, with five nuclear lobes and hypogranular cytoplasm, is somewhat dysmorphic.

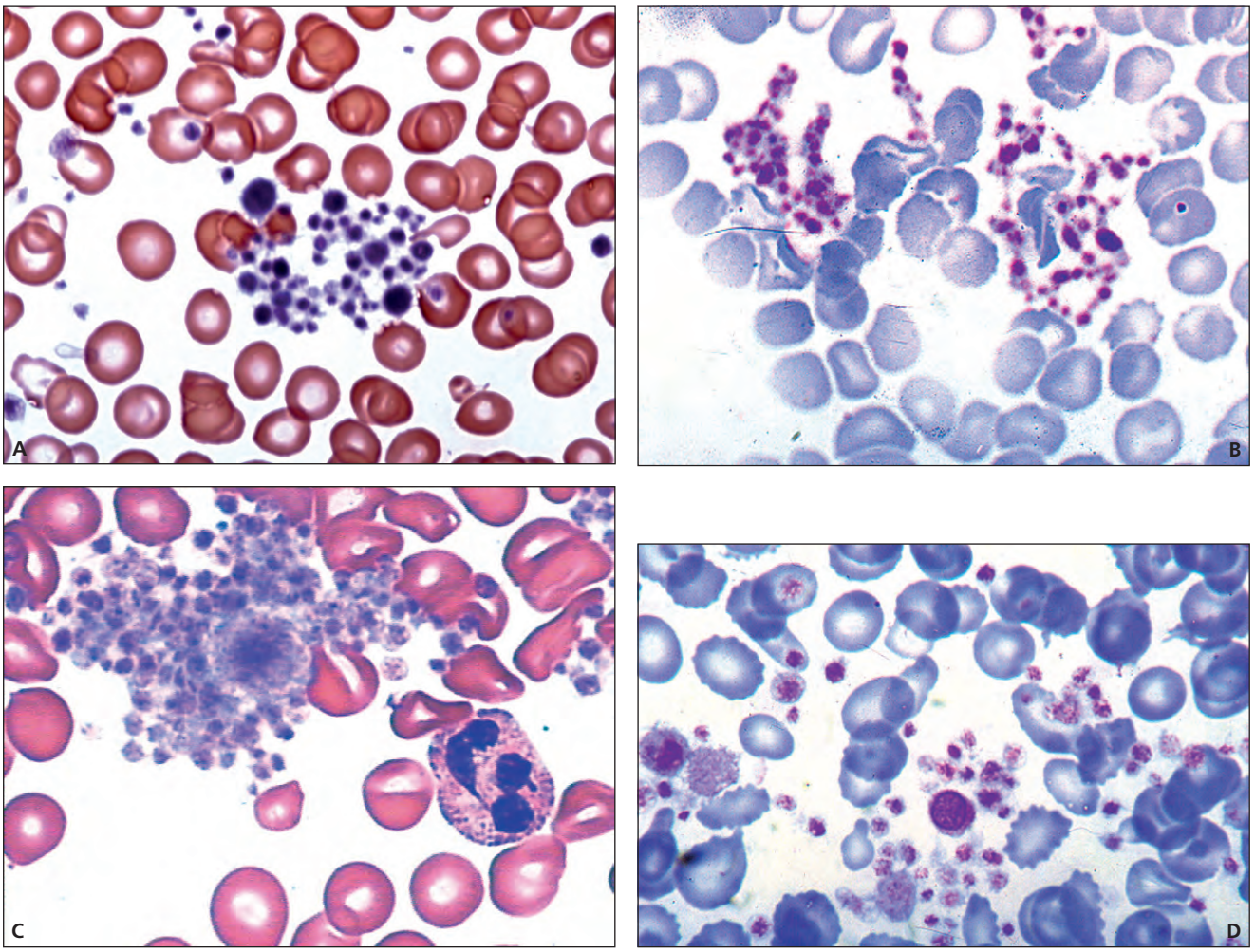



**Fig. 5.48** Masses of platelets that have not separated from each other; peripheral blood films of different patients with ET. It is not possible to be sure, in cases such as these, whether these interplatelet links are primary in nature (failure of separation) or secondary (agglutination or aggregation), although the first of these mechanisms appears the more probable, the appearance being similar to the related anomaly of megakaryocyte cytoplasm that can be observed in the bone marrow. (A) A conglomerate of platelets characterised by marked heterogeneity of size, with giant forms and a generalised tendency to hyperchromia. (B) Small syncytia of platelets that are forming long chains, very evocative of a failure of individual platelets to detach at the moment when a strand of cytoplasm is released from a megakaryocyte. (C) A dense mass of platelets that are mainly fairly similar in size, with small basophilic hyalomeres and violet chromomeres that are sometimes hypertrophic. One giant platelet, larger that the adjacent stomatocytes, shows the same characteristics on a grander scale. (D) These platelets, more numerous than the red cells and partially attached to each other in small strands, are characterised by increased heterogeneity in shape, size and granule content: the granules are often condensed to form a central chromomere, surrounded by a rim of basophilic agranular hyalomere. There are four giant platelets, two of which have the same greyish colour and infrequency of granules, while one, in the centre, has a very dense pseudonucleus.

gastrointestinal tract. Splenic infarcts sometimes occur and can lead to functional hyposplenism with the appearance in the circulation of basophilic stippling and Howell-Jolly bodies. Report 5.5 shows a typical automated instrument printout in ET.

The bone marrow aspirate in ET, in comparison with that in PV, is somewhat more often inadequate, this being related to a tendency to fibrosis. The increase in reticulin fibres is usually proportional to the number of megakaryocytes in the bone marrow. These two features are also pathogenetically related since the megakaryocytes produce factors that stimulate the proliferation and function of fibroblasts. In satisfactory bone marrow films and in histological sections, the cytomorphological picture is characterised by increased numbers and anomalous morphology of megakaryocytes. The granulocyte and erythroid lineages do not appear hyperplastic and their morphology does not show atypia. Conversely, the characteristic markedly increased numbers of megakaryocytes is

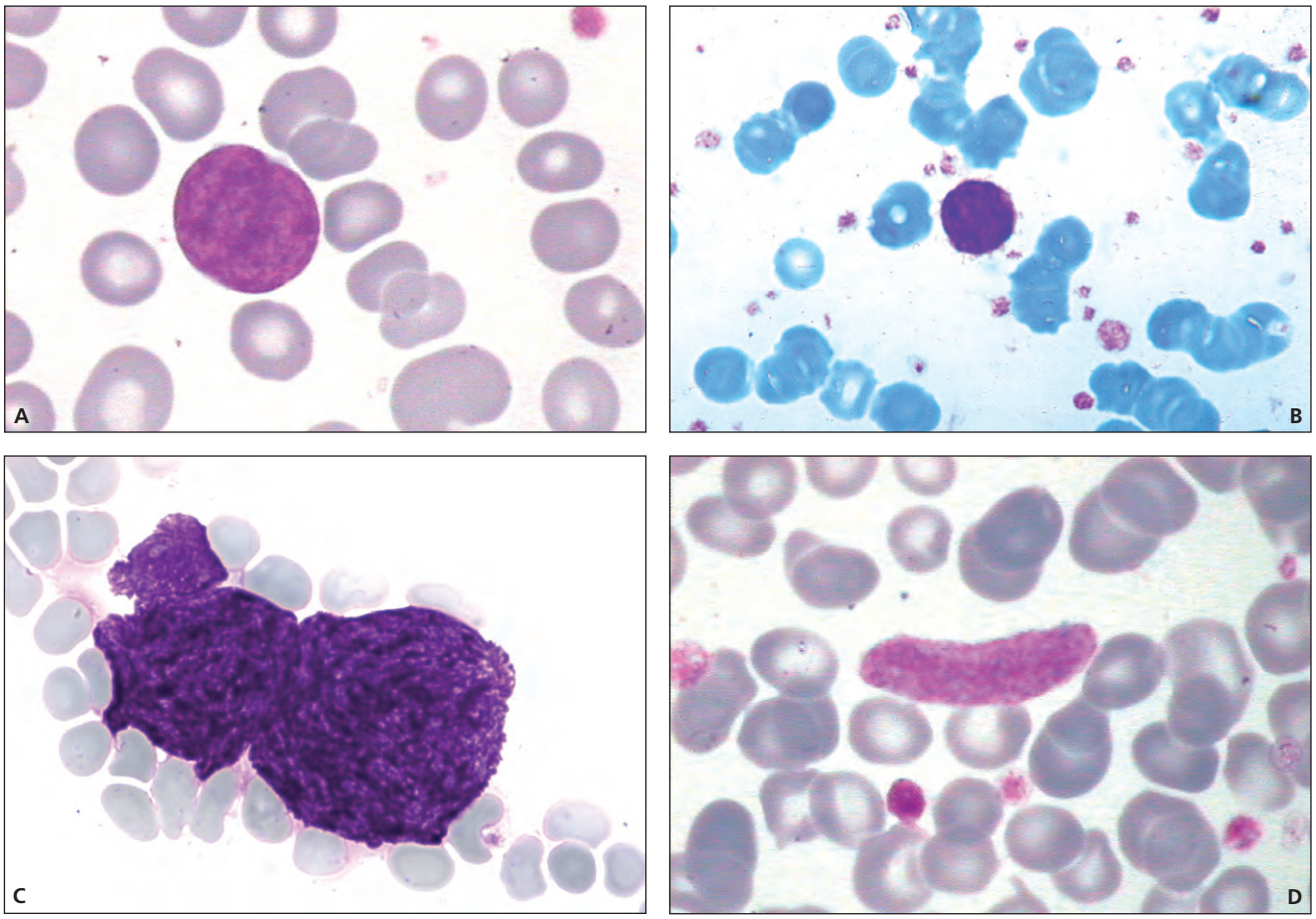



**Fig. 5.49** ET; atypical megakaryocytes, their nuclei and fragments of their cytoplasm in peripheral blood films of different patients. (A) Small circulating megakaryocyte with negligible cytoplasm in the peripheral blood of a patient with ET evolving to myelofibrosis. The nucleus is composed of two closely related lobes, like a coffee bean, as if cut in half along a curvilinear sulcus. The chromatin is compact with some areas of greater condensation. The nucleus is only apparently bare: the very fine rim of cytoplasm that surrounds it is just visible in the notch in the nucleus at the top of the nuclear sulcus. (B) In the clear spaces left by the red cells, which have formed short rouleaux, there are numerous platelets and, in the centre, a rounded micromegakaryocyte: its nucleus is perfectly round, slightly larger than a red cell, with very dense chromatin and a barely evident sulcus; the presence of the almost invisible cytoplasm can be appreciated by noting the cytoplasmic projections, thin and regularly distributed over the surface of the cell. (C) Large fragment of a hyperdiploid nucleus, torn and bare, derived from a giant bone marrow megakaryocyte. (D) A gigantic strip of megakaryocyte cytoplasm, of the length of three or four red cells, with a homogeneous structure and very fine granules, without any separation into hyalomeres and chromomeres.

associated with a particular tendency to gather together in clusters[76] (Figs 5.50 through to 5.59). The most typical feature of ET megakaryocytes is gigantism: the cytoplasm is hypertrophic, mature and granular, and the nucleus is hyperlobated and hyperdiploid, often with a fairly immature chromatin structure, sometimes with large nuclear fragments or entire lobes detached from the main nucleus. Emperipoiesis, the presence of leucocytes and haemopoietic precursors with well-preserved cellular features within the cytoplasm of megakaryocytes, is often observed. Many megakaryocytes show an accumulation of well-formed platelets at the periphery of the cytoplasm. Masses and ribbons of platelets are very common in the background of the films.

In accordance with the already cited concept of a prodromal diagnosis,[66] the recognition in the blood count of platelet numbers greater than the above-mentioned threshold, sustained over time and without any apparent cause, is an indication for bone marrow examination, with particular attention being paid to megakaryocyte morphology. The histological picture that is considered suggestive of the early phase of ET is the predominance of megakaryocytopoiesis in the context of a cellular marrow, and in the absence of erythroid or granulocytic hyperplasia. The markedly anomalous topographical distribution of the megakaryocytes and their tendency to form tight clusters are not yet present, while already apparent is the presence of pathological giant megakaryocytes with

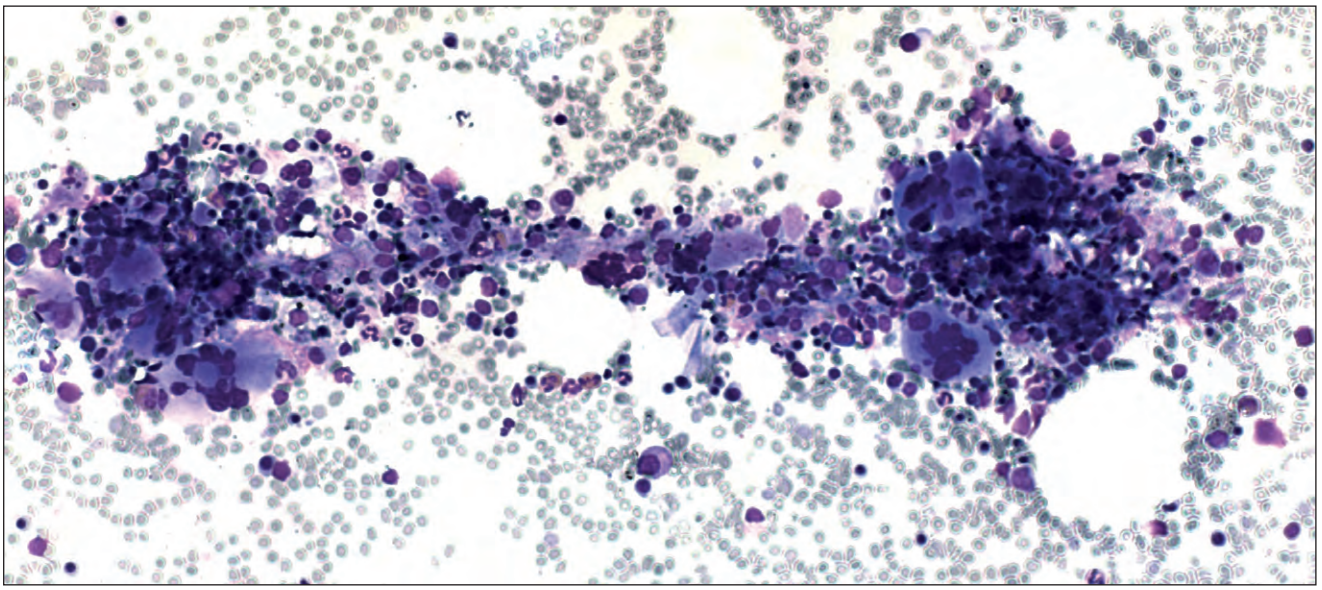


**Fig. 5.50** ET; bone marrow aspirate. There are small bone marrow fragments joined by a bridge of fibrohyaline material, which also forms the matrix of the fragments. In the context of a cellular and heterogeneous marrow there stand out, particularly, numerous large megakaryocytes with their hypersegmented, hyperdiploid nuclei and plentiful pink, mature cytoplasm, as in the megakaryocyte more to the left, or alternatively, with still basophilic cytoplasm despite the hyperlobation of the nucleus, as in the cells in the small particle on the right. In the areas surrounding the fragments, cellularity appears reduced with the cells present being erythroid and granulocytic precursors.

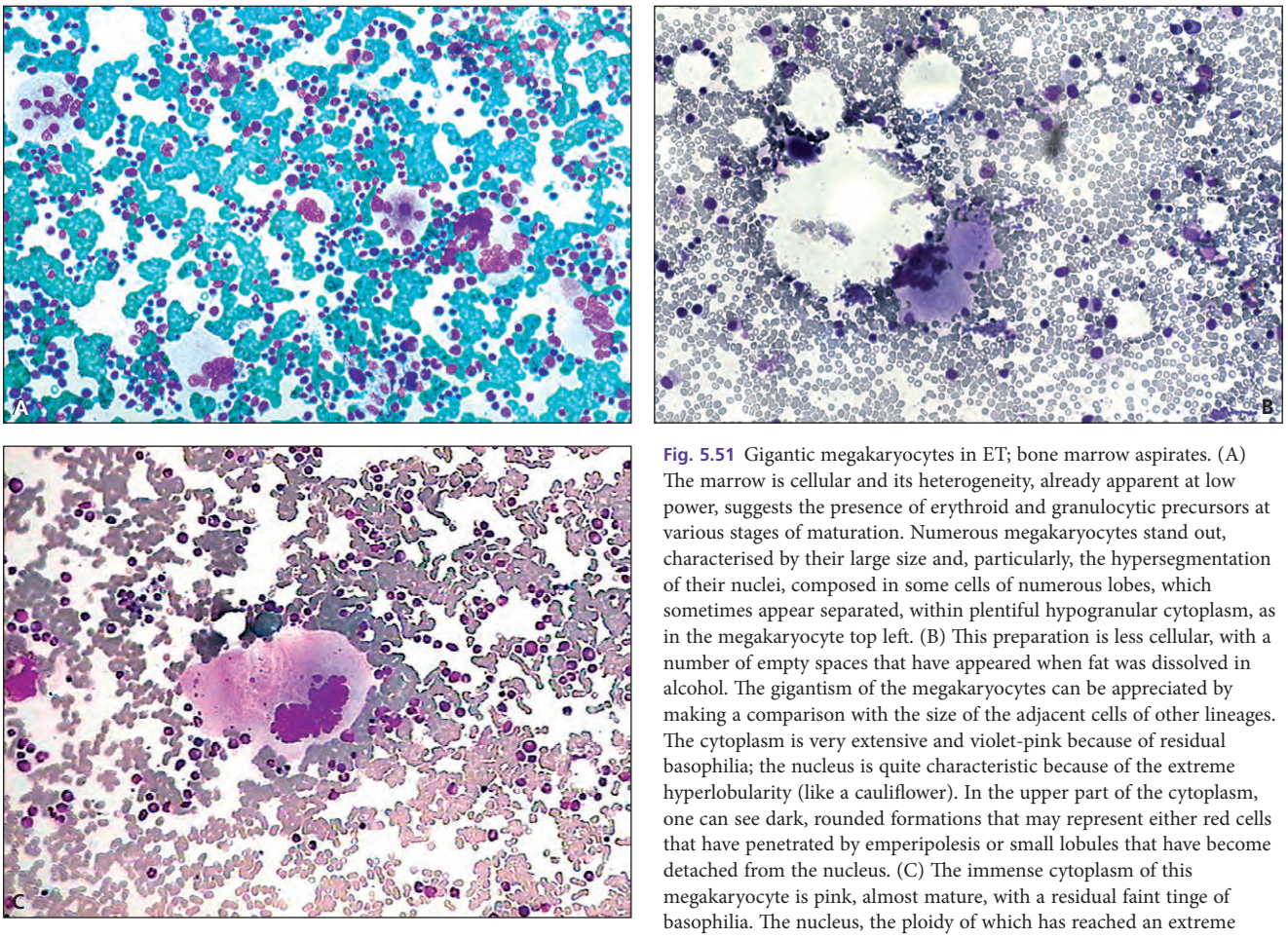



**Fig. 5.51** Gigantic megakaryocytes in ET; bone marrow aspirates. (A) The marrow is cellular and its heterogeneity, already apparent at low power, suggests the presence of erythroid and granulocytic precursors at various stages of maturation. Numerous megakaryocytes stand out, characterised by their large size and, particularly, the hypersegmentation of their nuclei, composed in some cells of numerous lobes, which sometimes appear separated, within plentiful hypogranular cytoplasm, as in the megakaryocyte top left. (B) This preparation is less cellular, with a number of empty spaces that have appeared when fat was dissolved in alcohol. The gigantism of the megakaryocytes can be appreciated by making a comparison with the size of the adjacent cells of other lineages. The cytoplasm is very extensive and violet-pink because of residual basophilia; the nucleus is quite characteristic because of the extreme hyperlobularity (like a cauliflower). In the upper part of the cytoplasm, one can see dark, rounded formations that may represent either red cells that have penetrated by emperipolesis or small lobules that have become detached from the nucleus. (C) The immense cytoplasm of this megakaryocyte is pink, almost mature, with a residual faint tinge of basophilia. The nucleus, the ploidy of which has reached an extreme level, has the appearance of a giant knobbly tuber; various nuclear fragments representing detached lobes are scattered through the cytoplasm, which, moreover, shows fissuring. The surrounding marrow is normally cellular.

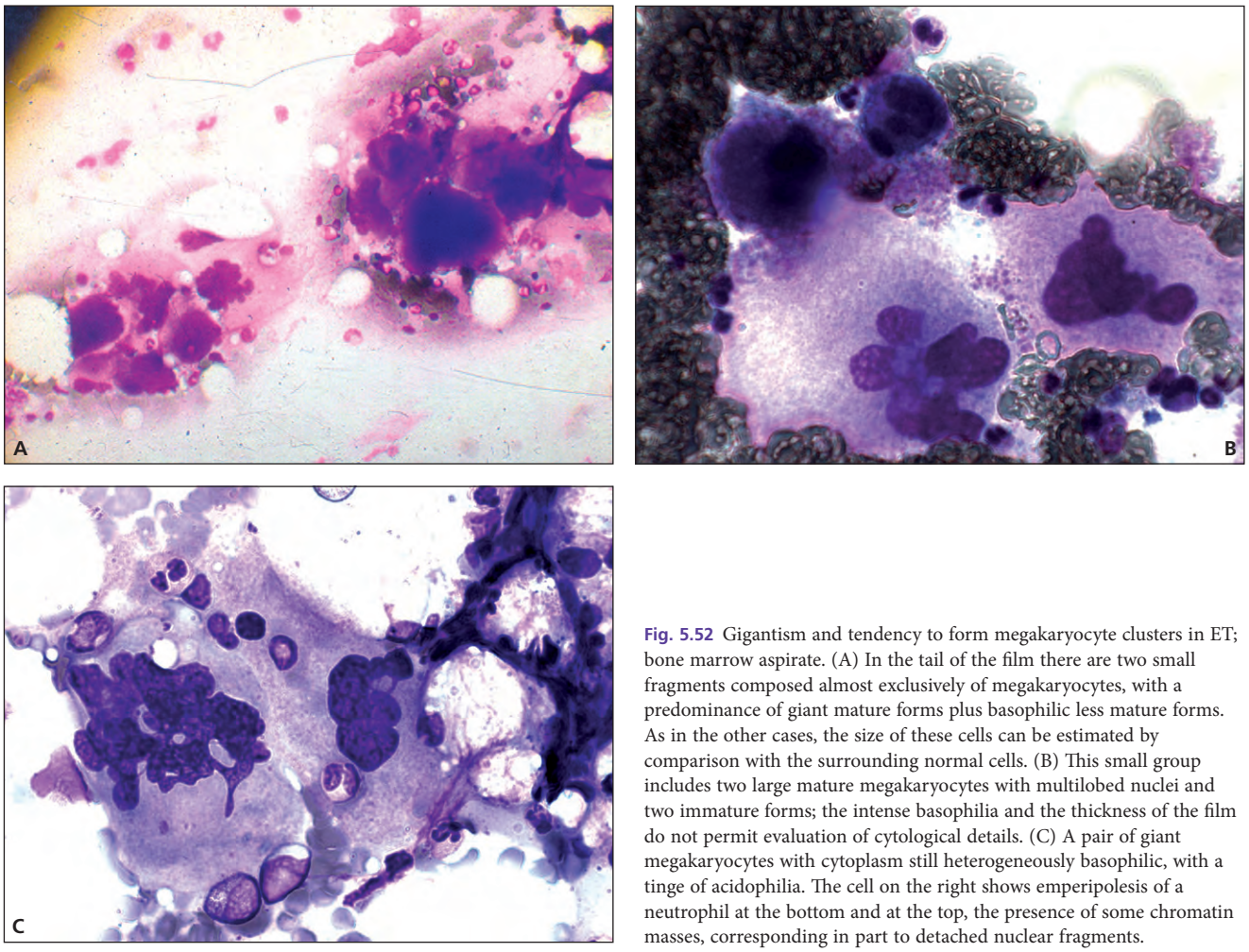



**Fig. 5.52** Gigantism and tendency to form megakaryocyte clusters in ET; bone marrow aspirate. (A) In the tail of the film there are two small fragments composed almost exclusively of megakaryocytes, with a predominance of giant mature forms plus basophilic less mature forms. As in the other cases, the size of these cells can be estimated by comparison with the surrounding normal cells. (B) This small group includes two large mature megakaryocytes with multilobed nuclei and two immature forms; the intense basophilia and the thickness of the film do not permit evaluation of cytological details. (C) A pair of giant megakaryocytes with cytoplasm still heterogeneously basophilic, with a tinge of acidophilia. The cell on the right shows emperipolesis of a neutrophil at the bottom and at the top, the presence of some chromatin masses, corresponding in part to detached nuclear fragments.

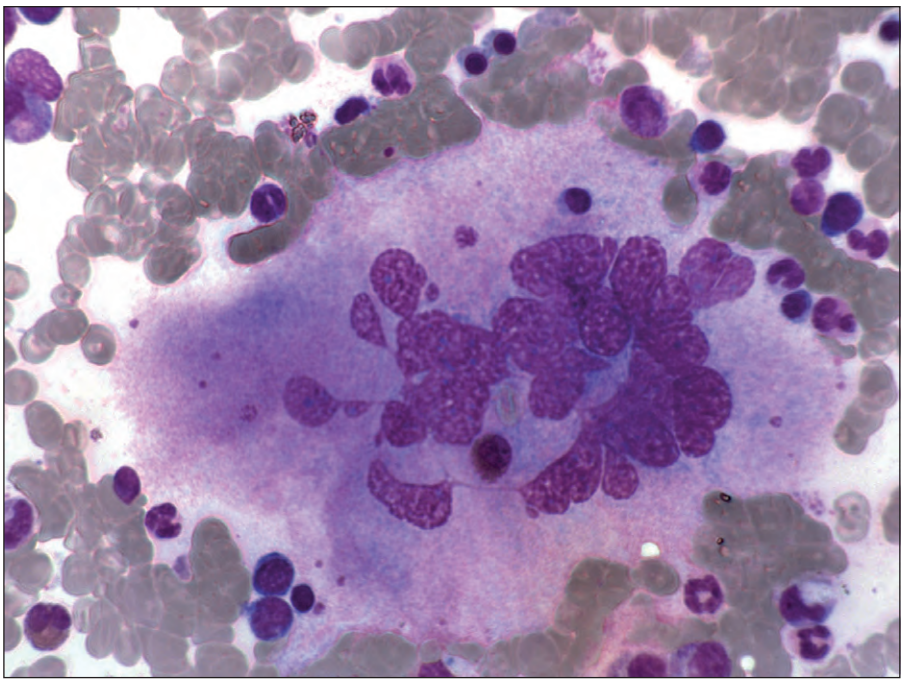


**Fig. 5.53** ET; bone marrow aspirate. In this colossal megakaryocyte with multicoloured cytoplasm the nucleus is formed of a dozen or so lobes, often quite distinct, teardrop shaped and joined by a thin chromatin filament or, sometimes (as on the right), joined to form a denser mass in the form of a festoon. The chromatin structure is airy with many pale spaces, less compact than in the majority of nuclei in the preceding figures.

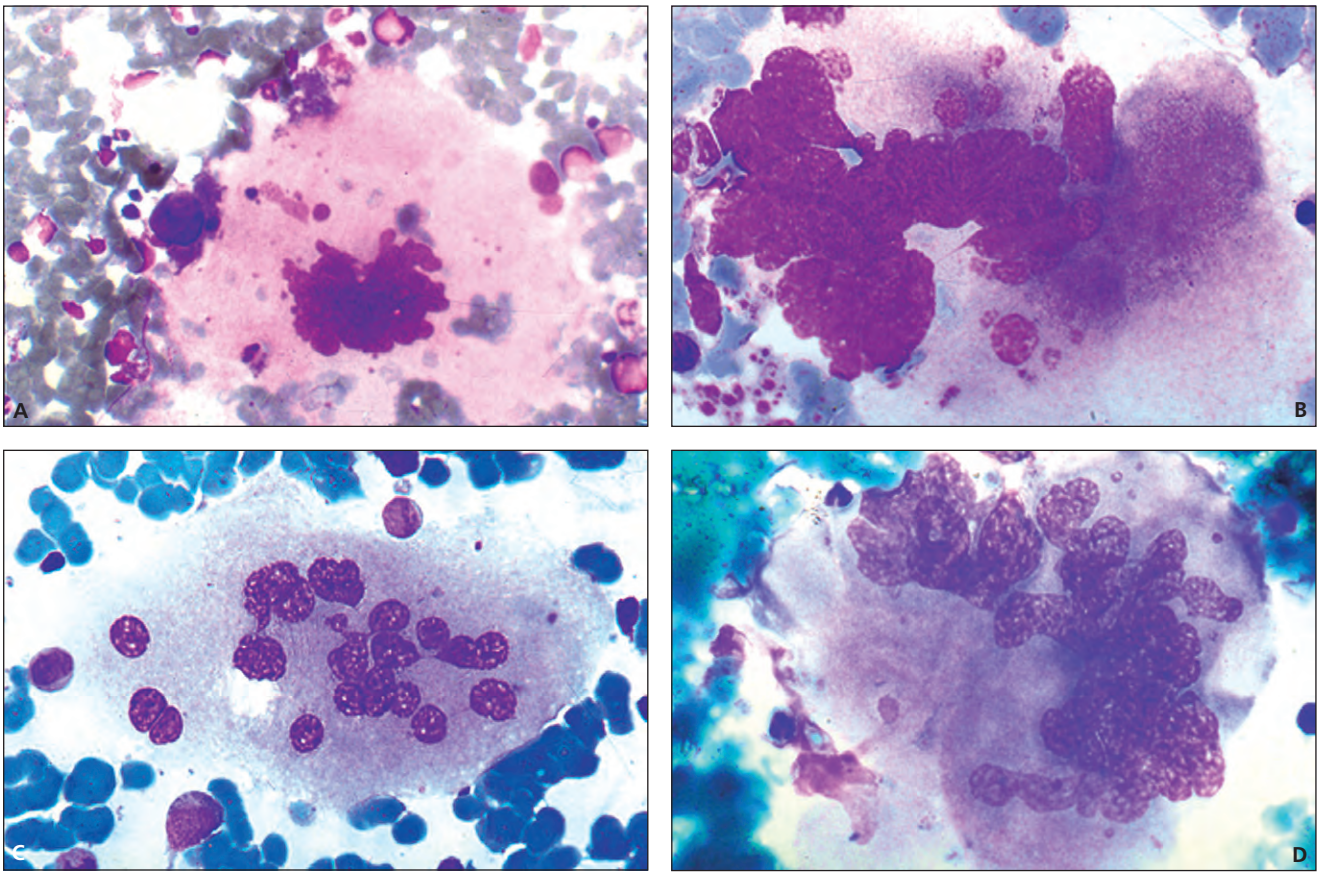



**Fig. 5.54** Other giant megakaryocytes in the bone marrow aspirates of patients with ET. (A) At low to medium power, this enormous, quite mature megakaryocyte stands out from among the normal haemopoietic precursors; its nucleus recalls the shape of a sessile tumour or a cauliflower, which is expanding within a giant pink blister. Some nuclear lobes have become detached and scattered through the cytoplasm, which also seems to contain a number of red cells. Immediately to the left, a smaller darker mass that represents a promegakaryocyte with a peripheral mass of already formed platelets. (B) Giant megakaryocyte with a diameter of more than 100 μm, and a multilobed nucleus. At a higher power one notes the spongy structure of the nuclear chromatin, which appears relatively immature in relation to the cytoplasm: the cytoplasm is granular and on the right side the granules are concentrating in small clusters to form a large area of platelet formation with the platelets almost ready to be liberated from the cell. Here also there are some rounded nuclear lobes and other ill-formed conformations of nuclear material. (C) In this cell, the predominant morphological feature is the presence of numerous nuclear lobes, some joined by thin filaments and some detached, apparently wandering around the vast, weakly basophilic and not very granular cytoplasm. This appearance is reminiscent of a form of dysmegakaryopoiesis observed in MDS. At the top, a blast cell. At the bottom, a promyelocyte. (D) The cytoplasm is quite plentiful and violet in colour, with numerous fine granules scattered on a weakly basophilic background. The hyperlobated nucleus is composed of heterogeneous spongy chromatin with an abundance of pale spaces. This type of nucleus has been called 'cloud like' because of its resemblance to large cumulus clouds.

hyperdiploid, hyperlobated nuclei surrounded by ample mature and well-differentiated cytoplasm.[77,78]

The increase in platelet numbers in ET is much more marked than in reactive or secondary thrombocytosis associated with chronic inflammation, neoplasia, physical exercise, haemolytic anaemia or iron deficiency. As well as in ET, values above $1000 \times 10^9$/l can be seen in rare and well-defined clinical situations, such as post-splenectomy. Particular attention must be paid to the recognition of pseudothrombocytosis, which may be observed with automated counters as a result of cryoglobulinaemia[79] because of interference by the cryoprecipitate, and also in microcytic anaemia with a very low MCV, in some microangiopathic haemolytic anaemias and in acute leukaemia with hyperleucocytosis, as a result of invasion of the platelet channel by microcytes, red cell fragments and detached fragments of leucocyte cytoplasm.[80]

As in PV, the transition to a spent phase of myelofibrosis (Table 5.7) is indicated by the appearance of morphological erythrocyte abnormalities (dacrocytes, anisopoikilocytosis, basophilic stippling, Howell-Jolly bodies) and by an increase in granulocyte precursors and erythroblasts in the circulation.[81] When this evolution occurs, an attempted bone marrow

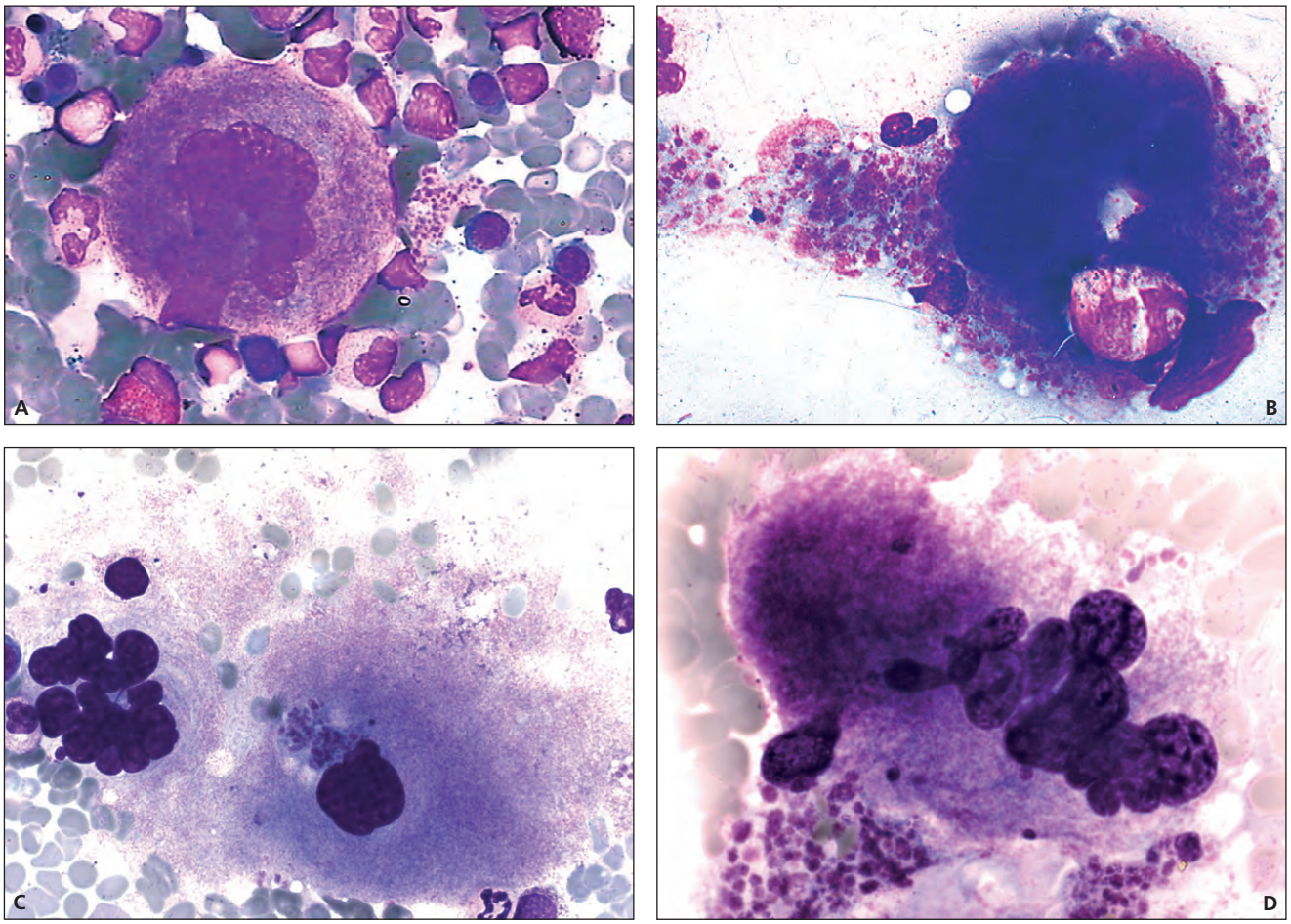



**Fig. 5.55** Cytoplasmic maturation and atypical platelet formation in ET. (A) The cytoplasm of this megakaryocyte is rich in granules, which are superimposed on a weakly basophilic background and, in some areas, are already condensed to form proplatelets. On electron microscopy this is referred to as 'demarcation of platelet fields'. (B) The complete transformation of megakaryocyte cytoplasm to platelets that are still attached to the nucleus has been referred to as 'a megakaryocyte burst'. At the bottom, an example of emperipolesis. (C) The megakaryocyte of the left has a nucleus composed of a dozen or so lobes with compact chromatin; the cytoplasm, damaged during the spreading of the film or released during platelet formation, has an indistinct outline. The megakaryocyte on the right has fewer lobes and multicoloured cytoplasm as a result of the alternation of basophilic areas and areas where there is a variable degree of concentration of granules. In contact with the nucleus, on the left, is a portion of cytoplasm that appears to have differentiated into a compact mass of well-formed platelets. (D) Also in this megakaryocyte, the nucleus is multilobated and the cytoplasm shows a degree of maturation that differs between different parts of the cell, with large strands and clumps of platelets along the lower border that are ready to be released.

aspiration usually yields only blood with infrequent fibronecrotic particles, in which one can still recognise dysmorphic megakaryocytes (Fig. 5.60). Histological sections still show numerous megakaryocytes and specific stains confirm the presence of fibrosis. In some cases, the appearance of blast cells in the peripheral blood, possibly associated with a sudden fall in the platelet count, indicates a rare transformation to acute megakaryocytic leukaemia (Fig. 5.61).

## Primary myelofibrosis

PMF is characterised by fibroblastic transformation of the bone marrow matrix as a result of the proliferation of fibroblasts: this is associated with extramedullary haemopoiesis, known as myeloid metaplasia, particularly in the spleen. The *JAK2* V617F mutation is present in around 50% of patients. Table 5.8 shows the diagnostic criteria proposed in the 2008 WHO classification.

The haematological picture at presentation of PMF is very variable, but shows various typical features which, in association with the clinical picture of marked splenomegaly, enable the diagnosis to be made: important among these is the presence of granulocyte precursors, erythroblasts and dacrocytes in the peripheral blood film. At the time of diagnosis there is often a moderate anaemia, associated with leucocytosis (the WBC not being above 20–30 $\times$ $10^9$/l); in a quarter of cases the leucocyte

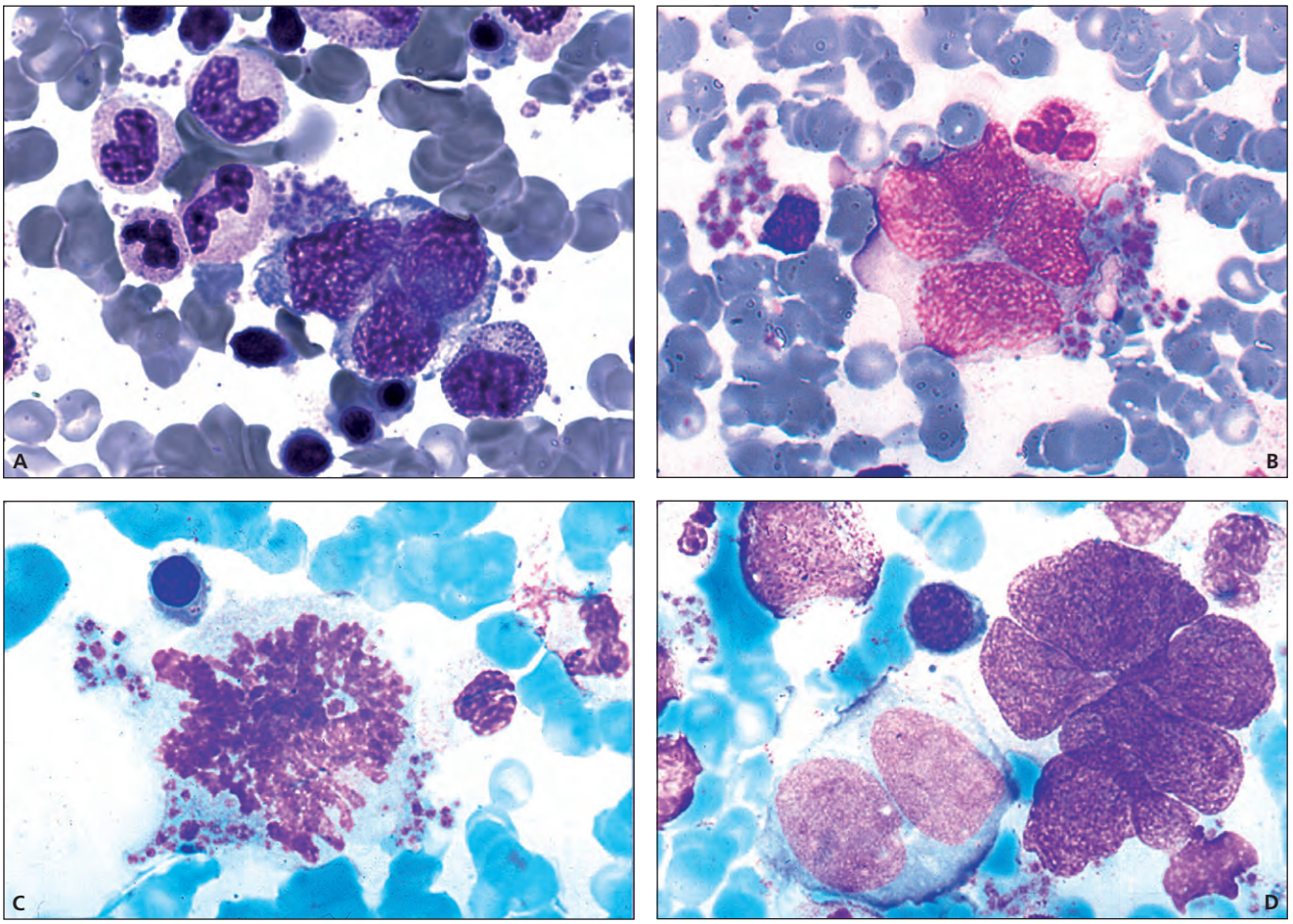



**Fig. 5.56** Atypia of megakaryocyte precursors in ET; bone marrow aspirate. (A) Dysmorphic promegakaryocyte with three large nuclear lobes, each of which is at least tetraploid, formed of spongy chromatin, distinguished by numerous pale fissures alternating with areas of condensation. The cytoplasm is intensely basophilic with round, optically empty areas in the cytoplasm, and appears to be differentiating at the top into an irregular clump of platelets that are ready to detach. (B) This cell also, which is quite similar to the preceding one, can be regarded as a promegakaryocyte because of the presence of areas of cytoplasm, on the right side, which have completed their maturation. (C) Megakaryocyte or atypical promegakaryocyte with a nucleus in mitosis within mature cytoplasm with peripheral platelet formation. The mitotic figure is hyperdiploid, as is shown by the large number of identifiable chromosomes. As in the cell in (B), there is asynchronous maturation, shown by the peripheral strands of cytoplasm that are forming easily identifiable individual platelets in the presence of a nucleus that is not yet mature. It is often difficult to establish whether a mass of platelets such as this has been formed by the megakaryocyte itself of is simply a clump of platelets that has adhered to its cytoplasmic membrane: in this cell, however, at least bottom left, the gradual passage from the main body of the cytoplasm to the outer part where platelets are forming is clearly apparent. (D) Binucleated megakaryoblast adjacent to a bare four-lobed nucleus. Note the delicate chromatin structure of the megakaryoblast, in which the two oval nuclei contain rather different incomplete nucleoli, without peripheral reinforcement; the cytoplasm is weakly basophilic, with fine vacuoles and peripheral enhancement of the basophilia. The bare multilobated nucleus results from the complete transformation of cytoplasm into platelets, and yet the chromatin is very immature for a cell in the last stages of platelet formation. These features underline again the maturational asynchrony typical of this type of MPN.

count is normal, while more rarely there is leucopenia. The platelet count is moderately increased in 30–50% of patients. With progression of the disorder, the WBC tends to increase, while the platelet count falls. In the peripheral blood film, red cell morphology is abnormal and characteristic (Figs 5.62, 5.63 and 5.64), with anisocytosis and poikilocytosis, which is usually marked, and, particularly, the presence or teardrop cells or dacrocytes; these are red cells with an outline that is deformed into a point, as if pulled by pincers, as a result of bone marrow fibrosis or, more probably, is deformed in the spleen, representing a trustworthy clue to the presence of extramedullary haemopoiesis. Peripheral blood manifestations of extramedullary haemopoiesis include:

- Polychromatic erythrocytes or erythrocytes with basophilic stippling, both corresponding to immature reticulocytes that still have large numbers of ribosomes;
- Erythrocytes with Howell-Jolly bodies, which are small spherical bodies composed of nuclear material and positive on a Feulgen reaction, generally eccentrically situated and on

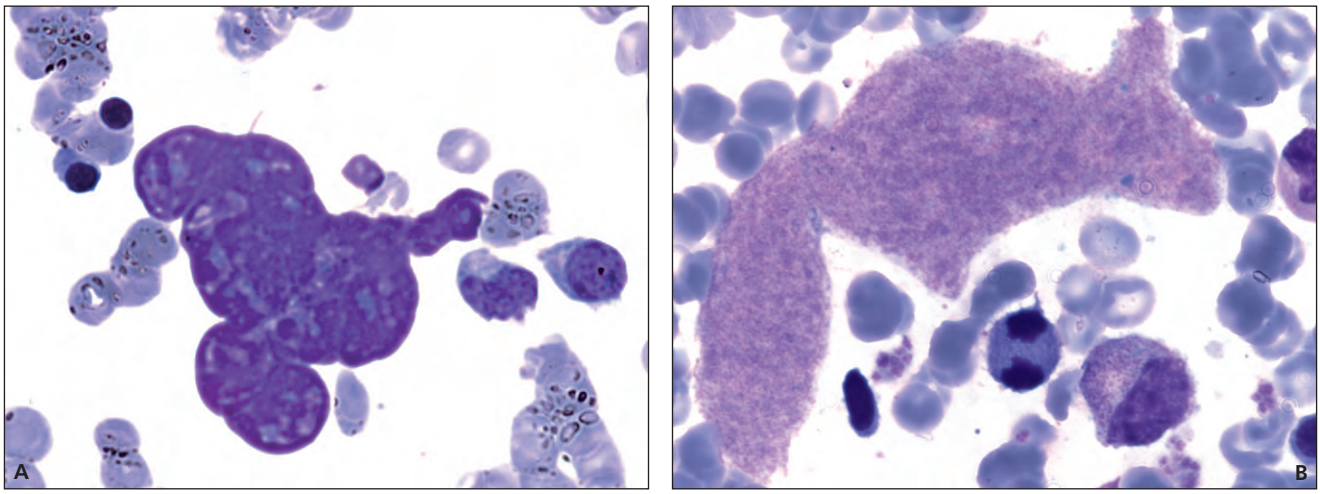



**Fig. 5.57** Final stages of megakaryocyte maturation and platelet formation in ET; bone marrow aspirate. (A) This gigantic bare nucleus, composed of a mass of numerous lobes with compact chromatin, is now completely free of cytoplasm. The adjacent cells, erythroblasts to the left and granulocyte precursors to the right, being of normal size, emphasize the gigantism of this residual nucleus. (B) Enormous fragment of megakaryocyte cytoplasm which has become detached from the cell of origin before completion of the process of platelet formation. Although there are visible microaggregates of granules that represent platelet fields, it is probable that there will never be demarcation and detachment of true platelets.

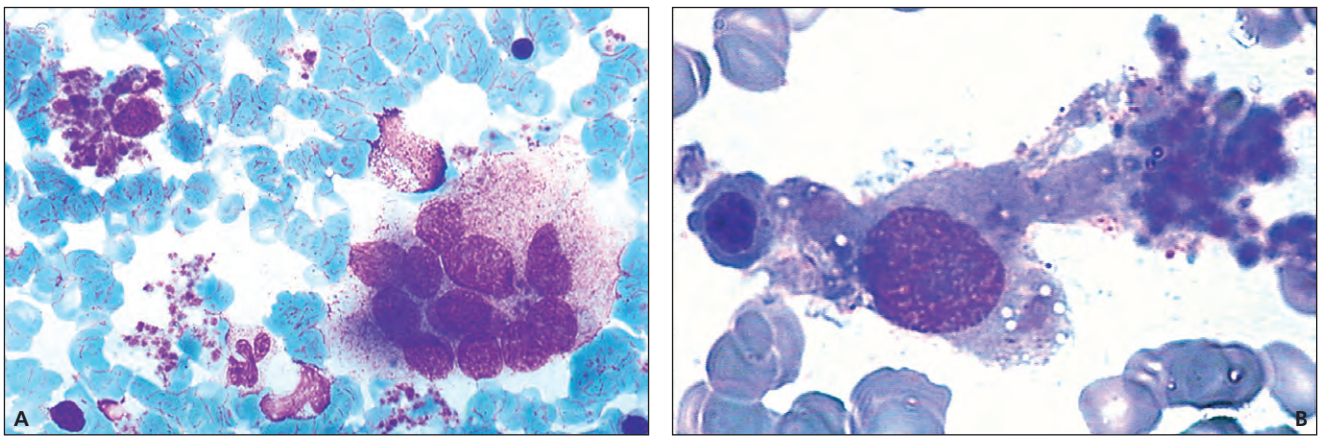



**Fig. 5.58** ET; bone marrow aspirate. (A) Usual morphology of megakaryocytes can sometimes make them resemble other giant multinucleated cells, that can often also be observed in the aspirates of these patients. Osteoclasts, such as the one represented here, have numerous separate nuclei, which typically occupy almost all the cytoplasm; although the nuclei are superimposed on each other, their delicate chromatin structure lets the outline of the underlying nucleus be seen, as can clearly be observed in the centre of this cell. Such transparency is generally not found with the denser nuclei of megakaryocytes. The cytoplasm is also different because of the presence in the osteoclast of large regularly distributed granules. These characteristics are also useful for distinguishing osteoclasts from the megakaryocytes with multiple separate nuclei that may be seen in MDS. One notes the presence of a number of masses of platelets spread in the background of the film. (B) This larger cell could appear to be a promegakaryocyte with the cytoplasm differentiating, in the projection at the right, into hyperchromatic platelets that are still attached to each other. However, it is actually a macrophage with a spongy nucleus to which platelets have adhered.

electron microscopy having the appearance of true micronuclei with a nuclear membrane; they correspond to chromosomes that have separated from the mitotic spindle during abnormal mitosis or are the result of karyorrhexis prior to nuclear expulsion;

- Erythrocytes containing Cabot rings, which are thin, bright red azurophilic filaments in the form of a ring or a figure of eight or the bow of a violin, negative on a Feulgen reaction and corresponding to the persistence of fibres of the mitotic spindle which have not disintegrated at the end of mitosis.

The peripheral blood film often shows giant platelets or actual fragments of megakaryocyte cytoplasm or their bare, often pyknotic, nuclei (Fig. 5.65). There is also often a relative and absolute neutrophilia, which parallels the increased WBC, with

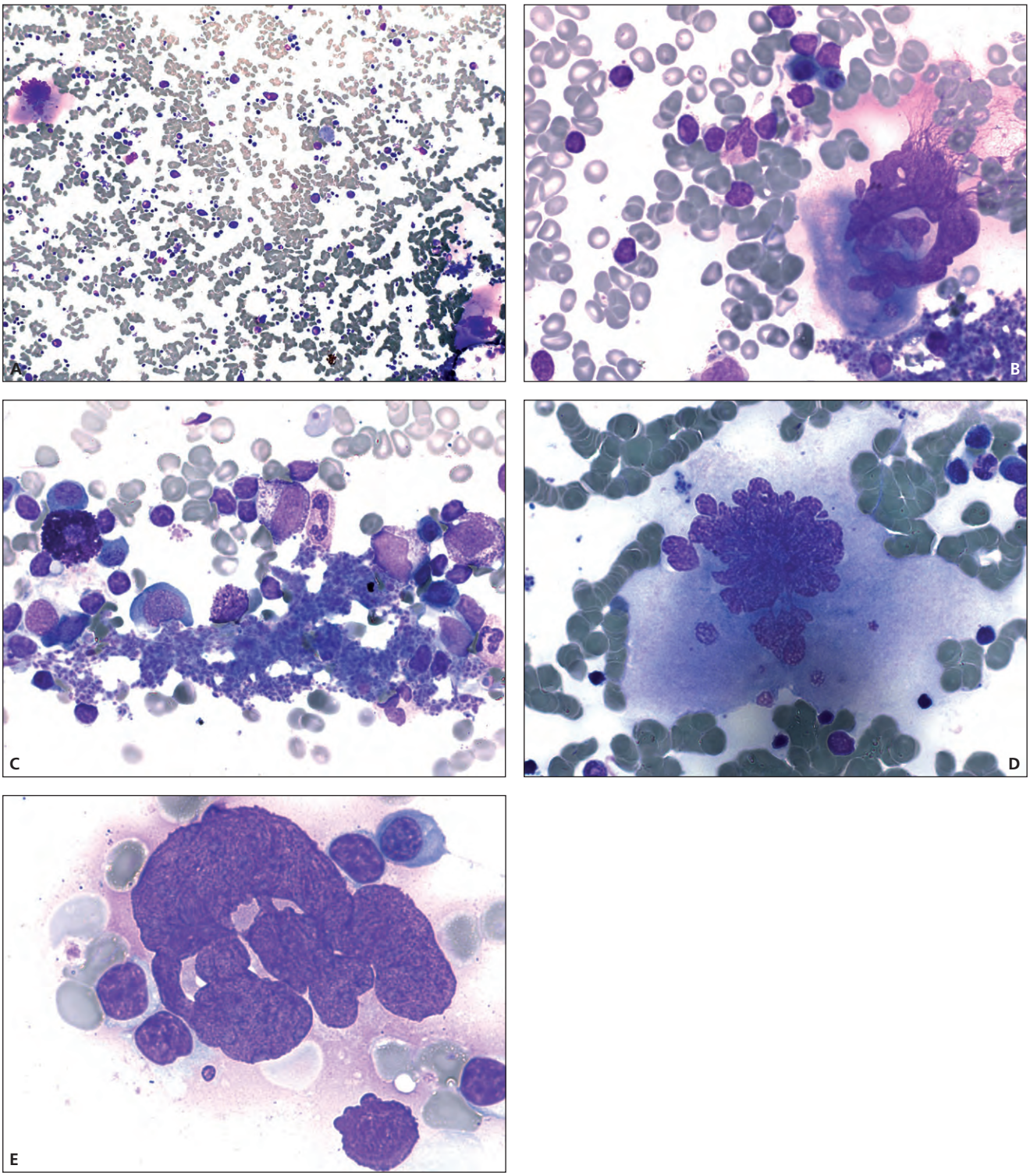



**Fig. 5.59** Bone marrow aspirate from a patient with both ET and chronic lymphocytic leukaemia (B lineage). (A) In this microscopic field with preserved cellularity and with heterogeneity of cell types, one can observe two gigantic megakaryocytes with cytoplasmic characteristics (maturity) and nuclear characteristics (hyperdiploidy and multilobation) typical of ET. (B) At higher power, a large part of the cells found in proximity to a giant megakaryocyte with a torn nucleus and basophilic cytoplasm are small lymphocytes with compact homogeneous chromatin and very scanty cytoplasm. (C) From the same film. A long, ample festoon of platelets, each one well formed but still attached to each other, are spread across the entire image. Top left, a number of mature lymphocytes. In the band across the centre, from the left, there is a mast cell, a proerythroblast, a basophil myelocyte and a number of neutrophil granulocytes in various stages of maturation. (D) Giant megakaryocyte with a cauliflower-shaped nucleus, isolated nuclear lobes and very extensive cytoplasm showing little differentiation. A number of lymphocytes are also visible. (E) This swollen nucleus, with tuberosities and protrusions, appears to be still surrounded by frayed, pink cytoplasm. Close to it are four small mature lymphocytes with chromatin condensed into a mosaic pattern and, top right, a plasma cell.

**Table 5.7** Diagnostic criteria for post-essential thrombocythaemia myelofibrosis according to the 2008 WHO classification (adapted from Swerdlow 2008,[13] with permission of IARC)

**Essential criteria**

1. Previous documented diagnosis of essential thrombocythaemia according to the WHO criteria
2. Bone marrow fibrosis shown on bone marrow biopsy, histopathological grade 2–3 (on a scale of 0–3) or 3–4 (on a scale of 0–4)

**Additional criteria** (at least two are necessary for the diagnosis)

1. Anaemia (haemoglobin concentration below the reference interval); or reduction of haemoglobin concentration by at least 20 g/l
2. Leucoerythroblastic blood film
3. Significant increase in splenomegaly
4. Increase in serum lactate dehydrogenase (LDH) above the reference interval
5. Appearance of at least two constitutional symptoms (weight loss, nocturnal sweating, fever)

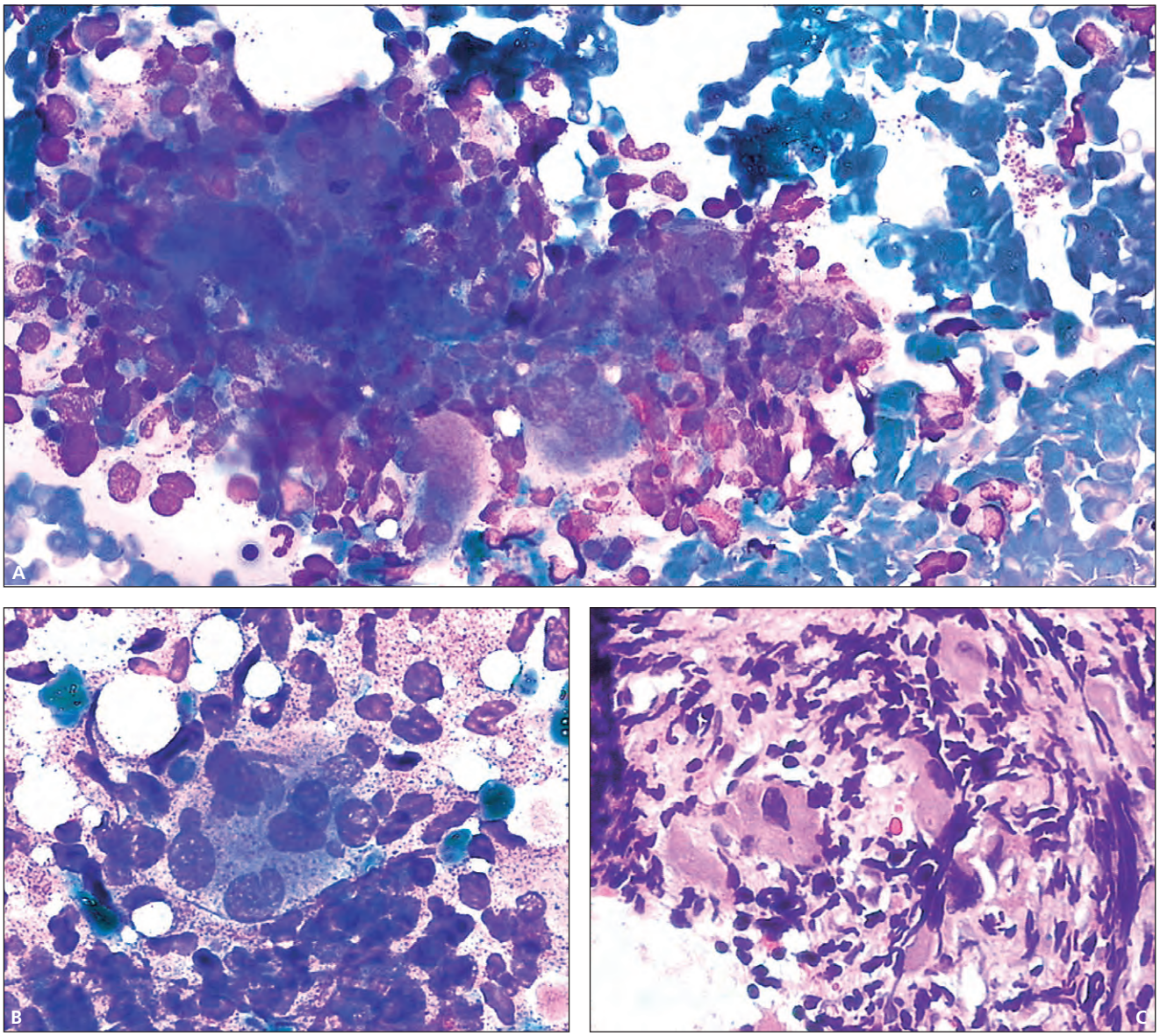



**Fig. 5.60** ET; from a case with evolution to a spent myelofibrotic phase. (A) Bone marrow aspirate, at low power. This small fragment is formed of a compact agglomerate of cells in which can be seen numerous neutrophils, some eosinophils and a number of medium-sized megakaryocytes. In the surrounding area, cellularity is reduced. (B) At higher power, there are many damaged unrecognisable cells; the nuclei appear merged with a hyaline and granular background mass. In the centre, a dysmorphic megakaryocyte with separate nuclei. (C) Histological section of a trephine biopsy specimen. There are numerous megakaryocytes with well-differentiated cytoplasm within fibrous tissue.

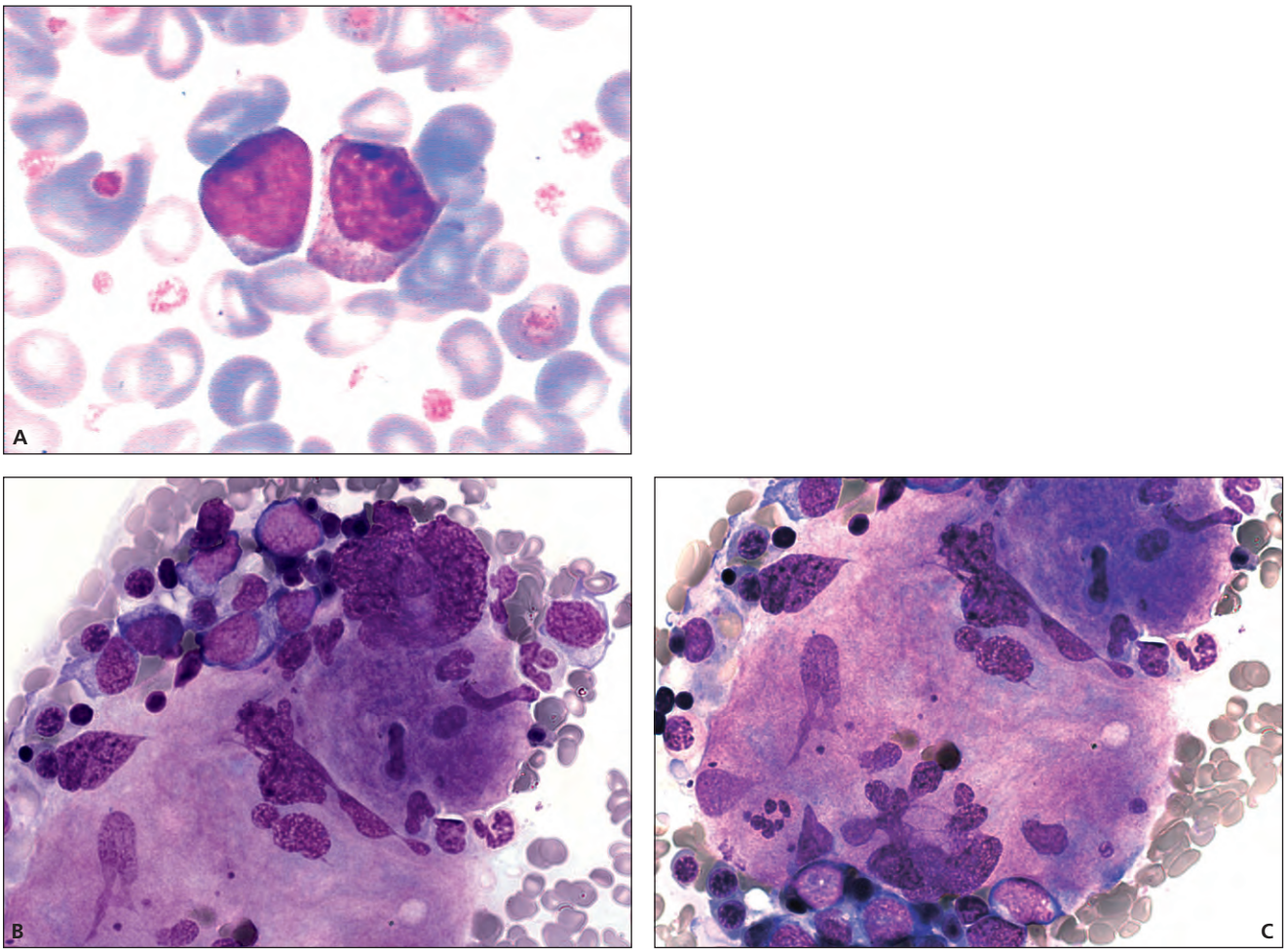



**Fig. 5.61** ET; from a patient with evolution into acute leukaemia. (A) In the peripheral blood, among the many platelets is an agranular blast cell, with homogeneous dense chromatin and a nucleolus, together with a neutrophil myelocyte. (B) Bone marrow aspirate. Two gigantic megakaryocytes, one more mature with numerous fine granules scattered through the cytoplasm and a number of isolated nuclear lobes, the other with more intensely basophilic cytoplasm and showing emperipolesis. In the upper part of the image, a number of agranular blast cells, with nuclei that have delicate chromatin and a number of nucleoli. On the left, polychromatic erythroblasts; on the right, neutrophils that are in part hypogranular and a small orthochromatic erythroblast. (C) This image shows the lower part of the megakaryocyte discussed in (B), which contains a nucleus formed of many spongy lobes joined by filaments and thicker chromatin bridges; a neutrophil has entered the megakaryocyte by emperipolesis. At the bottom, some polychromatic erythroblasts and a number of blast cells.

**Table 5.8** Diagnostic criteria for primary myelofibrosis according to the 2008 WHO classification* (adapted from Swerdlow 2008,[13] with permission of IARC)

**Major criteria**

1. Proliferation of atypical megakaryocytes in the bone marrow, associated with:
   - Reticulin and/or collagen fibrosis; or (in the absence of fibrosis)
   - Increase of bone marrow cellularity mainly in the granulocytic lineages (indicative of the prefibrotic phase)
2. Absence of WHO diagnostic criteria for PV, *BCR-ABL1*-positive CML, MDS or other haematological neoplasm
3. Presence of the *JAK2* V617F mutation or another relevant mutation (e.g. *MPL* W515K/L); or (in the absence of demonstrable clonality) exclusion of all causes of secondary fibrosis (inflammatory myelopathy, toxic, infective, autoimmune, lymphoid neoplasia or metastasis of solid tumour)

**Minor criteria**

1. Leucoerythroblastic blood film
2. Increase in serum lactate dehydrogenase (LDH)
3. Anaemia
4. Splenomegaly

*Diagnosis requires meeting the three major criteria and two minor criteria.

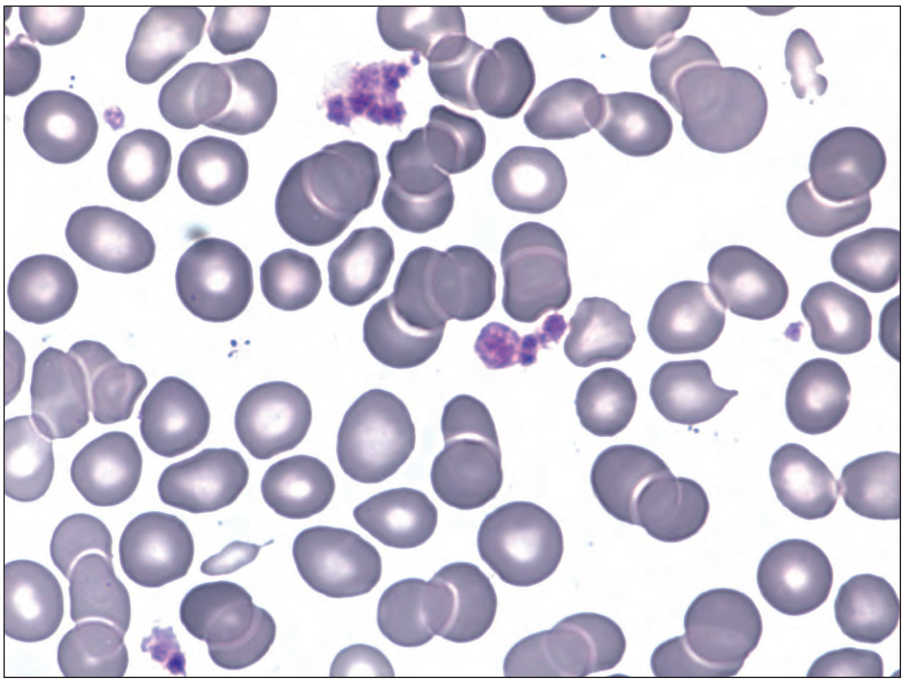


**Fig. 5.62** Initial phase of primary myelofibrosis (PMF); peripheral blood film. The red cells show moderate anisopoikilocytosis and the presence of two small dysmorphic microcytes and two dacrocytes. The platelets are hyperchromic, heterogeneous and adhering to each other in small groups.

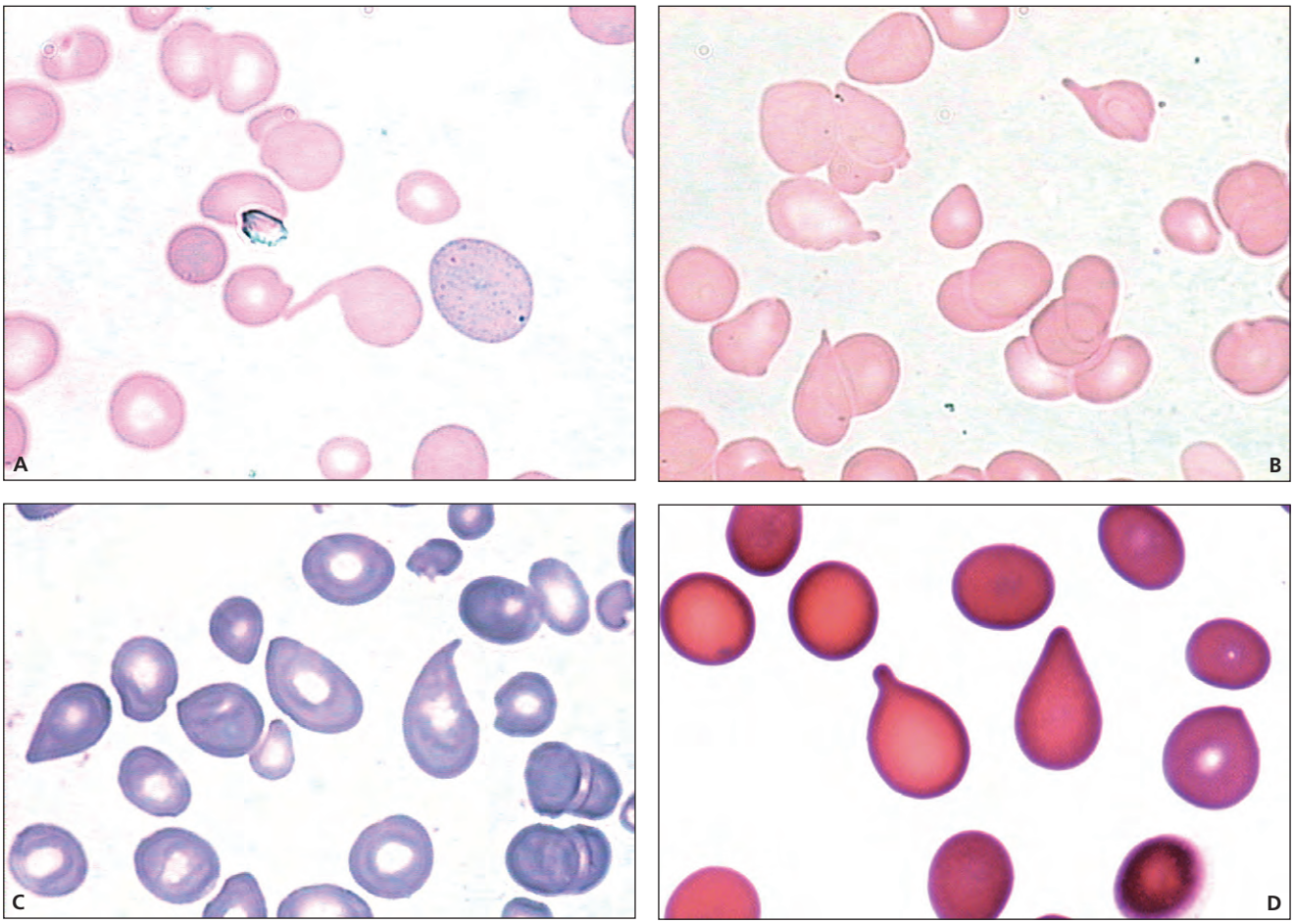



**Fig. 5.63** Alterations in peripheral blood red cell morphology in patients with overt PMF. (A) On the midline, a spherocyte, a dacrocyte with a long thin tail and a polychromatic macrocyte with fine basophilic stippling. (B) Numerous dacrocytes, anisochromasia and a tendency to rouleaux formation. (C) Marked anisocytosis with some very small red cells, the result of intramedullary or splenic fragmentation. Dacrocytes and microdacrocytes. (D) Two typical dacrocytes.

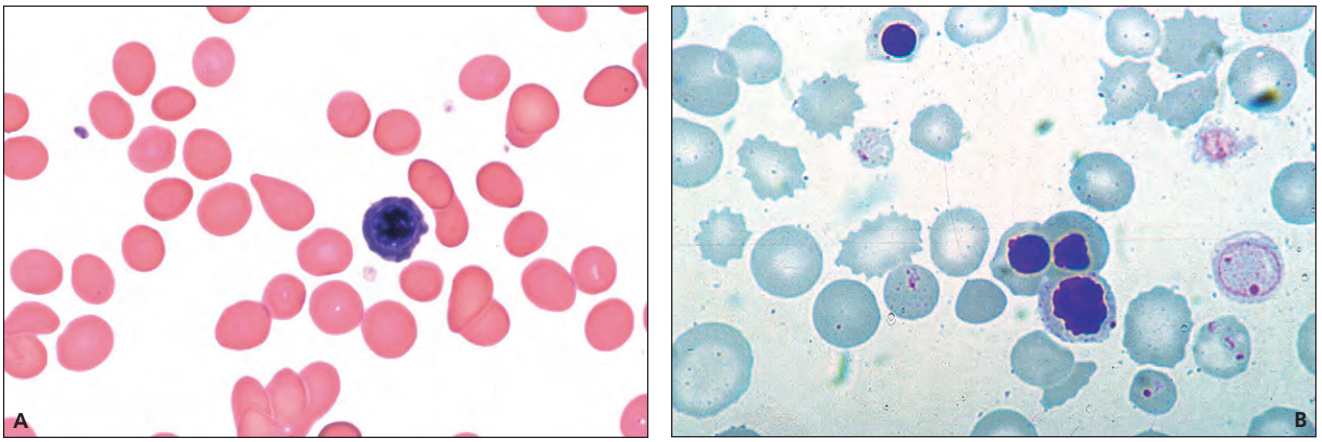



**Fig. 5.64** PMF; peripheral blood film. (A) A dacrocyte, a generalised tendency to hyperchromia and a circulating polychromatic erythroblast. (B) Marked anisopoikilocytosis. On the right, a red cell with a Cabot ring, a thin bright red azurophilic circle concentric with the cytoplasmic membrane; there are also granules with the same staining characteristics, both these and the Cabot ring representing remnants of the mitotic spindle. Nearer to the centre, an apparent syncytium of three orthochromatic erythroblasts with irregular nuclei; the lower one appears to contain basophilic granules and a small Howell-Jolly body. At the top, numerous echinocytes, a small orthochromatic erythroblast and a large platelet with the hyalomere forming pseudopodia.

the consistent presence of dysmorphic neutrophils, myelocytes, and polychromatic and orthochromatic erythroblasts (Figs 5.66 and 5.67). Eosinophilia, basophilia or both is observed in a small percentage of patients. Neutrophil alkaline phosphatase is usually increased. In two thirds of patients there is reticulocytosis, which is not proportional to the severity of the anaemia.

Report 5.6 shows a typical automated instrument printout in PMF.

Bone marrow cytology in PMF is difficult to observe because the frequency of fibrosis makes it impossible to obtain an adequate aspirate (*punctio sicca*, dry tap) or provides only blood with characteristics that are usually analogous to those of the peripheral blood (blood tap) or contains a larger proportion of dysmorphic precursors (Figs 5.68 through to 5.72). Only in a proportion of patients, who are probably still in the early cellular phase of PMF in which there is only early fibrosis, does bone marrow aspiration yield cellular material containing particles, distinguished by granulocytic and megakaryocytic hyperplasia with reduced erythropoiesis. In these cases, the megakaryocytes may be particularly increased and dysmorphic, often clustered and very atypical; the evaluation of these cells is always uncertain in cytological preparations. Mast cells may be increased.

In trephine biopsy sections, cellularity is very variable, from hypercellularity with disappearance of fat cells to a hypocellular picture in which only stromal components remain (Fig. 5.73). Hyperplasia, typical of the earliest phase of the disease,[66] involves the granulocytic series and the megakaryocytes, while erythroblasts are often reduced and dysmorphic. Histological evaluation is ideal for assessing the degree and characteristics of reticulin and collagen fibrosis and the features of megakaryocytes, which are typically increased in number, formed into tight clusters, and very dysmorphic, with bizarre hyposegmented or irregularly hyperlobated nuclei, with 'cloud-like' nuclei, or with hyperchromatic nuclei.[82] In the fibrotic stage, there may be residual foci of hypercellularity, with small aggregates of myeloblasts, more easily visualised with a CD34 stain, the number of which, however, is always below 10%. There is proliferation and tortuosity of small vessels and appositional new bone formation; finally the stage of osteosclerosis is reached with the bone trabeculae occupying more than 50% of the marrow cavity.

In PMF, transforming growth factor beta-1 (TGF-beta1) originating from megakaryocytes has been identified as the cytokine primarily responsible for the stromal changes, such as bone marrow fibrosis, osteosclerosis and angiogenesis. The megakaryocytes in PMF have an altered distribution of P-selectin, which promotes a pathological interaction between megakaryocytes and neutrophils; this leads to release both of angiogenic and fibrogenic cytokines, TGF-beta, PDGF and vascular endothelial growth factor (VEGF), and also elastase and other proteases of neutrophil derivation. It has been shown that the abnormal distribution of P-selectin in the megakaryocytes in PMF, e.g. in proximity to demarcation membranes, induces the selective sequestration of neutrophils by the megakaryocytes, as is evidenced by the frequent observation of emperipolesis of neutrophils in the bone marrow of these patients.[83] Furthermore, the release of proteases increases the number of CD34-positive blast cells released into the peripheral blood. This phenomenon is specific for established myelofibrosis (see Fig. 5.67), not being seen in non-fibrotic PV or ET.[84] This is in part analogous to the recognition of haematogones in patients with leukaemia. Evolution to AML occurs in about a third of patients.

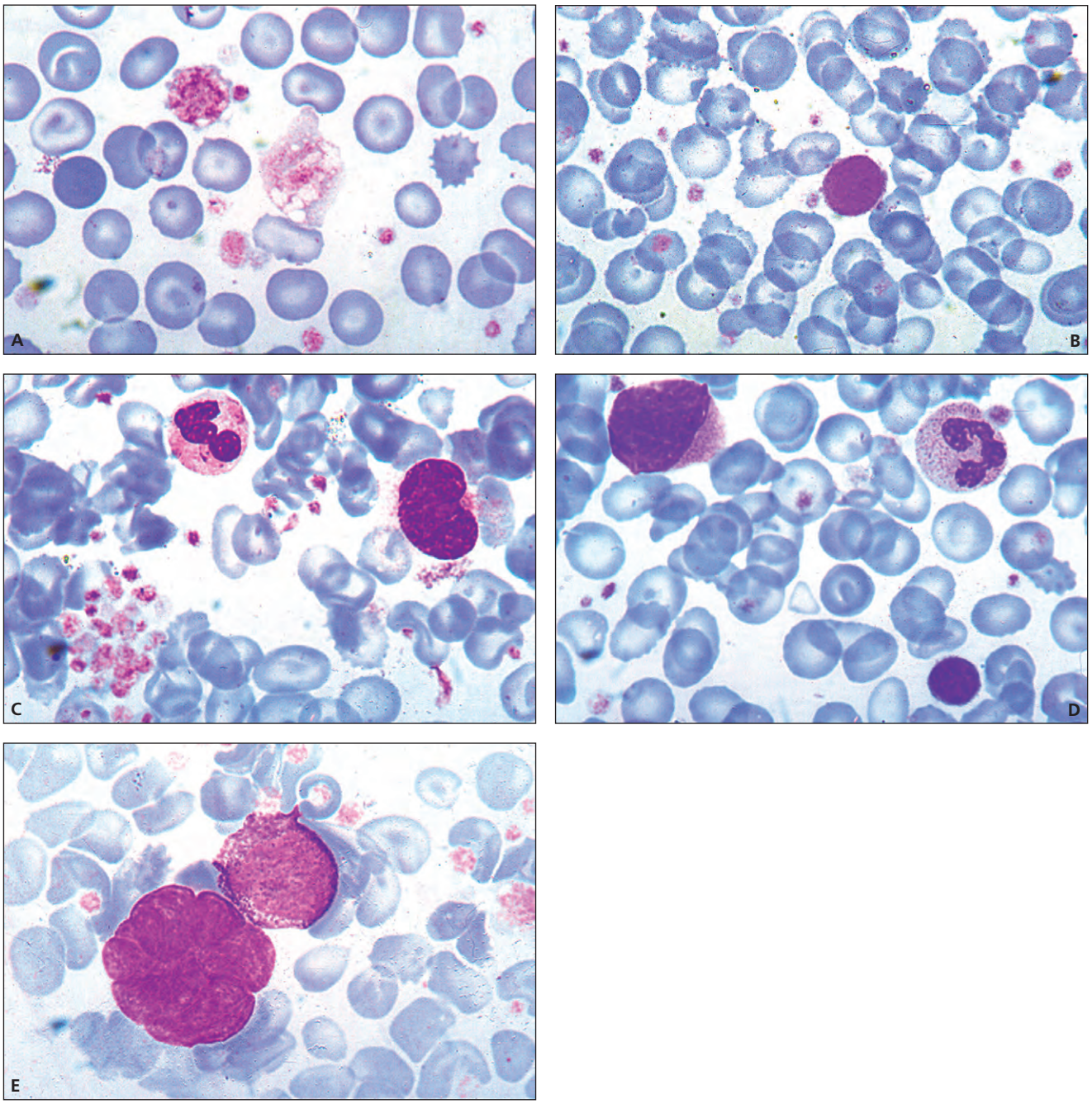



**Fig. 5.65** Platelet anomalies in PMF; peripheral blood film. (A) Anisocytosis and anisochromasia or both erythrocytes and platelets. On the left, a spherocyte; various target cells; two giant platelets (one hyperchromic and the other vacuolated and hypogranular); on the right, a small echinocyte. (B) The red cells are hypochromic, with moderate anisopoikilocytosis. The platelets are normal in size and structure, with the exception of a giant platelet similar to a lymphocyte, situated in the centre and comprising an enormous chromomere, formed by concentration and coalescence of numerous granules, with a thin hyalomere that is extending into fine vesicular protrusions. (C) Platelets, numerous and well formed, even if their granule content is variable, are concentrated on the left in a loose group. At the top, a mature neutrophil. On the right, a circulating micromegakaryocyte with a bean-shaped nucleus and scanty indistinct cytoplasm. (D) Top left, a small megakaryocyte with an almost triangular nucleus and scanty granular cytoplasm. On the right, a not completely segmented, granular neutrophil with a platelet adherent to its surface. At the bottom, a hyperchromic cell, too small in relation to the red cells and the neutrophil to be a lymphocyte and which must be a giant platelet with a pseudonucleus, similar to that seen in (B). In the centre, a hypochromic triangular schistocyte. (E) A large multilobated megakaryocyte nucleus with numerous closely related lobes, similar to the petals of a daisy. Beside this, a granulocyte precursor with cytoplasm that is very rich in azurophilic granules and a large nucleus with fairly immature chromatin, characteristic of a promyelocyte, without nucleoli. At the top, numerous platelets of various sizes.

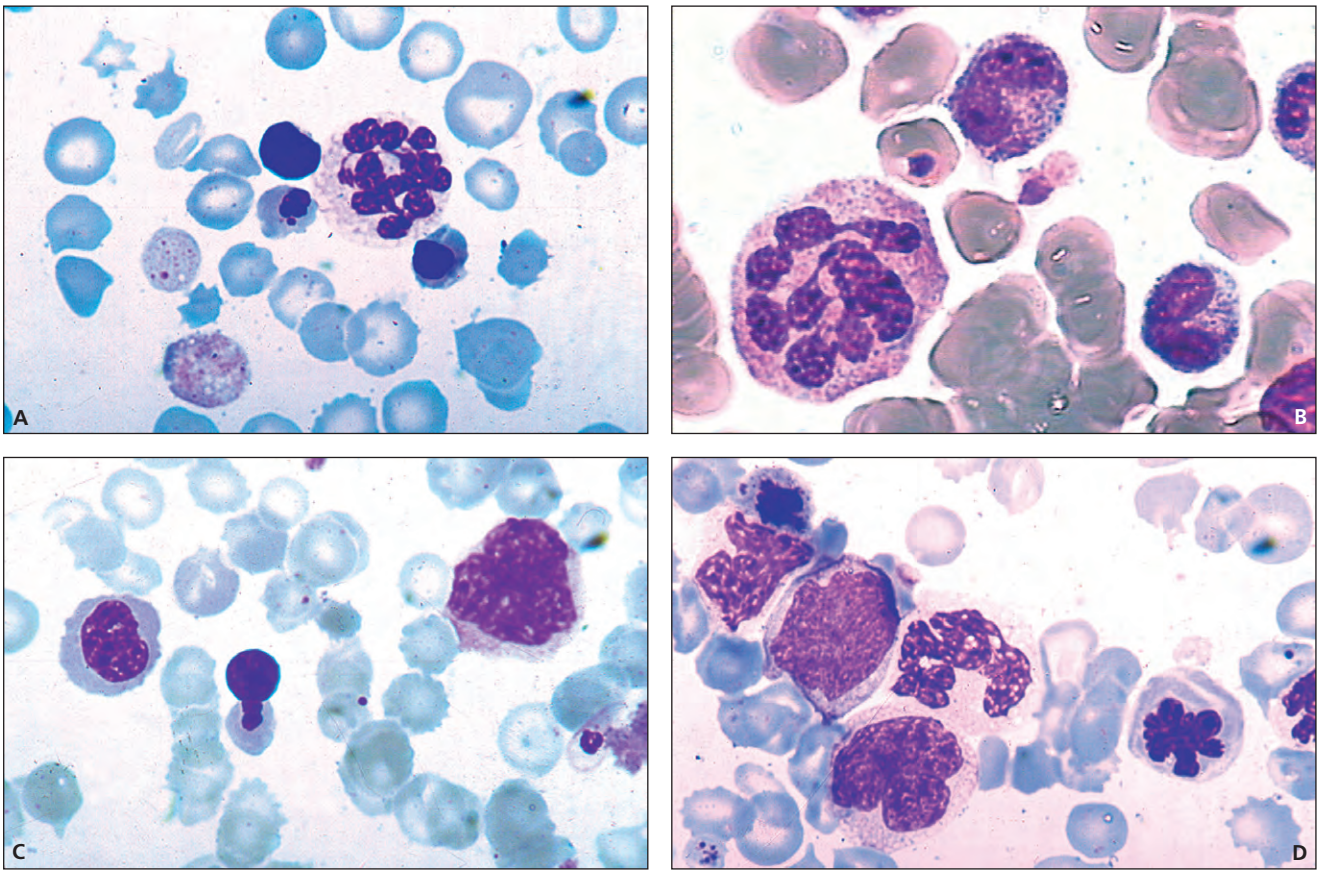



**Fig. 5.66** Anomalies of the granulocytic series in PMF; peripheral blood film. (A) Hypersegmented neutrophil with ten nuclear lobes and finely vacuolated cytoplasm. Surrounding it, a lymphocyte, an orthochromatic erythroblast with a fragmented nucleus and a polychromatic erythroblast. Marked anisopoikilocytosis: adjacent to a small hyperchromic dacrocyte is an erythrocyte with azurophilic granules in which one can just make out an incomplete Cabot ring. Lower down, a larger oval structure with azurophilic granules, numerous vacuoles and a half-moon of basophilic cytoplasm on the left margin: it may be a giant platelet or a degenerating macrocyte. (B) A gigantic macropolycyte with cytoplasm that is rich in granules and with residual basophilic areas, and two small metamyelocytes with an abnormal distribution of granules. (C) From left to right: a polychromatic erythroblast with an irregular nucleus and clumped chromatin; an orthochromatic erythroblast with a pyknotic chromatin mass that is partly extruded from the cell, but appears still to be anchored by a knobbly tail; an erythrocyte with a Howell-Jolly body adherent to the membrane; a hypogranular myelocyte with an extremely irregular nucleus; a thin elliptical filament corresponding to a Cabot ring from a haemolysed erythrocyte. (D) Other neutrophil granulocytes and their precursors with markedly dysplastic features (nuclear deformity and hypogranularity). The two erythroblasts are dyserythropoietic (karyorrhexis, nuclear lobulation and defective haemoglobinisation); on the right, an erythrocyte with a Howell-Jolly body and, immediately above, another with a granule of dust that is out of focus.

Acute panmyelosis with myelofibrosis is a rare condition, which is categorised in the AML subgroup, AML, not otherwise specified (AML, NOS), characterised by the rapid fibrous transformation of the bone marrow associated with pancytopenia, blastic proliferation, absence of splenomegaly and a rapidly fatal clinical course. In the peripheral blood in these cases one observes mild anisopoikilocytosis in the absence of dacrocytes (see Chapter 4).

## Chronic eosinophilic leukaemia, not otherwise specified

Chronic eosinophilic leukaemia, not otherwise specified (CEL, NOS) is defined in the 2008 WHO classification as a MPN characterised by an autonomous eosinophil expansion in the blood and marrow, in the absence of certain specified molecular abnormalities – *BCR-ABL1* fusion or rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*. The number of eosinophils in the peripheral blood must be at least $1.5 \times 10^9$/l and the percentage of blast cells in the peripheral blood and bone marrow must be less than 20%. If the clonality of the eosinophils is not demonstrable and blast cells are not increased, the diagnosis of idiopathic hypereosinophilic syndrome may be made. These conditions are dealt with in Chapter 6, together with other pathological processes involving eosinophils.

## Mastocytosis

This term designates a heterogeneous group of disorders resulting from clonal expansion of mast cells and their precursors.

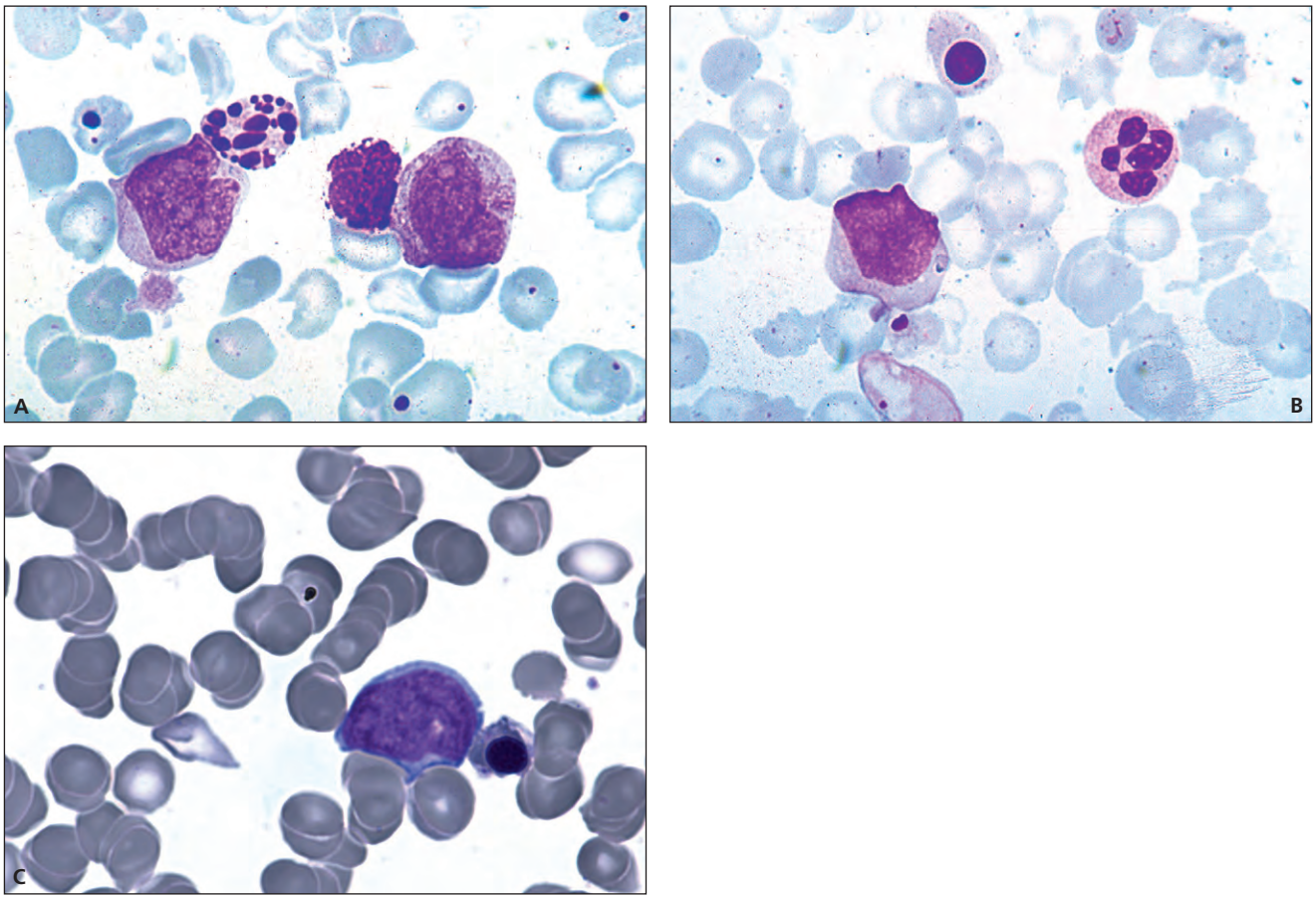



**Fig. 5.67** Blast cells and trilineage dysmorphism in PMF; peripheral blood film. The presence of immature cells in the peripheral blood in these patients does not have the same unfavourable prognostic significance as it has in the MDS, being a result of extramedullary erythropoiesis. (A) From left to right: a myeloblast with greyish, finely granulated cytoplasm and an indented nucleus with coarsely-woven chromatin and several nucleoli; a neutrophil with an apoptotic nucleus composed of numerous masses of pyknotic chromatin; a basophil granulocyte; a dysplastic promyelocyte, which can be distinguished from a myeloblast by the more numerous granules and the greater prominence of the nucleoli. Marked anisopoikilocytosis with frequent Howell-Jolly bodies. Beneath the myeloblast, a large star-shaped platelet. (B) From the bottom, clockwise: a poorly haemoglobinised macrocyte with a long Cabot ring and a single azurophilic granule; a blast cell with plentiful cytoplasm containing very fine granules, and an angulated nucleus with finely condensed chromatin and large indistinct nucleoli; a dyserythropoietic orthochromatic erythroblast and a neutrophil with five nuclear lobes. (C) An agranular blast cell with a notched nucleus with a prominent nucleolus. Beside it, an orthochromatic erythroblast with poorly haemoglobinised cytoplasm. The surrounding red cells are tending to form rouleaux. One of the red cells, a little above the centre, seems to contain a blackish body; this is not, however, a Howell-Jolly body, but rather is a stain deposit, as can be deduced from the pale refractile halo surrounding it.

Mast cells are normally localised mainly adjacent to epithelial membranes and serous cavities and around blood vessels, including bone marrow sinusoids. They are particularly frequent in areas of damaged bone marrow. They are medium to large mobile cells (diameter 20–30 μm), which are oval, trapezoidal or elongated in shape; they have prolonged villiform extensions that are difficult to visualise by light microscopy. The nucleus can be round to oval or bilobed; the chromatin structure differs from that of basophils, and other granulocytes, which have peripheral chromatin condensation, well visualised on electron microscopy. The cytoplasm is packed with dark purple-black granules, similar to those of basophils, but in general smaller; they are very numerous but do not usually obscure the nucleus. Mast cell granules have specific ultrastructural characteristics.

The granules of mast cells contain glycosaminoglycan sulphates, which stain with alcian blue and are responsible, as is the case for basophil granules, for the characteristic metachromasia with basic stains such as toluidine blue and azure blue. The granule contents are released from the mast cell by exocytosis following fusion of the granule membrane with the cytoplasmic membrane.

Mast cells and basophil granulocytes, although both originate from multipotent haemopoietic progenitors, follow different differentiation pathways.[85] Basophils are derived from a progenitor that is already committed to myeloid differentiation and which differentiates and matures in the bone marrow; they are released from the marrow into the peripheral blood from which they can migrate into tissues. There is so far no evidence that

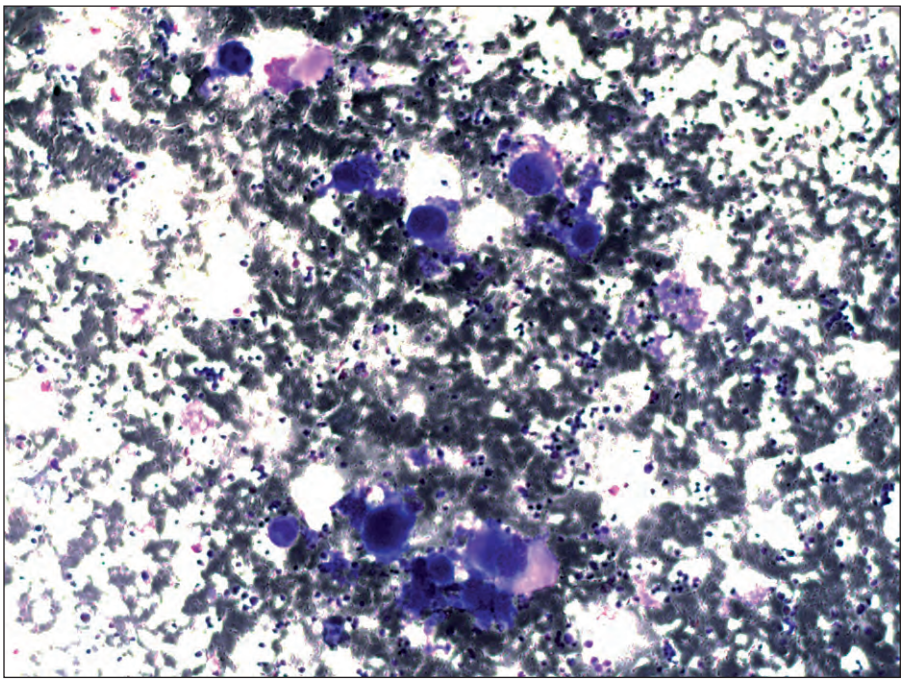


**Fig. 5.68** PMF at presentation; bone marrow aspirate at low power. In a marrow of reduced cellularity there are numerous megakaryocytes at various stages of maturation: some (at the bottom towards the right and at the top towards the left) resemble those of ET because of the plentiful cytoplasm and hyperlobated nucleus; many others are smaller and appear immature because of the intense basophilia of the cytoplasm, which obscures the nucleus.

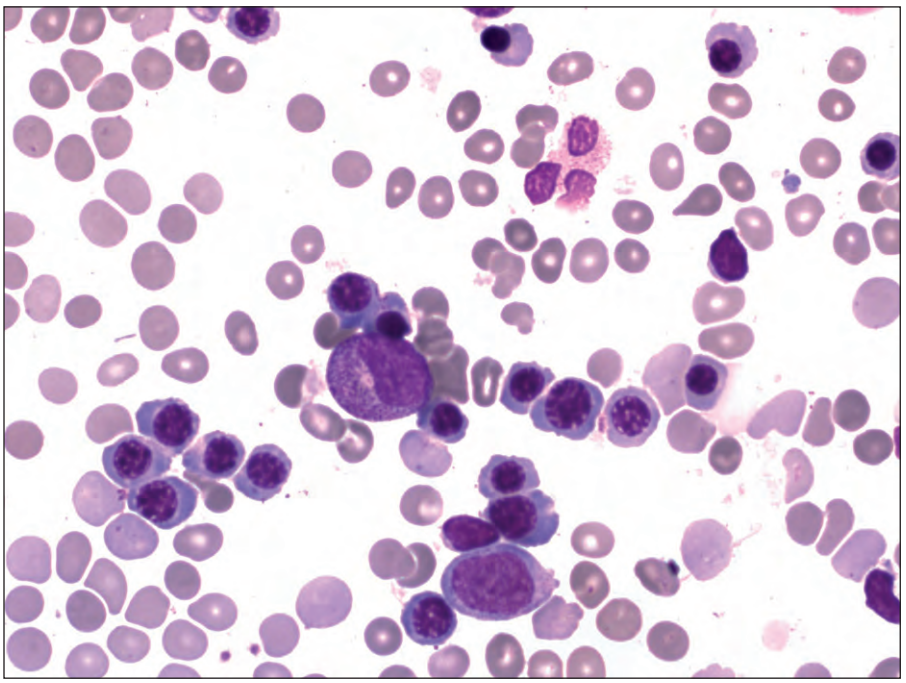


**Fig. 5.69** PMF; bone marrow aspirate. The film is hypocellular. There are numerous polychromatic erythroblasts with a clear delay in nuclear maturation, a promyelocyte in the centre and, lower down, a small lymphocyte and a blast with rare fine granules in its basophilic cytoplasm. On the right, higher up, a dacrocyte and a lymphocyte. Red cell morphology, not easily evaluable on this aspirate, does however show anisocytosis and anisochromasia, with polychromasia and the presence of hyperchromic spherocytes; it should, however, be noted that the apparent spherocytes may be artefactual, resulting from the thinness of the film.

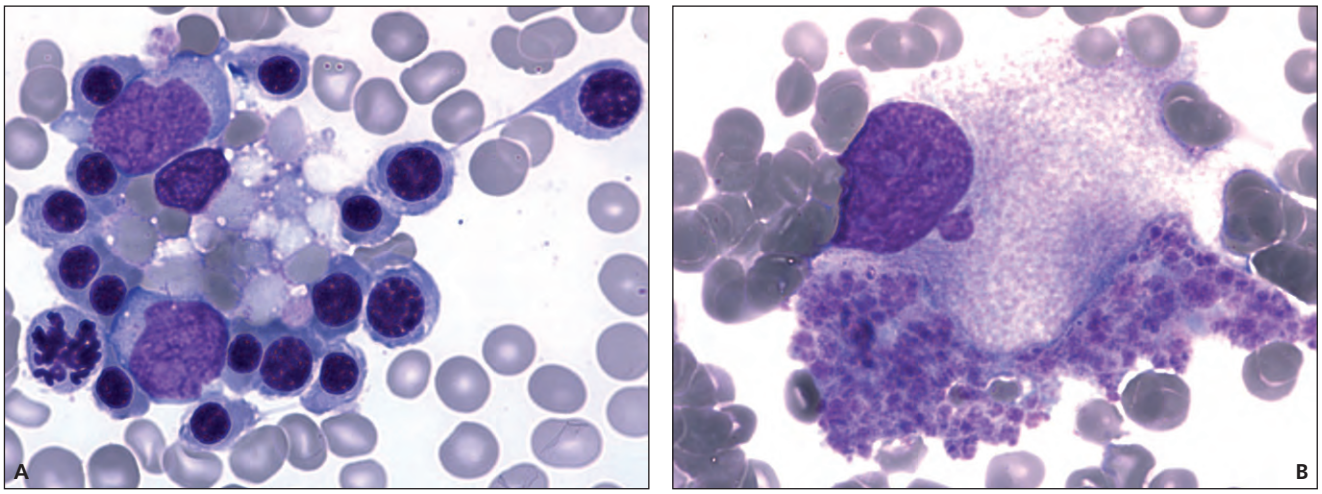



**Fig. 5.70** PMF; cytological features in hypocellular bone marrow aspirates. (A) An erythroid island that is atypical because of the presence of two blast cells that do not appear to be erythroid; even though the cytoplasm is intensely basophilic they are not proerythroblasts, as is indicated by the nuclear morphology: an irregular outline with notches, very large prominent nucleoli and eccentricity of the nucleus which appears adherent to the cytoplasmic membrane. The macrophage, in which the nucleus is visible in contact with the lower border of the upper blast cell, has its cytoplasm distended by red cells that are partly haemolysed and partly well preserved (massive erythrophagocytosis). The polychromatic and orthochromatic erythroblasts have clear features of dyserythropoiesis: asynchronous maturation with delayed nuclear development, intercellular bridges, poorly haemoglobinised cytoplasm and atypical mitotic figures. (B) A non-lobed megakaryocyte, although with a small round nuclear projection. The chromatin is condensed into small clumps and there are pale nucleoli with indistinct margins. The plentiful cytoplasm is clearly divided into two sections delimited by a blue margin: a weakly basophilic network occupies the paranuclear area, while the periphery is completely differentiated to form an extensive carpet of mature platelets, with large intensely violet chromomeres.

basophils have mitotic capacity. Mast cells, on the other hand, are derived from a multipotent stem cell, able to give rise to the common myeloid or granulocyte-macrophage progenitors.[86] These are released early from the bone marrow and reach vascular areas of peripheral tissues – connective tissue and serous cavities: here they mature, conserving, however, a limited or latent proliferative capacity. In the resting state they spontaneously secrete, as an inactive proenzyme, a neutral protease, protryptase, in two forms designated alpha and beta, the serum level of which correlates with the mass of mast cells present in the individual.[87] A serum tryptase concentration above 20 ng/ml is a criterion in the diagnostic algorithm for mastocytosis.

With regard to immunophenotype, normal mast cells express CD9, CD33, CD45, CD68 and CD117; they can also be recognised with antitryptase antibodies. Neoplastic mast cells have a similar profile, but can also express CD2, CD25 or both.

Given the origin of the mast cell from a multipotent myeloid stem cell, the mastocytoses were included in the group of MPN in the 2008 WHO classification. Among the mastocytic disorders, three main groups are recognised:

1. The cutaneous form, which occurs mainly in infancy with the clinical picture of urticaria pigmentosa, which has a clinical course that tends to be benign; a more aggressive form is associated with vesicle formation (bullous mastocytosis).
2. Systemic mastocytosis (SM), which generally affects adults and which in turn can be subdivided into the following forms:
   - Indolent SM, with localisation in the skin, the bone marrow or both;
   - SM associated with clonal non-mast cell haematological neoplasia;
   - Aggressive SM, in general lacking cutaneous lesions;
   - Mast cell leukaemia, a rare and aggressive neoplasm.
3. Two forms that arise in non-cutaneous tissues (most often the lymph nodes, spleen, liver or gastrointestinal tract), with a malignant form, mast cell sarcoma, and a benign form, designated extracutaneous mastocytosis.

Table 5.9 shows the 2008 WHO diagnostic criteria for mastocytosis.[88] Mastocytosis is associated with numerous systemic symptoms attributable to the release of chemical mediators: fever, profuse sweating, flushing, abdominal or bone pain, headache, syncope, hypotension, tachycardia and tachypnoea, arthralgia and myalgia.

The clinical variability of mastocytosis is associated, as one would expect, with a marked variability in laboratory features.[89] The peripheral blood may show unilineage or multilineage cytopenia and eosinophilia is common. Sometimes there are dysplastic features. It is very rare to see mast cells in the circulation and their presence raises the suspicion of mast cell leukaemia. However, in the leukaemic variants, mast cells of variable mor-

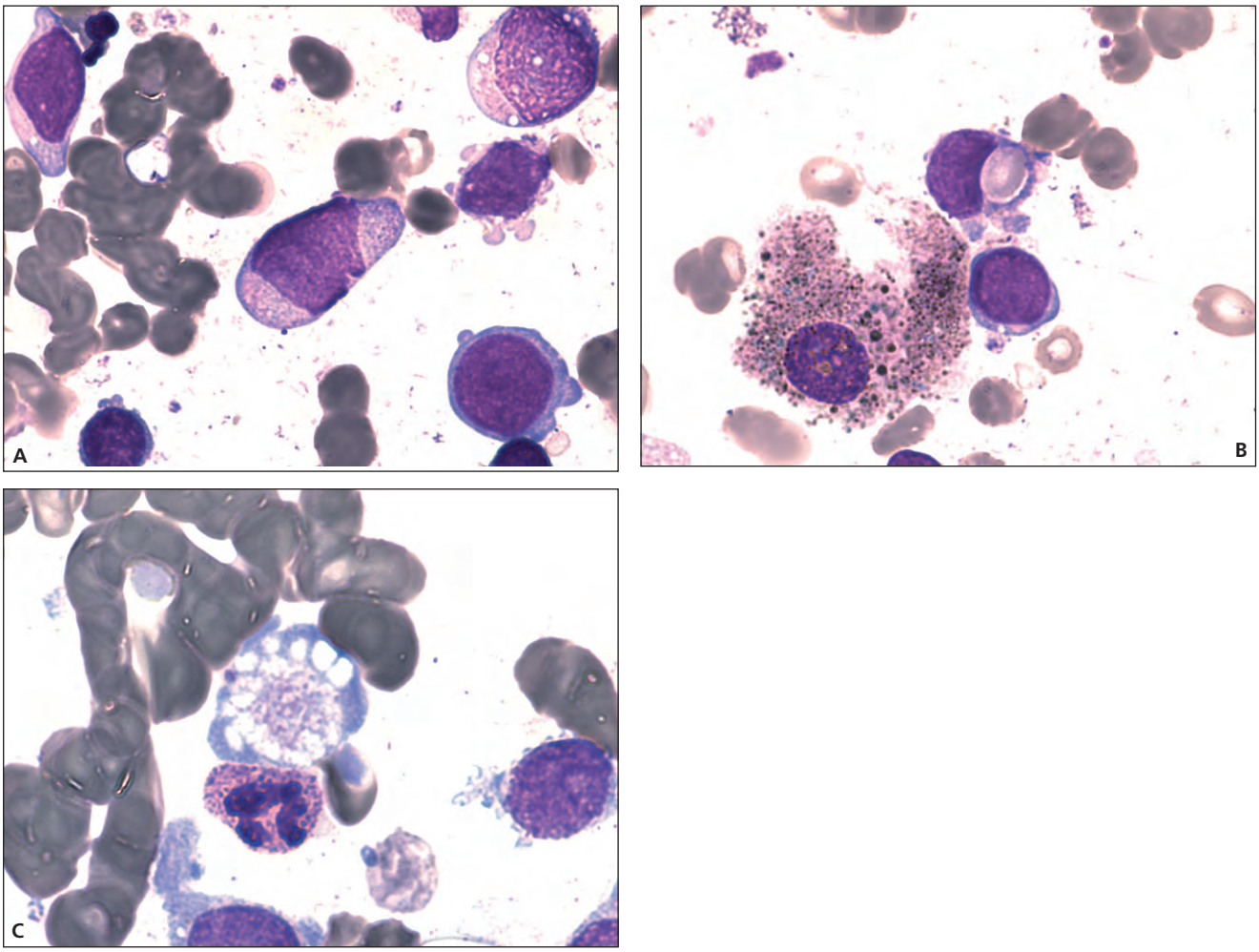



**Fig. 5.71** PMF; blast cells and dysmorphism in hypocellular bone marrow aspirates. (A) In the centre, a myeloid blast cell with fine cytoplasmic granules, an angulated and irregularly notched nucleus with thickly woven heterogeneous chromatin, with an indistinct nucleolus in contact with the straight left edge of the nucleus. On its right, a small cell with a hyperchromatic nucleus with dense chromatin and scanty cytoplasm which is extending into numerous swollen projections: this is certainly a micromegakaryocyte. Bottom right, a proerythroblast with a perfectly round nucleus with granular megaloblastoid chromatin and blebs at the periphery of the basophilic cytoplasm. (B) A small agranular blast cell, a mononuclear cell with more clumped chromatin that has phagocytosed an erythrocyte, and a macrophage with plentiful pink cytoplasm filled with dark grey granules of varying sizes; between these granules are small blue deposits similar to those seen in sea-blue histiocytes. (C) On the right, a small blast with scanty ragged cytoplasm. In the centre, a neutrophil and a giant vacuolated platelet.

phology are present in the peripheral blood in significant numbers, not less than 10%: they range from typical forms with a round or lobated nucleus and typical granules to atypical forms with few granules and features evocative of the monocyte lineage or of villous lymphocytes. Major alterations in the peripheral blood are present in mastocytosis associated with clonal non-mast cell haematological neoplasia, reflecting the nature of the latter: among the many possible forms, the associated neoplasm most often seen is chronic myelomonocytic leukaemia.

The bone marrow in SM may appear normal or show varying degrees of hyperplasia involving the neutrophil and eosinophil lineages, and also the megakaryocytes, but in the absence of a corresponding increase in mature cells in the peripheral blood. There can also be dysplastic features in various lineages. The mast cells can be morphologically normal, or can have a bilobed nucleus, a characteristic spindle shape with a long cytoplasmic tail, or a cigar-shaped nucleus with hypogranular cytoplasm (Figs 5.74 through to 5.79). The chromatin structure can be very immature, that of a blast cell, despite the cytoplasm showing typical granulation. If a bone marrow aspirate shows increased mast cells, it is always important to proceed to a trephine biopsy for a more accurate quantification of their numbers. Except in cases with an increased number of mast cells that are immature, atypical or both, morphological evaluation is not conclusive: the

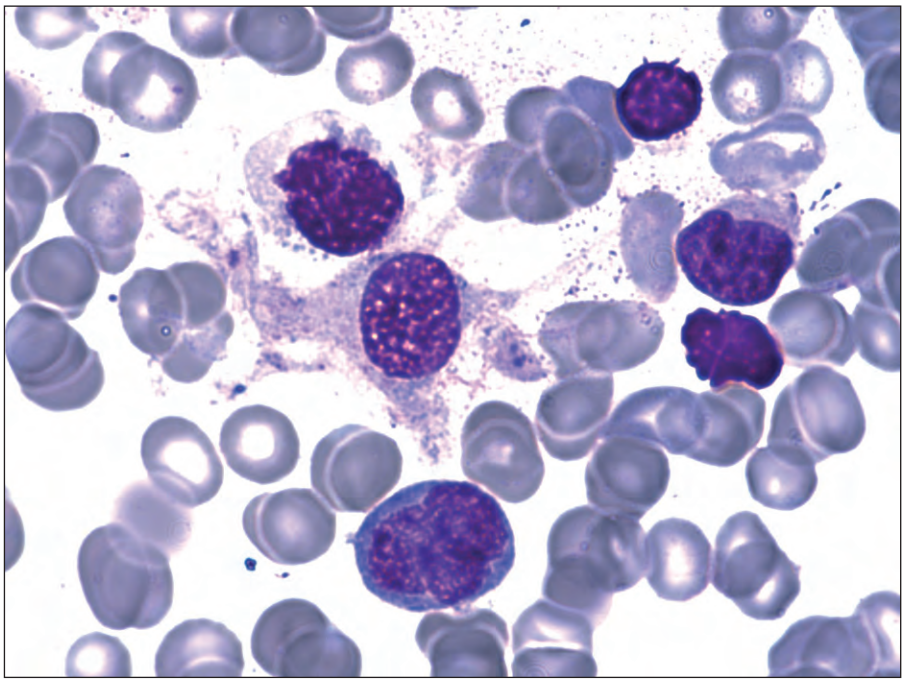


**Fig. 5.72** PMF; bone marrow aspirate. At the top, two damaged nuclei of unrecognisable cells. In the centre, a reticular adventitial cell: the nucleus, oval like those of macrophages, has spongy chromatin with visible pale fissures; the basophilic and heterogeneous cytoplasm is spreading out into tentacle-like projections. On the right, a micromegakaryocyte with a bean-shaped nucleus and scanty basophilic cytoplasm, similar to that in Fig. 5.65D. At the bottom, an unrecognisable cell with a trilobed nucleus and clumped chromatin.

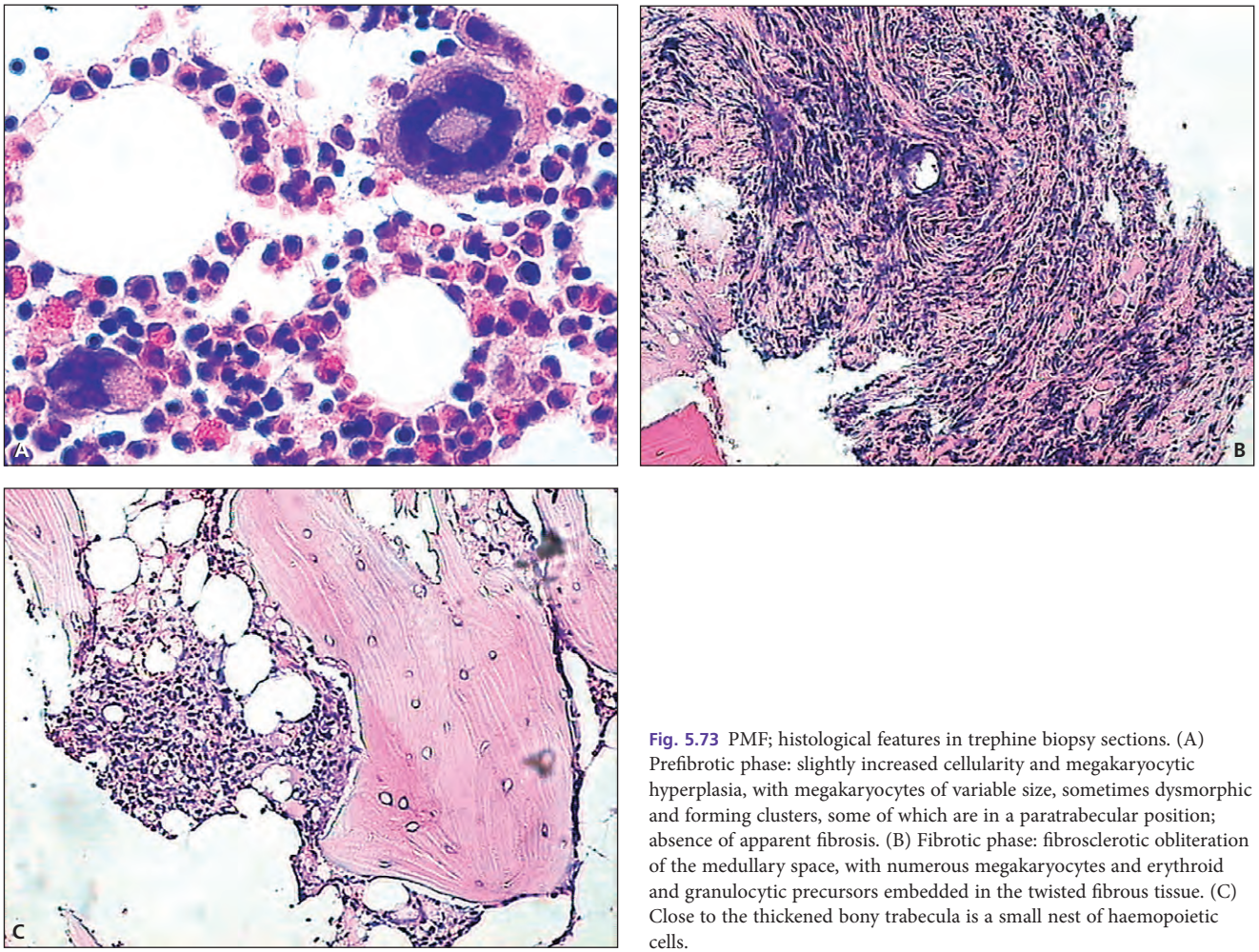



**Fig. 5.73** PMF; histological features in trephine biopsy sections. (A) Prefibrotic phase: slightly increased cellularity and megakaryocytic hyperplasia, with megakaryocytes of variable size, sometimes dysmorphic and forming clusters, some of which are in a paratrabecular position; absence of apparent fibrosis. (B) Fibrotic phase: fibrosclerotic obliteration of the medullary space, with numerous megakaryocytes and erythroid and granulocytic precursors embedded in the twisted fibrous tissue. (C) Close to the thickened bony trabecula is a small nest of haemopoietic cells.

**Table 5.9** Systemic mastocytosis: diagnostic criteria according to the 2008 WHO classification* and classification of the variant forms (adapted from Swerdlow 2008,[13] with permission of IARC)

**Diagnostic criteria**

*Major criteria*

Presence of multifocal mast cell aggregates (>15 cells per aggregate) in the bone marrow, other extracutaneous organs or both

*Minor criteria*

1. Infiltration of the bone marrow or other extracutaneous organs by >25% of atypical or immature mast cells
2. Mutation in codon 816 of the *KIT* gene detected in the bone marrow, peripheral blood or other extracutaneous organ
3. Mast cell expression of CD2, CD25 or both associated with normal lineage-related antigens (CD117)
4. Serum tryptase >20 ng/ml, in the absence of other clonal myeloid neoplasm

*Diagnosis requires meeting the major criterion and one minor criterion or three minor criteria

**Variant forms**

- Indolent systemic mastocytosis: the criteria for systemic mastocytosis are met and there are associated cutaneous lesions in the absence of bone marrow insufficiency, associated haematological neoplasm, hepatosplenomegaly, bone lesions or malabsorption related to mast cell infiltration. Variant forms are bone marrow mastocytosis, in which there are no cutaneous lesions, and smouldering systemic mastocytosis, in which at least two of the following criteria are met:
  - Bone marrow mast cell infiltration >30% and/or serum tryptase >200 ng/ml
  - Dysplasia or proliferation of mast cells associated with a normal peripheral blood count
  - Hepatomegaly without hepatic insufficiency, splenomegaly without hypersplenism or lymphadenopathy
- Aggressive systemic mastocytosis: the criteria for systemic mastocytosis are met in the absence of cutaneous localisation and in the presence of bone marrow insufficiency, associated haematological neoplasm, hepatosplenomegaly, bone marrow lesions or malabsorption due to mast cell infiltration. A variant form is lymphadenopathic mastocytosis with eosinophilia
- Systemic mastocytosis with associated clonal non-mast cell haematological neoplasm: the criteria for systemic mastocytosis are met and also those of another haematological neoplasm (MDS, MPN, MDS/MPN, AML or lymphoma)
- Mast cell leukaemia: the criteria for systemic mastocytosis are met in the presence of diffuse infiltration of the bone marrow by immature atypical mast cells and the presence in the peripheral blood of at least 10% of mast cells

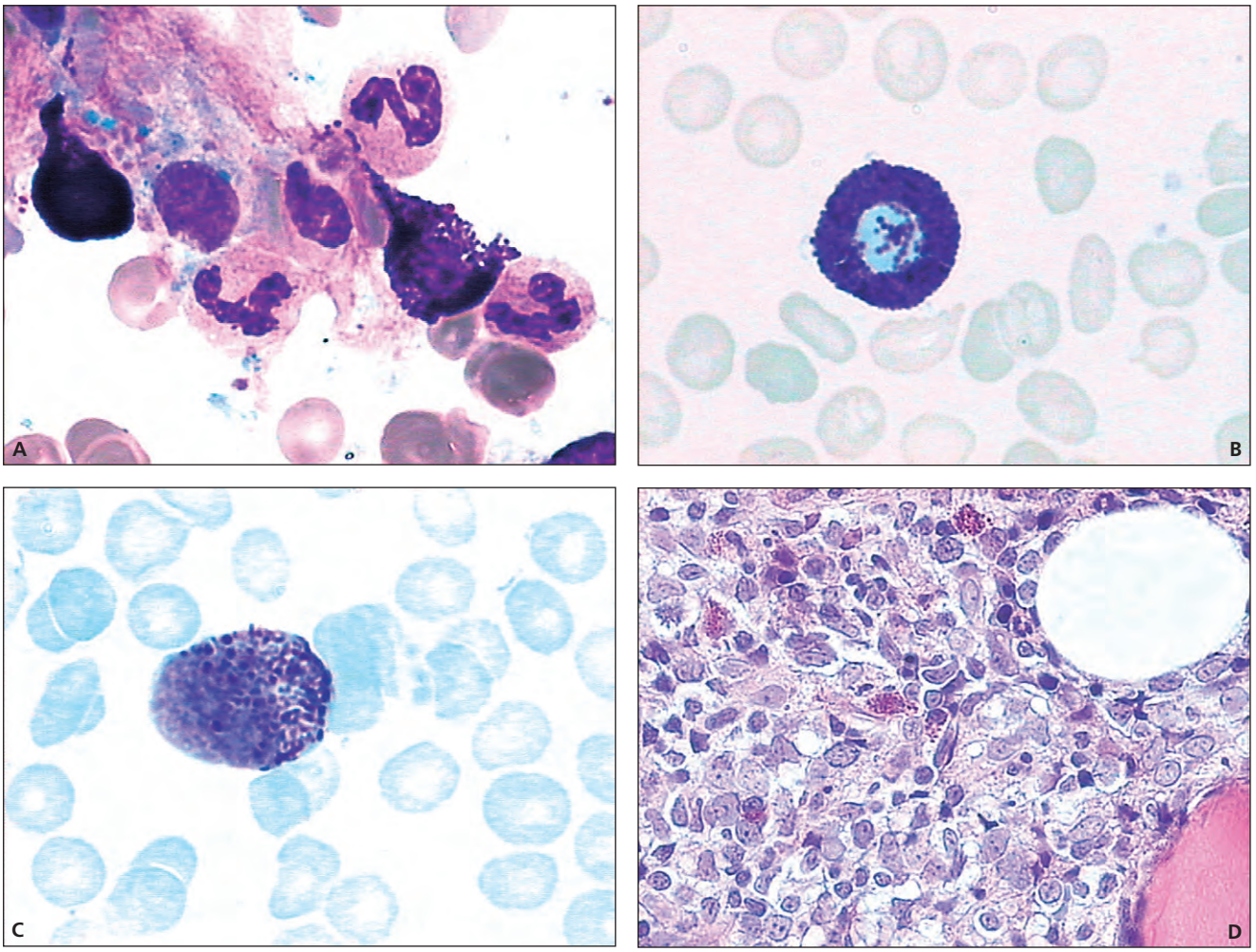



**Fig. 5.74** Systemic mastocytosis associated with T-cell non-Hodgkin lymphoma. (A) In the hypocellular material obtained by bone marrow aspiration there are numerous, morphologically normal mast cells. The mast cell on the right shows the typical visibility of the nucleus, which is not covered by the thickly packed cytoplasmic granules. (B) This latter feature is even more evident in this cell, in which the granules have stained, metachromatically, dark purple with toluidine blue. (C) In some mast cell precursors at an intermediate stage of maturation, the toluidine blue-positive granules are less numerous and the nucleus shows little chromatin condensation. (D) In this histological section, the mast cells appear as hypogranular reddish cells, scattered through the population of lymphoma cells, which include a high proportion of blast cells.

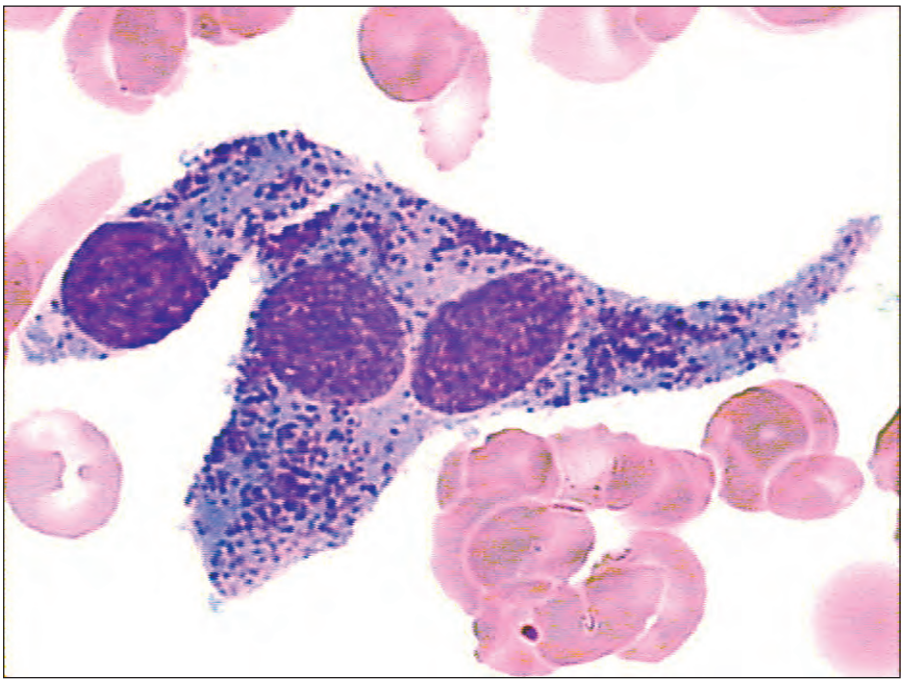


**Fig. 5.75** Systemic mastocytosis; bone marrow aspirate. Two atypical mast cells with granules spread through the plentiful basophilic cytoplasm. The cell on the right is binucleated and it appears to have cytoplasm that is double normal size, as in a syncytium: the two nuclei have less condensed chromatin.

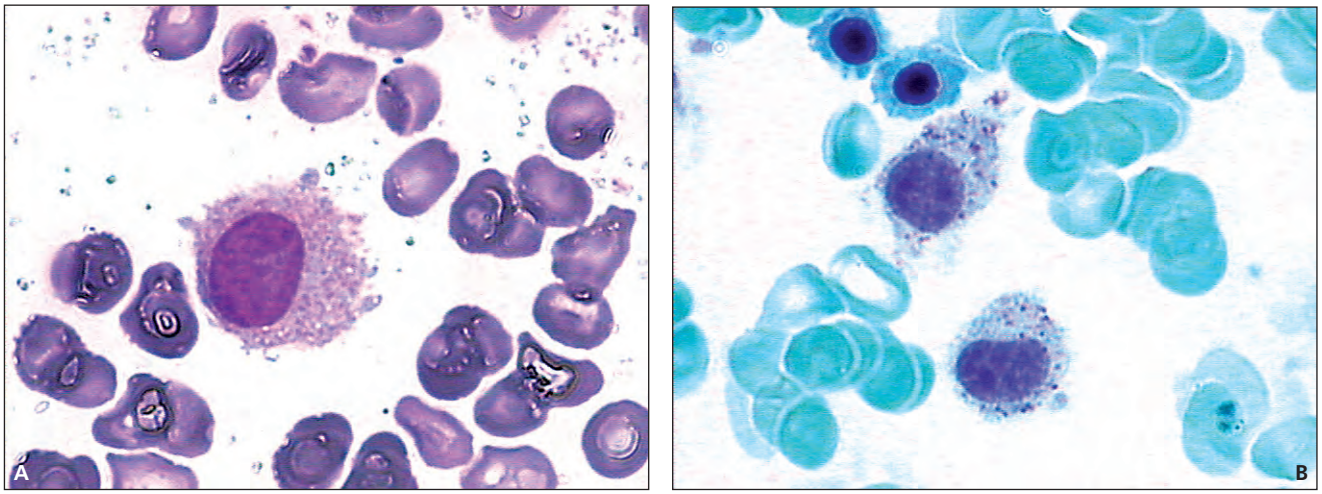



**Fig. 5.76** Systemic mastocytosis; bone marrow aspirate. (A) Immature mast cell with an ovoid nucleus, homogeneous chromatin and plentiful fringed cytoplasm containing fine, dispersed granules on a basophilic background. (B) A toluidine blue stain demonstrates the mast cell nature of these cells, in which the granules show the characteristic metachromasia.

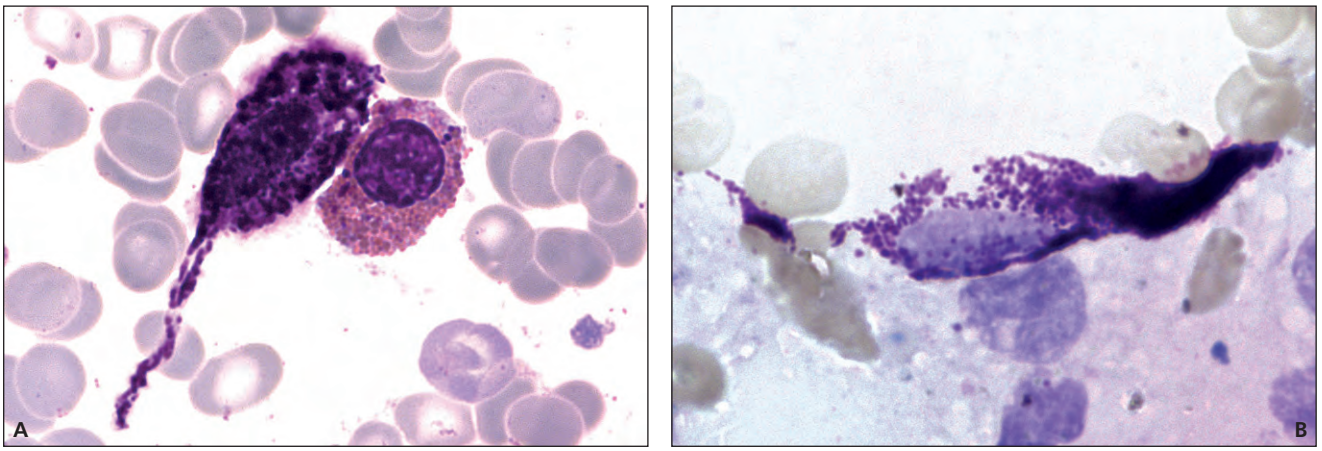



**Fig. 5.77** Systemic mastocytosis; bone marrow aspirate. (A) Beside an eosinophil myelocyte with a small nucleus and some dark proeosinophilic granules is a mast cell with fusiform cytoplasm that is elongated into a long thin tail, laden with typical granules that let the nucleus remain evident. (B) Another fusiform mast cell showing metachromatic staining with toluidine blue.

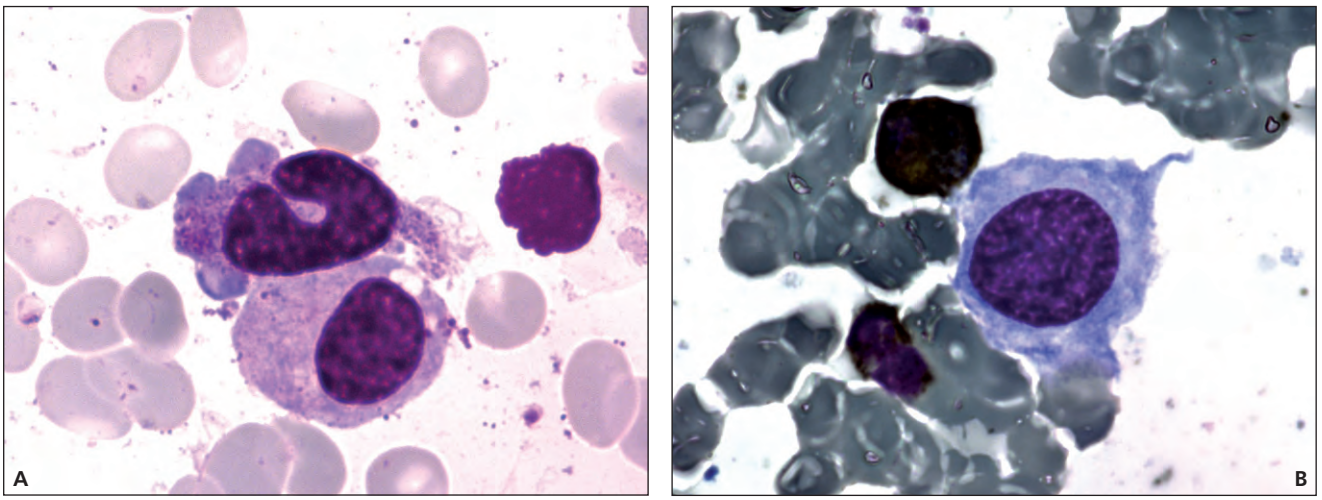



**Fig. 5.78** Systemic mastocytosis; bone marrow aspirate. (A) Immature and dysmorphic mast cells with hypogranular cytoplasm. The nuclear chromatin is condensed into hyperchromic masses, separated by paler areas. (B) A mast cell of blastic type (a 'mast blast'): the plentiful, basophilic, agranular cytoplasm has some villous projections. The nucleus is round with a clearly outlined nuclear membrane and heterogeneous chromatin in which the paler areas predominate over the dark foci of condensation.

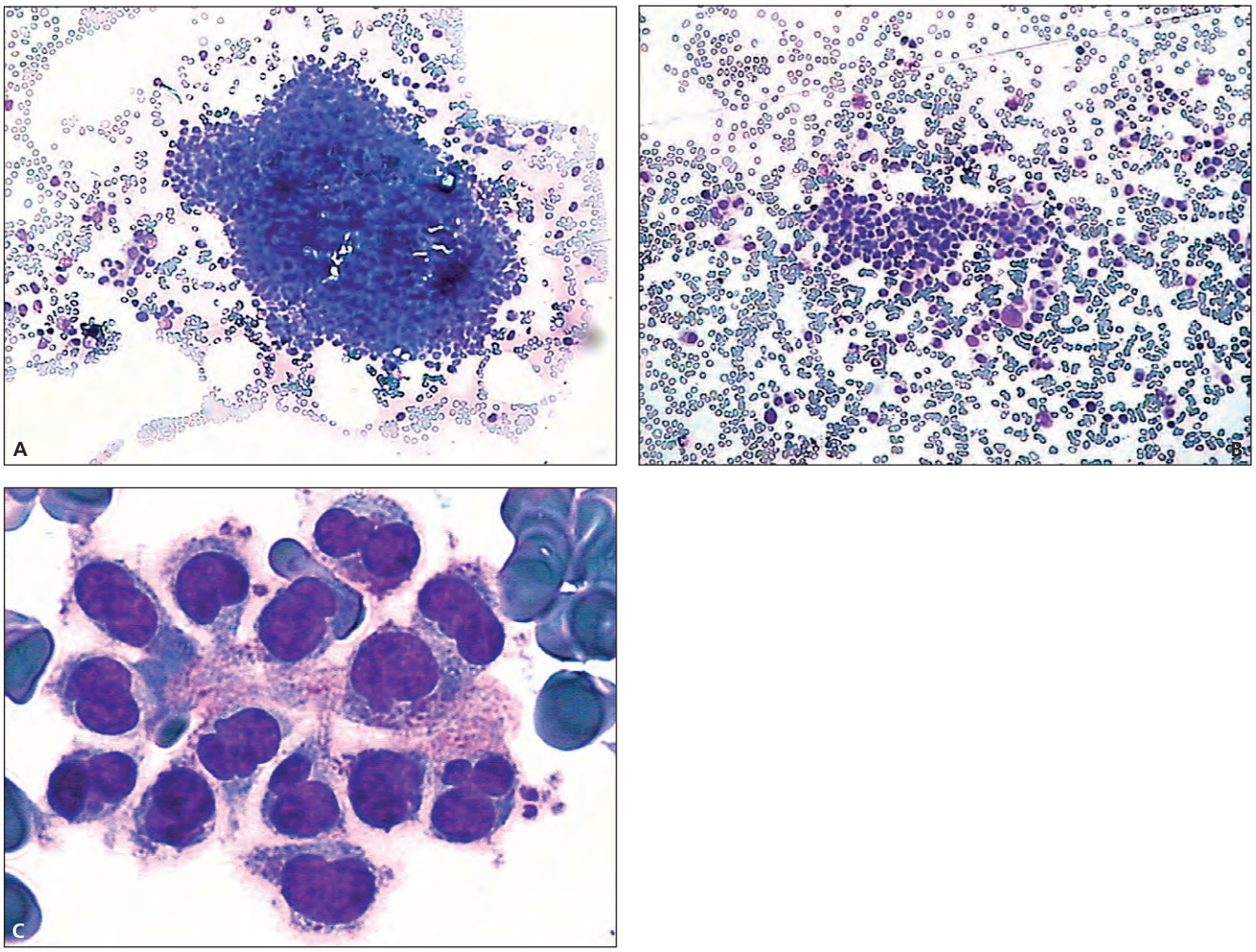



**Fig. 5.79** Systemic mastocytosis; bone marrow aspirate. (A) In this patient the massive bone marrow infiltration by mast cells was in the form of monomorphic nodules of variable size. The cells outside these nodules are also largely atypical mast cells. (B) Another small mass of hypogranular mast cells, with ragged cytoplasm and nuclei that are often reniform. (C) At higher power, the characteristic morphology of these atypical mast cells is more clearly evident. The nuclei are reniform or lobulated and their chromatin appears condensed in a fairly compact manner. The cytoplasm is quite plentiful and intensely basophilic, sometimes showing some peripheral granules. A consistent feature is the raggedness of the cytoplasmic margin.

differential diagnosis is reactive mastocytosis or mast cell hyperplasia, which can be associated with haematological disorders but also with non-haematological pathological processes. It is also necessary to exclude an underlying lymphoproliferative neoplasm, particularly hairy cell leukaemia, which can have cytomorphological analogies with mastocytosis (Figs 5.76 and 5.80): in addition, these two conditions may co-exist.

From the strictly morphological point of view, the differential diagnosis must also include:

- Haematological neoplasms with eosinophilia;
- The very rare chronic basophilic leukaemia, in which the basophils generally have segmented nuclei, a low expression of CD38, negativity for CD117 and rearrangement of *ETV6*[90];
- The rare eosinophilic or basophilic progression/crisis of MDS.[91]

In SM there is often a point mutation in the *KIT* gene, which encodes the receptor for the tyrosine kinase growth factor, stem cell factor (SCF). In addition, there can be cytogenetic aberrations among which are trisomy 8 or 9, monosomy 7 and 20q-. *JAK2* mutation is occasionally observed. The recognition of genetic alterations such as *BCR-ABL1* or *FIP1L1-PDGFRA* indicates an alternative diagnosis.

An atypical mast cell proliferation, designated myelomastocytic leukaemia or myelodysplastic syndrome with mastocytosis, is characterised by mast cells that are positive for CD117 and tryptase, but do not form multifocal infiltrates in the bone marrow, are CD2 and CD25 negative, and do not show the typical *KIT* mutation.[92] Finally, a condition analogous to monoclonal gammopathy of undetermined significance (MGUS), designated monoclonal mast cell activation, is characterised by

infiltration by clonal KIT-positive mast cells with an associated increased risk of anaphylaxis; the mast cells do not show any phenotypic markers of mastocytosis nor are other diagnostic criteria of mastocytosis met.[93]

### Myeloproliferative neoplasm, not classifiable

The 2008 WHO classification retains a category for cases that present morphological and laboratory features that are compatible with an MPN without meeting the criteria for a conclusive diagnosis of one of the more specific categories of MPN. Such patients should be observed every four to six months with a detailed description being made of the diagnostic features that are lacking, with the aim of promptly recognising any progression that occurs. In the majority of cases, one is dealing with a very early stage of one of the MPN, a prodromal phase of a disease that will subsequently become overt.

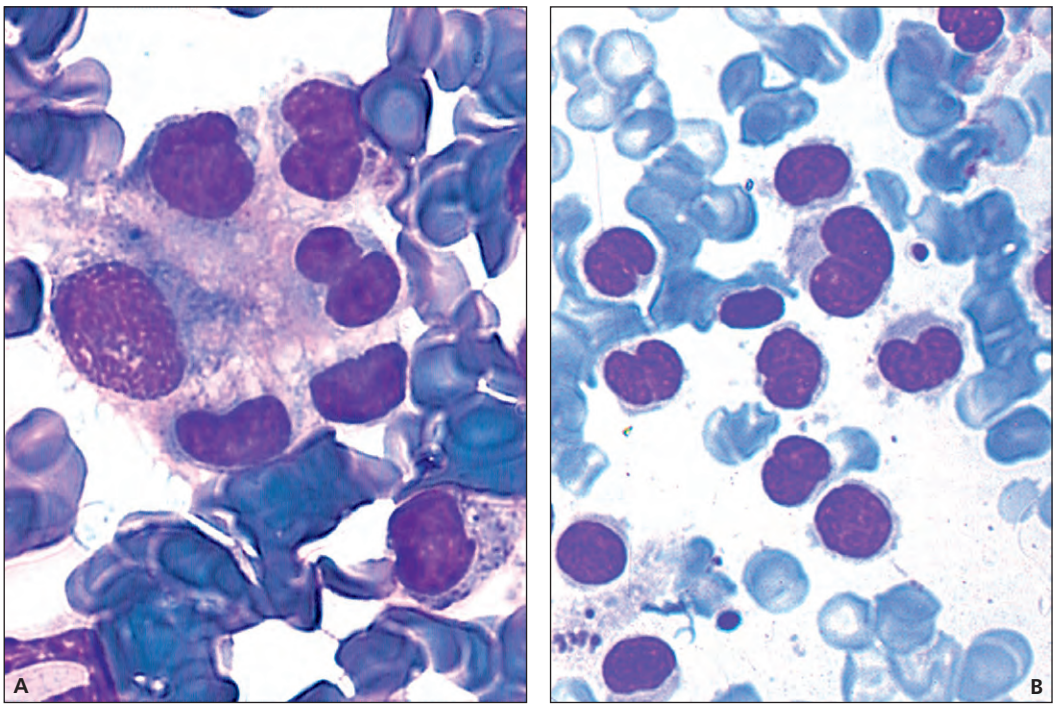
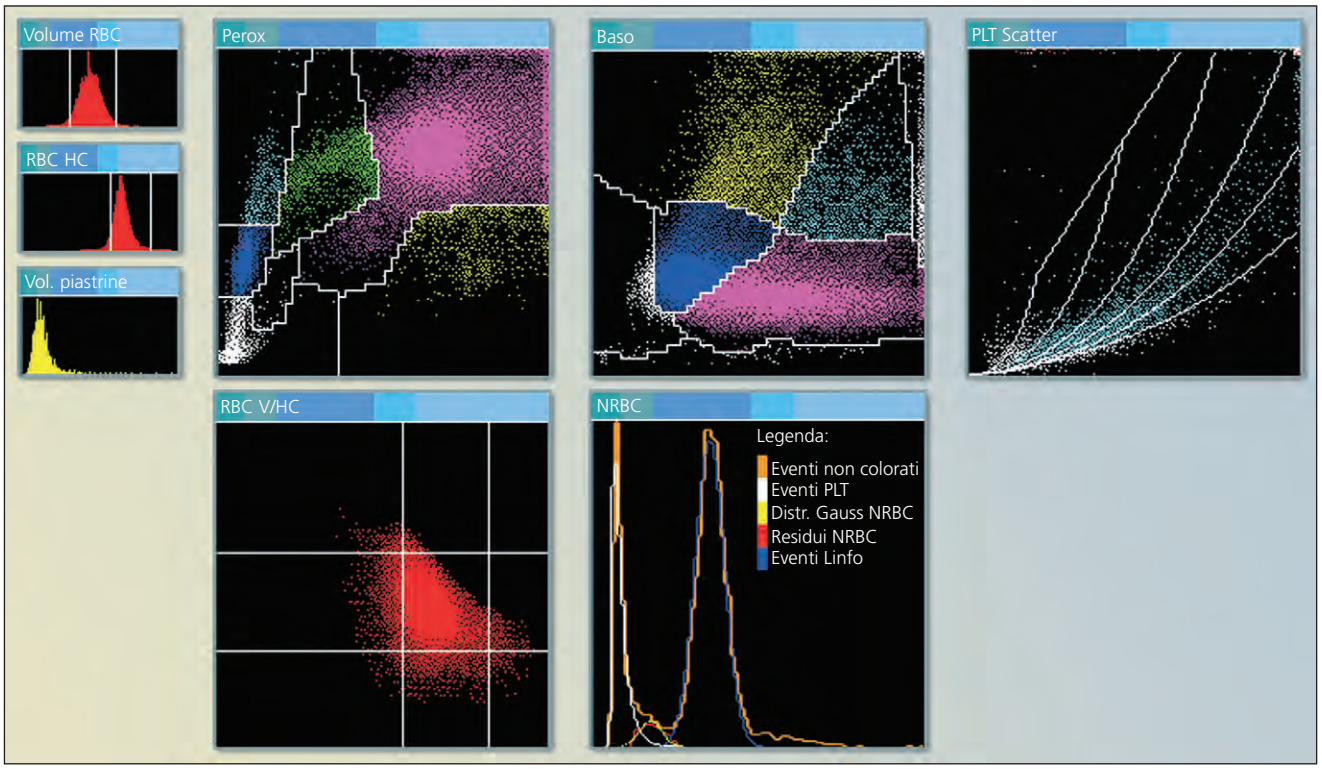



**Fig. 5.80** Cytomorphological differential diagnosis between systemic mastocytosis and hairy cell leukaemia; bone marrow aspirates. (A) Systemic mastocytosis; same preparation as Fig. 5.79. The atypical mast cells have morphology that is practically indistinguishable from that of hairy cell leukaemia. The cell at the top and another lower down have in their cytoplasm a number of granules that point towards the correct diagnosis, although this requires confirmation by immunophenotyping. (B) Hairy cell leukaemia. Hairy cells also have ragged cytoplasm and nuclei with chromatin that is fairly dense and homogeneous; often the nuclei are reniform or bean shaped. Mast cells, conversely, do not in general have longitudinal fissures that create a 'coffee-bean' appearance. The colour of the cytoplasm, varying from sky-blue to grey, can however be very similar.

## REPORT 5.1 Chronic myeloid leukaemia, *BCR-ABL1*-positive, at presentation



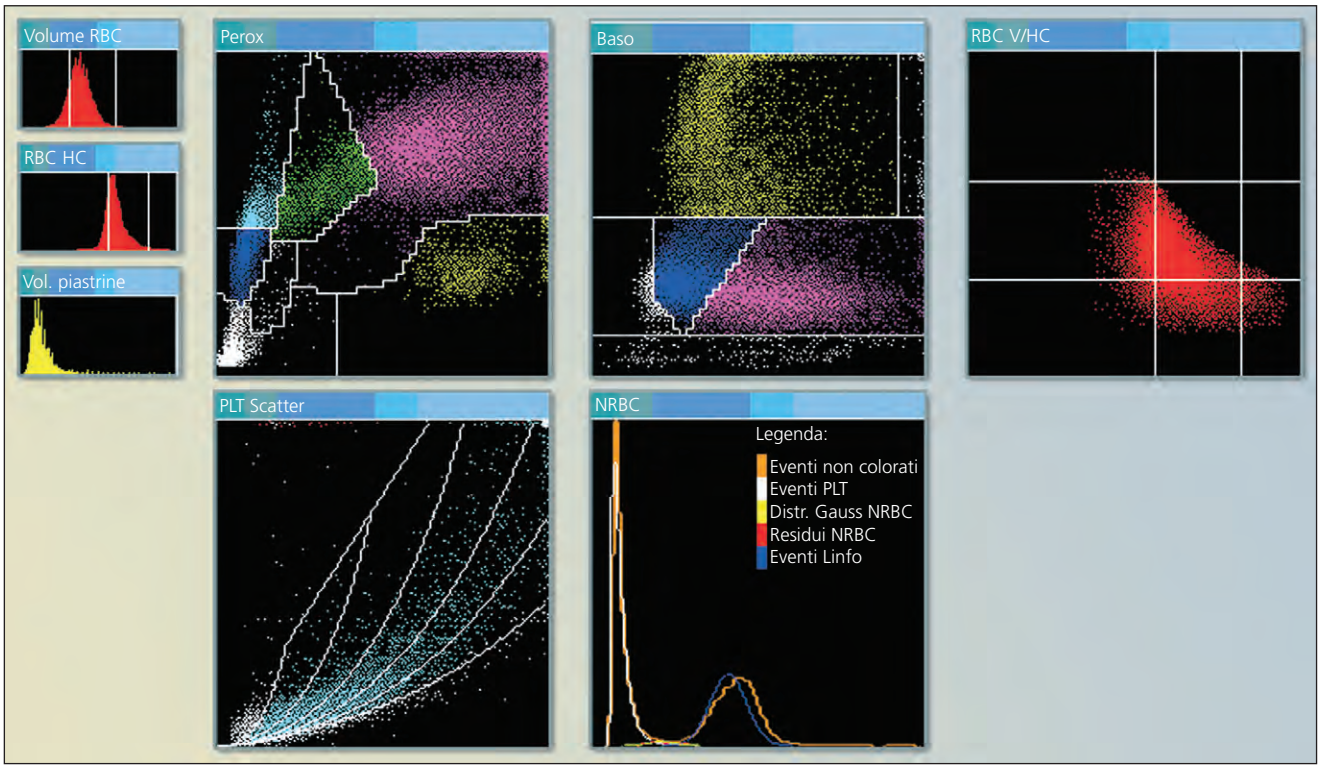

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 128 | g/l | |
| RBC | 4.42 | $\times 10^{12}$/l | |
| HCT | 0.385 | l/l | |
| MCV | 87.2 | fl | |
| MCH | 28.9 | pg | |
| MCHC | 331 | g/l | |
| CHCM | 320 | g/l | |
| RDW | 16.2 | % | |
| HDW | 2.81 | g/dl | |
| HYPO | 5.1 | % | Hypochromia + |
| HYPER | 0.7 | % | |
| MICRO | 2.6 | % | Microcytosis + |
| MACRO | 0.9 | % | |
| RBC fragments | 0.03 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 1.3 | % | |
| RET | 61.9 | $\times 10^{9}$/l | |
| CHr | 29.4 | pg | |
| IRF (M+H) | 6.3 | % | |
| PLT | 279 | $\times 10^{9}$/l | |
| MPV | 7.9 | fl | |
| PDW | 60.0 | % | |
| Large platelets | 6 | $\times 10^{9}$/l | |
| MPC | 28.0 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 88.25 | | * |
| WBC Perox | 81.80 | | * |
| NEUT | 75.81 | 85.4 | |
| LYMPH | 8.55 | 9.6 | |
| MONO | 2.36 | 2.7 | |
| EOS | 1.35 | 1.5 | |
| BASO | 3.89 | 4.4 | B-SUSP |
| LUC | 0.71 | 0.8 | |
| NRBC | 0.55 | 0.6 | NRBC + |
| LI | 1.93 | | LSH +, B-NV |
| MPXI | 9.0 | | |
| MN | | 44.9 | IG +++ |
| PMN | | 47.7 | |
| BLASTS | | 0.7 | |
| Perox HPX | | 15.13 | |
| Perox SAT | | 10.4 | |

Leucocytosis as a result of the presence of peroxidase-rich cells which, in the Perox cytogram, fall into the neutrophil box forming a triangle which extends along the right-hand border in saturation, causing an increased MPXI and HPX (events with absorption ≥4.1 times the average of the neutrophil cluster on the X axis) and with a SAT flag (indicating events that fall into the area of saturation in channels 97–99 on the X axis of the Perox cytogram). LUC are almost absent. The Baso cytogram does not show a MN/PMN valley and there is an IG flag caused by the invasion of the MN area by numerous nuclei of cells that in the Perox channel are peroxidase positive, giving a discrepancy between the two leucocyte channels; the number of cells classified as blasts is not significant and the related blast flag has not been triggered. The increase of basophils is due in part to pseudobasophilia, secondary to the leucocytosis, and in part to the presence of a true basophilia, which can be recognised from the separate cluster in the highest area of the Baso cytogram. The PANDA classification is P4, D0. There is neither anaemia nor thrombocytopenia and the red cell indices are normal.

**Morphology.** Differential white cell count: neutrophils 39%, neutrophil myelocytes and metamyelocytes 38%, promyelocytes 8%, eosinophils 1%, basophils 3%, lymphocytes 11%. The appearance of the cytogram is very suggestive of the diagnosis of chronic myeloid leukaemia in chronic phase, confirmed by cytogenetic and molecular analysis.

## REPORT 5.2 Chronic myeloid leukaemia, *BCR-ABL1*-positive, accelerated phase



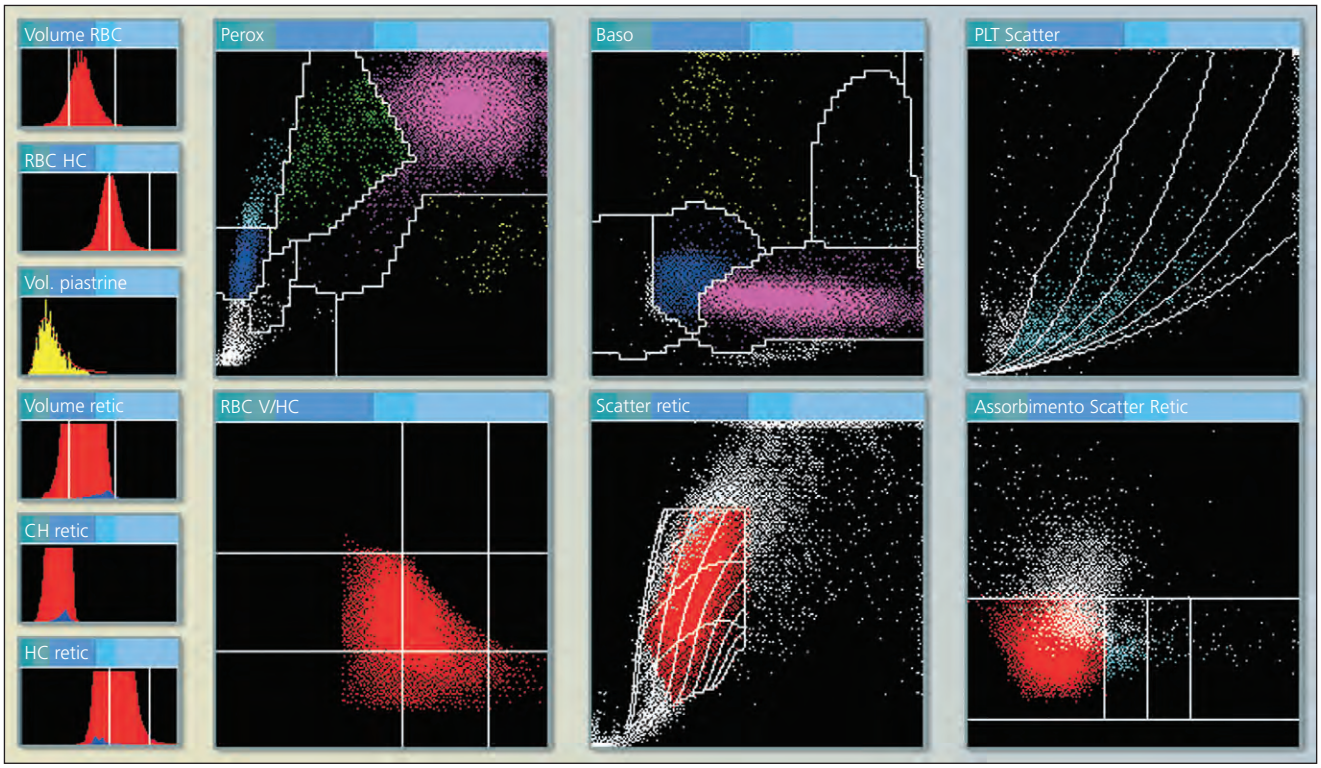

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 112 | g/l | |
| RBC | 4.85 | $\times 10^{12}$/l | |
| HCT | 0.36 | l/l | |
| MCV | 74.3 | fl | |
| MCH | 23.0 | pg | |
| MCHC | 310 | g/l | |
| CHCM | 306 | g/l | |
| RDW | 18.7 | % | Anisocytosis ++ |
| HDW | 3.25 | g/dl | |
| HYPO | 17.6 | % | Hypochromia +++ |
| HYPER | 0.8 | % | |
| MICRO | 14.7 | % | Microcytosis +++ |
| MACRO | 0.1 | % | |
| RBC fragments | 0.05 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 0.98 | % | |
| RET | 47.5 | $\times 10^{9}$/l | |
| CHr | 27.6 | pg | |
| IRF (M+H) | 13.2 | % | |
| PLT | 541 | $\times 10^{9}$/l | |
| MPV | 8.4 | fl | |
| PDW | 61 | % | |
| Large platelets | 14 | $\times 10^{9}$/l | LPLT ++ |
| MPC | 24.8 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 31.34 | | |
| WBC Perox | 29.13 | | |
| NEUT | 21.39 | 68.3 | |
| LYMPH | 5.00 | 16.0 | * |
| MONO | 1.65 | 5.3 | |
| EOS | 1.02 | 3.2 | |
| BASO | 4.34 | 13.8 | * B-SUSP |
| LUC | 2.28 | 7.3 | *, Atyp + |
| NRBC | 0.00 | 0.0 | |
| LI | 1.31 | | * B-NV, LSH +++ |
| MPXI | 7.2 | | |
| MN | | 47.3 | IG +++ |
| PMN | | 38.6 | |
| BLASTS | | 1.3 | Blasts ++ |
| Perox HPX | | 13.2 | |
| Perox SAT | | 5.8 | |

The distribution of the leukaemic population in the Perox cytogram is analogous to that of Report 5.1, with the cells with a high peroxidase activity distributed in a wedge that invades the zone of saturation. However, the peroxidase-negative population of lymphocytes and LUC (increased with an Atyp flag) is denser and shorter. In the Baso cytogram, the basophil area is occupied by signals that appear for the major part to be distinct from those in the MN and PMN areas, with an appearance that suggests a true increase in basophils; the MN/PMN valley is obscured. The events classified as blasts are only 1.3%, but the Blast flag is activated because of an excess of signals classified as basophils in accordance with the formula %BASO + %BASO Susp + %BASO Sat $\geq$ 10%.

**Morphology.** Differential leucocyte count: neutrophils 38%, neutrophil myelocytes and metamyelocytes 16%, promyelocytes 8%, blasts 2%, eosinophils 3%, basophils 14% dysmorphic, lymphocytes 17%, monocytes 2%. Numerous giant platelets. The very evident increase of promyelocytes and basophilia, with the morphological confirmation of the cytogram, indicates that the disease is in accelerated phase.

## REPORT 5.3 Polycythaemia vera being treated by phlebotomy



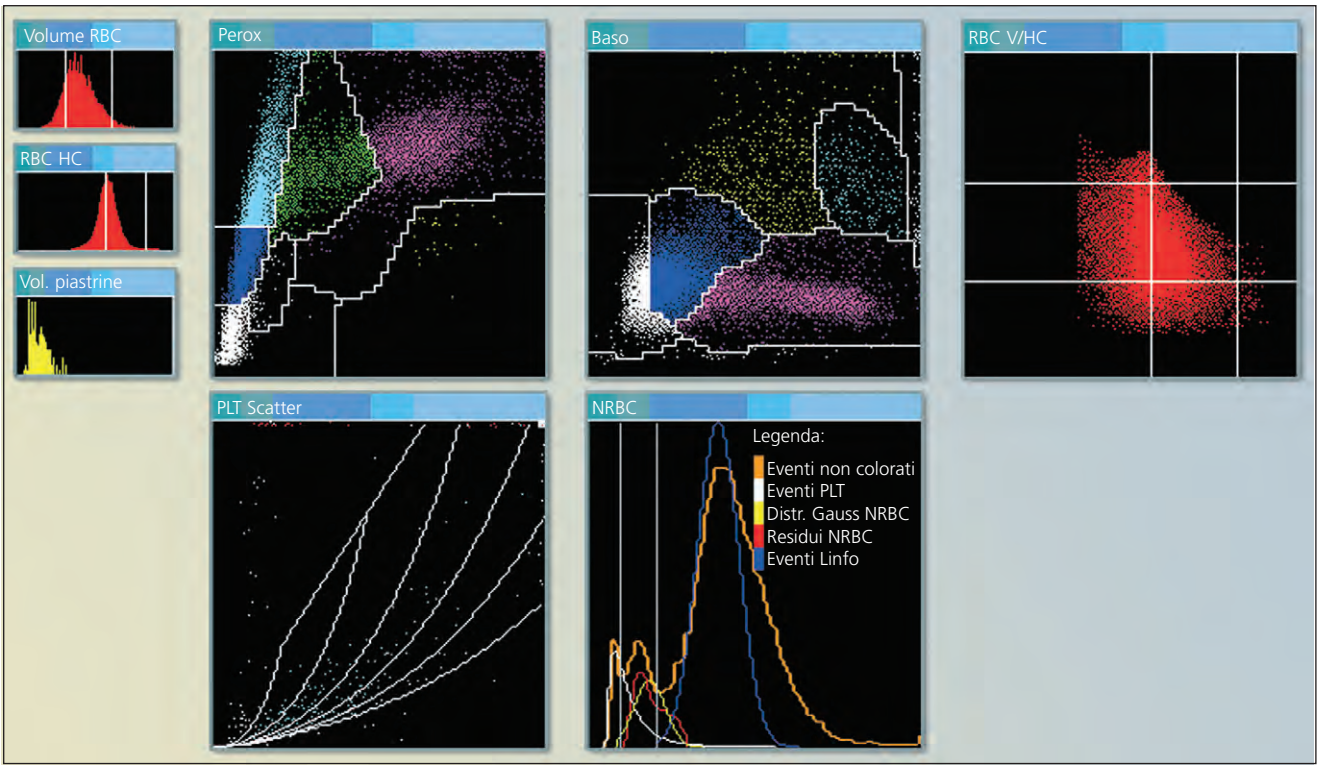

| Parameter | Value | Unit | Flags |
|---|---|---|---|
| HGB | 164 | g/l | |
| RBC | 7.24 | $\times 10^{12}$/l | |
| HCT | 0.559 | l/l | |
| MCV | 77.3 | fl | |
| MCH | 22.7 | pg | |
| MCHC | 294 | g/l | |
| CHCM | 288 | g/l | Anisocytosis ++ |
| RDW | 20.0 | % | |
| HDW | 3.36 | g/dl | Hypochromia +++ |
| HYPO | 42.3 | % | |
| HYPER | 0.3 | % | Microcytosis +++ |
| MICRO | 12.4 | % | |
| MACRO | 0.0 | % | |
| RBC fragments | 0.05 | $\times 10^{12}$/l | |
| RBC ghosts | 0.3 | $\times 10^{12}$/l | |
| RET | 0.58 | % | |
| RET | 41.8 | $\times 10^{9}$/l | |
| CHr | 27.0 | pg | |
| IRF (M+H) | 14.1 | % | |
| PLT | 158 | $\times 10^{9}$/l | |
| MPV | 10.6 | fl | |
| PDW | 54 | % | |
| Large platelets | 8 | $\times 10^{9}$/l | |
| MPC | 25.3 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flags |
|---|---|---|---|
| WBC Baso | 28.86 | | |
| WBC Perox | 31.02 | | |
| NEUT | 26.06 | 90.3 | |
| LYMPH | 1.51 | 5.2 | |
| MONO | 0.58 | 2.0 | |
| EOS | 0.11 | 0.4 | |
| BASO | 0.30 | 1.0 | |
| LUC | 0.31 | 1.1 | |
| NRBC | 0.00 | 0.0 | |
| LI | 2.01 | | LSH + |
| MPXI | 15.3 | | |
| MN | | 9.3 | |
| PMN | | 88.9 | |
| BLASTS | | 0.3 | |
| Perox HPX | | 1.4 | |
| Perox SAT | | 4.6 | |

The values for haemoglobin concentration and haematocrit are fairly elevated. The measured parameters for the conspicuous erythrocyte population indicate anisocytosis, microcytosis and, particularly, marked hypochromia, which are quite evident from the red cell V/HC cytogram and the red cell HC histogram: the picture is typical or iron-deficient erythropoiesis, which is common in patients with polycythaemia vera, particularly when treated by phlebotomy. The CHr is also reduced. There is leucocytosis with marked neutrophilia, with no flags other than a slight left shift: the population of neutrophils in the Perox cytogram and that of the PMN in the Baso cytogram are dense, distinct and compact, without scattering of signals that could suggest the presence of immature granulocytes. In the Baso cytogram there is a clear population of basophil granulocytes with intact cytoplasm high on the Y axis.

**Morphology.** Differential leucocyte count: neutrophils 89%, metamyelocytes 2%, eosinophils 0.5%, basophils 1.5%, lymphocytes 7%. The red cell series shows hypochromia and anisocytosis, with mild poikilocytosis and some dacrocytes. The platelets do not show any morphological anomalies.

## REPORT 5.4 Secondary acute myeloid leukaemia in a patient with polycythaemia treated in the past with alkylating agents and hydroxycarbamide



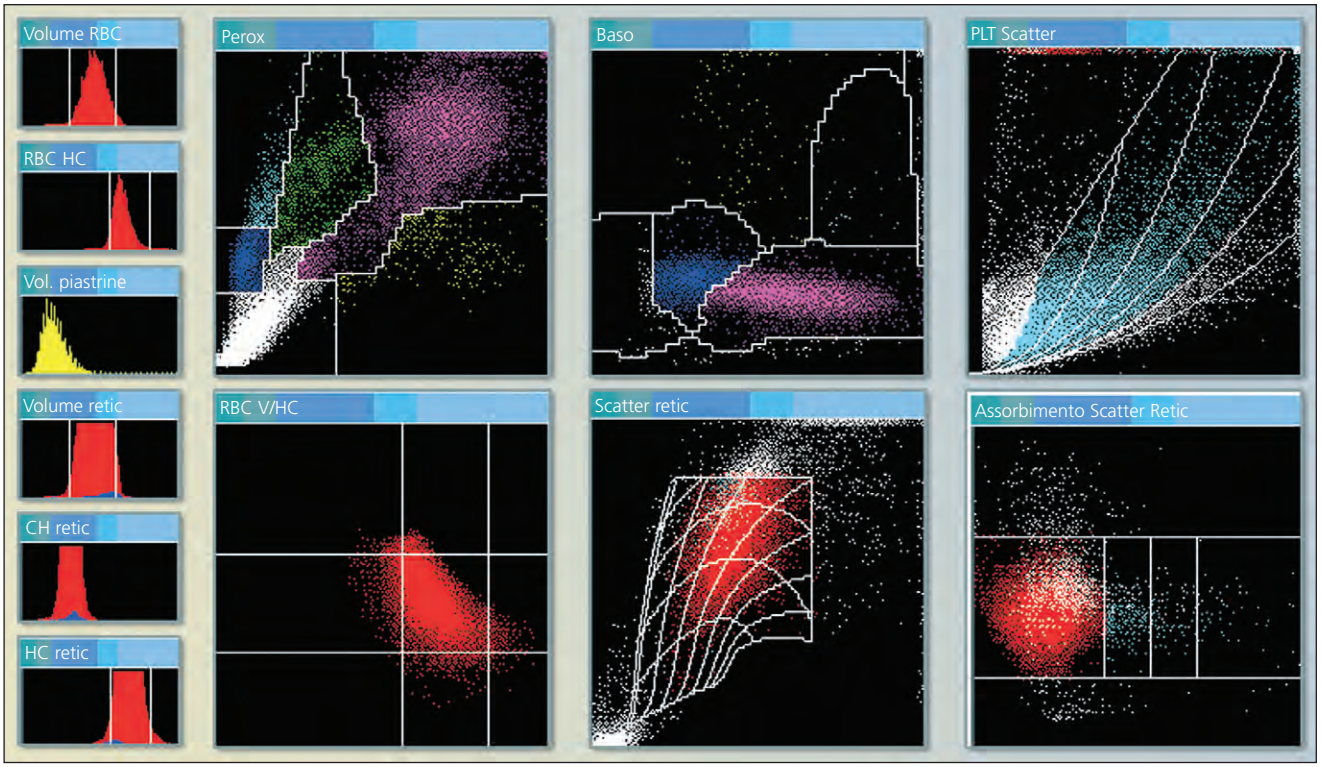

| Parameter | Value | Unit | Flags |
|---|---|---|---|
| HGB | 136 | g/l | |
| RBC | 5.64 | $\times 10^{12}$/l | |
| HCT | 0.449 | l/l | |
| MCV | 79.7 | fl | |
| MCH | 24.1 | pg | |
| MCHC | 303 | g/l | |
| CHCM | 289 | g/l | |
| RDW | 23.8 | % | Anisocytosis +++ |
| HDW | 3.28 | g/dl | |
| HYPO | 38.2 | % | Hypochromia +++ |
| HYPER | 0.2 | % | |
| MICRO | 14.6 | % | Microcytosis +++ |
| MACRO | 0.02 | % | |
| RBC fragments | 0.02 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 1.2 | % | |
| RET | 67.7 | $\times 10^{9}$/l | |
| CHr | 25.4 | pg | |
| IRF (M+H) | 7.9 | % | |
| PLT | 26 | $\times 10^{9}$/l | |
| MPV | 8.5 | fl | |
| PDW | 40.0 | % | |
| Large platelets | 0 | $\times 10^{9}$/l | |
| MPC | 24.0 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flags |
|---|---|---|---|
| WBC Baso | 28.88 | * | |
| WBC Perox | 28.19 | * | |
| NEUT | 3.18 | 11.0 | * |
| LYMPH | 14.86 | 51.5 | * |
| MONO | 1.41 | 4.9 | * |
| EOS | 0.04 | 0.1 | * |
| BASO | 0.65 | 2.3 | * |
| LUC | 8.74 | 30.3 | * |
| NRBC | 1.95 | 6.7/100 WBC | * N-RBC, NRBC + |
| LI | 1.99 | | |
| MPXI | −3.0 | | |
| MN | | 88.7 | |
| PMN | | 7.5 | |
| BLASTS | | 17.2 | Blasts +++ |
| Perox HPX | | 0.52 | |
| Perox SAT | | 0.2 | |

The erythroid series is characterised by very marked hypochromia, with the MCV at the lower limit of normal: the histograms show a great heterogeneity of size, which reflects both the effects of the cytotoxic agents and dyserythropoiesis. The morphology of the cytogram, according to the PANDA classification is P1 (because of the presence of a high peak of LUC which are dispersed towards the monocyte box because of weak peroxidase activity), D1 (because of the deformation of the MN head in the Baso channel, attributable to 17% of low density signals to the left of the channel 8 threshold on the X axis). There is a significant proportion of erythroblasts, which are identified on the NRBC cytogram as a small intermediate peak and which are also found in the PMN tail in the Baso channel, which is denser towards the right. There is severe thrombocytopenia.

**Morphology.** Leucocyte differential count: neutrophils 9%, lymphocytes 8%, monocytes 1%, blasts 82%, erythroblasts 5 per 100 leucocytes. Anisopoikilocytosis with Howell-Jolly bodies and basophilic stippling. The blast cells lack granules and show weak peroxidase activity; flow cytometric immunophenotyping shows MPO+, CD34+ and CD33+. The haemoglobin concentration is still good, despite the presence of massive blastic infiltration of the bone marrow, and the characteristics of the red cell series are testimony to the underlying myeloproliferative neoplasm.

# REPORT 5.5 Essential thrombocythaemia



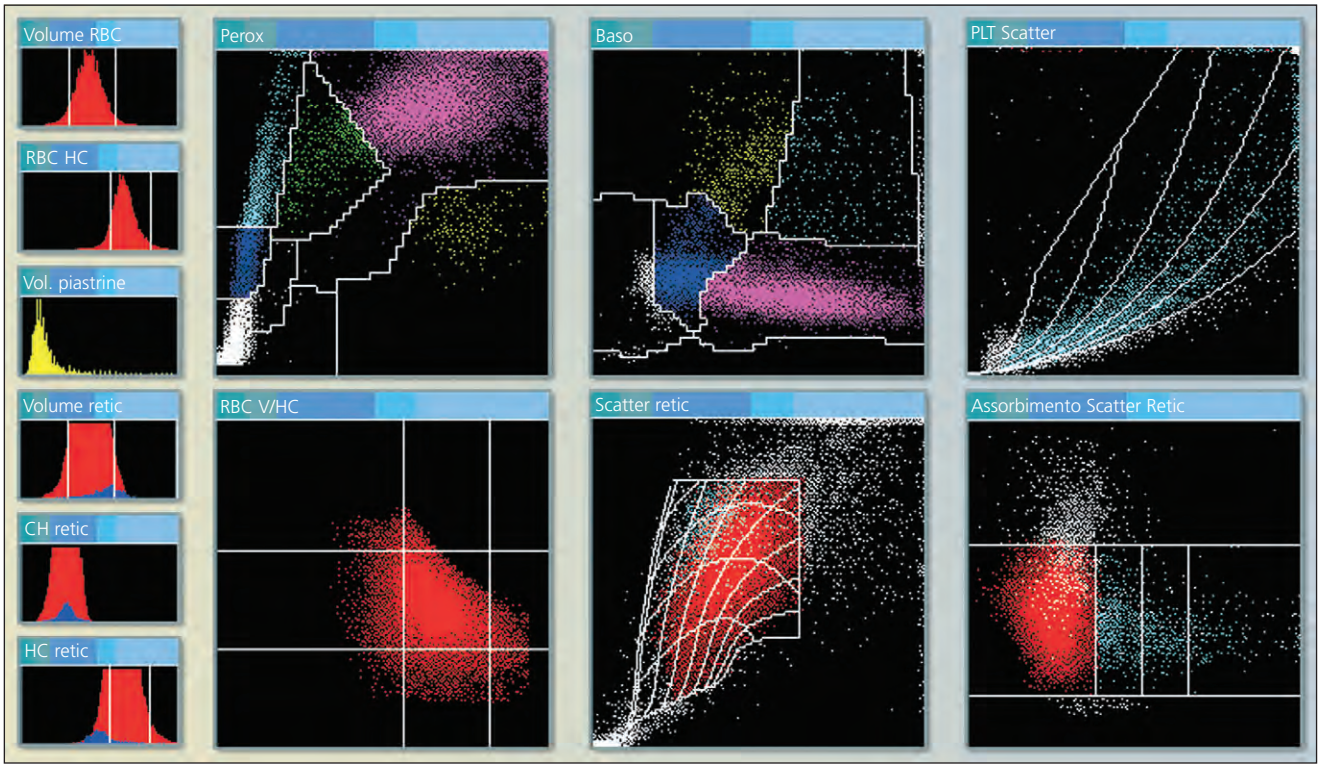

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 122 | g/l | |
| RBC | 5.11 | $\times 10^{12}$/l | |
| HCT | 0.424 | % | * |
| MCV | 82.9 | fl | * |
| MCH | 23.8 | pg | * |
| MCHC | 287 | g/l | * |
| CHCM | 296 | g/l | * |
| RDW | 15.4 | % | * |
| HDW | 2.53 | g/dl | * |
| HYPO | 23.1 | % | Hypochromia +++ |
| HYPER | 0.3 | % | |
| MICRO | 3.0 | % | Microcytosis + |
| MACRO | 0.1 | % | |
| RBC fragments | 0.19 | $\times 10^{12}$/l | RBC fragments + |
| RBC ghosts | 0.06 | $\times 10^{12}$/l | |
| RET | 2.57 | % | |
| RET | 131.5 | $\times 10^{9}$/l | |
| CHr | 26.9 | pg | |
| IRF (M+H) | 27.9 | % | |
| PLT | 2410 | $\times 10^{9}$/l | *, PLT-CLM, |
| MPV | 8.3 | fl | * PLT aggregate |
| PDW | 51.6 | % | PLT-ORN, NRLPLT, |
| Large platelets | 46 | $\times 10^{9}$/l | LPLT +++ |
| MPC | 22.8 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 18.65 | * | |
| WBC Perox | 19.38 | * | |
| NEUT | 13.51 | 72.5 | * |
| LYMPH | 2.96 | 15.9 | * |
| MONO | 0.53 | 2.8 | * |
| EOS | 0.54 | 2.9 | * |
| BASO | 0.27 | 1.5 | * |
| LUC | 0.83 | 4.5 | * |
| NRBC | 1.03 | 5.5/100 WBC | * NRBC, N-RBC |
| LI | 1.82 | | LSH ++ |
| MPXI | 2.5 | | |
| MN | | 24.8 | |
| PMN | | 72.6 | |
| BLASTS | | 1.5 | |
| Perox HPX | | 1.5 | |
| Perox SAT | | 0.3 | |

Leucocytosis with marked neutrophilia, haemoglobin concentration at the lower limit of normal for a man and marked increase in platelets. The red cells are normocytic and mainly hypochromic; reticulocytes are increased with a high IRF. The Perox cytogram shows a compact population of neutrophil granulocytes, the nuclei of which are found in the PMN tail in the Baso cytogram. LUC are at the upper limit of normal and the system signals the presence of erythroblasts. The excess of platelets generates a flag, which leads to an asterisk on almost all parameters: PLT-CLM indicates the invasion of the small clusters of platelet agglutinates (on the right in the lymphocyte box); NRLPLT indicates that the count of large platelets is above 40; PLTORN indicates the presence of increased background noise at the origin of the platelet histogram.

**Morphology.** The leucocyte differential count on a blood film confirms the instrument values. There are some activated lymphocytes, but there are no immature cells or erythroblasts. Platelets are very numerous, mainly morphologically normal but with a tendency to form small agglutinates. It is possible that the erythrocyte hypochromia indicates a latent iron deficiency due to a subclinical lack that is frequent as a result of the platelet dysfunction that is often associated with thrombocytosis in these myeloproliferative neoplasms.

## REPORT 5.6 Primary myelofibrosis in initial phase




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 124 | g/l | |
| RBC | 4.53 | $\times 10^{12}$/l | |
| HCT | 0.393 | l/l | |
| MCV | 86.7 | fl | |
| MCH | 27.4 | pg | |
| MCHC | 316 | g/l | |
| CHCM | 327 | g/l | |
| RDW | 19.2 | % | Anisocytosis ++ |
| HDW | 3.70 | g/dl | Anisochromia + |
| HYPO | 7.9 | % | Hypochromia + |
| HYPER | 2.0 | % | |
| MICRO | 5.3 | % | Microcytosis + |
| MACRO | 1.9 | % | |
| RBC fragments | 0.06 | $\times 10^{12}$/l | |
| RBC ghosts | 0.03 | $\times 10^{12}$/l | |
| RET | 2.24 | % | |
| RET | 101.5 | $\times 10^{9}$/l | |
| CHr | 28.6 | pg | |
| IRF (M+H) | 38.7 | % | |
| PLT | 517 | $\times 10^{9}$/l | |
| MPV | 9.1 | fl | |
| PDW | 53.9 | % | |
| Large platelets | 27 | $\times 10^{9}$/l | LPLT +++ |
| MPC | 26.2 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 25.74 | * | |
| WBC Perox | 26.02 | * | |
| NEUT | 21.62 | 84.0 | * |
| LYMPH | 1.47 | 5.7 | * |
| MONO | 0.51 | 2.0 | * |
| EOS | 0.25 | 1.0 | * |
| BASO | 0.62 | 2.4 | * |
| LUC | 1.26 | 4.9 | * Atyp + |
| NRBC | 0.76 | 2.9/100 WBC | * NRBC +, N-RBC |
| LI | 2.01 | | |
| MPXI | −0.3 | | |
| MN | | 26.5 | IG +++ |
| PMN | | 69.1 | |
| BLASTS | | 1.6 | Blasts + |
| Perox HPX | | 2.9 | |
| Perox SAT | | 1.6 | |

Leucocytosis with neutrophilia, increase of LUC and basophils. The Perox cytogram shows dispersion towards the right and the top of the granulocyte population and, to the left, a spindle-shaped peak of peroxidase-negative cells; there is an excess of background noise in the bottom left corner, related to thrombocytosis and the possible presence of erythroblasts. In the Baso cytogram there is a dense PMN tail with a number of signals that start from the MN head and invade the basophil zone. The IG flag is attributable to the fact that the neutrophil count in the Perox channel is clearly greater than the PMN count in the Baso channel. The erythrocyte population is very heterogeneous with regard to size and haemoglobin concentration; there is a slight increase in reticulocytes.

**Morphology.** Leucocyte differential count on the blood film: neutrophils 64%, neutrophil myelocytes and metamyelocytes 12%, eosinophils 1%, basophils 0.5%, lymphocytes 8.5%, blasts 4%, erythroblasts 2 per 100 leucocytes. Marked anisopoikilocytosis with numerous dacrocytes and erythrocytes containing Howell-Jolly bodies. The blast cells are medium sized with the undifferentiated morphology of haematogones: it is possible that they are responsible for the pseudobasophilia.

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CHAPTER 6

# Hypereosinophilia

In the peripheral blood, eosinophil granulocytes are normally present in a concentration of 0.05–0.5 $\times$ $10^9$/l, equal to 0.2–5% of circulating leucocytes. Classical criteria identify hypereosinophilia (or simply eosinophilia) as mild, moderate or severe when the concentration of eosinophils is respectively greater than an absolute count of 0.5, 1.5 or 5.0 $\times$ $10^9$/l.[1] The value of 1.5 $\times$ $10^9$/l is also that established as a threshold for classification of neoplastic disorders with eosinophilia in the 2008 World Health Organization (WHO) classification.[2]

Table 6.1 shows the principal causes of hypereosinophilia subdivided into:

- Reactive;
- Clonal;
- Idiopathic.[3–10]

In considering a differential diagnosis it is important to underline certain fundamental concepts:

- There are no unequivocal morphological or flow cytometric criteria that distinguish between reactive, clonal and idiopathic hypereosinophilias;
- The search for a possible cause of reactive eosinophilia is usually preliminary to other diagnostic approaches and complements the diagnostic approach to eosinophilia associated with haematological neoplasia;
- The identification of molecular genetic lesions is fundamental in the diagnostic pathway in cases that are not obviously reactive;
- Tissue and organ damage resulting from eosinophil infiltration occur independently of the cause of the eosinophilia.

Reactive hypereosinophilia can be associated with pathological processes such as infections (particularly parasitic infections), allergies, autoimmune disorders and neoplasms (Figs 6.1 through to 6.4). Hypereosinophilia is regulated by haemopoietic growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and various interleukins (IL-2, IL-3, IL-5), IL-5 regulating specifically the production of eosinophils. Reactive eosinophilia results from the release of these cytokines in diverse pathological conditions or their release by various pathological or neoplastic cells. In the presence of a haematological neoplasm with associated eosinophilia, it is important, although not always possible, to establish whether or not the eosinophils are part of the neoplastic clone. A reactive eosinophilia is often seen in lymphoid neoplasms, including T-lineage lymphoproliferative disorders such as Sézary syndrome and angioimmunoblastic T-cell lymphoma,[11] B- or T-lineage lymphoblastic leukaemia/lymphoma (see Fig. 8.5), Hodgkin lymphoma and multiple myeloma; it is less common in other B-lineage lymphomas. A particular form of reactive eosinophilia is that secondary to the presence of a T-lymphoid population, which may or may not be clonal, but which often has an anomalous phenotype and is producing cytokines, in particular IL-5: this form of hypereosinophilia was originally described as 'idiopathic',[12] but nowadays must be recognised as reactive.

Clonal eosinophilias are subdivided into two groups on the basis of the presence or absence of molecular rearrangement of the *PDGFRA*, *PDGFRB* or *FGFR1* genes, which encode tyrosine kinase receptors involved in eosinophil proliferation.[13] The first group comprises the neoplasms, either myeloid or myeloid–lymphoid, in which it is possible to demonstrate molecular alteration of *PDFRA*, *PDGFRB* or *FGFR1*. The second group includes chronic eosinophilic leukaemia, not otherwise specified (CEL, NOS) of the 2008 WHO classification and all the other conditions – myeloproliferative, acute myeloid or acute biphenotypic leukaemia described in other chapters of this book, in which the eosinophils in the peripheral blood do not represent the principal alteration, but are an integral part of the clonal expansion.

The term 'idiopathic hypereosinophilia' is used to designate the clinicopathological picture when neither the diagnostic criteria for reactive hypereosinophilia nor those for clonal eosinophilia are met.[14,15] It is thus a diagnosis of exclusion based on the presence of an eosinophil count of at least 1.5 $\times$ $10^9$/l without an increase of blast cells, persisting for more than six months, in subjects in whom all the causes of reactive eosinophilia (see Table 6.1) have been excluded and, similarly, all the possible clonal lesions described below have been excluded. Idiopathic hypereosinophilia that is not demonstrably clonal or reactive, and is associated with signs of tissue infiltration and

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**Table 6.1** Principal causes of eosinophilia

**Reactive eosinophilia**

*Non-neoplastic conditions*

- Allergic and atopic disorders (asthma, allergic rhinitis, allergic pneumonitis, hard metal pneumoconiosis, urticaria, eczema, atopic dermatitis, milk protein allergy, cyclical oedema with eosinophilia, drug reactions)
- Parasitic infections (visceral larva migrans, trichinosis, ascariasis, strongyloidiasis, ankylostomiasis, tapeworm infestation, echinococcosis, filariasis and tropical pulmonary eosinophilia, schistosomiasis, amoebiasis, fasciolaiasis, *Pneumocystis jirovecii* infection, *Toxocara canis* infection, toxoplasmosis)
- Non-parasitic infections (aspergillosis, coccidioidomycosis, infectious mononucleosis, mycobacterial infection, scarlet fever, brucellosis, cat scratch disease, infective lymphocytosis)
- Respiratory disorders (Churg-Strauss syndrome, Loeffler syndrome, cystic fibrosis, bronchiectasis)
- Endocrine disorders (adrenal insufficiency)
- Vasculitis and connective tissue disorders (rheumatoid arthritis, polyarteritis nodosa, Wegener granulomatosis, eosinophilic fasciitis)
- Gastrointestinal disorders (allergic gastroenteritis, coeliac disease)
- Cutaneous disorders (atopic dermatitis, eczema, pemphigus)
- Familial eosinophilia, including hereditary immunodeficiency associated with increased IgE
- Rare syndromes associated with eosinophilia such as Omenn syndrome
- Syndrome of L-tryptophane exposure
- Other causes (cirrhosis, radiotherapy, peritoneal dialysis)

*Paraneoplastic conditions*

- Carcinoma or sarcoma (lung, pancreas, colon, cervix, ovary)
- Hodgkin lymphoma
- T-cell leukaemia/lymphoma, including that due to the presence of an occult T-cell clone
- B-cell acute leukaemia/lymphoblastic lymphoma with t(5;14)(q31;q32)
- Langerhans cell histiocytosis
- Multiple myeloma and other haematological neoplasms (release of eosinophilopoietic cytokines by tumour cells)

**Clonal eosinophilia**

- Chronic eosinophilic leukaemia, not otherwise specified
- Haematological neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*
- Chronic myeloid leukaemia *BCR-ABL1*-positive with eosinophilia
- Acute myelomonocytic leukaemia with inv(16) or t(16;16) and eosinophilia
- Acute myeloid leukaemia with t(8;21) or not otherwise specified with (eosinophilic) maturation
- Myeloproliferative neoplasm, *JAK2* V617F-positive, with eosinophilia
- Aggressive systemic mastocytosis with eosinophilia
- Indolent systemic mastocytosis with eosinophilia
- Myelodysplastic syndrome with eosinophilia
- Myelodysplastic/myeloproliferative neoplasm with eosinophilia

**Idiopathic eosinophilia**

- Idiopathic hypereosinophilia (without organ damage)
- Idiopathic hypereosinophilic syndrome (with organ damage)

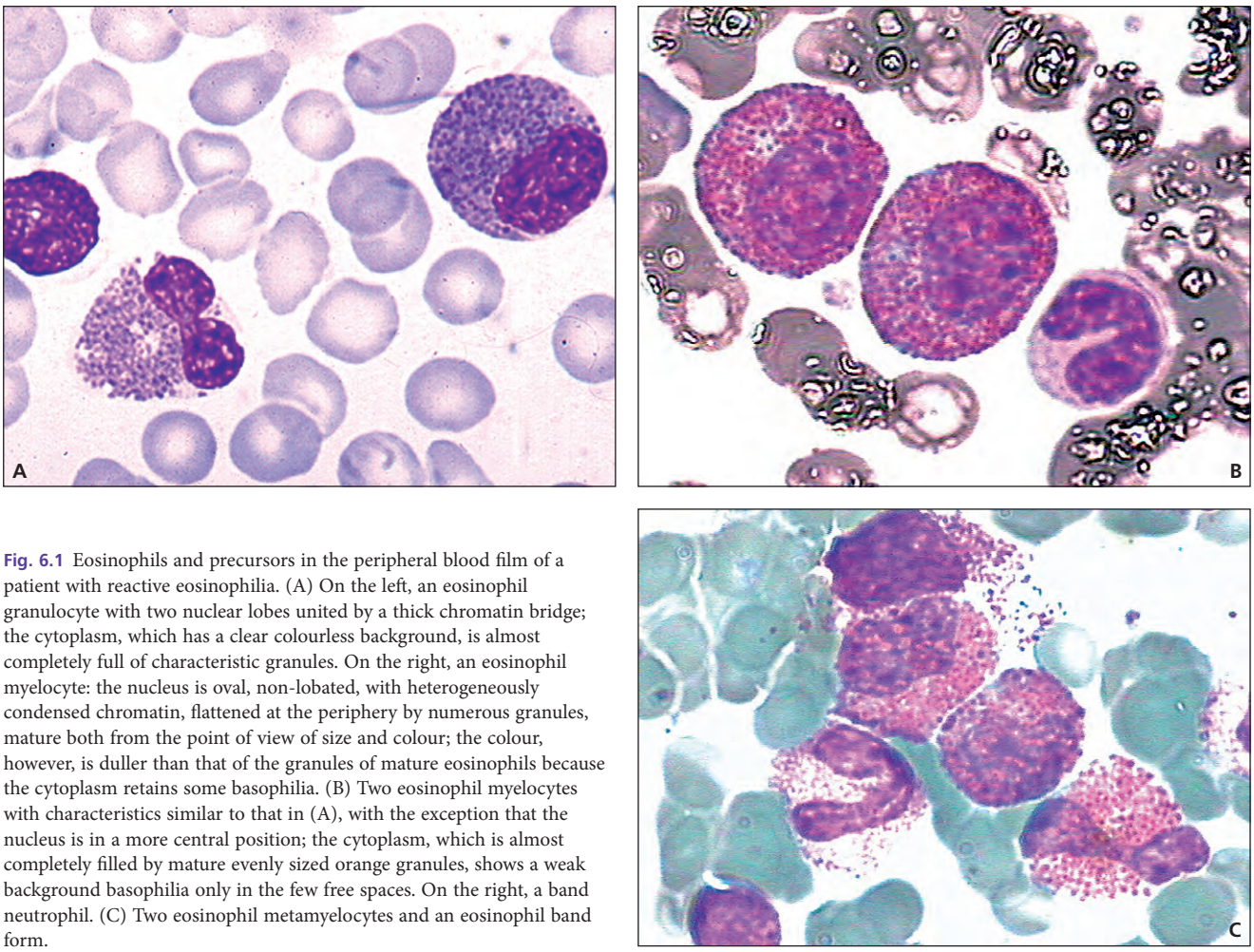



**Fig. 6.1** Eosinophils and precursors in the peripheral blood film of a patient with reactive eosinophilia. (A) On the left, an eosinophil granulocyte with two nuclear lobes united by a thick chromatin bridge; the cytoplasm, which has a clear colourless background, is almost completely full of characteristic granules. On the right, an eosinophil myelocyte: the nucleus is oval, non-lobated, with heterogeneously condensed chromatin, flattened at the periphery by numerous granules, mature both from the point of view of size and colour; the colour, however, is duller than that of the granules of mature eosinophils because the cytoplasm retains some basophilia. (B) Two eosinophil myelocytes with characteristics similar to that in (A), with the exception that the nucleus is in a more central position; the cytoplasm, which is almost completely filled by mature evenly sized orange granules, shows a weak background basophilia only in the few free spaces. On the right, a band neutrophil. (C) Two eosinophil metamyelocytes and an eosinophil band form.

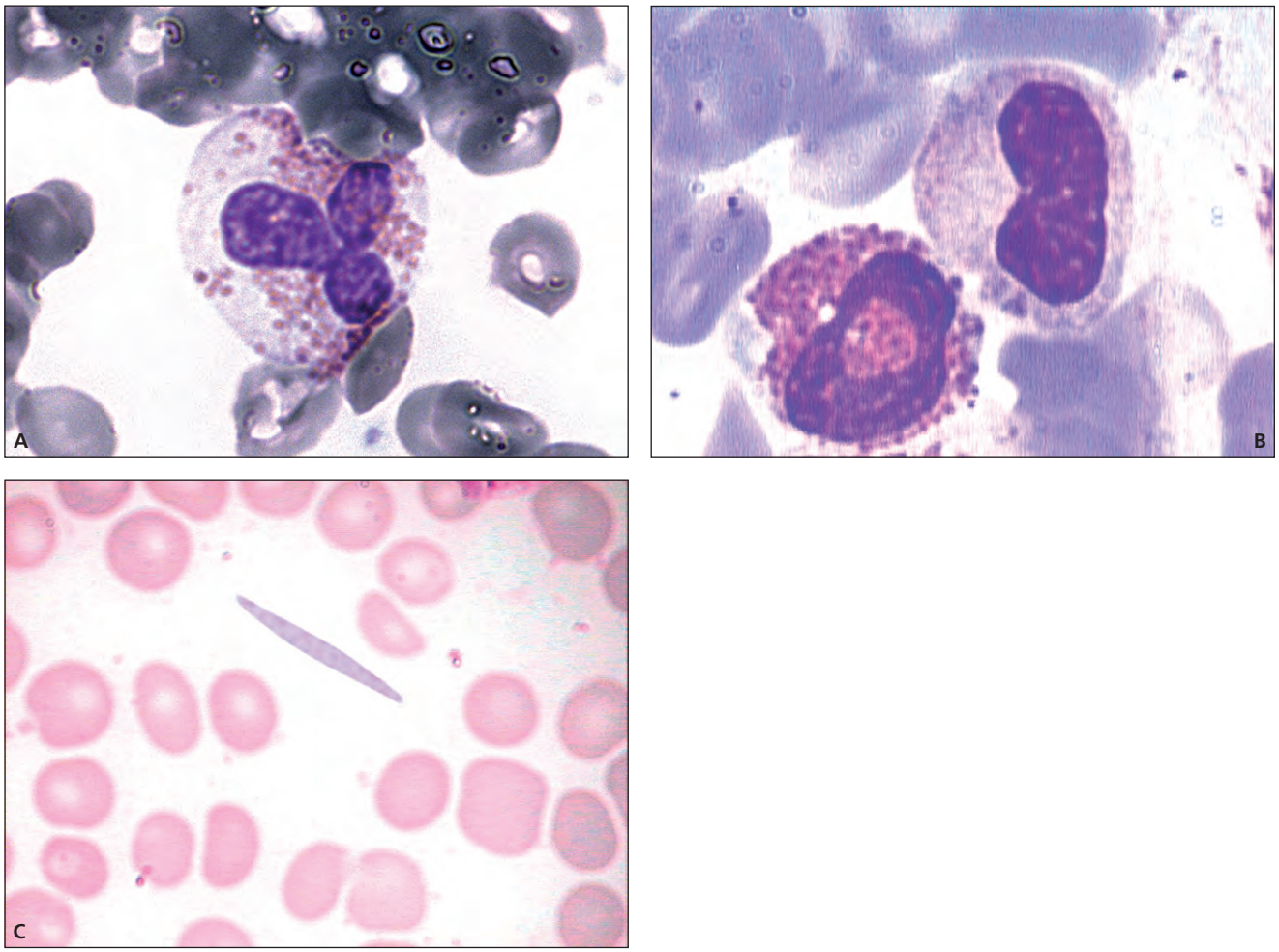



**Fig. 6.2** Dismorphism of mature eosinophils in patients with eosinophilia of various origins; peripheral blood film. Similar abnormalities are seen in reactive eosinophilia, but with a lower frequency than in clonal eosinophilia, associated with myelodysplasia (see also Figs 3.18 and 4.71) or with specific molecular rearrangements. (A) Eosinophil with a trilobed nucleus, in the form of a clover leaf; one of the lobes is twice the size of the other two. The granules appear mature but are scanty and irregularly distributed in cytoplasm that is grey with a bluish tinge. From the same blood film as Fig. 7.9. (B) Eosinophil with a ring nucleus in the peripheral blood of a patient with reactive eosinophilia. (C) Charcot-Leyden crystal in the bone marrow aspirate of a patient with a hypereosinophilic syndrome.

organ damage similar to those described for CEL, NOS, is designated the 'idiopathic hypereosinophilic syndrome' (Fig. 6.5, Report 6.1). It is possible that this group may include both some cases of eosinophilic leukaemia in which it is not currently possible to recognise the presence of clonal alterations, and some cases of reactive eosinophilia, secondary to release of cytokines as a result of a non-identifiable underlying condition.

The incidence of myeloproliferative hypereosinophilia in a study that included both clonal and 'essential' cases was 0.036 per 100 000 individuals per year, with a male predominance (M : F ratio of 1.47) and a peak incidence between 65 and 74 years, although some cases were observed in a younger group. Around 20% of patients with a diagnosis of 'hypereosinophilic syndrome' have rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*.[16]

## Chronic eosinophilic leukaemia, not otherwise specified

This is a rare haematological neoplasm included under this designation in the MPN group in the 2008 WHO classification, characterised by a persistent clonal proliferation of progenitors showing eosinophilic differentiation: as a consequence there is an expansion of the eosinophil component, often with well-preserved maturation, in the bone marrow, in the peripheral

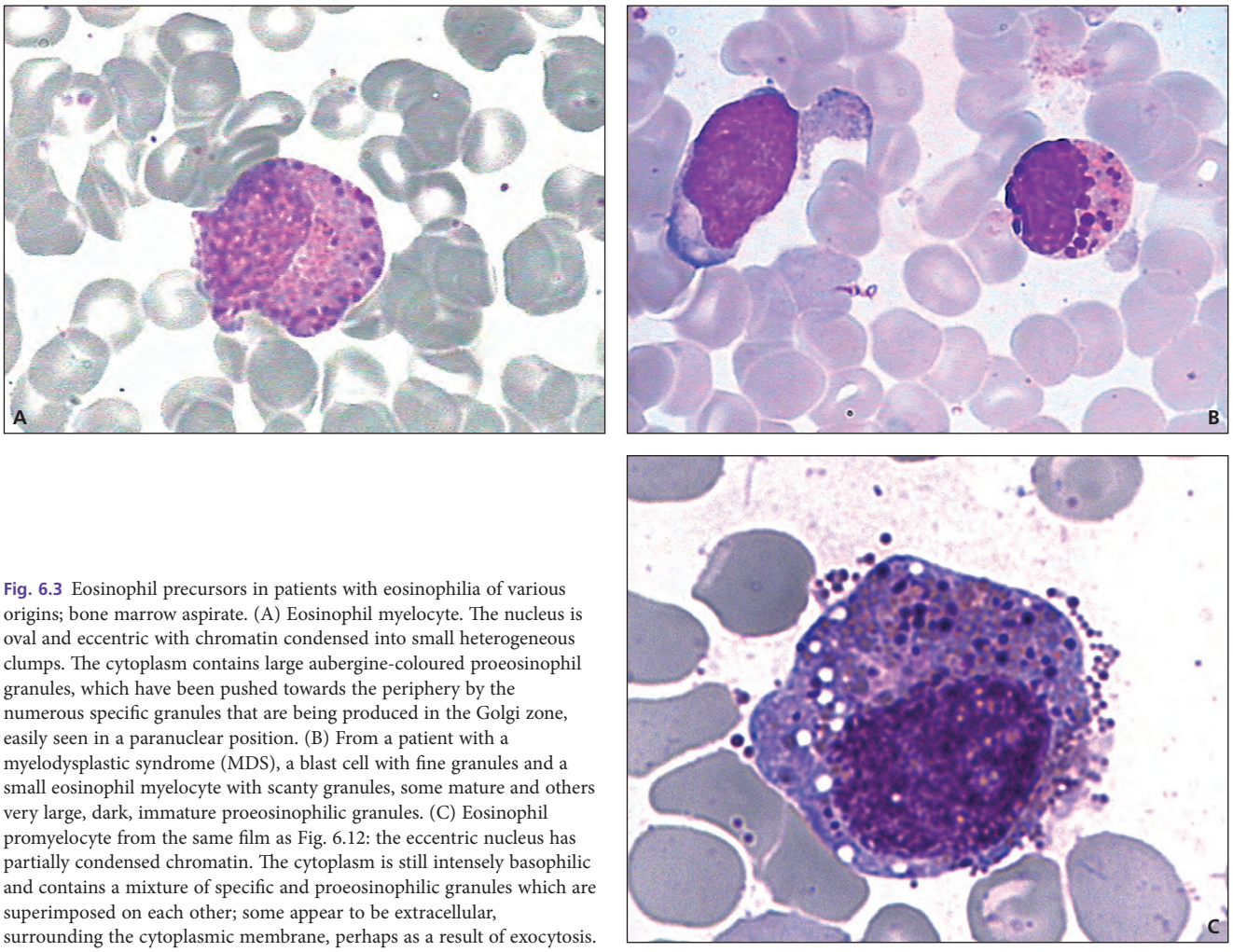



**Fig. 6.3** Eosinophil precursors in patients with eosinophilia of various origins; bone marrow aspirate. (A) Eosinophil myelocyte. The nucleus is oval and eccentric with chromatin condensed into small heterogeneous clumps. The cytoplasm contains large aubergine-coloured proeosinophil granules, which have been pushed towards the periphery by the numerous specific granules that are being produced in the Golgi zone, easily seen in a paranuclear position. (B) From a patient with a myelodysplastic syndrome (MDS), a blast cell with fine granules and a small eosinophil myelocyte with scanty granules, some mature and others very large, dark, immature proeosinophilic granules. (C) Eosinophil promyelocyte from the same film as Fig. 6.12: the eccentric nucleus has partially condensed chromatin. The cytoplasm is still intensely basophilic and contains a mixture of specific and proeosinophilic granules which are superimposed on each other; some appear to be extracellular, surrounding the cytoplasmic membrane, perhaps as a result of exocytosis.

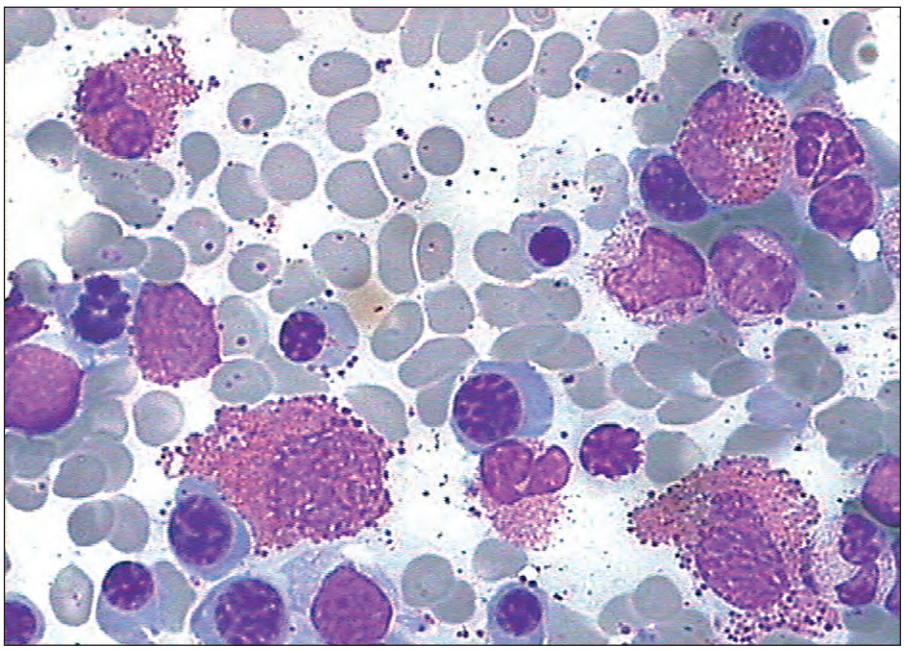


**Fig. 6.4** Reactive hypereosinophilia; bone marrow aspirate. There are numerous polychromatophilic and orthochromatic erythroblasts with retardation of nuclear maturation and a mitotic figure on the left; top right, two small metamyelocytes, a neutrophil granulocyte, a lymphocyte and a large mature eosinophil with a bilobed nucleus. At the bottom, two very spread out eosinophil myelocytes with a partially damaged cytoplasmic membrane; there is another mature eosinophil top left.

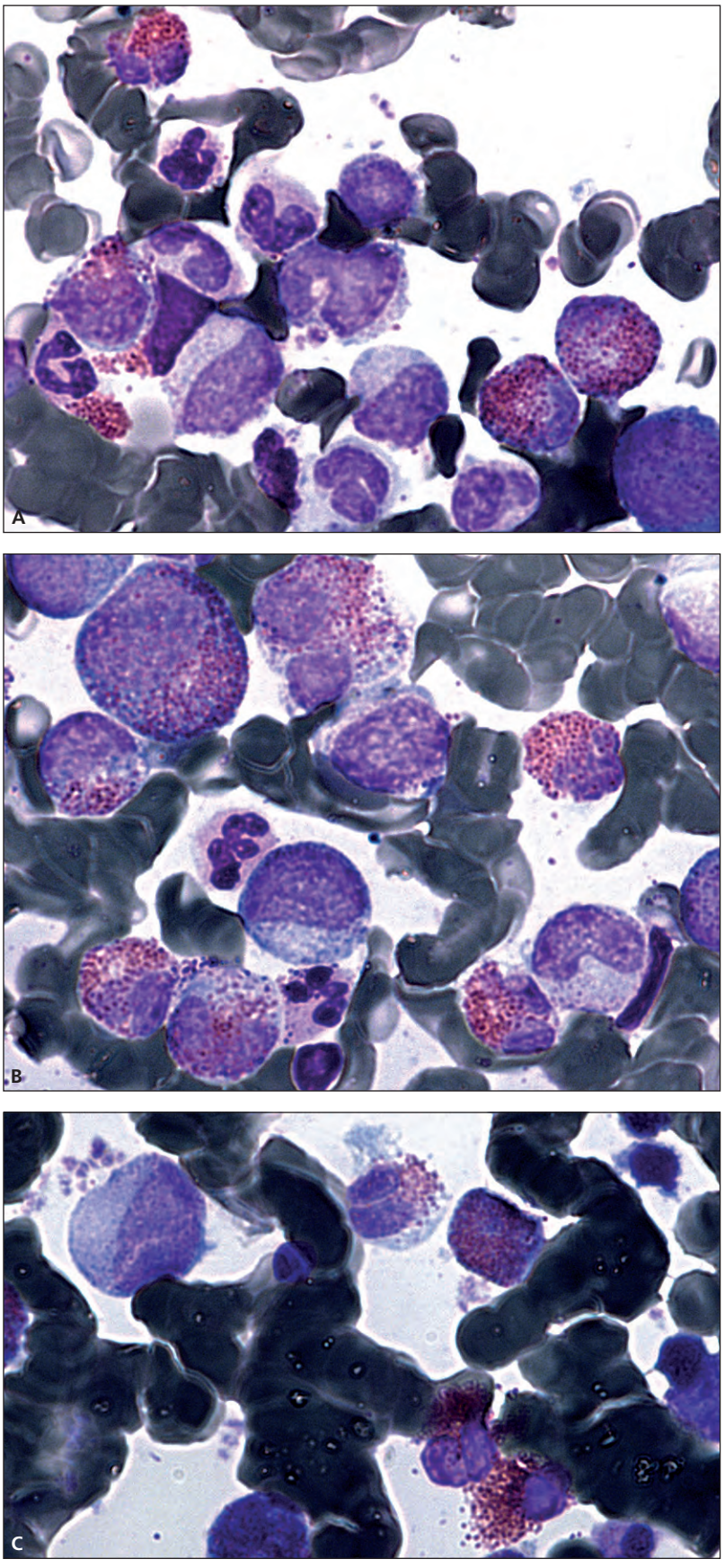



**Fig. 6.5** Idiopathic hypereosinophilic syndrome; bone marrow aspirate. (A) There are various neutrophil and eosinophil granulocytes at different stages of maturation. (B) Top left, a late promyelocyte with basophilic cytoplasm, loaded with specific granules; immediately beneath there is a small eosinophil metamyelocyte. At the top, a large bilobed eosinophil, incompletely granulated; another similar cell is found near to the lower edge of the image. The other mature eosinophils have a more normal appearance. (C) Mature dysmorphic eosinophils from the same film. The upper one has two elongated nuclear lobes like a saddlebag and scanty granules concentrated in a paranuclear position, leaving free large areas of blue cytoplasm. At the bottom, an eosinophil with a non-lobed nucleus.

blood and in tissues.[17] The pathological effects are systemic since degranulation of eosinophils leads to release of cytotoxic proteins, cytokines and humoral factors that cause damage to numerous organs and systems. Among these effects, there can be fibrosis and cardiac valvular lesions, cough resulting from bronchospasm, pulmonary fibrosis, involvement of the central nervous system, peripheral neuropathy, cutaneous lesions such as angio-oedema, urticaria and erythema, venous and arterial thrombosis, diarrhoea, joint disorders of a rheumatic type and ocular alterations.[18,19] In a manner analogous to other MPN, around a half of patients present with hepatosplenomegaly.

The WHO 2008 diagnostic criteria for CEL, NOS are met when there are[2]:

- Peripheral blood eosinophils of at least $1.5 \times 10^9$/l;
- Absence of the Philadelphia chromosome, *BCR-ABL1* fusion or both;
- Absence of the criteria required for a diagnosis of other MPN: polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF);
- Absence of the criteria required for a diagnosis of other myelodysplastic/myeloproliferative neoplasms (MDS/MPN): chronic myelomonocytic leukaemia (CMML) and atypical chronic myeloid leukaemia (aCML);
- Peripheral blood and bone marrow blast cells less than 20% (to exclude a diagnosis of acute myeloid leukaemia [AML]);
- Absence of inv(16)(p13q22) and t(16;16)(p13;q22) and of other alterations diagnostic of AML;
- Absence of *FIPL1-PDGFRA* and other rearrangements of *PDGFRA*;
- Absence of t(5;12)(q31–35;p13) and other rearrangements of *PDGFRB*;
- Absence of rearrangement of *FGFR1*;
- Recognition of a clonal cytogenetic or molecular genetic abnormality (other than those cited above) or an increase of blast cells, equal to or greater than 2% in the blood or 5% in the bone marrow, or evident dysplasia in one or more haemopoietic lineages;
- Exclusion of all causes or reactive eosinophilia, including paraneoplastic eosinophilia.

In summary, the diagnosis of CEL, NOS is made, on the one hand, by exclusion of the above-mentioned specific genetic lesions and other conditions and, on the other hand, by the demonstration of clonality or evidence of myeloid neoplasia furnished by an increase of blast cells or the association with severe dysplastic changes in other lineages. No molecular alterations have been identified that are pathognomonic for CEL, NOS: the cytogenetic abnormalities most often observed are non-specific, such as trisomy 8 or isochromosome 17p. The presence of clonality can sometimes be demonstrated in females by analysis of polymorphism in X chromosome genes such as *PGK2* or *AR* (HUMARA).[20]

With regard to morphology, in the peripheral blood one observes mainly mature eosinophils, with a variable percentage, not usually very high, of eosinophil metamyelocytes, myelocytes and promyelocytes. The eosinophils can be morphologically normal or they can be altered in size or show various cytomorphological abnormalities; the latter vary in degree and can include reduced segmentation (pseudo-Pelger type), increased segmentation and bizarre shapes such as ring nuclei.[21] The cytoplasm may show the presence of vacuoles, hypogranularity, irregular distribution of granules with clear or weakly basophilic agranular areas, reduced granule size or granule fusion to form small inclusions that resemble miniature Charcot-Leyden crystals. The precursors can have prominent violaceous proeosinophilic granules. All these types of eosinophil dysmorphism are non-specific and therefore cannot be used to assign a particular case to the clonal, reactive or idiopathic categories. The morphological observations that are useful for excluding a reactive or idiopathic condition are the recognition at the time of diagnosis of increased blast cells, either granular or agranular, in the peripheral blood or bone marrow or the recognition of dysplasia in other lineages. In addition to eosinophilia, neutrophilia is common; less frequent is monocytosis or basophilia. Anaemia and thrombocytopenia are seen in the more advanced stages of the disease.

The bone marrow is hypercellular with a marked increase in the percentage of eosinophils, generally with preservation of normal maturation. There can be dysmorphism of immature eosinophils (Fig. 6.6). The percentage of blast cells may be increased, but by definition is less than 20%. One may observe, as in other types of hypereosinophilia, Charcot-Leyden crystals of a typical elongated rhomboidal or diamond shape, like double pyramids joined by their bases, sometimes orange or rose in colour, which are often found in the expectorate or nasal secretions of allergic subjects and in the faeces of patients with parasitic infections; they are formed from phospholipase liberated from eosinophil granules.[22,23] Other bone marrow lineages are usually normal. An increase in myeloblasts and the recognition of dysgranulopoiesis, severe dyserythropoiesis or dysmegakaryopoiesis exclude the diagnosis of reactive eosinophilia and these are therefore important features that sustain a diagnosis of CEL. There is almost always reticulin fibrosis as a result of eosinophil degranulation.[24]

From the immunophenotypic point of view, clonal eosinophils do not show any specific markers.

## Hypereosinophilia associated with clonal myeloid disorders

In this form of hypereosinophilia, the eosinophils are almost always part of the neoplastic clone. Only in rare cases has a reactive nature been shown.[4] The neoplastic disorders that are most often characterised by involvement of the eosinophil lineage with hypereosinophilia are:

- AML with recurrent cytogenetic abnormalities (particularly acute myelomonocytic leukaemia with chromosome 16 aberrations; see Figs 4.25 and 4.31);

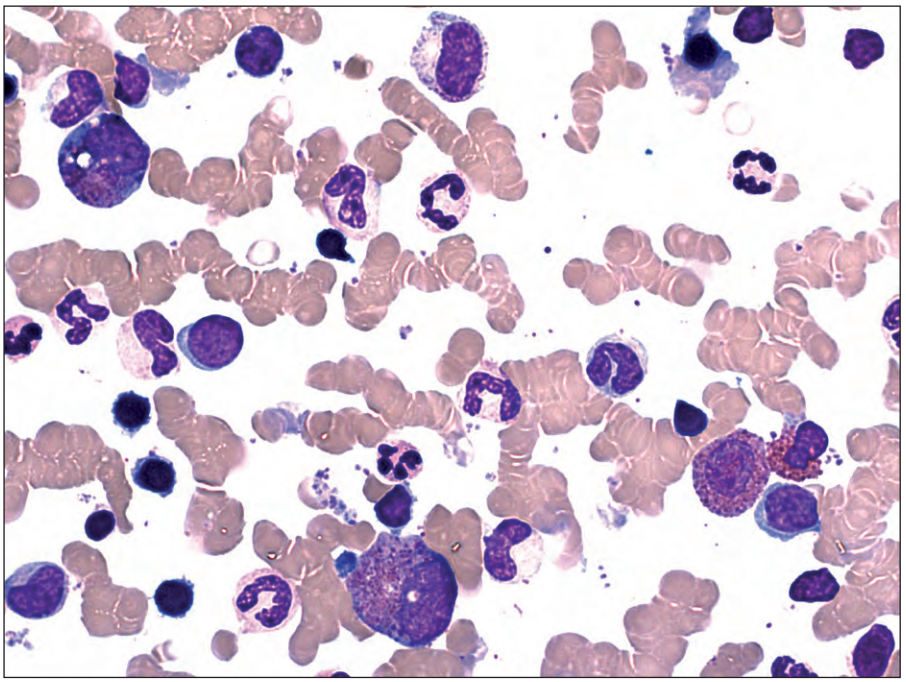


**Fig. 6.6** Infiltration by eosinophils at all stages of maturation in another patient with idiopathic hypereosinophilic syndrome; bone marrow aspirate. In the top left corner and in the centre at the bottom, two eosinophil promyelocytes; in the middle between them is a small agranular blast cell. On the right, an eosinophil myelocyte and an eosinophil promyelocyte. The red cells are forming long rouleaux.

- MPN, particularly *BCR-ABL1*-positive CML and systemic mastocytosis[25,26];
- MDS/MPN (CMML, juvenile myelomonocytic leukaemia [JMML] and aCML);
- Some MDS (see Fig. 3.18).

## Myeloid and lymphoid neoplasms with alteration of *PDGFRA*, *PDGFRB* or *FGFR1*

The neoplasms associated with rearrangement of one of the three genes that encode platelet-derived growth factors A (*PDGFRA*) or B (*PDGFRB*) or the receptor for fibroblast growth factor 1 (*FGFR1*) have variable characteristics, involving both myeloid and lymphoid cells; they thus have quite variable clinicopathological presentations, including AML, MPN, MDS/MPN and lymphoid neoplasms.

These disorders are dealt with in a specific chapter of the 2008 WHO classification.[27] The pathogenetic element that unites them is a molecular alteration that leads to the encoding of one of three different tyrosine kinases implicated in the neoplastic transformation. A fusion gene involving one of these three genes is created, each encoding a specific aberrant tyrosine kinase, which is activated and conveys a proliferative advantage and resistance to apoptosis; this favours the generation and expansion of an aberrant clone. Numerous partner genes involved in the generation of fusion genes have been described: the most frequent are *FIP1L1* as a partner of *PDGFRA*, *ETV6* (*TEL*) as a partner of *PDGFRB* and *ZNF198* as a partner of *FGFR1*.[28,29] The clinical picture and the course of the disease differ according to the type of rearrangement.

The recognition of rearrangement of *PDGFRA* or *FGFR1* in both myeloid and lymphoid neoplasms indicates derivation of these neoplasms from a mutated pluripotent stem cell. Further confirmation of the heterogeneity of the disorders included in this category is provided by their different responses to treatment with tyrosine kinase inhibitors, which are very efficacious when there is rearrangement of *PDGFRA* or *PDGFRB*, but not when there is a molecular alteration in *FGFR1*.[30]

### Neoplasms with rearrangement of *PDGFRA*

These conditions, in general, have very similar clinical and haematological features to CEL, NOS, with peripheral blood and bone marrow eosinophilia and the clinical picture of organ damage. They are much more common in men (M : F ratio of 17 : 1). Often there is a tendency to acute transformation. It is also possible for the initial presentation to be as AML, T-lineage lymphoblastic lymphoma or the simultaneous presentation of CEL and acute leukaemia or lymphoblastic lymphoma. According to the 2008 WHO classification, the presence in these neoplasms of rearrangement of *PDGFRA* is sufficient for inclusion of a case in this category.

The common feature is the presence of an often marked peripheral blood eosinophilia (at least $1.5 \times 10^9$/l). The eosinophils are mainly mature, but there can also be some eosinophil precursors at different stages of maturation (Figs 6.7 through to 6.10). As in CEL, NOS, the eosinophils can appear normal or can show cytoplasmic atypia (such as vacuolisation,

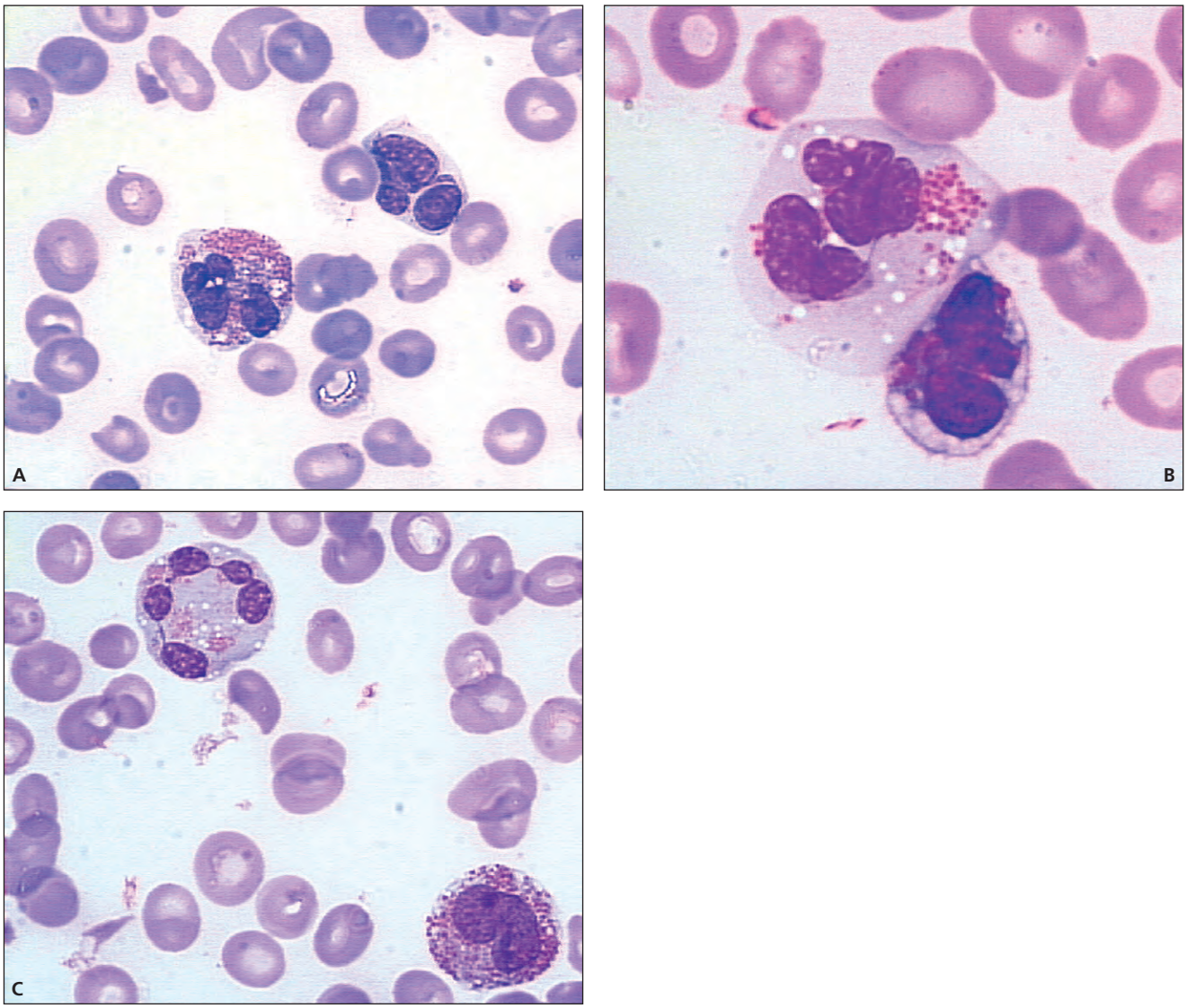



**Fig. 6.7** Myeloproliferative neoplasm (MPN) with hypereosinophilia and rearrangement of *PDGFRA*; peripheral blood film. The eosinophil series in this patient shows very striking dysmorphism. (A) On the left, an eosinophil granulocyte with an irregularly multilobated nucleus; the specific granules are small and numerous, but do not occupy all the cytoplasm, which is grey and contains some vacuoles. The cell on the right is certainly an eosinophil with similar nucleus and cytoplasm, but is completely agranular. (B) Two eosinophils, one giant and one contracted with an extreme grade of nuclear and cytoplasmic dysmorphism. For cells of this type, one can also use the designation 'dysplastic', which is generally not attributed to morphological abnormalities of eosinophils because of their lack of specificity. (C) Top left, a large eosinophil with a five-lobed nucleus, stretched out and almost forming a circle; the grey vacuolated cytoplasm contains a few clumps of fine specific granules. Bottom right, an eosinophil metamyelocyte, this also having very small granules.

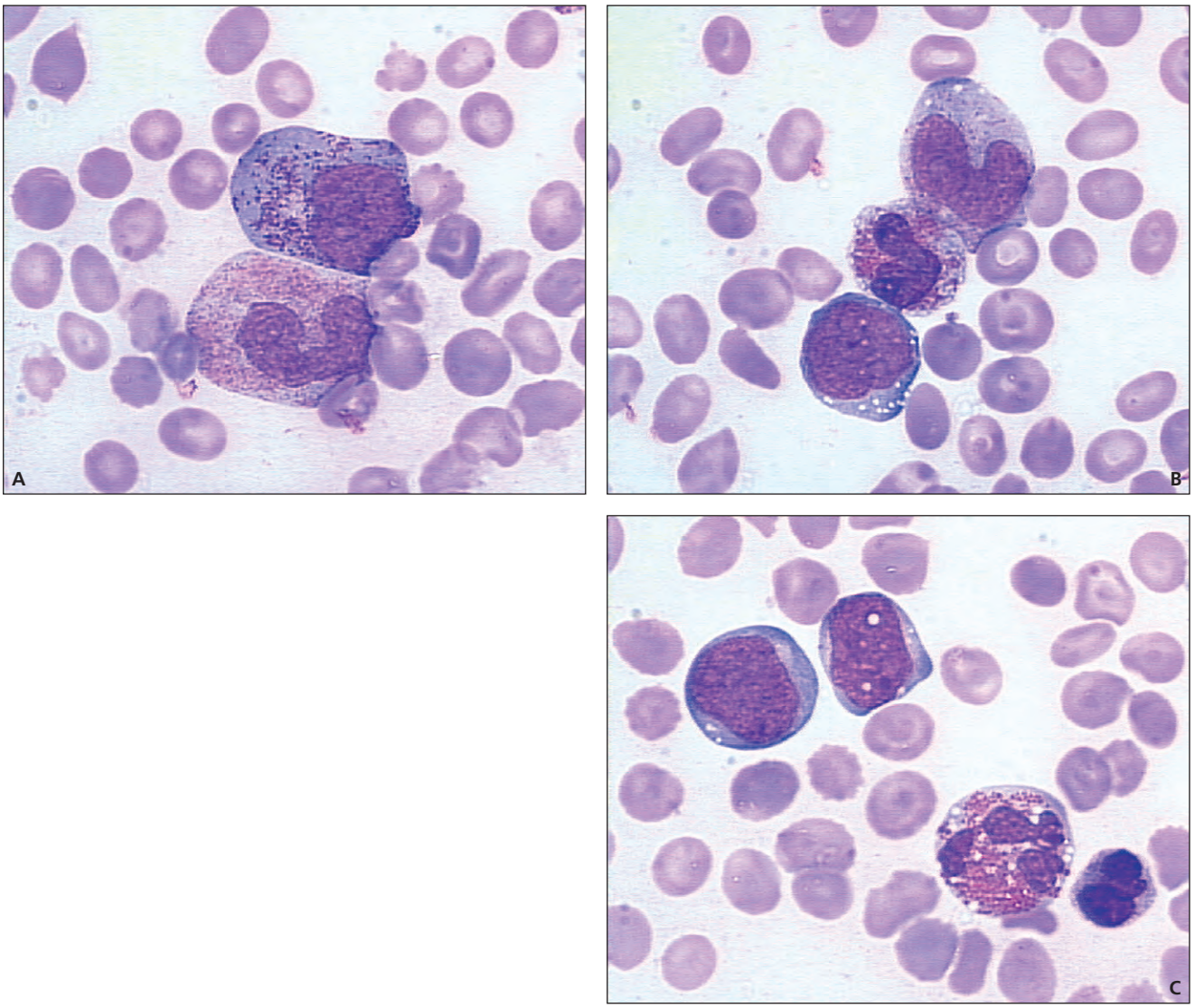



**Fig. 6.8** MPN with hypereosinophilia and rearrangement of *PDGFRA*; acute evolution in the patient shown in Fig. 6.7; peripheral blood film. (A) At the bottom, a dysplastic metamyelocyte with very fine granules dispersed in ample cytoplasm; the nucleus has immature chromatin. At the top, a dysplastic promyelocyte with ample basophilic cytoplasm and granules spread unevenly around an agranular paranuclear zone. The chromatin is reticular with some incompletely defined nucleoli. The possible eosinophil lineage of this cell is a supposition based on the general cytomorphological context of hypereosinophilia with severe dysmorphism. (B) At the top, an immature precursor with a horseshoe-shaped nucleus and reticular chromatin with nucleoli; it is not morphologically definable (granular blast cell or dysplastic promyelocyte?). At the bottom, an agranular blast cell with very fine homogeneous chromatin and two nucleoli. Between the two, an eosinophil with dysmorphism of the nucleus and granules that appear fine and dark. (C) On the left, two agranular blast cells with an angulated nuclear profile. On the right, an eosinophil with a four-lobed nucleus and an unrecognisable dwarf granulocyte.

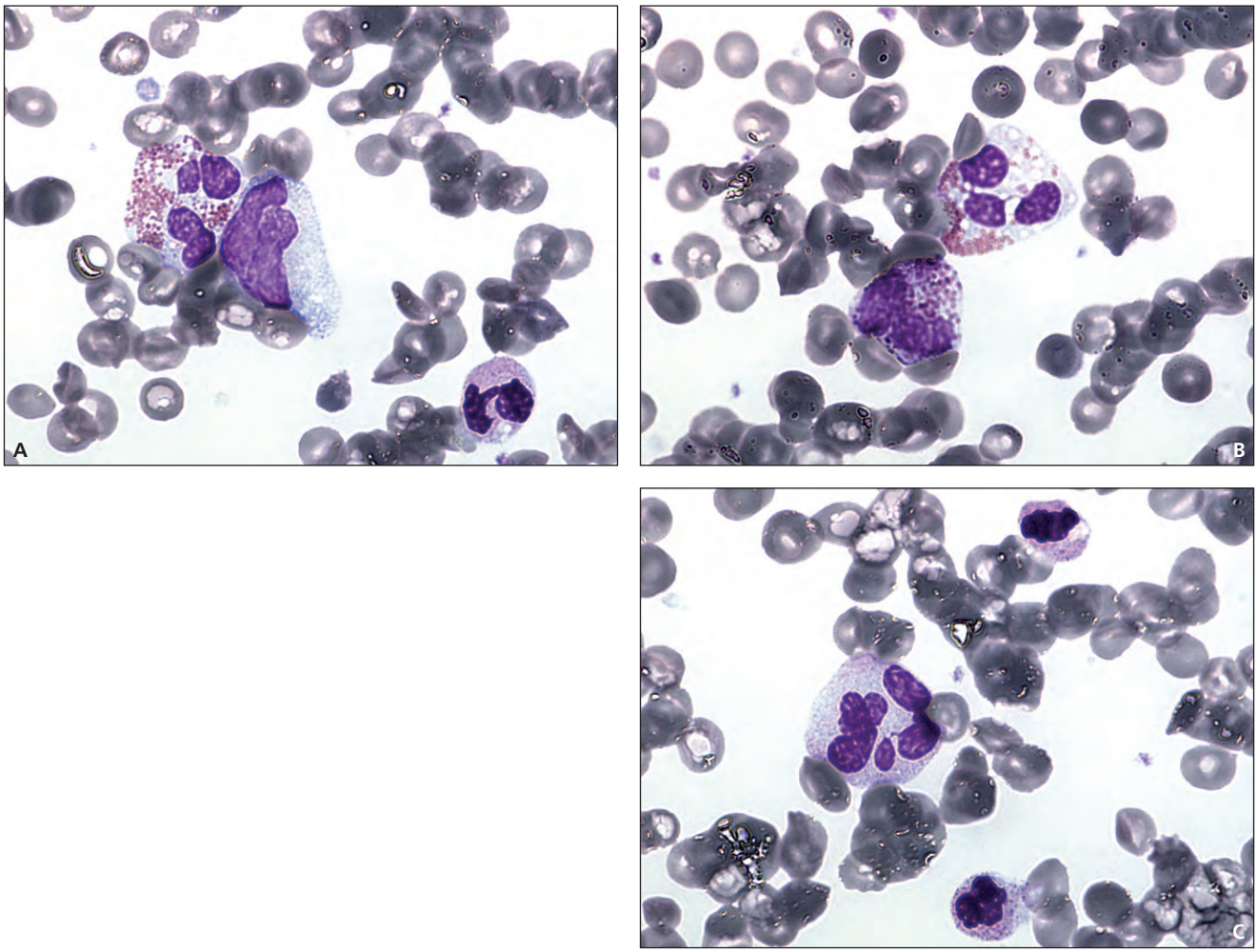



**Fig. 6.9** MPN with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; peripheral blood film. (A) On the left an eosinophil that has a multilobed nucleus and cytoplasm incompletely filled with granules; an atypical monocyte; a small neutrophil with very dense chromatin and abnormal segmentation. (B) A trilobed eosinophil with vacuolated cytoplasm and scanty granules almost all of which are clumped together in a limited area of the cytoplasm beneath the cytoplasmic membrane. At the bottom, an eosinophil metamyelocyte with proeosinophilic granules. (C) In the centre, a large dysplastic neutrophil granulocyte with pale hypogranular cytoplasm and a hyperlobated nucleus. At the top and at the bottom, another two dysplastic neutrophils, quite a deal smaller with almost pyknotic chromatin.

disorderly distribution of granules and immature purple granules), nuclear atypia or alterations in cell size. As already indicated, morphological alterations in eosinophils are non-specific and are found similarly in reactive and clonal eosinophilias. Sometimes at presentation there is anaemia, thrombocytopenia, neutrophilia or the presence of a small number of blast cells. Blast cells are more numerous when there is associated AML.

The bone marrow is usually hyperplastic with expansion of the eosinophil compartment, generally with retention of the capacity to mature (Figs 6.11 and 6.12). The percentage of blast cells is higher in cases with associated AML. There is often a marked increase in mast cells, so that the differential diagnosis includes systemic mastocytosis; the biological link between eosinophils and mast cells, as seen in allergic conditions and Hodgkin lymphoma, is evident.[26] In histological sections, the mast cells are usually dispersed, but they can form cohesive aggregates with fusiform cells, making the bone marrow picture sometimes indistinguishable from that of systemic mastocytosis.[31] Charcot-Leyden crystals may be observed both in the bone marrow aspirate and in histological sections.[32] Areas of necrosis, increased reticulin and an increased number of blast cells are characteristic of the disease in evolution.

Laboratory investigations show an increase in serum vitamin $B_{12}$ and, often, an increase in serum tryptase. Immunohistochemical investigation is sometimes useful to show the mast cells' immunophenotype, these cells often being positive for

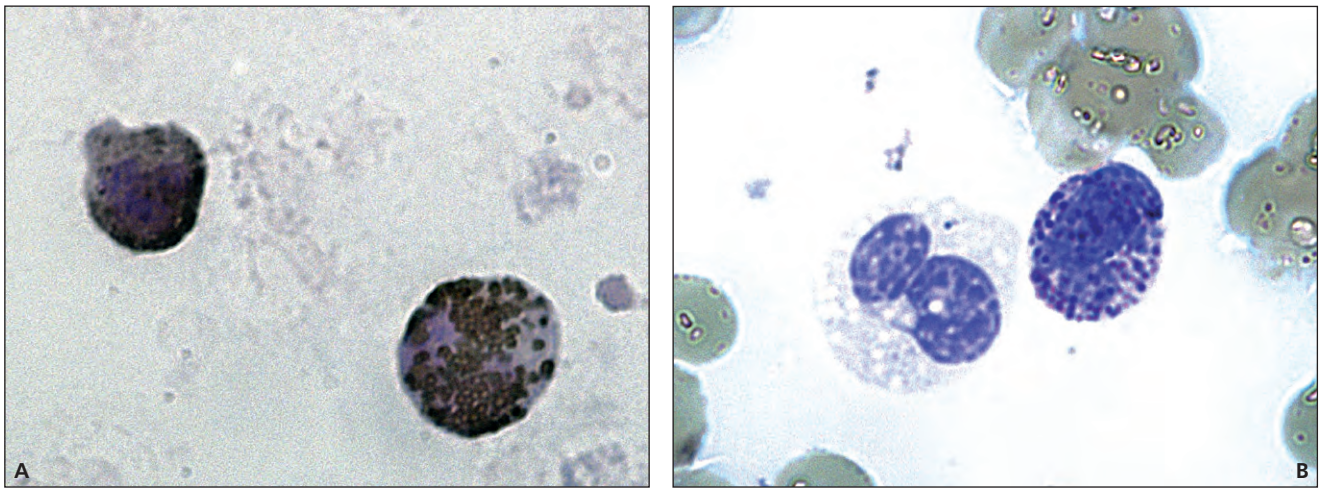



**Fig. 6.10** Myeloproliferative neoplasm with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; same case as Fig. 6.9, peripheral blood film. (A) Peroxidase reaction. The cell on the right shows the typical large granular positivity of the eosinophil lineage. (B) Toluidine blue. The granular metachromatic staining with a purplish tinge shown by the granulocyte on the right confirms basophil lineage. The granulocyte on the left has no positively-stained granules.

CD25 but, in contrast with the majority of cases of systemic mastocytosis, negative for CD2. Diagnosis requires the identification of the fusion gene by reverse transcription-polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridisation (FISH). In a minority of cases cytogenetic analysis shows a translocation such as t(1;4) or t(4;10) with a 4q22 breakpoint; in the majority of cases, however, the molecular lesion is cryptic and cytogenetic analysis is normal.

### Neoplasms with rearrangement of *PDGFRB*

In the presence of an *ETV6-PDGFRB* fusion gene resulting from t(5;12)(q31∼33;p12) there is a characteristic picture of an MPN with eosinophilia; other molecular variants also resulting in *PDGFRB* rearrangement have been described. From the clinicopathological point of view, the features are most often those of a myelodysplastic/proliferative neoplasm (MDS/MPN) (see Chapter 7): the most frequently observed picture is that of CMML with eosinophilia, but cases have also been described with features very similar to aCML or CEL or, rarely, AML (Fig. 6.13). These cytomorphological variants are associated with hypereosinophilia in the majority of cases, but this is not an obligatory feature.[33] The peripheral blood typically shows leucocytosis, resulting from neutrophilia, eosinophilia and monocytosis, usually in the absence of basophilia or abnormal maturation. The bone marrow is hypercellular because of expanded, effective neutrophil and eosinophil granulopoiesis, often associated with monocytosis: the percentage of blast cells, in the absence of acute evolution, must be, by definition, less than 20%. There may be an increase in mast cells and these may be fusiform.

The diagnosis is made with the aid of cytogenetic analysis and molecular analysis by RT-PCR. This disorder, like neoplasms with rearrangement of *PDGFRA*, responds well to treatment with tyrosine kinase inhibitors.

### Neoplasms with rearrangement of *FGFR1*

Rearrangement of *FGFR1* is associated with a very heterogeneous group of clinical disorders, suggesting that the mutation arises in a pluripotent haemopoietic stem cell. Depending on the lineage and the degree of differentiation of the expanding clone, these patients can present with an MPN, AML, T-lineage or B-lineage acute lymphoblastic leukaemia or an acute leukaemia of mixed phenotype. In the literature, patients with t(8;13)(p11;q12) and the *ZNF198-FGFR1* fusion gene have been designated as having the '8p11 myeloproliferative syndrome' or '8p11 stem cell syndrome'.

Eosinophilia has been recognised in more than 90% of the reported case, regardless of whether the clinicopathological presentation is as a chronic disorder, usually with associated neutrophilia and monocytosis, or as an acute leukaemia. Currently, the prognosis of these patients is grave; tyrosine kinase inhibitors are ineffective.

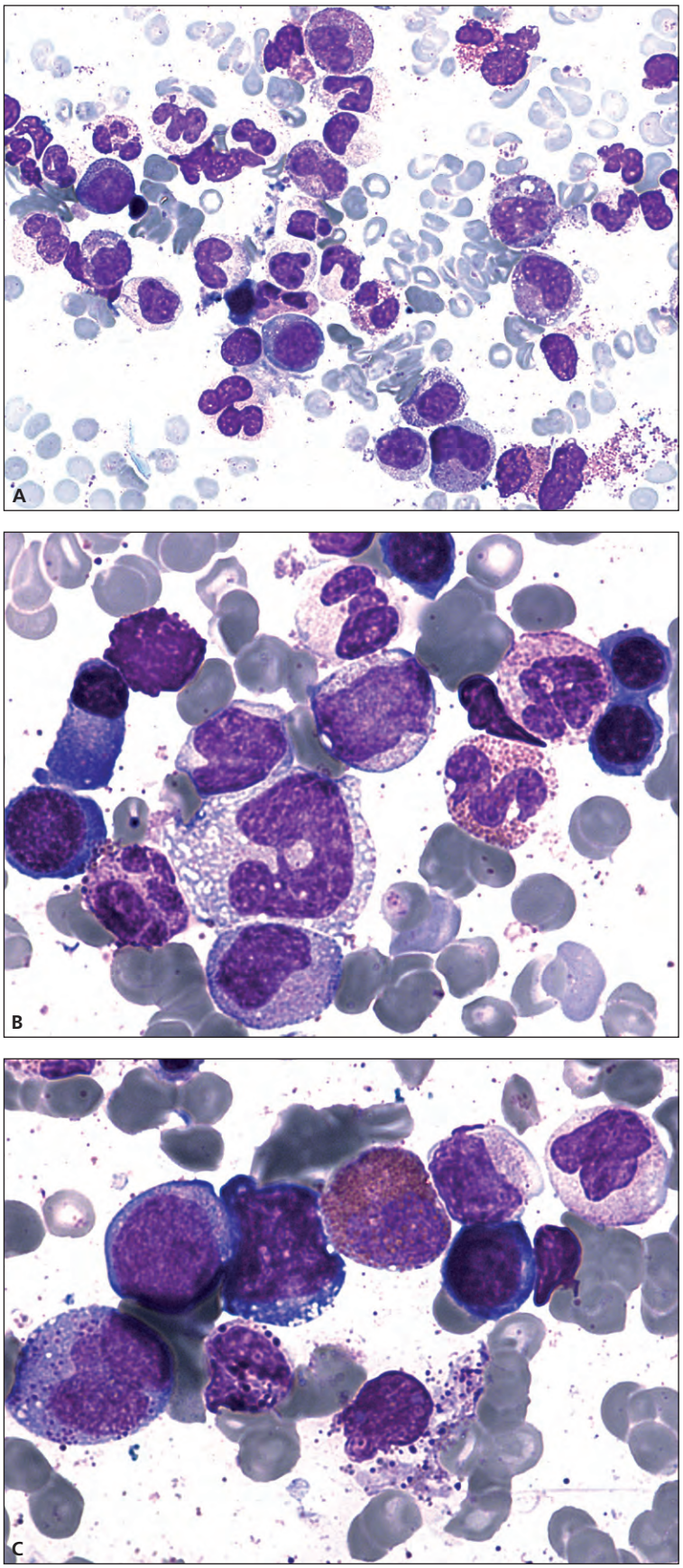



**Fig. 6.11** MPN with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; bone marrow aspirate of the same case as Figs 6.9 and 6.10. (A) The very cellular marrow is composed mainly of cells of neutrophil lineage at all stages of maturation: there are two promyelocytes (one just under the centre of the image, the other higher to the left) and various mature cells, in which dysplastic features are more evident. In the centre and on the right, several mature eosinophils, two of which have torn cytoplasm. (B) On the left, a basophil granulocyte, a small almost rectangular plasma cell and a basophilic erythroblast. In the central area, six dysplastic granulocytes in various stages of maturation. On the right, a mature neutrophil, an eosinophil band form and two polychromatic erythroblasts with cytoplasm not yet divided. (C) On the left, a dysplastic promyelocyte, a blast cell with fine granules, a large vacuolated and crushed basophilic cell (perhaps a proerythroblast), immediately beneath a small hypogranular basophil, an eosinophil metamyelocyte, a basophilic erythroblast and two dysplastic neutrophil metamyelocytes.

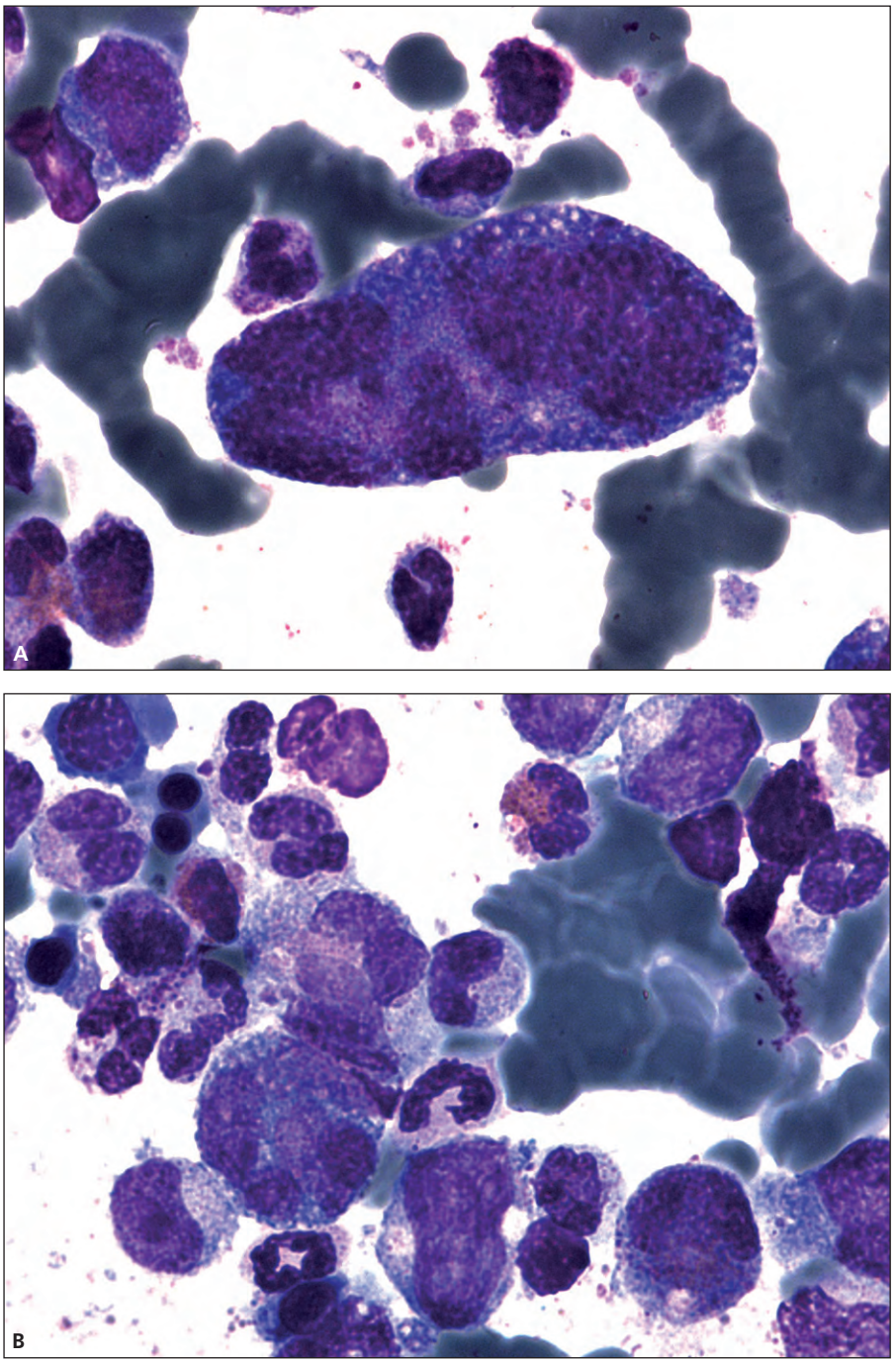



**Fig. 6.12** MPN with hypereosinophilia, basophilia, dysgranulopoiesis and rearrangement of *PDGFRA*; bone marrow aspirate, same case as Fig. 6.11. (A) A monstrous immature hyperdiploid and multinucleated granulocyte, with extensive intensely basophilic and granulated cytoplasm, certainly a dysplastic cell belonging to the neutrophil lineage. (B) Dysplastic neutrophil granulocyte, two eosinophils and two dysplastic erythroblasts. In the top right corner, a fusiform mast cell.

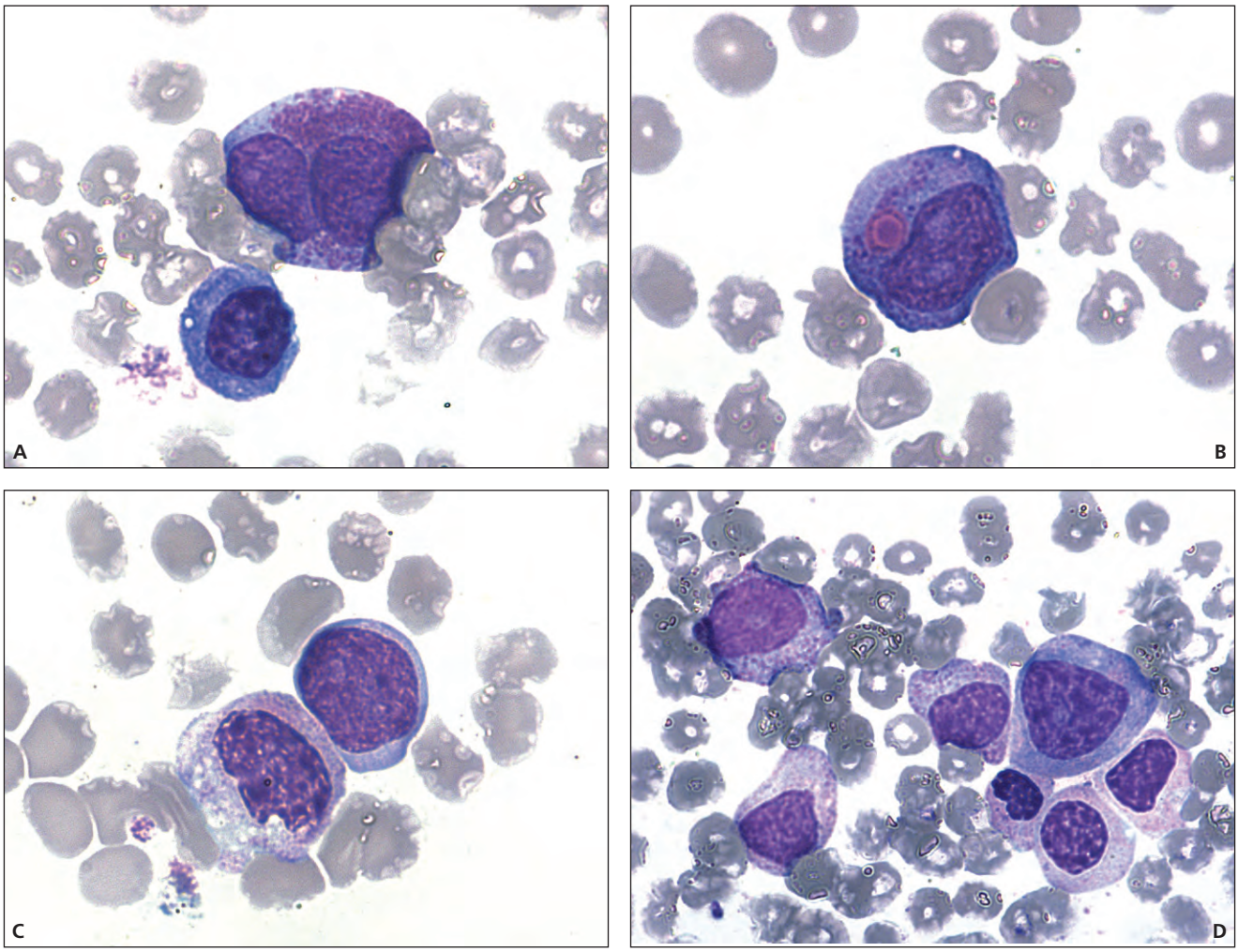
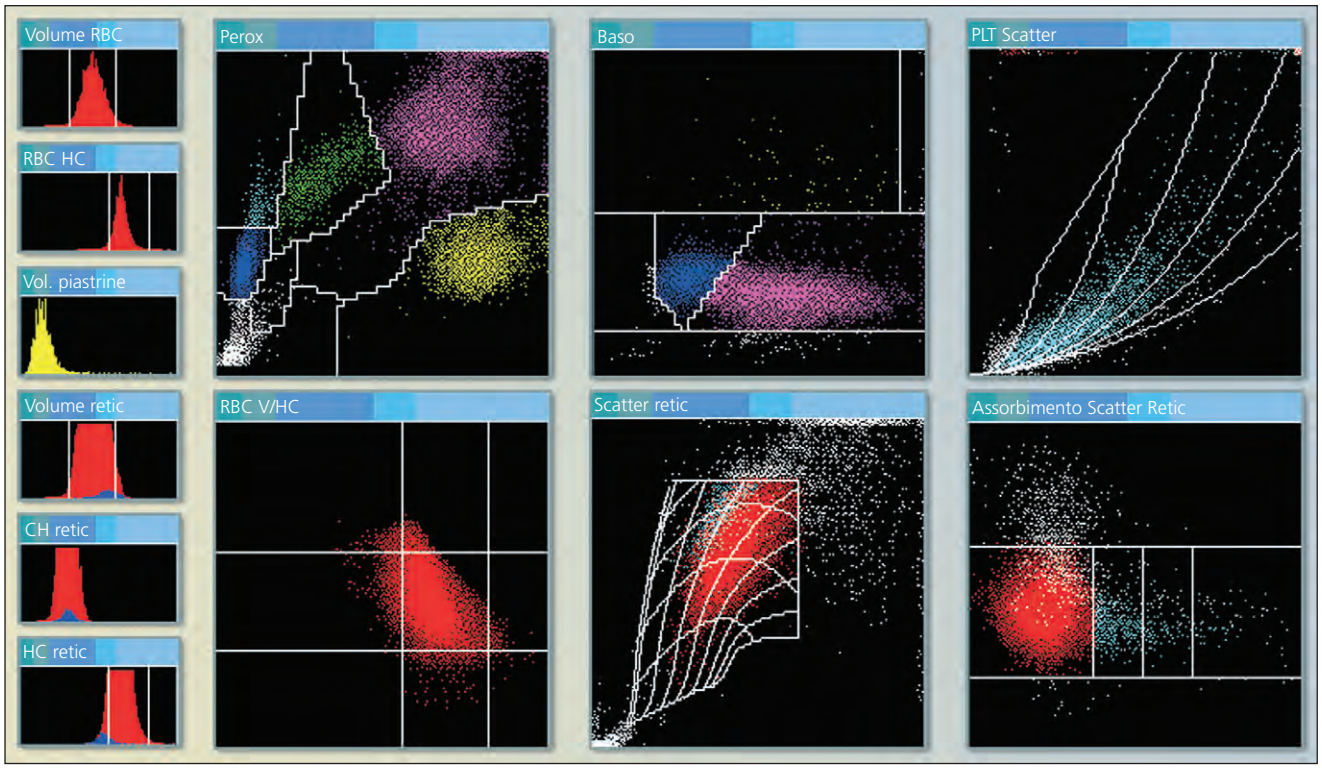



**Fig. 6.13** MDS/MPN with leucocytosis (WBC 14 × $10^9$/l), eosinophilia and rearrangement of *PDGFRB* in a patient previously treated for carcinoma of the colon and subsequently small cell carcinoma of the lung (therapy-related myeloid neoplasm); peripheral blood film. (A) Large dysplastic eosinophil with immature chromatin; a dyserythropoietic basophilic erythroblast with nuclear chromatin in blocks. (B) Circulating dysplastic eosinophil promyelocyte with two nucleoli; the basophilic cytoplasm contains azurophilic granules and an enormous eosinophil granule of pseudo-Chédiak-Higashi type. (C) From the left: two dysmorphic platelets, a neutrophil myelocyte with vacuolated cytoplasm and an agranular blast cell. (D) Dysplasia of neutrophil granulocytes; top left, a dysplastic promyelocyte.

# REPORT 6.1 Idiopathic hypereosinophilic syndrome




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 132 | g/l | |
| RBC | 4.47 | $\times 10^{12}$/l | |
| HCT | 0.412 | l/l | |
| MCV | 92.0 | fl | |
| MCH | 29.6 | pg | |
| MCHC | 322 | g/l | |
| CHCM | 322 | g/l | |
| RDW | 14.9 | % | Anisocytosis + |
| HDW | 2.29 | g/dl | |
| HYPO | 3.0 | % | |
| HYPER | 0.2 | % | |
| MICRO | 0.6 | % | |
| MACRO | 2.0 | % | |
| RBC fragments | 0.03 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 1.26 | % | |
| RET | 56.3 | $\times 10^{9}$/l | |
| CHr | 30.2 | pg | |
| IRF (M + H) | 28.2 | % | |
| PLT | 503 | $\times 10^{9}$/l | |
| MPV | 8.8 | fl | |
| PDW | 49.6 | % | |
| Large platelets | 5 | $\times 10^{9}$/l | |
| MPC | 22.6 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 15.07 | | |
| WBC Perox | 14.60 | | |
| NEUT | 7.16 | | |
| LYMPH | 3.60 | | |
| MONO | 0.85 | | |
| EOS | 3.14 | | |
| BASO | 0.09 | | |
| LUC | 0.24 | | |
| NRBC | 0.00 | | |
| LI | 1.93 | | |
| MPXI | 5.6 | | |
| MN | | 30.9 | |
| PMN | | 68.4 | |
| BLASTS | | 0.3 | |
| Perox HPX | | 1.9 | |
| Perox SAT | | 0.3 | |

Full blood count done on a patient with cough, bronchospasm and diarrhoea. All the haematological parameters are normal with the exception of a moderate leucocytosis and thrombocytosis, the latter attributable mainly to a marked increase in eosinophils, both in terms of percentage and absolute count. Clinical and laboratory assessment had not identified any possible cause of secondary eosinophilia. In the Perox cytogram, the eosinophils form a spherical group in the area where these cells are usually located, because of their intense peroxidase activity that leads to absorption of a proportion of the light so that they appear smaller than neutrophils. In the Baso cytogram, the nuclei of the eosinophils appear in the PMN tail, where, if they are numerous as in this case, they form a dense cloud extending downwards from the central portion of the PMN profile.

**Morphology.** The differential white cell count on the blood film gives very similar values to the instrument counts. The eosinophils are cytologically normal and there are no immature forms present. The platelets are normal and well formed. The diagnosis of idiopathic hypereosinophilic syndrome must be considered provisional, particularly because of the moderate thrombocytosis which indicates the need in this patient for further haematological investigations directed at excluding a myeloproliferative neoplasm.

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CHAPTER 7

# Myelodysplastic/Myeloproliferative Neoplasms

In the publications of the French-American-British (FAB) group there was already a description of four haematological neoplasms in which a proliferative expansion was associated with dysplasia: among these, chronic myelomonocytic leukaemia (CMML) was included in the myelodysplastic syndromes (MDS), whereas atypical chronic myeloid leukaemia (aCML) was included in what were then known as myeloproliferative disorders, now myeloproliferative neoplasms (MPN).[1–3]

Starting with the 2001 World Health Organization (WHO) classification,[4] the concept of myelodysplastic/myeloproliferative neoplasms was developed with the abbreviation MDS/MPN, also definable as dysmyeloproliferative disorders, which brought together four clinicopathological disorders, including CMML, juvenile myelomonocytic leukaemia (JMML), aCML and the group designated 'unclassifiable'. The aim of this innovation was to define precise criteria that would permit this group of disorders to be separated from their previous diagnostic context,[5] unifying them in a distinct group of neoplasms; this concept was confirmed in the 2008 WHO classification.[6,7] These clonal myeloid neoplasms are characterised by the simultaneous presence, at their onset, of both proliferative and dysplastic aspects, without the diagnostic criteria for specific MPN or MDS being satisfied. In MDS/MPN, features such as leucocytosis, thrombocytosis and organomegaly are associated with cytopenia and dysplastic maturation, with clinical features resulting from the complications of cytopenias, from leukaemic infiltration and from functional alterations in dysplastic cells. No specific molecular alteration in this group of neoplasms has yet been identified: in particular, the presence of a mutation in the Janus kinase 2 gene, *JAK2* V617F, which is frequent among *BCR-ABL1*-negative MPN, is less often recognised in this group of disorders. Some authors have suggested that they should be included within a larger generic group as 'variant forms of MPN'.[8,9] However, the recent recognition in these neoplasms of mutations in genes such as *RUNX1* and *CEBPA*, which are not observed in MPN, has reinforced the concept of MDS/MPN as effectively representing a group of haematological neoplasms.[10]

It is necessary to specify the diagnostic criteria that are essential for the correct identification of these cases:

1. The diagnosis is based on an evaluation of the peripheral blood and bone marrow; in the absence of adequate material, the diagnosis cannot be made; the blast percentage and a microscopic assessment of the number of promonocytes, which must be considered blast equivalents, is an essential prerequisite for the diagnosis;
2. Dysplasia and increased cell counts must be assessed at the time of diagnosis before patients have received any therapy (including growth factors) capable of modifying the cell counts or the morphological features; the variations in cell counts and morphology that can be observed during the course of treatment of other haematological neoplasms must not lead to an erroneous diagnosis of MDS/MPN;
3. The presence of immunophenotypic abnormalities, with more than one aberrant immunophenotypic marker expressed by blast cells and/or cells of the monocytic lineage can give support for the diagnosis, especially when there is diagnostic doubt[11];
4. Cytogenetic and molecular genetic analysis must always be performed:
   - To exclude the diagnosis of MPN associated with *BCR-ABL1* or rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*, and also for detection of alterations in *NRAS*, *KRAS*, *PTPN11* or *NF1*;
   - To confirm the diagnosis in certain specific contexts, such as alteration of *CBL* in JMML, the presence of *JAK2* V617F in refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T) or of isolated i(17q) indicating a clonal population.[12]

## Chronic myelomonocytic leukaemia

The principal characteristic feature of CMML is the presence in the peripheral blood of neutrophilia associated with monocytosis, with the monocyte count being above $1 \times 10^9$/l (and almost always at least 10% in the leucocyte differential count), which persists for more than three months[13]; this peripheral blood picture is not always associated with bone marrow monocytosis,

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particularly in the cases that present with marked granulocytic expansion.

The first publication of the FAB group, in 1976, gave, in addition to criteria for the diagnosis of acute leukaemia, a description of two specific clinicopathological conditions, distinguished by the presence of myelodysplasia and the frequent evolution to acute leukaemia: refractory anaemia with excess of blasts and CMML,[1] the latter defined by the presence in the peripheral blood of more than $1 \times 10^9$/l monocytes. The subsequent FAB paper from 1982 confirmed the inclusion of CMML in the MDS group,[2] while in 1994 they clarified diagnostic aspects in the differential diagnosis with myeloproliferative disorders.[3,14] The FAB group introduced a further subdivision of CMML into subtype 1 (prevalence of dysplastic features) and subtype 2 (prevalence of myeloproliferative features and a higher incidence of splenomegaly), using the criterion of the degree of peripheral blood leucocytosis, respectively above and below $13 \times 10^9$/l: this criterion was subsequently held to be arbitrary and prognostically inadequate.[15] In the WHO publications,[4,8] this division into two subtypes has been retained, but using as a differentiating criterion the percentage of blasts (including myeloblasts, monoblasts and promonocytes in the peripheral blood and/or the bone marrow), this being confirmed as the major prognostic parameter in these patients:

- In the subtype CMML-1, blast cells are less 5% in the blood and less than 10% in the bone marrow;
- In the subtype CMML-2, blasts cells are either between 5% and 19% in the blood or between 10% and 19% in the bone marrow.[16]

Table 7.1 shows the diagnostic criteria for CMML in the 2008 WHO classification: these include, as well as morphological criteria, the absence of cytogenetic and molecular genetic abnormalities specific for other haematological neoplasms.

A separate subgroup is CMML with hypereosinophilia (peripheral blood eosinophil count at least $1.5 \times 10^9$/l) in the absence of rearrangement of *PDGFRA* or *PDGFRB*: the clinical picture is characterised by a mild anaemia, a tendency to thrombocytosis with large morphologically atypical platelets and the frequency of specific complications related to degranulation of eosinophils.[13,17]

Cytogenetic and molecular investigations have not demonstrated any pathognomonic alteration in CMML, but these studies are fundamental for the exclusion of alterations characteristic of other disorders of a myeloproliferative type. Clonal alterations are found in 20–40% of cases; the most frequent are trisomy 8, monosomy 7, del(7q) and structural alterations of 12p and 20q. Various authors have suggested that cases with an isolated isochromosome of 17p, even if they meet the diagnostic criteria for CMML, should be assigned to the group of MDS/MPN, unclassifiable, both because of the aggressiveness of the clinical course and because of the specificity of the clinicomorphological picture: there is a low percentage of blast cells at diagnosis (<5%), trilineage dysplasia that is particularly marked in the neutrophil series (characteristic hyposegmentation of neutrophil nuclei, ring forms and/or pseudo-Pelger nuclei), expansion of the monocyte series, presence of i(17q) in all myeloid lineages but not in lymphocytes, a very high risk of evolution to acute myeloid leukaemia (AML) and a median survival of 2.5 years.[18] In addition to the dysplasia described above in the neutrophil lineage, dysplasia is also seen particularly in the megakaryocyte lineage.

*RAS* gene mutations have recently been identified in a high percentage of cases. In addition, an association of CMML with systemic mastocytosis has been described in patients with the *KIT* D816V mutation characteristic of this disorder[19]: the frequent association of this clonal mast cell disorder with monocytic leukaemia could be a clue to the existence of a common precursor of these two lineages.

In CMML there is usually expression of antigens characteristic of the myelomonocytic lineage such as CD33 and CD13, associated with a variable expression of CD14, CD11c, CD64 and CD68: immunophenotyping is useful in the confirmation of the diagnosis, rather than as a primary diagnostic investigation. The recognition of an aberrant immunophenotype, such as the hyperexpression of CD56, the expression of CD2 or low expression of HLA-DR, CD13, CD64 and/or CD36, provides useful supporting evidence of the diagnosis, particularly in cases with mainly bone marrow infiltration. In addition, the recognition of an increased percentage of CD34-positive cells or of a clone with an aberrant immunophenotype is useful in the follow-up of these cases and may indicate a likely transformation to AML.[20,21] In around 20% of cases of CMML there is an associated proliferation, observed in histological sections, of plasmacytoid dendritic cells; these are medium to large cells with a round or oval, sometimes indented, nucleus with finely

**Table 7.1** Diagnostic criteria for chronic myelomonocytic leukaemia and its subtypes according to the 2008 WHO classification (reproduced from Orazi 2008,[13] with permission of IARC)

1. Persistent peripheral blood monocytosis $>1 \times 10^9$/l
2. Absence of the Philadelphia chromosome or the *BCR-ABL1* fusion gene or both
3. Absence of rearrangement of *PDGFRA* and *PDGFRB*
4. Blast cells (myeloblasts, monoblasts and promonocytes) <20% in the peripheral blood and the bone marrow
5. Dysplasia of one or more lineages. In the absence of dysplasia, the diagnosis can still be made if one of two alternative criteria is met:
   - If there is a clonal cytogenetic or molecular genetic abnormality in haemopoietic cells, *or*
   - If the monocytosis persists for three months and is not attributable to other causes

**Subtypes**

- CMML-1: Blasts (myeloblasts, monoblasts and promonocytes) <5% in the peripheral blood and <10% in the bone marrow
- CMML-2: Blasts (myeloblasts, monoblasts and promonocytes) ≥5% to <20% in the peripheral blood or ≥10% to <20% in the bone marrow
- CMML with eosinophilia (type 1 or 2 on the basis of blast percentages): absolute eosinophil count at least $1.5 \times 10^9$/l in the peripheral blood

dispersed chromatin and abundant fairly clear cytoplasm[22,23] (see Fig. 7.37). From the morphological point of view, the identification of these cells in preparations stained with panoptic stains, in the context of expansion of the monocyte lineage, can be problematic even if with cytochemical stains they are always negative for myeloperoxidase and esterases. They can be better identified by the phenotype on immunohistochemistry, which is characterised by expression of CD14, CD123, CD68, CD68R, CD4 and granzyme B and absence of expression of lymphoid and myeloid markers (except for variable expression of CD56, CD2, CD5, CD13, CD11c, myeloperoxidase and CD34).[13,23]

The incidence and the prevalence of CMML have not yet been established with certainty, because of the different ways such patients have been classified in case series[23]: estimates vary from three cases per 100 000 individuals over 60 years of age[2] to 0.5 cases per 100 000 individuals in the total population, or 31% of cases of MDS when the incidence of MDS is around 13 cases per 100 000 individuals.[24] The median age at diagnosis varies from 65 to 75 years, with a male predominance of about 2:1; the frequency is higher in the western hemisphere.

CMML is a morphologically and clinically heterogeneous disorder, which is sometimes an incidental diagnosis following a report showing monocytosis. On other occasions there are presenting symptoms that result from the cytopenias (asthenia, infections and, rarely, haemorrhage), or physical examinations shows hepatomegaly, splenomegaly or both, these being detected at disease presentation in around 30–50% of patients, particularly in the group that is characterised from the outset by leucocytosis; leukaemic infiltration of the spleen is in the red pulp. Extramedullary localisation, such as lymphadenopathy, cutaneous infiltration, involvement of serous cavities and gum infiltration, is less frequent, but is seen more often in patients with leucocytosis, who can also present with symptoms of hypercatabolism such as weight loss, fever and night sweats. Lymph node infiltration is described particularly with blastic transformation and in cases with infiltration by plasmacytoid dendritic cells. Cases of CMML following cytotoxic chemotherapy for solid tumours have been described. The survival of patients with CMML is fairly heterogeneous in published series, varying from one to more than 100 months with an estimated median survival of 20–40 months[25,26]; around 15–30% of patients die following transformation to AML, often with a monocytic component (FAB M4 or M5).

## Peripheral blood

Anaemia and thrombocytopenia are common, but are rarely severe at presentation. The white blood cell count (WBC) is very variable, from normal values or even reduced (in rare cases with neutropenia) to marked leucocytosis similar to that seen in CML; in the majority of cases, however, the count at diagnosis is between 4 and 50 $\times$ $10^9$/l (see Report 7.1). Leucocytosis tends in general to increase during the evolution of the disease, often in parallel with increasing splenomegaly. The red cells may be normal or there may be macrocytosis, nuclear remnants or other signs of dyserythropooesis; similarly, the platelets show, although not always, anisocytosis and dysmorphism typical of dysplasia. It is not rare, particularly in cases with anaemia and dyserythropoiesis, to see occasional circulating erythroblasts.

By definition, the absolute monocyte count is above 1 $\times$ $10^9$/l: the frequent morphological anomalies will be described in the following paragraphs. In around 50% of cases, the monocytosis is accompanied by neutrophilia. The circulating neutrophils, in some cases very numerous, can appear normal or have morphological alterations of a dysplastic type, such as hypogranularity or either defective or increased nuclear lobulation, including a pseudo-Pelger appearance; some neutrophil myelocytes and metamyelocytes can almost always be found, particularly if the edges of the blood film are searched, but they are almost always less than 10%. Basophils and eosinophils can be absent, normal or somewhat increased. Patients who present with eosinophilia (at least 1.5 $\times$ $10^9$/l) require specific evaluation: as already indicated, before making a diagnosis of CMML with eosinophilia, the presence of rearrangement of *PDGFRA* or *PDGFRB* must be excluded.[17]

An increase of lysozyme in the serum and urine is common and correlates with the monocyte count. It is quite common for CMML to be associated with immunological anomalies, the most frequent of which is a polyclonal increase in serum immunoglobulin concentration, which is found in 30% of cases; in 5–10% of patients there is a monoclonal paraprotein and it is not rare to identify autoantibodies, cold agglutinins and Coombs positivity.

### Monocytes, promonocytes and blast cells

The typical picture that is observed in the stained blood film of a patient with CMML is that of a particular abundance of monocytes (Figs 7.1 through to 7.5), often more numerous in the tail and along the edges of the film, generally associated with neutrophilia (particularly when the leucocyte count is increased), together with a small number of lymphocytes and occasional myelocytes and metamyelocytes. However, the haematological picture at onset is very heterogeneous, varying from a simple isolated monocytosis, without anaemia or thrombocytopenia, to cases with very evident myeloproliferative features, with a very high WBC and the presence in the circulation of numerous immature neutrophils and monocytes. The appearance of the monocytes is often not very different from that of normal mature monocytes, even if the size is sometimes larger than normal; the voluminous nucleus can have an irregular and contorted form with hyperlobulation, which sometimes means that these cells resemble very large hypogranular neutrophils; the chromatin network can be spongy, paler and more delicate than normal, rarely with nucleoli being unusually evident in cells that otherwise appear mature. The cytoplasm is always abundant and of a blue-grey colour with a sandy appearance as the result of the presence of very fine azurophilic granules; sometimes these granules are absent or not apparent with an optical microscope and the cytoplasm is pale blue, like that of a lymphocyte;

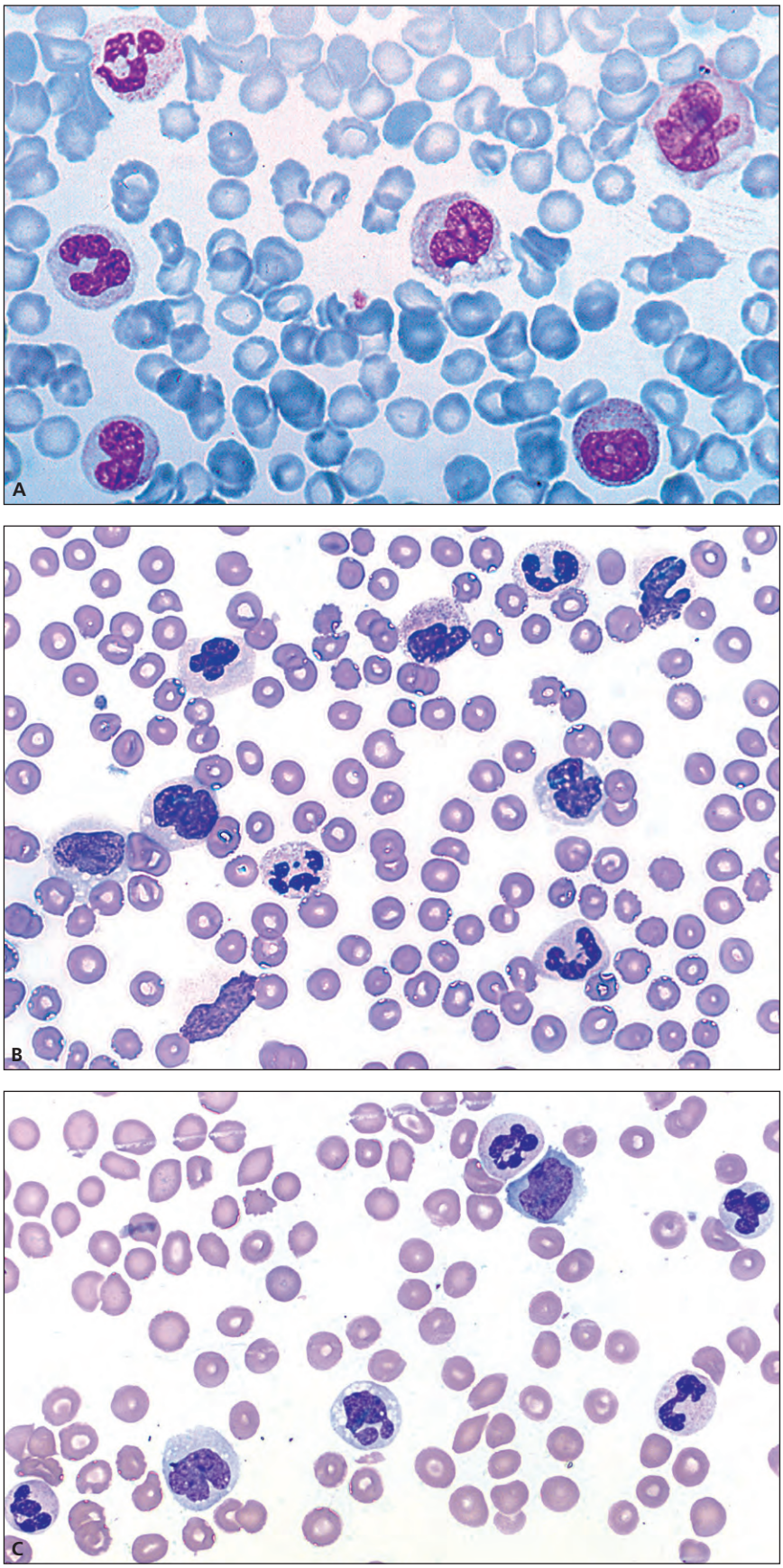



**Fig. 7.1** Chronic myelomonocytic leukaemia (CMML); peripheral blood of different patients at presentation. (A) Four monocytes with heterogeneous nuclear morphology. Top left, a well-formed mature neutrophil; bottom right, a neutrophil myelocyte. (B) From the left towards the centre, two monocytes with cytoplasmic vacuoles, a neutrophil with a nuclear appendage (of drumstick or Barr body type), at the top another monocyte and, at the bottom, another dysplastic neutrophil with the nucleus not completely segmented and hypogranular cytoplasm. Top left, a cell that cannot be unequivocally identified: both the greyish cytoplasm without evident granules and the nuclear shape could suggest a monocyte; the compact structure and large clumps of chromatin, however, suggest a dysplastic neutrophil. (C) Three cells of monocyte lineage; the one in the centre has bizarre nuclear morphology and cytoplasm that appears lace-like because of the presence of numerous vacuoles. The nucleocytoplasmic (N:C) ratio is low, as is typical of the monocyte lineage. At the edges, four neutrophils with heterogeneous segmentation; the one furthest to the right is hypogranular and non-segmented and could be mistaken for a monocyte if one had not noted the chromatin structure which is totally analogous to that of the other neutrophils.

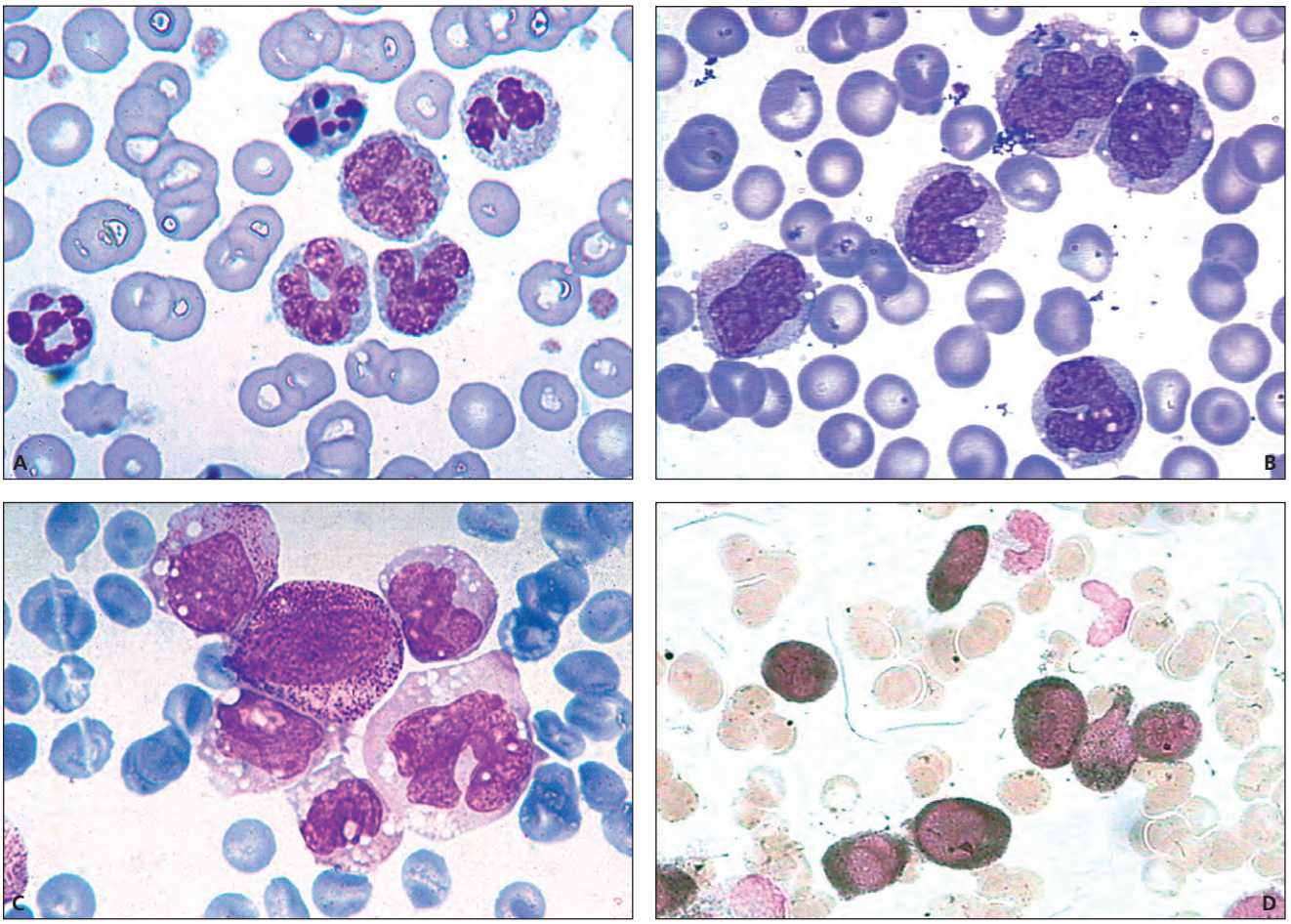



**Fig. 7.2** Heterogeneity of monocytes in different cases of CMML: these could seem a 'caricature' of normal monocytes because of the accentuation of the morphological characteristics; peripheral blood film. (A) The nuclei of the three central monocytes appear convoluted, almost multilobated. The two neutrophils are dysplastic. Top centre, an apoptotic cell, the nucleus of which is divided into small dark pyknotic masses; it is difficult to establish its original nature because of the tendency to lobulation of all the other cells, even if the resemblance to the neutrophil at the left makes it probable that it is a neutrophil. (B) Cells of monocyte lineage with variable curvature and indentation of the nuclear outline. The chromatin is open and spongy with internal folds in the two cells top right. In the cell on the left, the chromatin is more worm like, with the remains of a nucleolus. The sandy appearance of the cytoplasm is characteristic. (C) Three atypical monocytes, two promonocytes (at the top) and a neutrophil myelocyte. The mature monocytes are similar to each other with delicate chromatin and greyish cytoplasm with very fine azurophilic granules and heterogeneous vacuoles; one of these cells is double sized and has a tetraploid horseshoe-shaped nucleus. In the centre of the small group, an early neutrophil with a disorderly distribution of the numerous cytoplasmic granules. The cell top left, which could be classified erroneously as a myelocyte, is a promonocyte, as indicated by the grey vacuolated cytoplasm, similar to that of the monocytes but with more prominent bright red granulation and a chromatin pattern of fine dots, with a pale nucleolus with subtle condensation of the surrounding chromatin. The other promonocyte does not have prominent granules and its chromatin is forming a fine network. (D) Clear positivity for alpha-naphthyl acetate esterase in the cytoplasm of the leukaemic monocytes.

in other cases the azurophilic granules are less numerous, but are larger and stand out on the weakly basophilic cytoplasmic background; vacuoles are frequent, sometimes abundant. The peroxidase content of monocytes in CMML is usually normal or slightly reduced: these cells are generally classified either as monocytes or as LUC (large unstained cells) by automated counters based on automated cytochemistry.

The subdivision of monocytes and their precursors on the basis of morphology is not always easy, but is of fundamental importance, representing the principle diagnostic criterion in CMML. At present, four stages of morphologically recognisable maturation are acknowledged: monocytes, immature or atypical monocytes, promonocytes and monoblasts (Table 7.2).[27] In addition to mature monocytes, it is not rare in some patients to find monocytes with anomalous granulation or variable cytoplasmic basophilia or cells that are smaller than normal or that show heterogeneity in the folding and lobulation of the nucleus; the nucleus may have composite and non-homogeneous

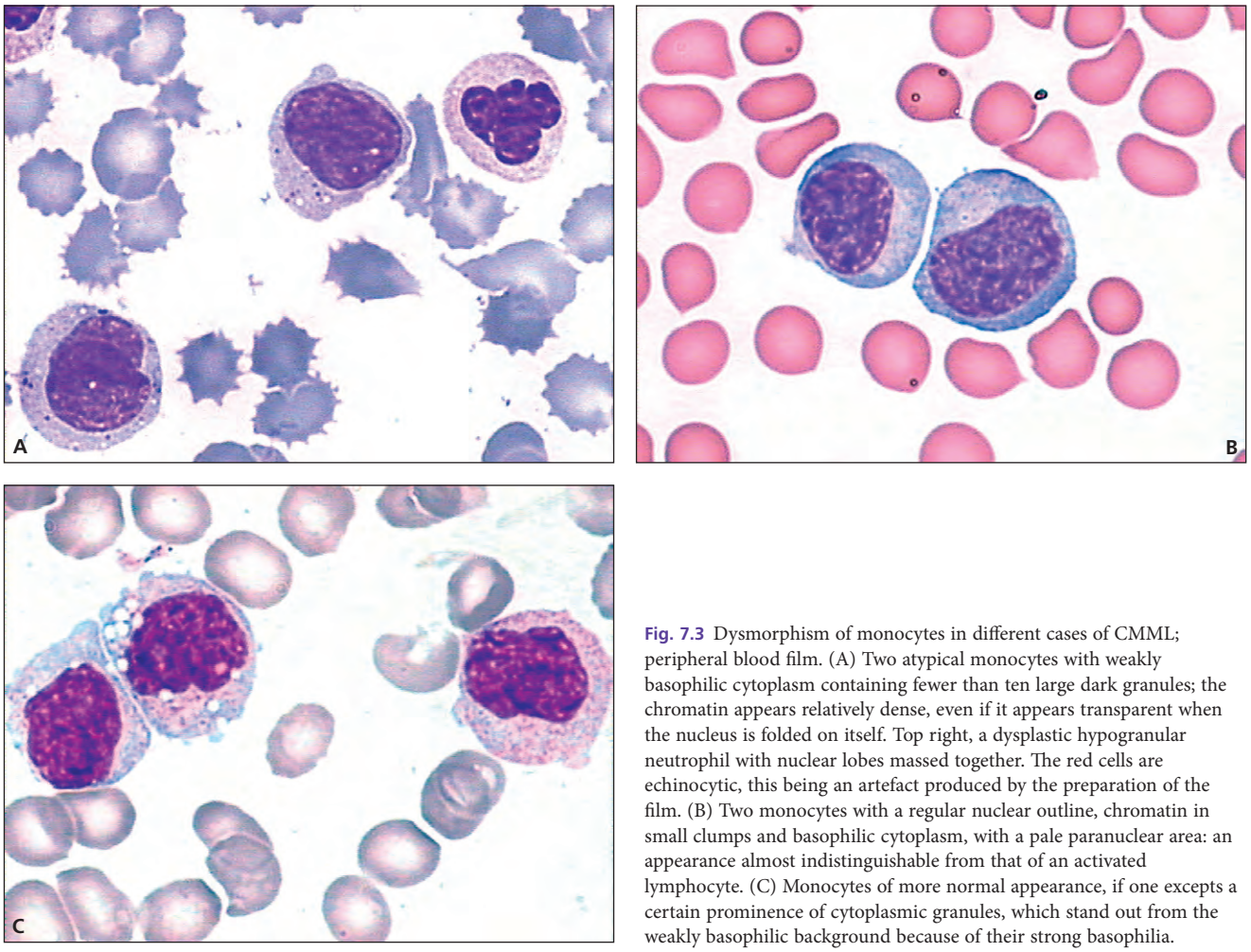



**Fig. 7.3** Dysmorphism of monocytes in different cases of CMML; peripheral blood film. (A) Two atypical monocytes with weakly basophilic cytoplasm containing fewer than ten large dark granules; the chromatin appears relatively dense, even if it appears transparent when the nucleus is folded on itself. Top right, a dysplastic hypogranular neutrophil with nuclear lobes massed together. The red cells are echinocytic, this being an artefact produced by the preparation of the film. (B) Two monocytes with a regular nuclear outline, chromatin in small clumps and basophilic cytoplasm, with a pale paranuclear area: an appearance almost indistinguishable from that of an activated lymphocyte. (C) Monocytes of more normal appearance, if one excepts a certain prominence of cytoplasmic granules, which stand out from the weakly basophilic background because of their strong basophilia.

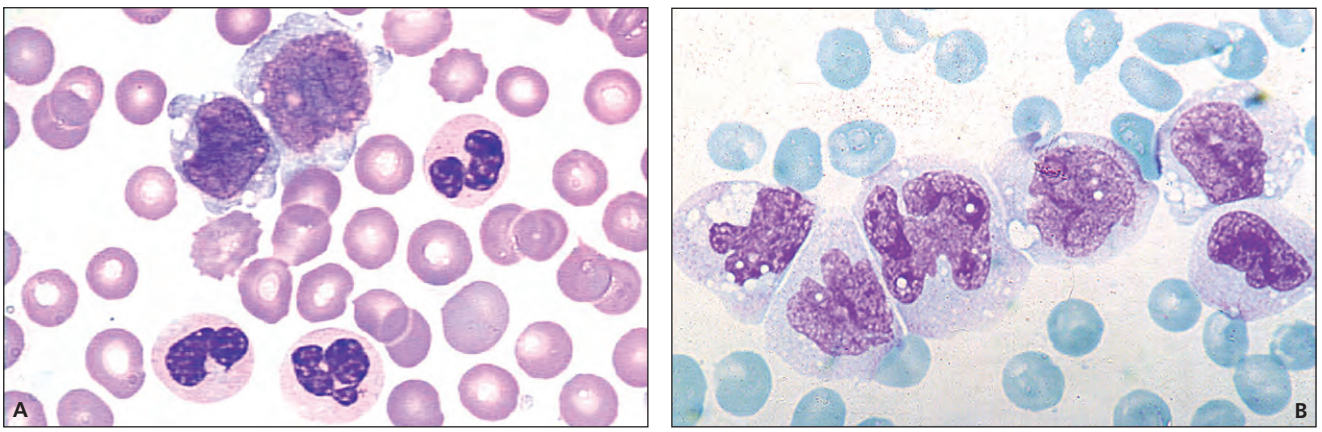



**Fig. 7.4** Dysmorphism of cells of monocytic lineage in different cases of CMML; peripheral blood film. (A) Bottom right, three hypogranular neutrophils with nuclear irregularity. Top, a monocyte and a larger promonocyte with fine chromatin and nucleoli, both of these cells having heavily vacuolated cytoplasm. (B) These atypical monocytes also show the moderate morphological anomalies that are often observed in this disorder. The cytoplasm is pale, with only a minimal basophilic tinge, and is perforated by numerous vacuoles of diverse sizes (the vacuolation tends to increase if there is prolonged storage of the EDTA-anticoagulated blood specimen). Azurophilic granules are apparent towards the periphery of some cells. The nuclei have a very irregular and convoluted outline; the chromatin is pale with the appearance of a loosely-woven net, like blown glass; one can see the remains of nucleoli.

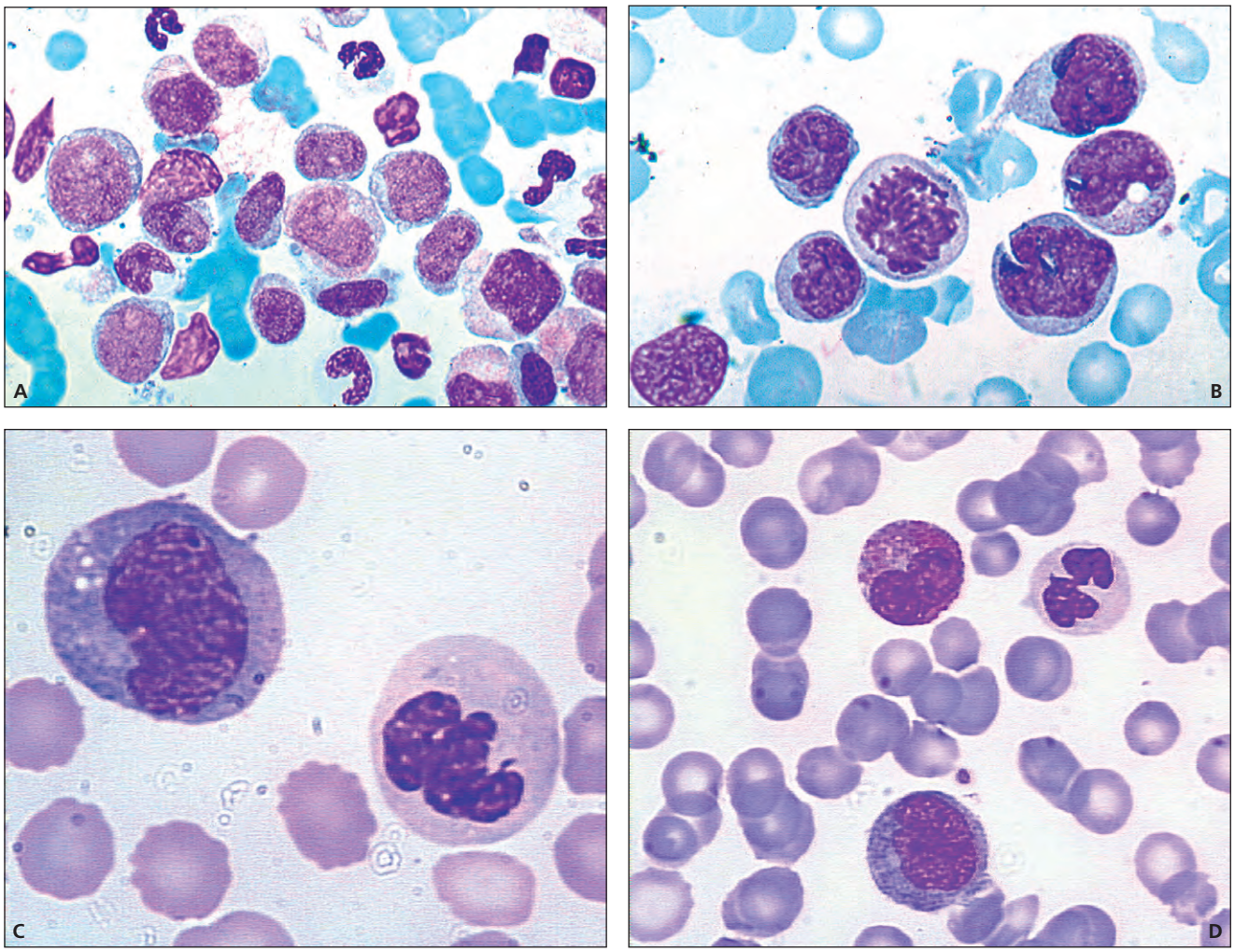



**Fig. 7.5** Neutrophil dysplasia and monocytosis in the blood films of patients with CMML. (A) From a case with marked leucocytosis and 16% circulating blast cells. On the left, two monoblasts with basophilic cytoplasm, regular oval nuclei, very fine chromatin and single prominent nucleoli. In the centre, between other smaller blasts, a promonocyte with a similar nucleus and chromatin pattern, but with an indented nuclear outline and fine cytoplasmic granules. Close to the edges of the image there are numerous dysplastic neutrophils with agranular cytoplasm. (B) Four monocytes with moderate nuclear atypia, a mitotic figure and an immature granulocyte precursor, still with a nucleolus, in which the Golgi zone has been transformed into a vacuole in the centre of the cell. (C) An immature monocyte with worm-like chromatin and an agranular neutrophil with a poorly segmented nucleus and chromatin condensed into atypical large clumps. (D) From the same film as (C), at the bottom, a monocyte; at the top, an eosinophil and, on its right, a dysplastic neutrophil.

**Table 7.2** Morphological characteristics identifying the four type of cell of monocyte lineage[27]

| | Monoblast | Promonocyte | Immature monocyte | Mature monocyte | Atypical monocyte |
|---|---|---|---|---|---|
| Nucleus | Round or oval | Convoluted, indented | Convoluted, indented | Lobulated, indented | Variably indented, folded |
| Chromatin | Delicate and finely dispersed | Delicate and finely dispersed | Intermediate density | Condensed | Less delicate and dispersed than in promonocytes |
| Nucleolus | Prominent | Prominent | Rare | Absent | Indistinct/absent |
| Cytoplasm | Basophilic with possibly rare azurophilic granules | Basophilic with variable azurophilic granules | Variable basophilia | Grey, granular, basophilia often with vacuoles | Grey and granular |
| Size | 20–30 μm | Variable | Larger than mature monocytes | 20–25 μm | Variable |

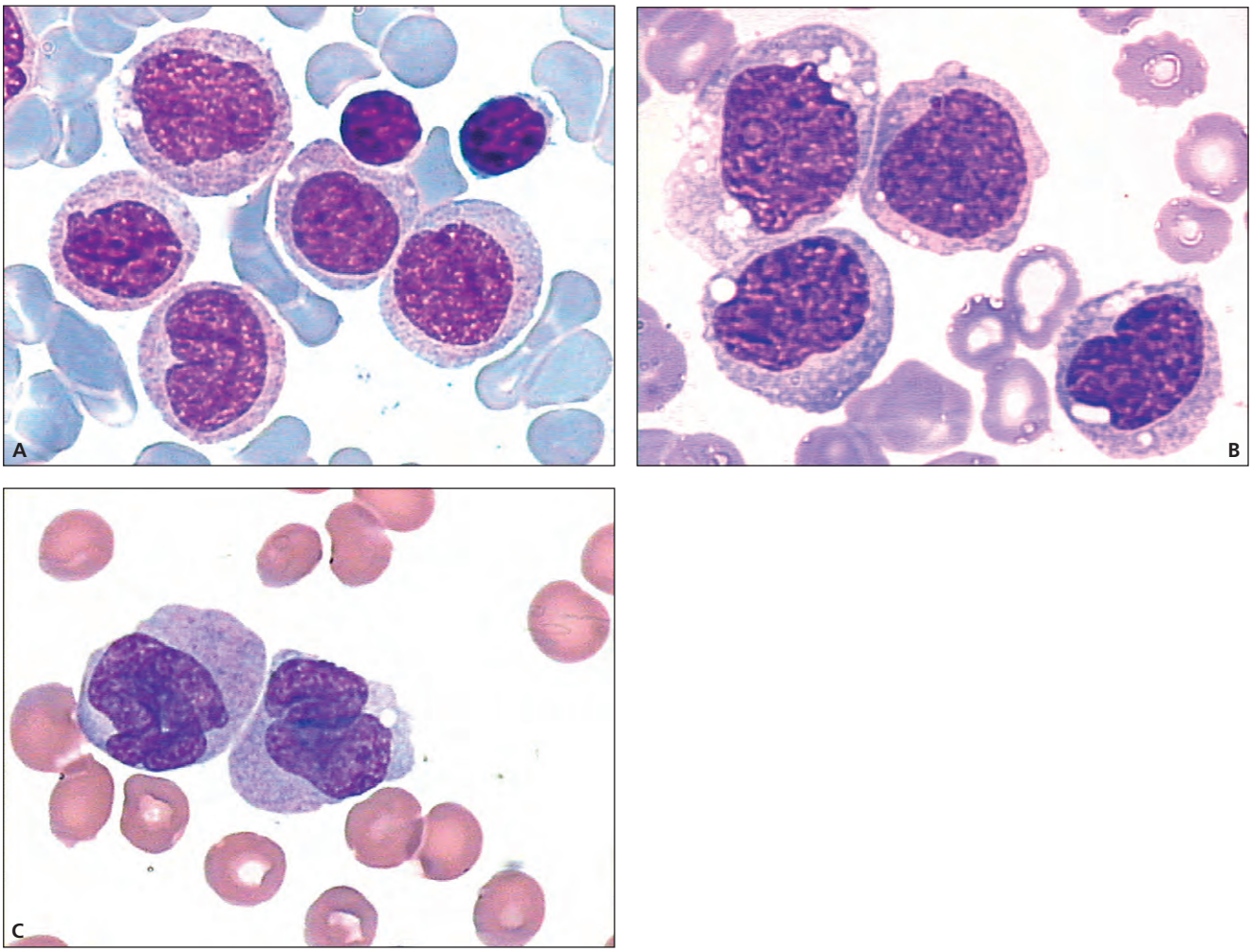



**Fig. 7.6** Monocytes with atypical morphology in CMML; peripheral blood film. (A) There is ample cytoplasm with areas of weak basophilia, particularly at the periphery, and numerous azurophilic granules of various sizes: the appearance is typically sandy. The nuclei have a fairly regular outline with indentations of variable depth and spongy chromatin with numerous small compact masses alternating with dot-like non-stained spaces. Top right, two small lymphocytes with a nucleus with large, quite compact chromatin masses. (B) The morphology of these four atypical monocytes, analogous to those of (A), is common in the indolent phase of this disorder. In some nuclei, with an outline that is barely notched, there are incomplete nucleolar remains. The cytoplasm is blotchy, with basophilic and acidophilic areas and vacuoles. (C) Differing from the previous cells, this atypical monocyte has a very convoluted nucleus and fairly mature chromatin. There is ample cytoplasm and, as usual, there is a background basophilia on which are superimposed a myriad of fine red granules.

chromatin, tending however to be compact, with nucleolar residues being rare, thus differing from the stippled, reticular or loose structure of the chromatin of promonocytes and monoblasts. Cells of this type can also be seen in non-neoplastic monocytosis, such as that observed in some infections, in bone marrow recovery after suppression of haemopoiesis and during treatment with growth factors.[28] These are designated immature monocytes[27] or, according to the suggestion of the 2008 WHO classification,[13] atypical (abnormal) monocytes (Figs 7.2 through to 7.8). From the diagnostic point of view, attention must be focused on the identification of promonocytes, which are of cardinal cytomorphological importance, particularly with regard to the simple differentiation between CMML and AML with a monocytic component.

The FAB group suggested a series of cytomorphological features in the peripheral blood film that would enable a distinction to be made between CMML and other chronic myeloid leukaemias: monocyte count above $1.0 \times 10^9$/l, basophils below 2%, dysplastic neutrophils, the possible presence of immature granulocytes but below 10–15% and of blast cells below 2–5%.[3] The 2008 WHO classification, in addition to specifying new criteria of prognostic and therapeutic relevance (see Table 7.1), introduced a new morphological requirement, which, although still debated, can be considered fundamental and applicable to

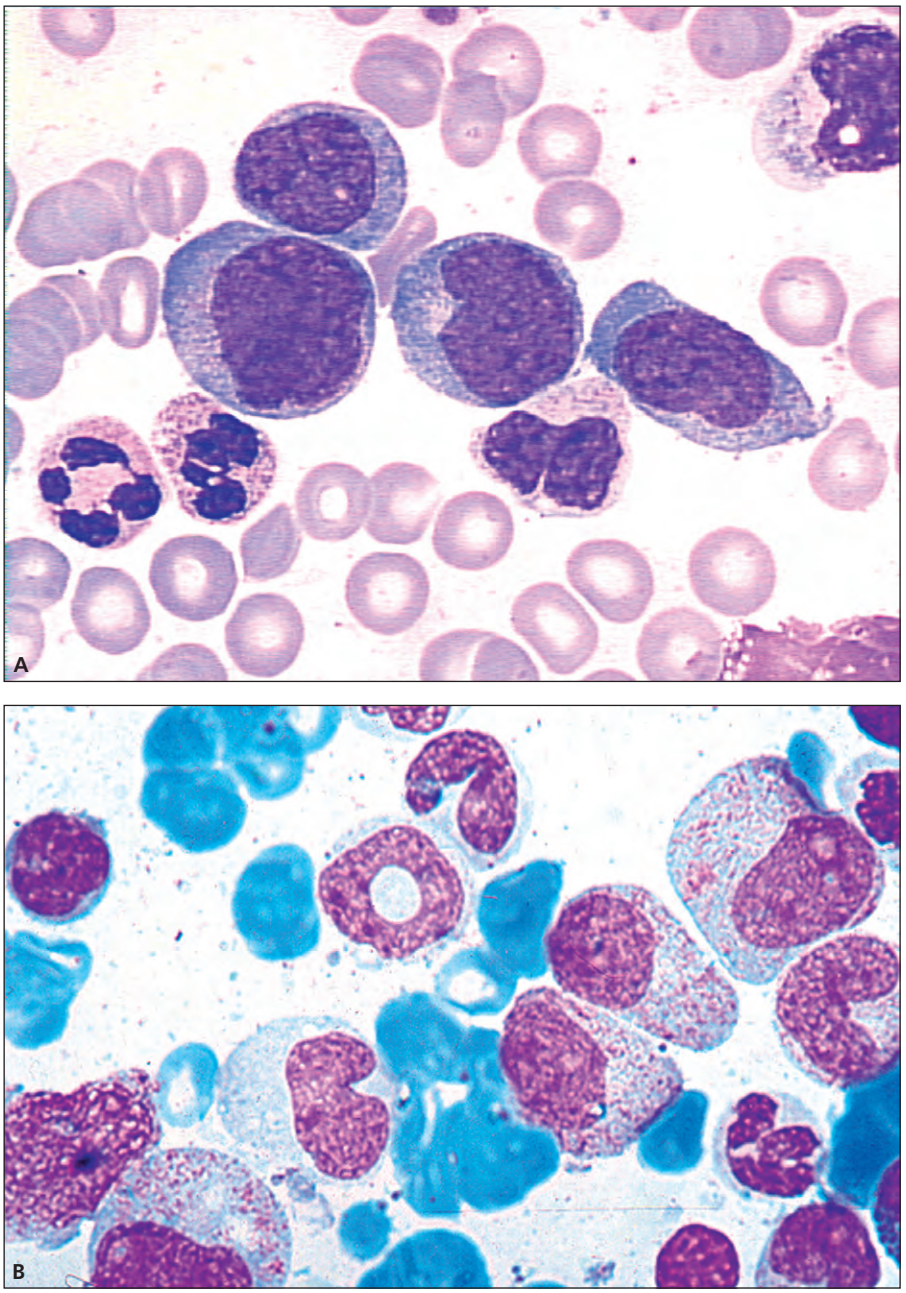



**Fig. 7.7** Morphology of promonocytes in CMML; peripheral blood film. (A) The three large cells in the central band can be defined as promonocytes (and thus will be included in the blast count): they have basophilic cytoplasm with fine granules, clear but not very deep nuclear notches and granular chromatin, not clumped, in which delicate nucleoli can be detected. The cell immediately above is an atypical monocyte. (B) In the top right corner, a promonocyte characterised by prominent granules (a feature that sometimes distinguishes promonocytes), fairly tightly woven reticular chromatin and nucleoli. The two smaller cells, to its left, also should be classified as promonocytes, particularly because of the appearance of the chromatin. Above in the centre, a monocyte with a ring nucleus. Bottom right, a dysplastic neutrophil.

all haematological neoplasms with monocytic expansion: an accurate characterisation of promonocytes and their inclusion in the blast cell count. To make a diagnosis of CMML, according to the 2008 WHO classification, the sum of myeloblasts, monoblasts and promonocytes in the peripheral blood and the bone marrow must be less than 20%. Whenever this threshold is reached or exceeded, the diagnosis of AML must be made. It is thus evident that, with the aim of unequivocally stratifying patients at diagnosis, the precise morphological criteria for categorising cells as blast cells must be respected, distinguishing monoblasts and promonocytes from immature or atypical monocytes and from mature monocytes, which must be

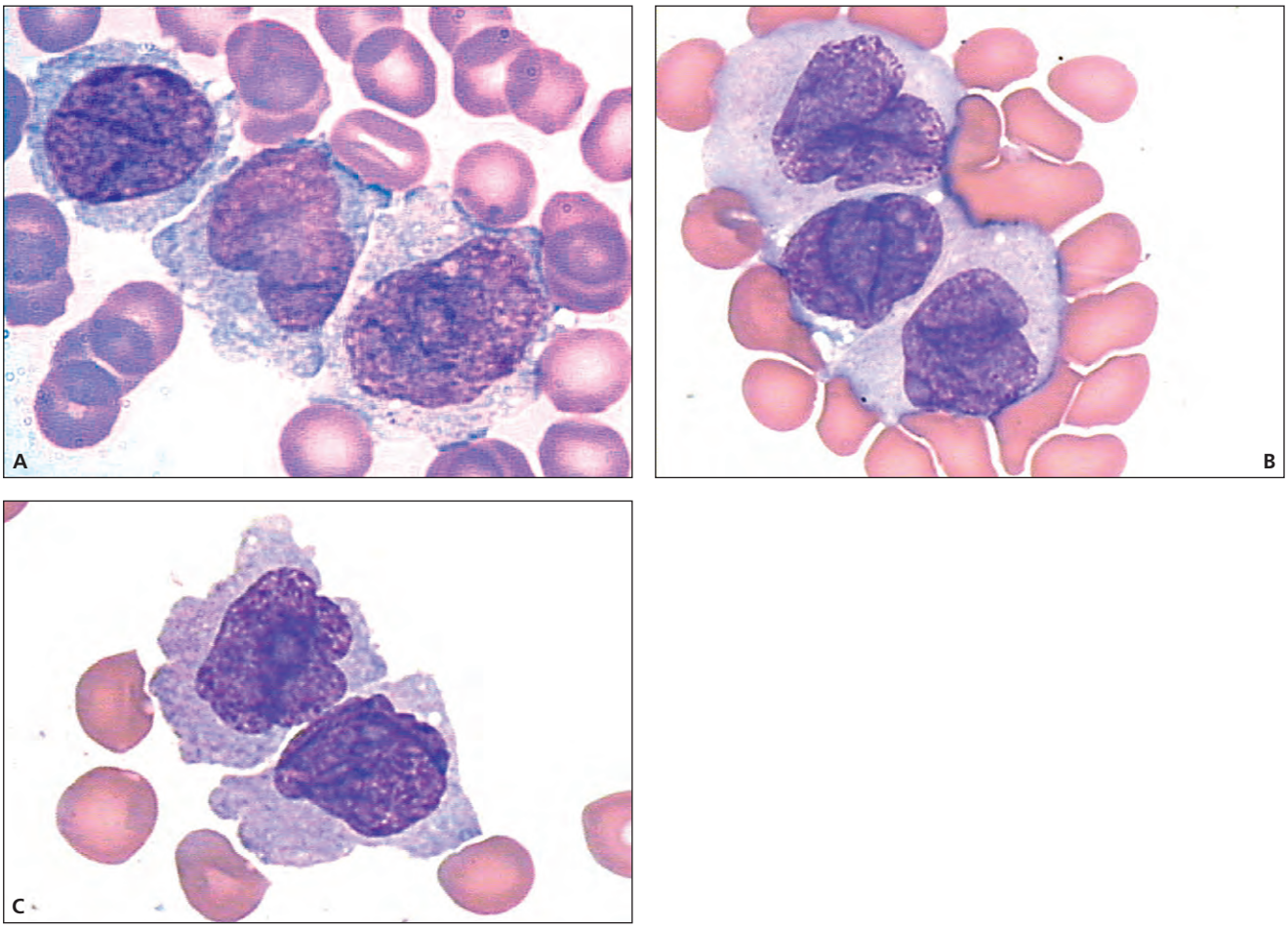



**Fig. 7.8** Morphology of promonocytes and atypical monocytes in CMML; peripheral blood film. (A) Two promonocytes, centre and right, with a nucleus that is folded on itself and has chromatin that is fine, loose and delicately spongy; the nucleoli are incomplete and not very distinct, but the immaturity of these cells is evident. The nuclear outline is tortuous in the cell at the centre, while that below and to the right has some straight sections. The cytoplasm is basophilic and dusty with small peripheral fringes and fine granules visible particularly in the bottom right cell. Top left, an atypical monocyte, with denser and more folded chromatin. (B) The nuclear chromatin in these promonocytes is spongy, open, transparent, letting the folding of the nuclear material and some pale blue nucleoli be seen. The nuclear outline is twisted and the ample cytoplasm is pale and blue-grey with acidophilic blotches (same patient as Fig. 7.6C). (C) From the same film as (B), two cells that are similar to the preceding ones but with denser chromatin, which suggests they should be classified as atypical monocytes. The nucleoli are still visible, but inclusion of these cells in the blast count would be inappropriate.

excluded from the blast count[13,27] (Figs 7.8, 7.9 and 7.10). This highlights how in diagnostic microscopy, attention must be paid to the specific cytomorphological features of individual cells and also to the context, a comparison being made to other cells that are 'similar' but not identical, with the knowledge that recognising the similarities but detecting the differences is the key to categorisation. In particular, monoblasts and mature monocytes, which are found at the two extremes, can be recognised fairly easily, since they have morphological characteristics that are unequivocal and thus recognisable.

Mature monocytes have been described previously; monoblasts are usually large, with cytoplasm that is usually abundant and weakly basophilic, a nucleus that is regular and round or oval, dispersed, fine and delicate chromatin and often evident nucleoli. Between these two extremes one must consider the other less typical cells of the monocyte lineage, evaluating precisely their shape and structure. In particular, delicate and finely dispersed chromatin, regardless of the shape of the nucleus, the colour of the cytoplasm and the possible presence of granules, identifies a promonocyte, which in this context must be included in the blast count, together with granular and agranular myeloblasts and monoblasts. Put simply, promonocytes can be described as cells that have the chromatin structure and nucleolus of a blast cell, while the nuclear shape may be irregular and the cytoplasm may have staining characteristics more similar to those of monocytes, and can contain granules that are more evident than those of mature monocytes.

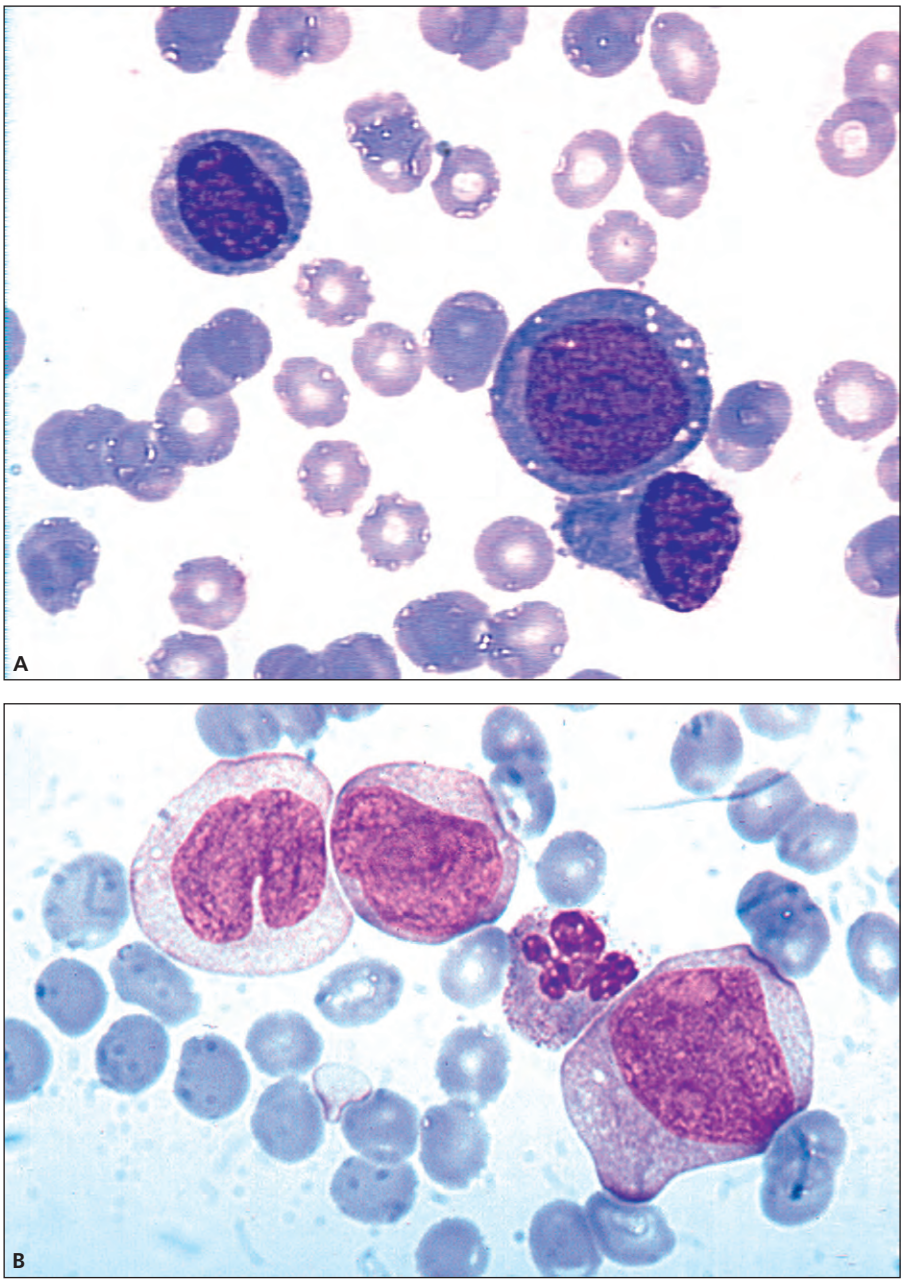



**Fig. 7.9** Morphology of monoblasts and promonocytes in CMML. (A) The larger cell is a monoblast with characteristic morphology: the N:C ratio is low with respect to blast cells of other types, but is higher than in promonocytes and monocytes; the nuclear profile is regular with a circumference showing only minimal angulation; the chromatin forms a loose network with a very fine weave; nucleoli are easily seen; the cytoplasm is basophilic with small vacuoles. Top left, an atypical monocyte. (B) The large cell bottom right, with two prominent nucleoli, has characteristics intermediate between a monoblast (delicacy of the chromatin network) and a promonocyte (clear nuclear angulation and folding of the nucleus visible as a dark line, bottom right). Towards the left, a hyperlobated dysplastic neutrophil, a promonocyte and a large atypical monocyte, with a nucleus that has light chromatin, like blown glass, and ample pale cytoplasm.

## Bone marrow

The bone marrow aspirate in CMML is always hypercellular (Fig. 7.11). The population that predominates is the neutrophil series, often with a left shift of the maturation curve and the presence of a variable number of blast cells, between 5% and 19%. The percentage of monocytes and promonocytes is always increased (Figs 7.12 through to 7.16), but generally relatively less than is observed in the peripheral blood, so that the neutrophil:monocyte ratio is almost always greater in the marrow than in the blood. Cytochemical staining for non-specific

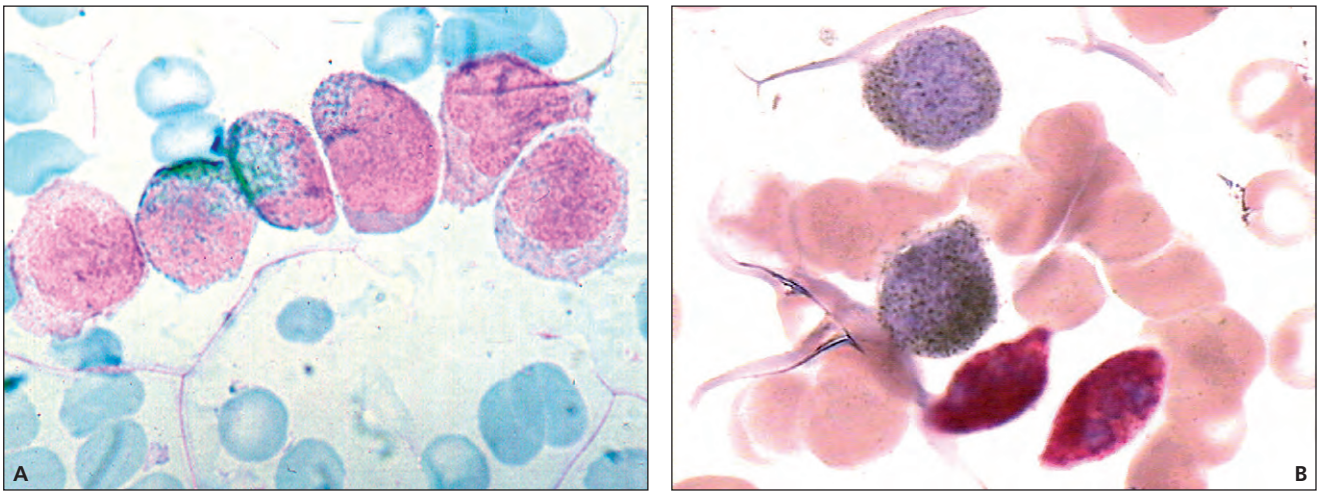



**Fig. 7.10** Cytochemistry of cells of monocyte lineage in CMML. (A) Peroxidase reaction: the positivity is heterogeneous. Monoblasts, such as that on the right, are usually negative. (B) Double esterase stain: the monocytes and their precursors show the typical diffuse black granular cytoplasmic positivity for alpha-naphthyl acetate esterase; two neutrophils, on the other hand, show the red staining indicating positivity for chloroacetate esterase.

esterase is clearly positive (see Figs 7.2C and 7.10), whereas peroxidase is negative or weakly positive, this being useful to demonstrate clearly the increase in the monocytic component. In cases with peripheral eosinophilia, there is an increase in eosinophil precursors. Possible, but infrequent, is a slight increase in basophils.

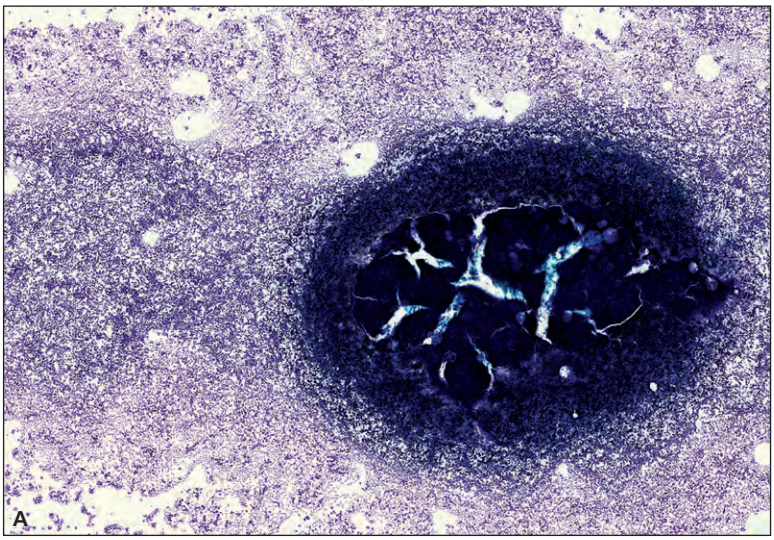
Because of the abundance of neutrophil precursors and the more minor monocytic component, the bone marrow appearance of many patients with CMML can be more difficult to distinguish, in comparison with the peripheral blood, from *BCR-ABL1*-positive CML, at least when there is no evident dysplasia, or from aCML. For this reason, the cytomorphological diagnosis of MDS/MPN and MPN must be based, more than in other conditions, on an accurate morphological assessment of the peripheral blood. The erythroid series, on the other hand, is in general better preserved in CMML than in CML, with the percentage of erythroid cells generally being above 10% and the myeloid:erythroid (M : E) ratio being less elevated than in CML.

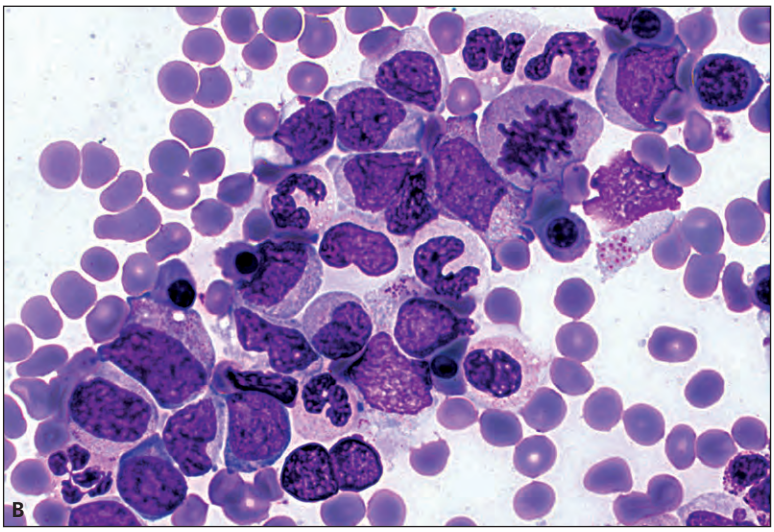
There is great variability between patients with regard to the frequency and severity of dysplasia in bone marrow precursors of granulocyte, erythroid and megakaryocyte lineages (Figs 7.17 through to 7.20): this ranges from cases in which dysplasia is virtually absent to cases that have so much dysplasia that they are close to refractory anaemia with excess blasts (RAEB). Trilineage dysplasia is more marked in cases with a lower leucocyte count. In the majority of patients, the morphological changes that identify dyserythropoiesis are megaloblastosis, nuclear protrusions and the presence of ring sideroblasts. The megakaryocytes are generally small with a tendency to hypolobulation[29]; micromegakaryocytes are particularly frequent. In a third of patients, bone marrow trephine biopsy, which is always diagnostically useful, shows fibrosis or variable grades.

## Prognostic aspects and differential diagnosis

There is variability in many clinical, haematological and morphological aspects of CMML: the degree of leucocytosis, the severity of dysplasia and the length of survival; there are forms that are rapidly progressive and others that show little disease evolution. This has led many authors to suggest that, although monocytosis is a unifying element, various pathological entities are included under the designation of CMML: some are characterised by the prevalence of a myeloproliferative component, as shown in the monocytosis, the hyperleucocytosis and the presence of an increased percentage of immature granulocytes or blast cells[14]; conversely, in others the myelodysplastic element predominates, identifiable from morphological alterations and a tendency to pancytopenia. According to other authors, however, one is not dealing with homogeneous separate entities, but with a continuous spectrum of haematological disorders with characteristics that vary from solely myelodysplastic features to a chronic myeloid leukaemia with intermediate cases with mixed features.[30] The subdivision of cases on the basis of the leucocyte count, with the identification of two distinct groups, respectively designated MDS type and MPN type, has recently been reproposed as a valid prognostic indicator,[31] the MPN type, with more marked leucocytosis, being characterised by a tendency to progression and a shorter survival.

The variability of the clinical course of CMML means that it is very important to find prognostic factors that can identify the cases in which a particularly aggressive course can be predicted in order to plan more efficacious treatment strategies. Evolution to myelofibrosis is rare. The most important morphological feature associated with a short survival is the percentage of blast cells in the peripheral blood and bone marrow at diagnosis.[17,29,32] Anaemia and more severe thrombocytopenia, more marked leucocytosis, a younger age and marked splenomegaly are other



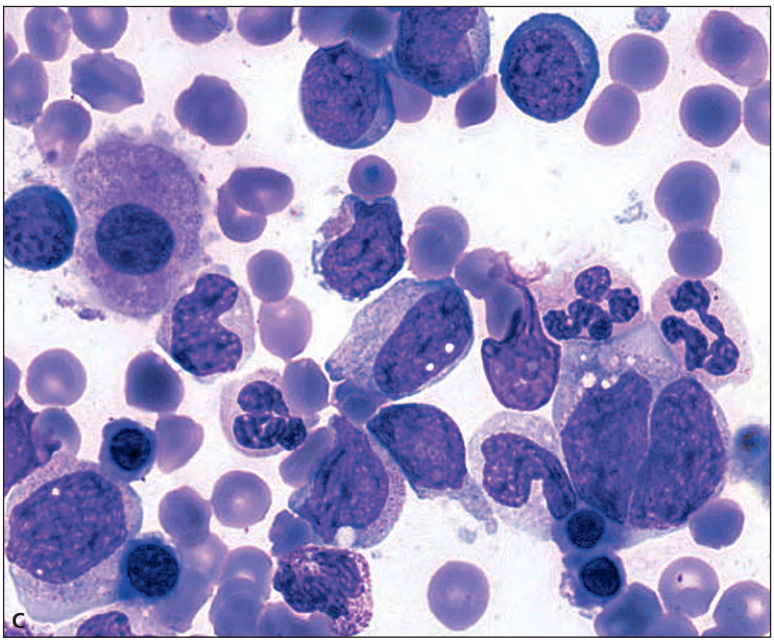
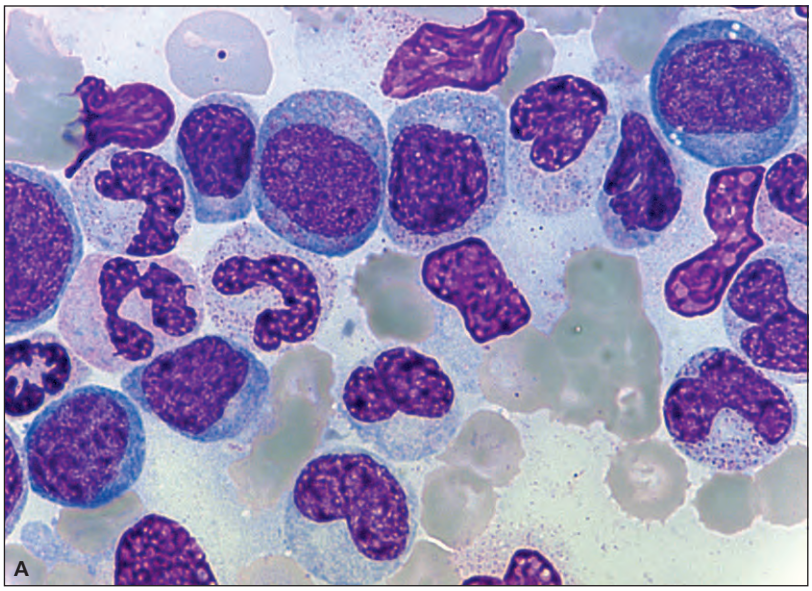
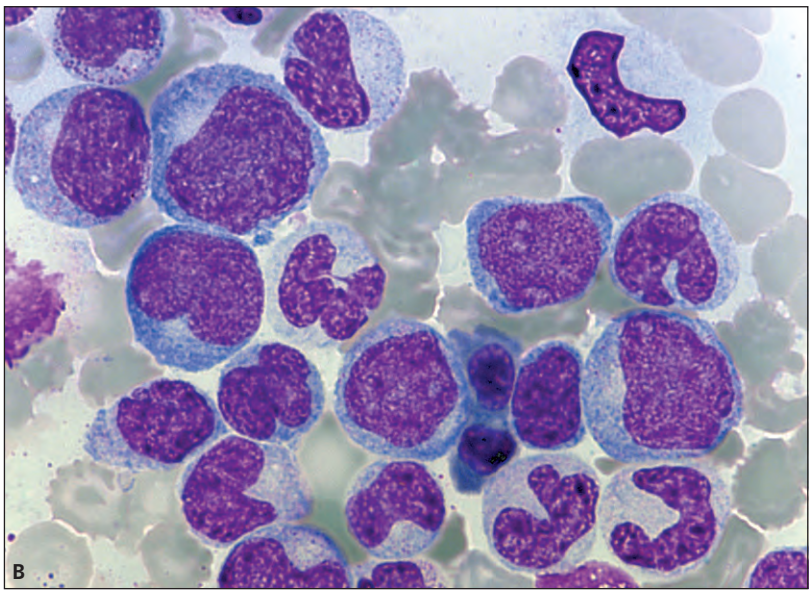




**Fig. 7.11** Bone marrow aspirate in a case of CMML. (A) Hypercellular fragments close to the tail of the film. The marrow appears very cellular in the surrounding area. (B) In this group of cells, numerous monocytes, mainly atypical (such as the two high in the centre), various dysplastic neutrophils (such as the two high up immediately to the right of the preceding cells, recognisable from the clumping of their nuclear chromatin), some small blast cells (such as the two cells low on the left, to the right of a five-lobed neutrophil and separated by an atypical monocyte), a basophilic erythroblast (top right corner), two polychromatophilic erythroblasts and three orthochromatic erythroblasts. (C) To the left, a micromegakaryocyte: a not unusual observation, together with other aspects of dysmegakaryopoiesis, in this disorder. High in the centre, three blast cells (one cut off at the edge) with scanty basophilic cytoplasm. Also various atypical monocytes, with ample grey-blue cytoplasm containing easily seen azurophilic granules, and another blast cell; one of the promonocytes, on the right, is large and binucleate.



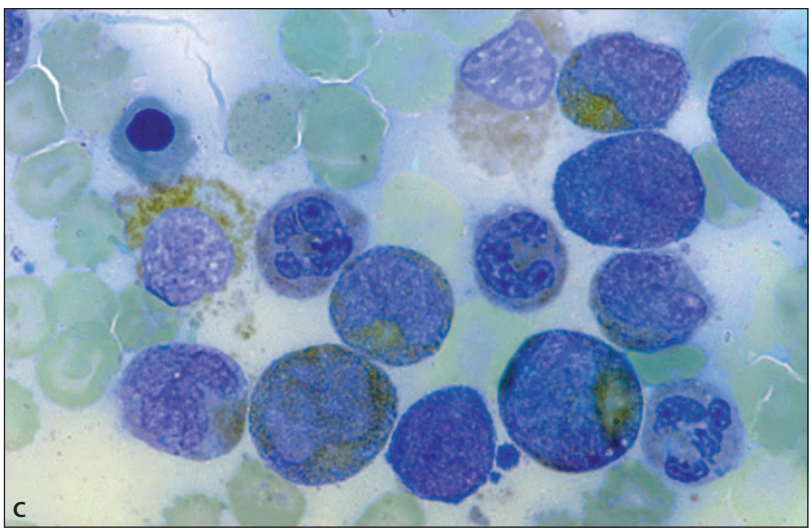




**Fig. 7.12** CMML; bone marrow aspirate. The cellularity of the specimen causes crowding together of cells that are not cytologically very dissimilar – hypogranular neutrophils and atypical monocytes – making detailed cytological analysis rather difficult. Often the morphology of monocytes is more readily appreciated in peripheral blood films in which they are better spread. (A) In the upper band, from left to right, a monocyte with pale granular cytoplasm (immediately below which are two neutrophil granulocytes, one a band form); a monocyte with basophilic cytoplasm and a compact nucleus (an appearance that is common in bone marrow aspirates); a monocyte with cytoplasmic granules; a large atypical monocyte (immature, as previously discussed); a crushed monocyte and another blast cell with basophilic cytoplasm. (B) A similar picture to (A). Some of the five cells with a blastic appearance (such as the larger one on the right) are better classified as promonocytes because of the presence of indentations in the nuclear outline. Close to the lower border there is a row of four mature monocytes. (C) Peroxidase reaction. The reaction is weak in the monocyte lineage. On the right, a large myeloblast with focal positivity in the Golgi zone and, adjacent, a mature neutrophil that is almost negative, apart from faint diffuse background staining (a feature of dysgranulopoiesis).

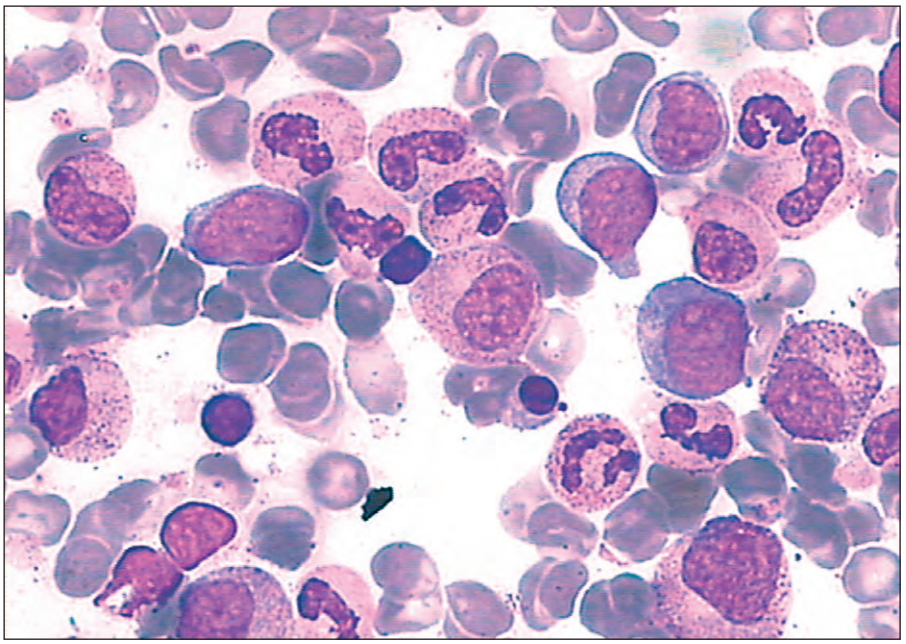


**Fig. 7.13** CMML; bone marrow aspirate. A case with the monocytic component expressed predominantly in the peripheral blood (WBC 18.7 × $10^9$/l; neutrophils 51%, myelocytes 3%, monocytes 28.2%, equivalent to 5.29 × $10^9$/l; haemoglobin concentration [Hb] 133 g/l, platelets 273 × $10^9$/l). The monocytic component in the marrow is not above 4%: in this field one observes only one monocyte, top right, together with numerous myelocytes and neutrophil band forms, three blast cells, two lymphocytes and an orthochromatic erythroblast.

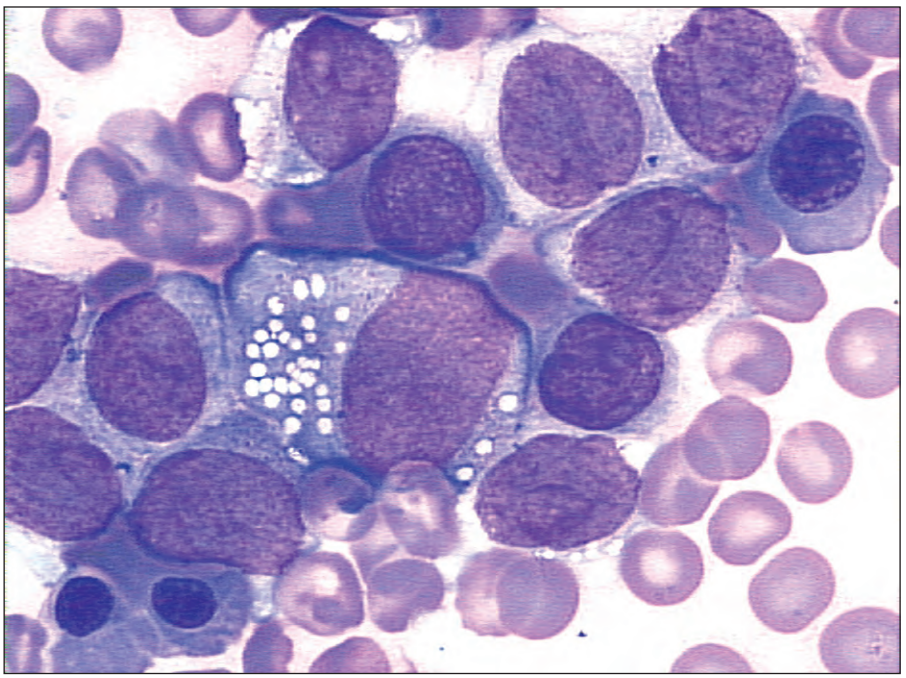


**Fig. 7.14** CMML with massive bone marrow invasion by much less mature cells; bone marrow aspirate. The largest cell, vacuolated with fine chromatin and a regular nuclear outline, can be classified as a monoblast, as can the smaller cell adjacent to it on the left. The three cells high up on their right, however, are promonocytes by virtue of their folded nuclei. Immediately beneath, an atypical monocyte with scanty cytoplasm. The percentage of blasts plus promonocytes is 17%. With this picture the differential diagnosis with acute monocytic and acute myelomonocytic leukaemia is difficult, and depends mainly on a cytological distinction between promonocytes and atypical monocytes, which requires judgement. In general, however, one is dealing with a disorder with a strong tendency to disease evolution. Bottom left, two orthochromatic erythroblasts.

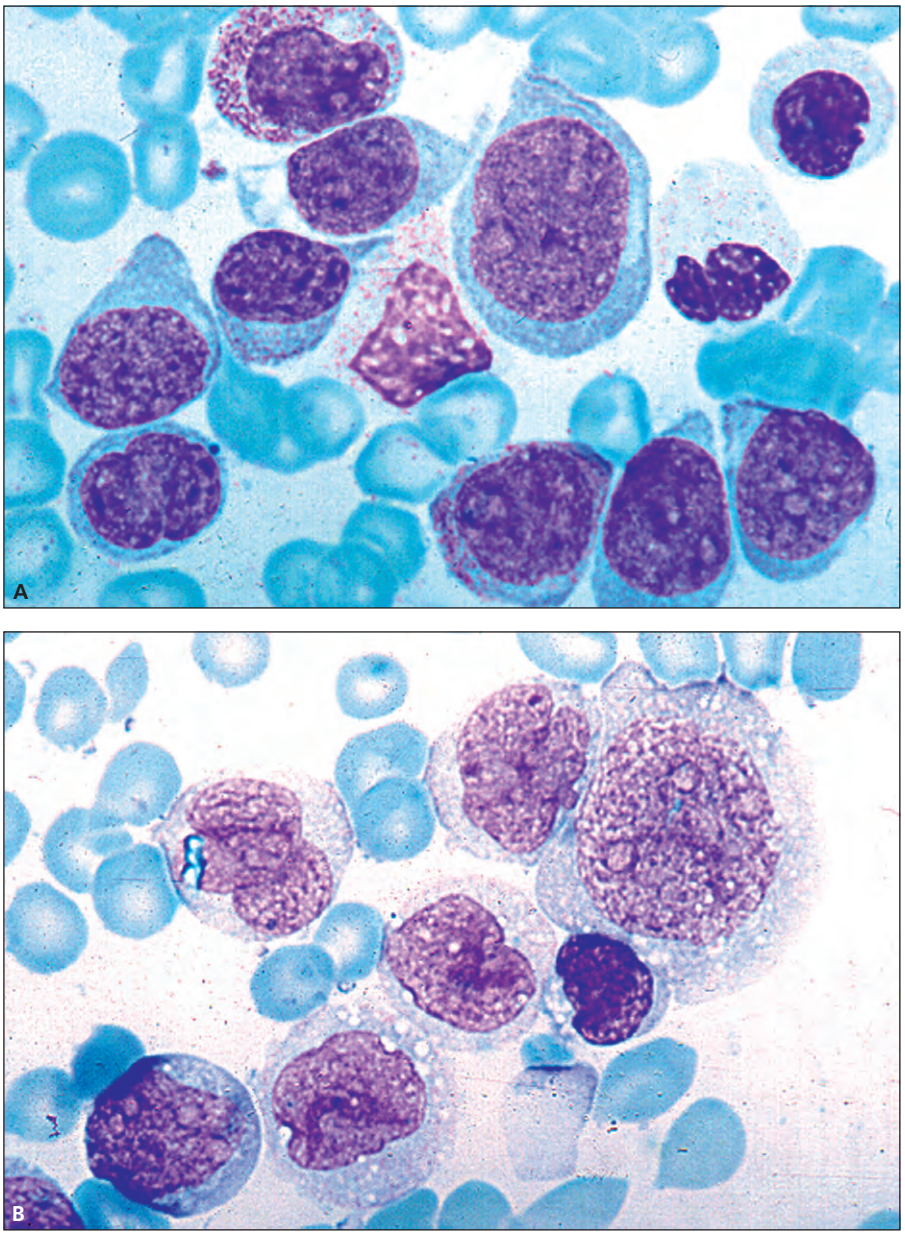



**Fig. 7.15** Another two cases of CMML with an increased component of immature cells; peripheral blood film. (A) Top right, a dysplastic neutrophil and a small monocyte. Immediately to their left, a large immature cell, with fairly abundant, agranular, basophilic cytoplasm; the nucleus is oval with a small notch on the left, chromatin in a regular very delicate network and three nucleoli, the one on the left with a circle of denser chromatin surrounding it, and the two on the right, one above the other, with a less well-defined outline; the delicacy of the chromatin in these cells is typical of monoblasts. The monocytic nature of the two smaller blasts situated in the bottom right corner is less evident, even though the characteristics of the cytoplasm are similar to those of monoblasts; to their left, a promonocyte: all three of these cells are included in the blast count. However, the two cells further to the left are excluded, these being classified as atypical monocytes because of their denser chromatin. (B) This film was obtained from a patient during acute evolution, with an increase in the leucocyte count and the appearance in the circulation of immature and highly atypical cells of monocyte lineage. On the right, a large monoblast, certainly tetraploid, with extremely reticular chromatin and a number of nucleoli; its cytoplasm is weakly basophilic. Immediately beneath, a small monocyte with compact chromatin and a larger one with paler chromatin, surrounded by three promonocytes which are quite similar to the latter but have less compact chromatin. In examples such as this the distinction can appear artificial, since there is a continuum of maturation: nevertheless, making this distinction is indispensable for assigning a case to the correct diagnostic category. Bottom left, another blast cell, with less certain signs of differentiation.

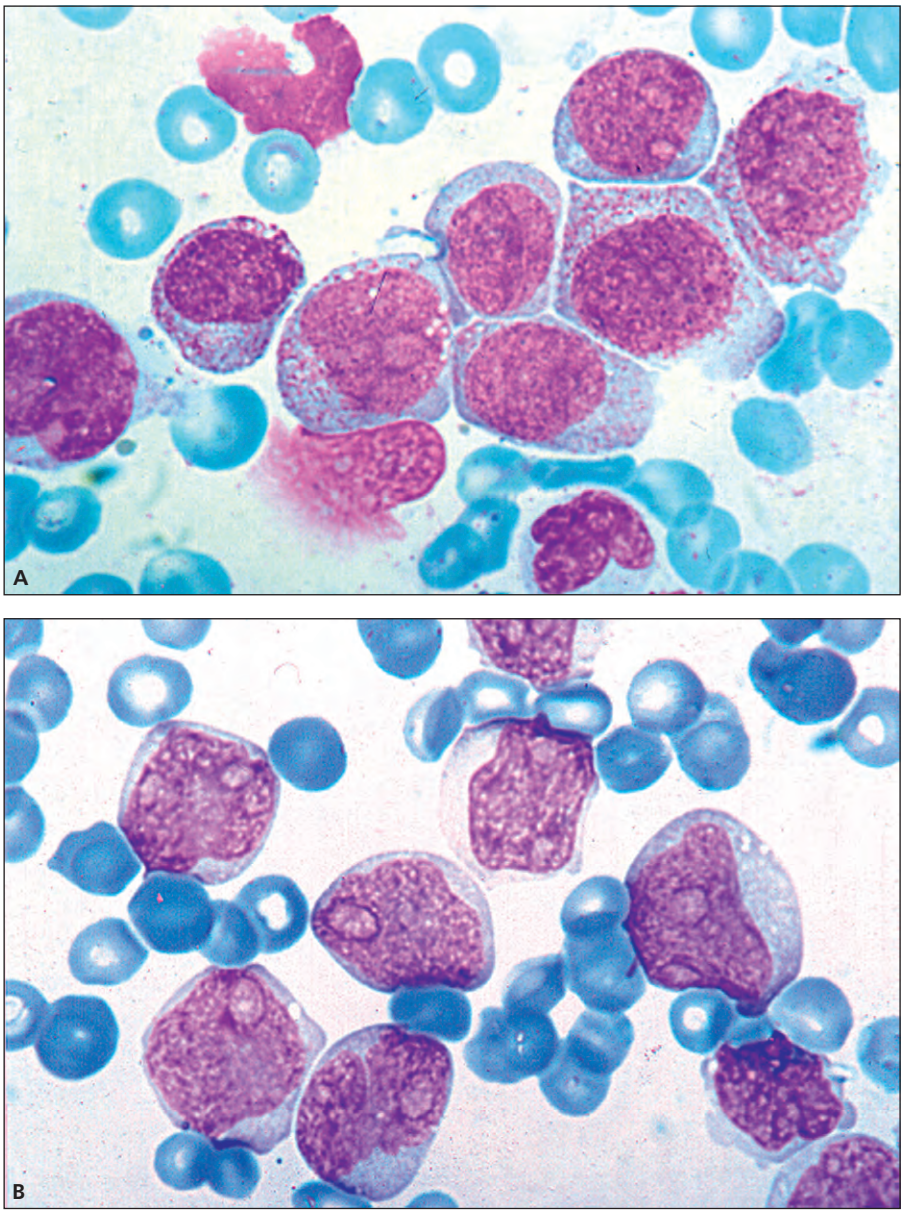



**Fig. 7.16** Leukaemic evolution during the clinical course of two patients with CMML. (A) Bone marrow aspirate. Top right, a monoblast. Below the monoblast, five promonocytes with conspicuous cytoplasmic granules and, to the left, a smaller neutrophil myelocyte. (B) WBC 205 $\times$ $10^9$/l. All the cells are medium-sized blasts with moderately abundant basophilic agranular cytoplasm. The nuclei are heterogeneous in shape with an irregular angular outline and loose-knit chromatin, somewhat coarser than that of typical monoblasts. In each nucleus there is at least one large, well-defined, very pale nucleolus, which stands out from the matrix of the nucleus because of the perinucleolar chromatin condensation. Top left, a smaller mature monocyte. Cytochemical staining for alpha-naphthyl butyrate esterase was strongly positive in all these cells, which were, however, completely negative for peroxidase. The picture in this phase of the disease thus corresponds to that of acute monoblastic leukaemia.

features that are noted among patients with a worse prognosis.[29,33–37] Recently, certain molecular features of prognostic importance have been recognised[38]: alterations in *TET2*, found in about a half of patients with CMML, correlate with a worse prognosis in the CMML-1 subgroup.[39] Around 40% of patients have an alteration in *RUNX1*: the presence of alteration in the C-terminal part of this gene is associated with leukaemic progression[40]; around 13% of patients have a mutation in *EZH2*, associated with a grave prognosis.[41]

On the basis of current knowledge it is useful to emphasise the following considerations in the differential diagnosis:

- Absolute monocytosis (>1.0 $\times$ $10^9$/l) remains the principal criterion for the differential diagnosis with MDS, in which-however, particularly because of neutropenia, there is often

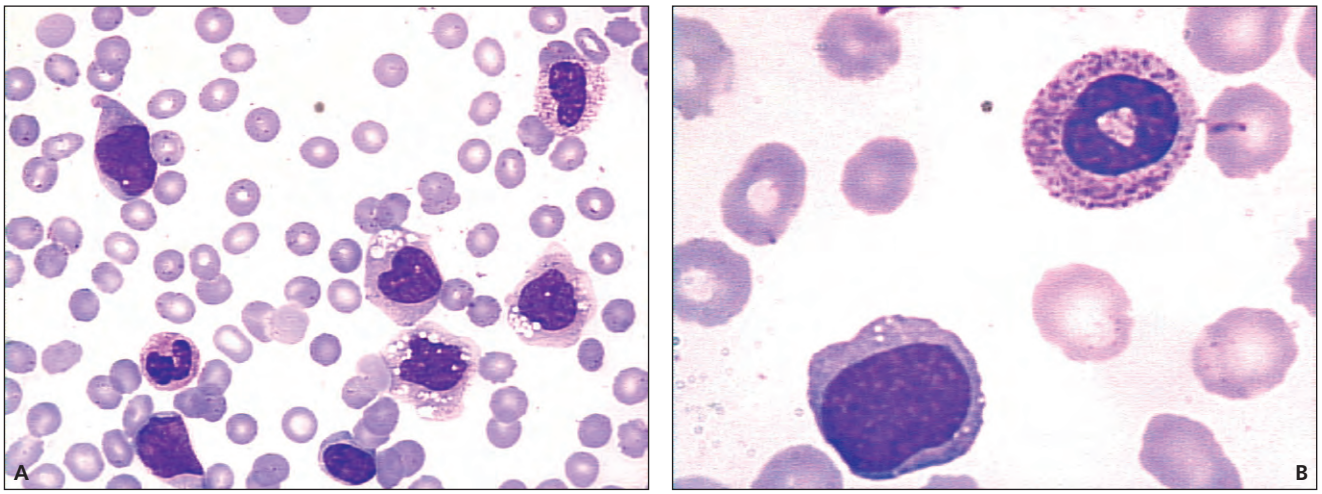



**Fig. 7.17** Peripheral blood film of a patient with CMML at presentation (same patient as Fig. 7.6B). (A) On the right, three mature monocytes with vacuolated cytoplasm; on the left, a promonocyte, a neutrophil and, at the bottom, a small blast cell. One also observes a neutrophil band form, top right, and at the bottom a lymphocyte. (B) A dysplastic neutrophil with a ring nucleus and an atypical monocyte with compact chromatin.

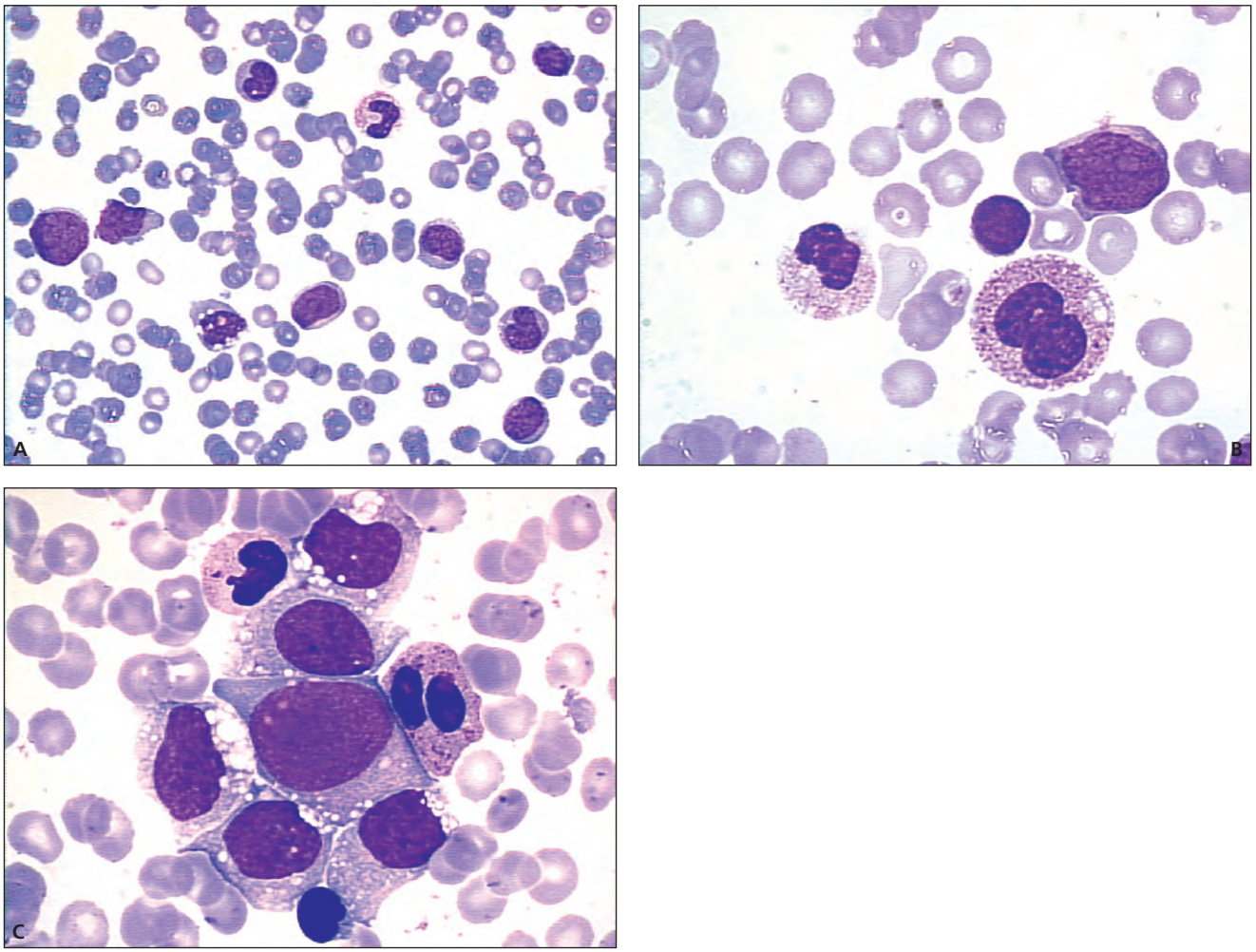



**Fig. 7.18** Same case as Fig. 7.17, six months later. (A) Peripheral blood film. From the left, a monoblast, a promonocyte, a small monocyte at the top and another, more spread out and vacuolated, at the bottom; beside this is a second promonocyte. The other three cells, bottom right, are atypical monocytes. At the top, a neutrophil band form and a lymphocyte. (B) Peripheral blood film. Two dysplastic neutrophils, a lymphocyte and a blast cell with scanty cytoplasm and a fairly thick chromatin network, thus morphologically not typical of the monocyte lineage. (C) Bone marrow aspirate. A large monoblast, centre, has very delicate homogeneous chromatin and a very regular oval nucleus; the nucleoli are not prominent. Around this cell, five atypical monocytes with greyish vacuolated cytoplasm and fairly compact chromatin. There are two dysplastic neutrophils with pyknotic chromatin (that on the right has separated lobes because of early apoptosis) and a small lymphocyte at the bottom.

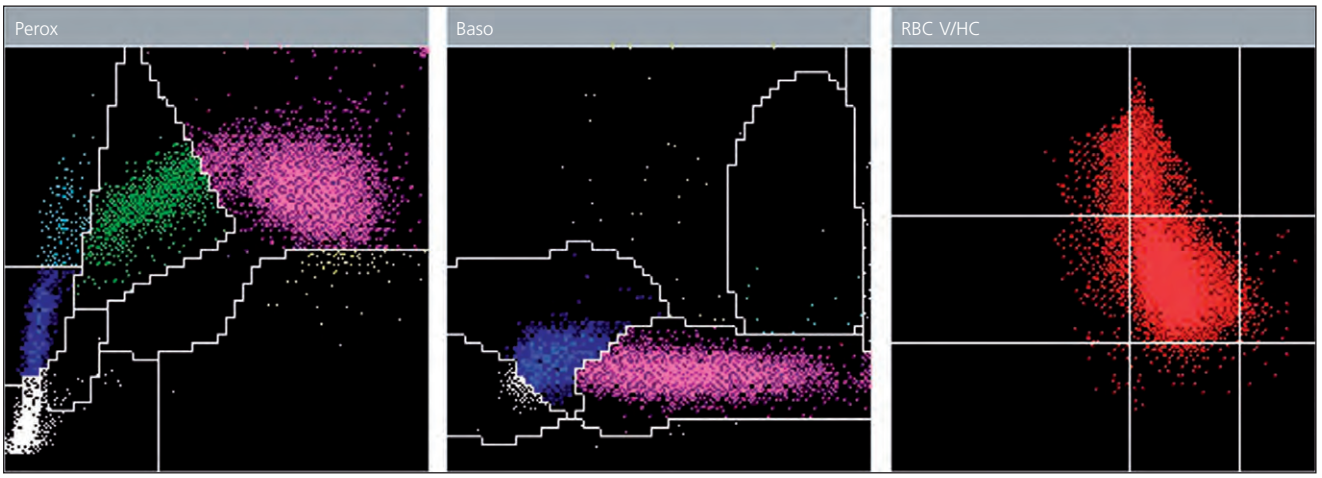



**Fig. 7.19** Automated instrument output of a case of CMML presenting with the incidental recognition of thrombocytopenia: there is moderate monocytosis (monocytes 1.34 × $10^9$/l, equal to 13.5%) and marked dysplasia with bilineage ineffective haemopoiesis (Hb 80 g/l; platelets 38 × $10^9$/l). The cytograms do not show any anomalies of the leucocyte population (LUC 1.8%, suspected blast cells 1.5%). Macrocytic red cells are 7.8%.

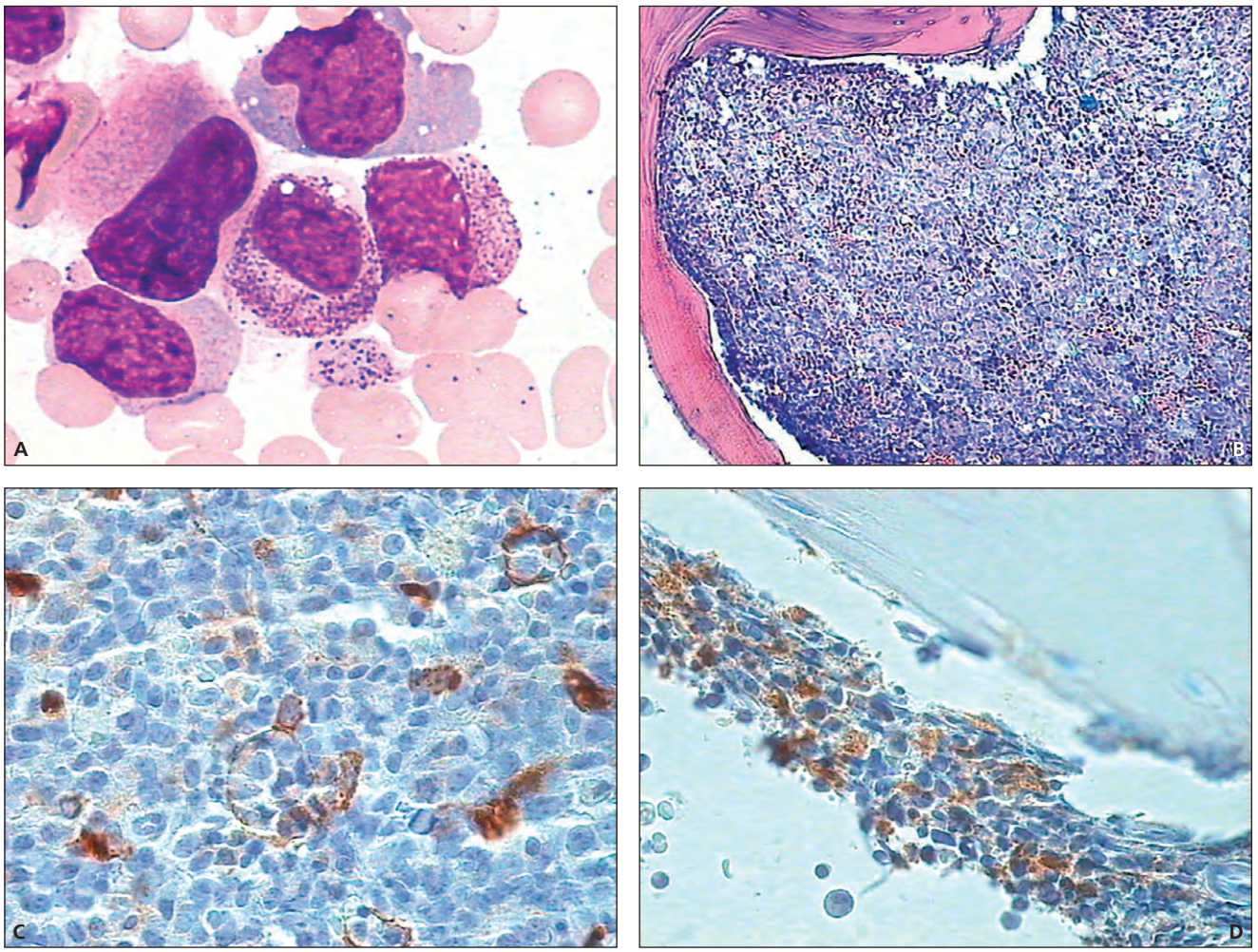



**Fig. 7.20** Bone marrow aspirate and trephine biopsy of the patient in Fig. 7.19. (A) Cytological examination of the aspirate film shows a cellular marrow with an increase in cells of the monocyte series (a promonocyte at the top, an atypical monocyte at the bottom) and trilineage dysplastic features: on the left, a dwarf megakarocyte. The other two cells could be classified as neutrophil metamyelocytes, except that the nucleus is not curved; they are small with mature cytoplasm. (B) Histological section, Giemsa stain, confirms a hypercellular marrow resulting from marked granulocytic hyperplasia with left shift and ALIP (abnormal localisation of immature precursors), together with monocytic hyperplasia with cells in various stages of maturation, including some monoblasts. (C) Immunohistochemistry (immunoperoxidase stain) shows that less than 1% of cells are CD34 positive. (D) Positivity for CD68 is consistent with the monocytic component (yellowish granularity). Cytogenetic analysis showed the presence of two anomalies (+8, 16p+). The haematological picture showed rapid deterioration with evolution to acute leukaemia and death in less than a year.

an increase in the percentage of monocytes (a relative monocytosis only);
- Some patients develop the features of CMML following a previous diagnosis of MDS. Published data[42] show that both patients with CMML evolved from a preceding MDS and patients with MDS with a relative monocytosis have a more aggressive clinical course;
- According to the 2008 WHO classification, CMML in patients previously treated with chemotherapy and/or radiotherapy for other diseases, even non-neoplastic, should be classified as therapy-related MDS/AML; an *MLL* rearrangement may be present, a molecular alteration also characteristic of *de novo* acute leukaemia;
- In cases with a marked increase and dysmorphism of the monocytic component, the diagnostic decision between CMML and AML with monocytic differentiation (FAB M5b) may not be easy (see Fig. 7.15A): the diagnosis is based, not only on clinical and blood count features, but on the presence of a double population of granulocytes and monocytes in CMML, while acute leukaemia with atypical monocytosis is associated mainly with neutropenia;
- Diagnostic difficulty can also arise in relation to other clinical conditions that can be associated with peripheral blood monocytosis, such as Hodgkin lymphoma and numerous infective and inflammatory conditions. Reactive monocytosis, resulting from subacute and chronic stimulation of the reticuloendothelial system has been described in connective tissue disorders such as systemic lupus erythematosus, sarcoidosis and lipid-storage disorders; in infective conditions such as tuberculosis, typhoid, bacterial endocarditis, long-standing fungal infection and during convalescence after an infection; and in certain tropical diseases such as malaria, kala-azar and trypanosomiasis. In these forms the monocytes can show morphological features of activation which make them similar to the 'atypical monocytes' of the most recent WHO classification (accentuation of nuclear irregularity, azurophilic granules and vacuoles).

## Atypical chronic myeloid leukaemia, *BCR-ABL1* negative

Atypical chronic myeloid leukaemia (aCML) is a rare neoplasm derived from a haemopoietic stem cell and characterised by neutrophil leucocytosis and dysgranulopoiesis, sometimes associated with splenomegaly. It has an aggressive clinical course with a median survival of two years and is characterised by evolution towards bone marrow failure or, in about 40% of cases, towards leukaemic transformation, which is observed particularly in patients with splenomegaly and transfusion dependency. It occurs in the elderly (70–80 years) without any gender predisposition. The reported incidence is one per million individuals per year, or one to two cases for every 100 new diagnoses of *BCR-ABL1*-positive CML.[43] An age above 65 years and a leucocyte count equal to or greater than $50 \times 10^9$/l are considered independent negative prognostic factors.[44]

Before the inclusion of this condition in the MDS/MPN group in the 2001 WHO classification, aCML was considered as a Philadelphia-negative variant of CML. The updated diagnostic criteria of the 2008 WHO classification are[6,45]:
- Persistent leucocytosis, equal to or greater than $13 \times 10^9$/l, with absolute neutrophilia associated with prominent granulocytic dysplasia;
- Absence of the Philadelphia chromosome and of the *BCR-ABL1* fusion gene;
- Absence of rearrangement of *PDGFRA* and *PDGFRB*;
- Absence of or only mild monocytosis (<10%);
- Absence of basophilia (<2%);
- Increased bone marrow cellularity with granulocytic hyperplasia and dysgranulopoiesis;
- Blast cells in the bone marrow and the peripheral blood less than 20%.

The haematological picture is sufficiently uniform and reproducible that, in the majority of cases, the diagnosis can be anticipated on the basis of morphology in advance of the molecular and cytogenetic confirmation that relevant abnormalities specific for other disorders are absent. Basic features that enable the distinction of aCML from *BCR-ABL1*-positive CML on the basis of peripheral blood examination are, particularly, the dysplastic aspects of the neutrophil series, the often modest number of circulating precursors, the lack of the classical basophilia and the presence of a larger proportion of monocytes.

The characteristic and distinctive peripheral blood features are the presence of neutrophil leucocytosis with marked dysgranulopoiesis (see Report 7.2; Figs 7.21, 7.22 and 7.23): in general, the count is between 20 and $100 \times 10^9$/l, although cases have been described with hyperleucocytosis with values for the WBC above $300 \times 10^9$/l. Anaemia and thrombocytopenia are often present; rare cases of thrombocytosis have been reported with values above $2000 \times 10^9$/l. Promyelocytes, myelocytes and metamyelocytes are between 10% and 20%, with little evidence of the neutrophil-myelocyte double peak that is seen in classical *BCR-ABL1*-positive CML. Blast cells are generally less than 5% and basophils less than 2%: there may be an absolute monocytosis, even though the WHO 2008 criteria indicate that monocytosis is 'absent or minimal', but monocytes are always less than 10%. The dysplastic features most often shown by neutrophils are anomalies of segmentation, which can be increased or decreased, abnormalities of chromatin condensation and abnormalities in the quantity of granules. The red cell population may show features of dyserythropoiesis and there can be macro-ovalocytes.

Bone marrow cellularity is very increased, mainly with a granulocytic expansion with retention of the ability to mature, but with consistent dysplasia, the features seen being the same as those observed in the peripheral blood (Figs 7.24 through to 7.30). Blast cells are, by definition, less than 20%, but are occasionally above 5%. The erythroid series, dyserythropoietic in

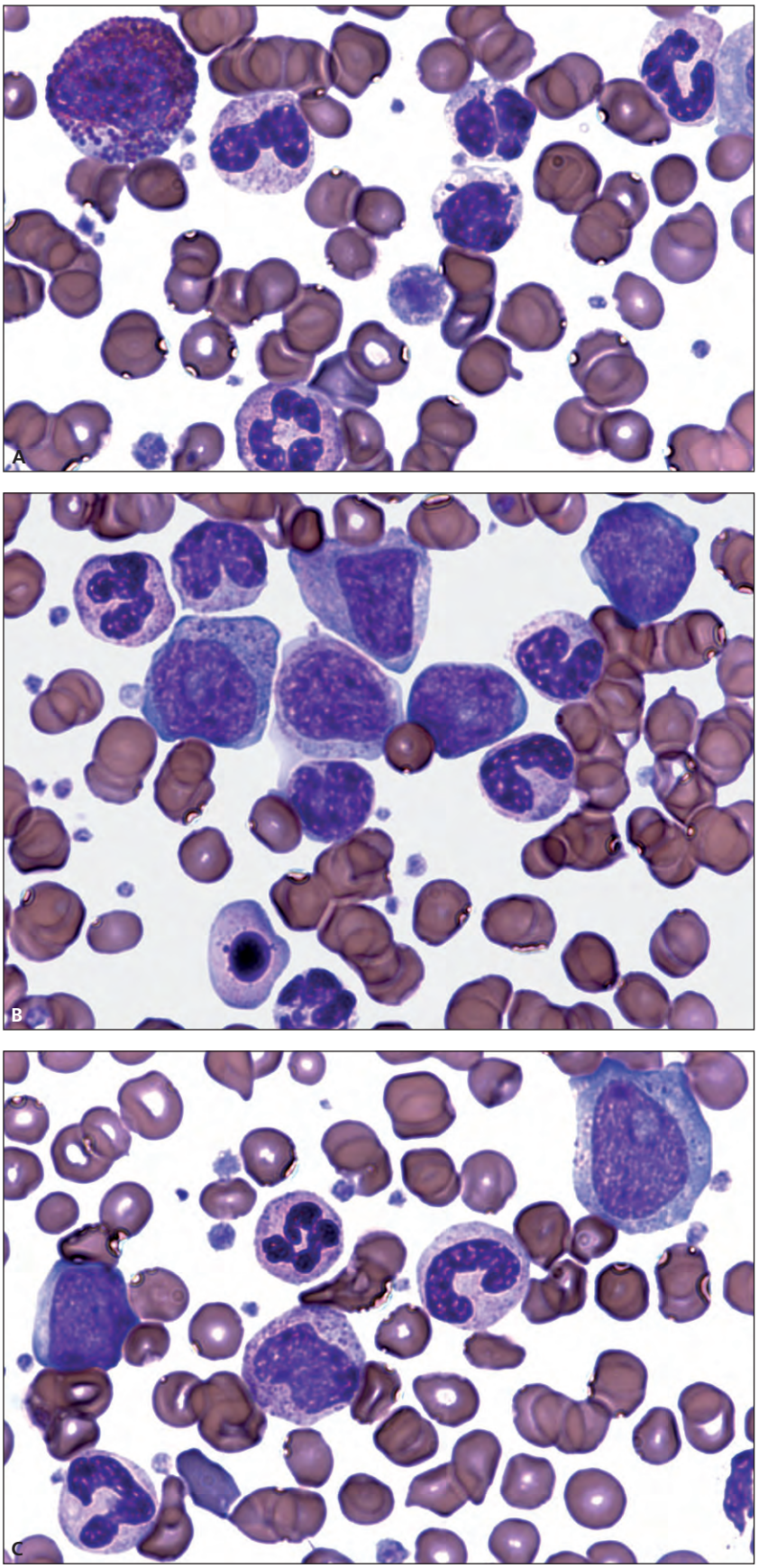



**Fig. 7.21** Atypical chronic myeloid leukaemia (aCML); the peripheral blood film, notable for neutrophilia and the presence of immature granulocytes, is distinguished in this field from the blood film of classical *BCR-ABL1*-positive chronic myeloid leukaemia (CML) by the presence of marked signs of dysgranulopoiesis. (A) In addition to the large eosinophil myelocyte on the left, one observes various dysplastic neutrophils. In particular, the two cells in the centre, above a giant platelet, appear monstrous because of the scantiness of the cytoplasm, the hypogranularity, the extreme compactness of the chromatin and the obvious nuclear appendages. (B) Top right, a blast cell with scanty basophilic cytoplasm; another is visible lower down towards the centre. The other cells are dysplastic neutrophils in various stages of maturation, from promyelocytes (with nucleoli) to mature neutrophils. The cell adjacent to the lower edge, which appears similar to a small erythroblast is, in fact, an apoptotic granulocyte, as can be deduced from the presence of violaceous cytoplasmic granules. (C) A blast on the left, three dysplastic neutrophils and a metamyelocyte. The large cell in the top right corner offers alternative cytological identifications, between a granular blast and a dysplastic promyelocyte, a common and important problem in the myelodysplastic syndromes: the choice in this case is a granular blast cell.

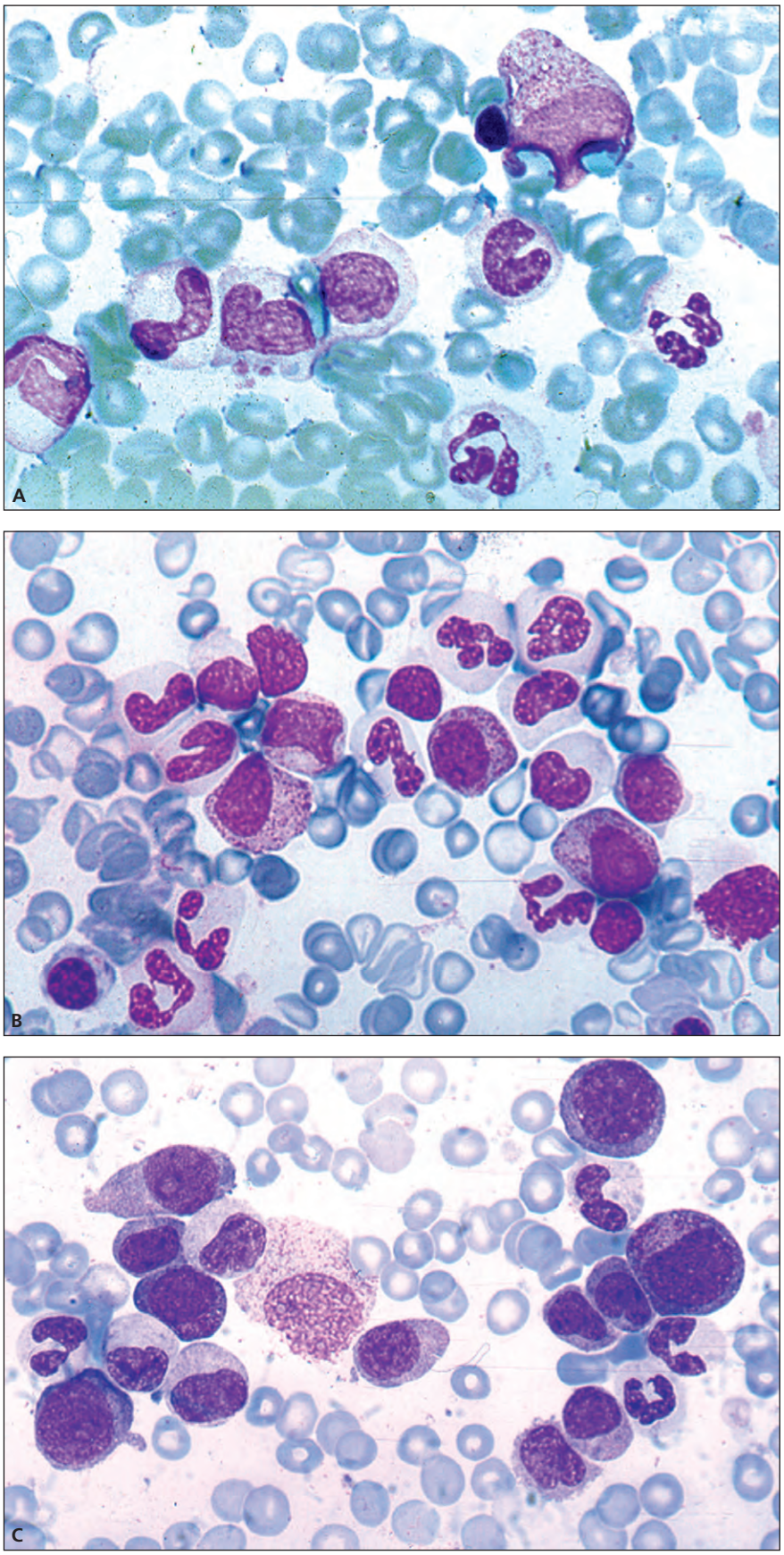



**Fig. 7.22** Peripheral blood films of three patients with aCML. (A) Lower right, two segmented neutrophils; in the central area, myelocytes and metamyelocytes, all with pale hypogranular cytoplasm. Top right, a promyelocyte, the lower edge of which shows invagination by the underlying erythrocytes: the nuclear chromatin is delicate, with a nucleolus on the right; numerous azurophilic granules stand out from the still basophilic background of the cytoplasm. On its left, a small orthochromatic erythroblast with the nucleus being expelled. (B) Numerous dysplastic neutrophils, with mainly non-segmented nuclei; the totally agranular cytoplasm retains a weakly basophilic tonality. The granulocyte precursors, on the other hand, have a normal number of cytoplasmic granules. On the right, towards the bottom, there is a promyelocyte, recognisable because of the large nucleolus almost in the centre of the nucleus; above this, a small undifferentiated agranular blast cell, with scanty cytoplasm and a nucleus with a fairly coarse chromatin network in which a nucleolus is barely visible. In the bottom left corner, an erythroblast with an area of non-haemoglobinised cytoplasm. The anisopoikilocytosis and hypochromia shown by the red cells complete the picture of dyserythropoiesis. (C) On the left, at the top and the bottom, two blast cells with scanty granules, the nuclei having a loose chromatin network and very obvious nucleoli. The neutrophils and some of their precursors are hypogranular and dysplastic. The two larger cells, top right, are a promyelocyte and a neutrophil myelocyte.

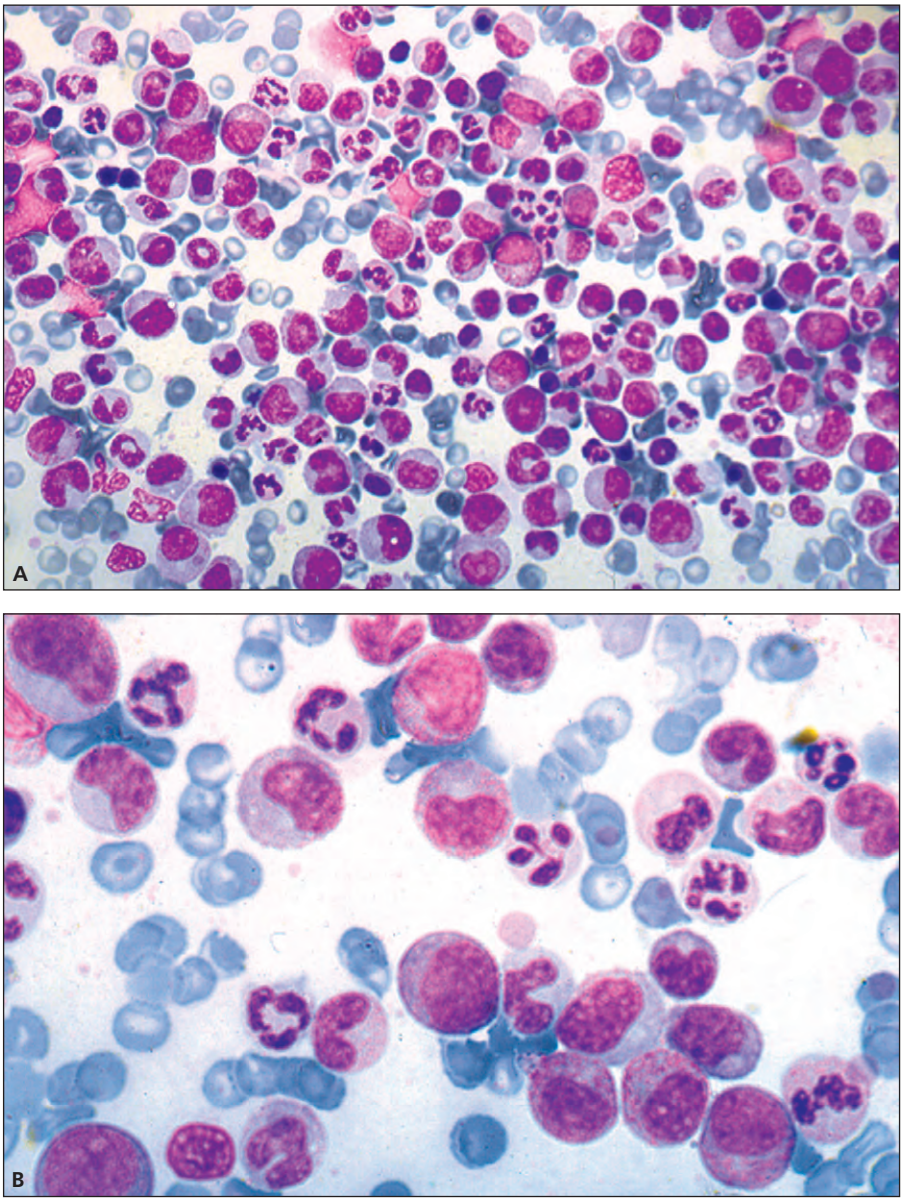



**Fig. 7.23** aCML; peripheral blood film of a patient with massive leucocytosis (WBC $302 \times 10^9$/l) and moderate anaemia and thrombocytopenia (Hb 92 g/l; platelets $78 \times 10^9$/l). (A) The leucocyte population, which occupies the entire film, shows a striking predominance of mature multilobated neutrophils and neutrophil precursors at all stages of maturation. One recognises also monocytes and lymphocytes. Some orthochromatic erythroblasts are also present. (B) The mature neutrophils have nuclei that are tending to hypersegmentation with very dark chromatin. The cytoplasm in some cells, such as those seen lower right, is pink with an apparently normal number of granules, but more often the cytoplasm is greyish and agranular. The other cells are promyelocytes, myelocytes and metamyelocytes of neutrophil lineage, these also showing variable cytoplasmic granulation. There is no basophilia, whereas some monocytes are present, such as that top left.

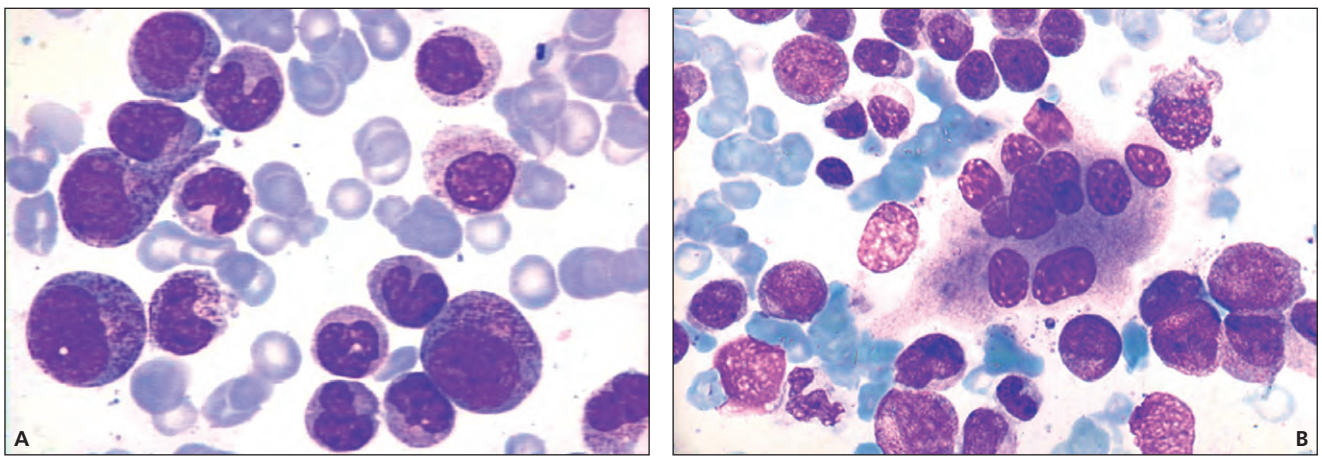



**Fig. 7.24** aCML; bone marrow aspirate. (A) The marrow is very cellular with granulocytic hyperplasia and features of dysgranulopoiesis, particularly in the more mature cells. One notes, also, three promyelocytes with normal morphological features such as the pale Golgi zone and the orderly distribution of cytoplasmic granules. (B) From the same film as (A), a dysplastic megakaryocyte with separated nuclear lobes, surrounded by neutrophil precursors.

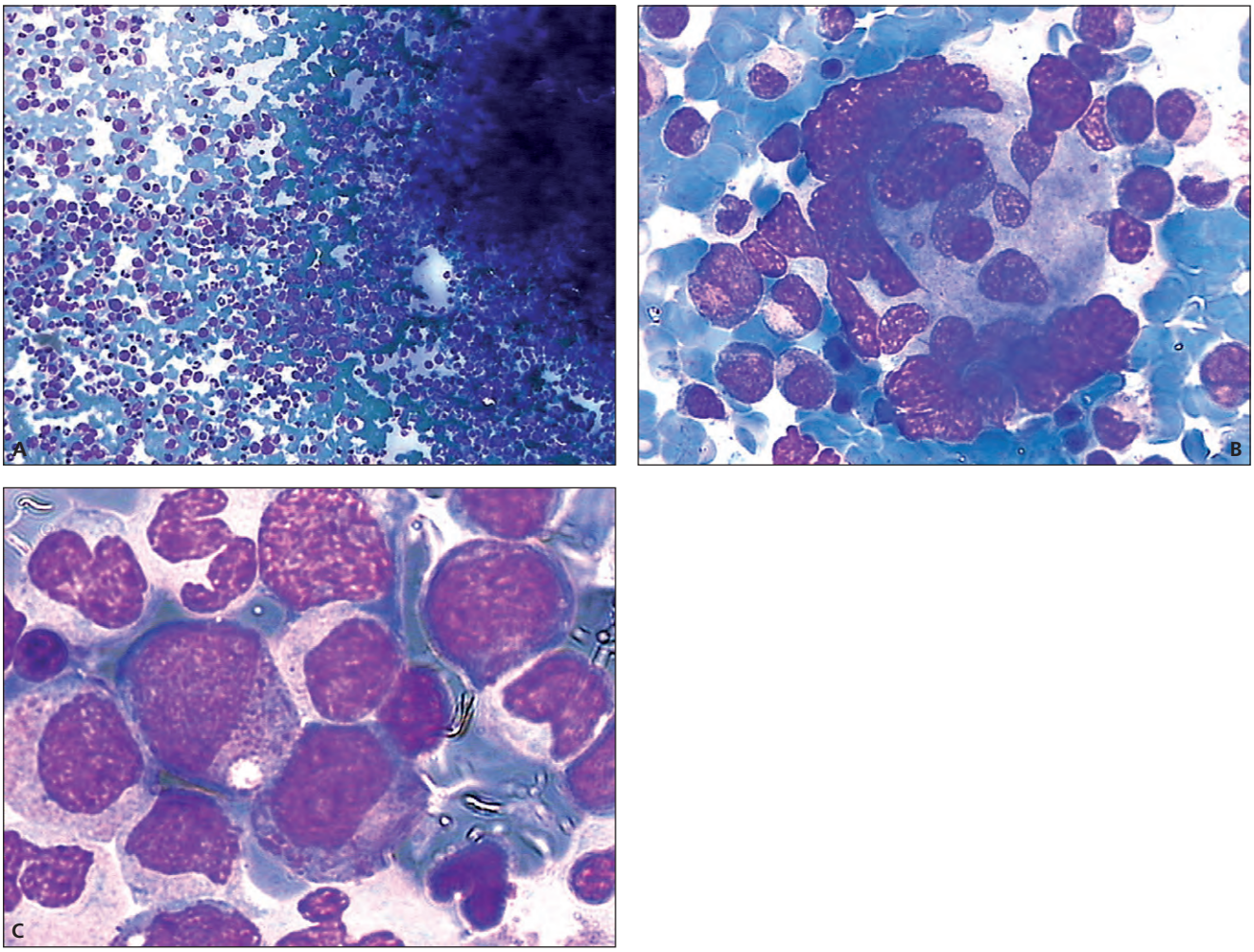



**Fig. 7.25** aCML; bone marrow aspirate. (A) At low power, the marrow at the edge of a fragment is hypercellular with heterogeneous cell types. (B) Dysplastic megakaryocyte with basophilic cytoplasm and a contorted nucleus showing partial detachment of nuclear lobes. (C) The cellular population is mainly immature granulocytes.

around a half of patients, in general represents around 10% of bone marrow cells with an M : E ratio greater than 10; however, in contrast to the bone marrow appearance in patients with *BCR-ABL1*-positive CML, it is much more common for the erythroid component to be increased, even above 30% with normalisation of the M:E ratio. Megakaryocytes are variable in number and dysplastic, in the majority of cases showing hypolobated or non-lobated nuclei and the presence of micromegakaryocytes. There is often increased reticulin deposition both at diagnosis and during progression of the disorder.

Other important aspects of the differential diagnosis between aCML and MPN are:

- The distinction between aCML and the hypercellular phase of primary myelofibrosis can be very difficult morphologically: in certain cases it is indeed investigation for mutation of *JAK2* and *MPL*, which are seen in myelofibrosis, that helps to make the distinction;
- For the distinction between aCML and neutrophilic leukaemia, a fundamental morphological criterion is the percentage of immature granulocyte precursors in the circulation, this being 10% or more in aCML while it must be less than 10% in chronic neutrophilic leukaemia;
- This same criterion can be useful in the differential diagnosis between cases of aCML with a moderate increase in monocytes and CMML, in which the proportion of immature granulocytes in the peripheral film is generally less than 10%.

Around 80% of patients have non-specific chromosomal alterations,[38] particularly trisomy 8 and del(20q); cases have also been described with alteration of chromosomes 12, 13, 14, 17

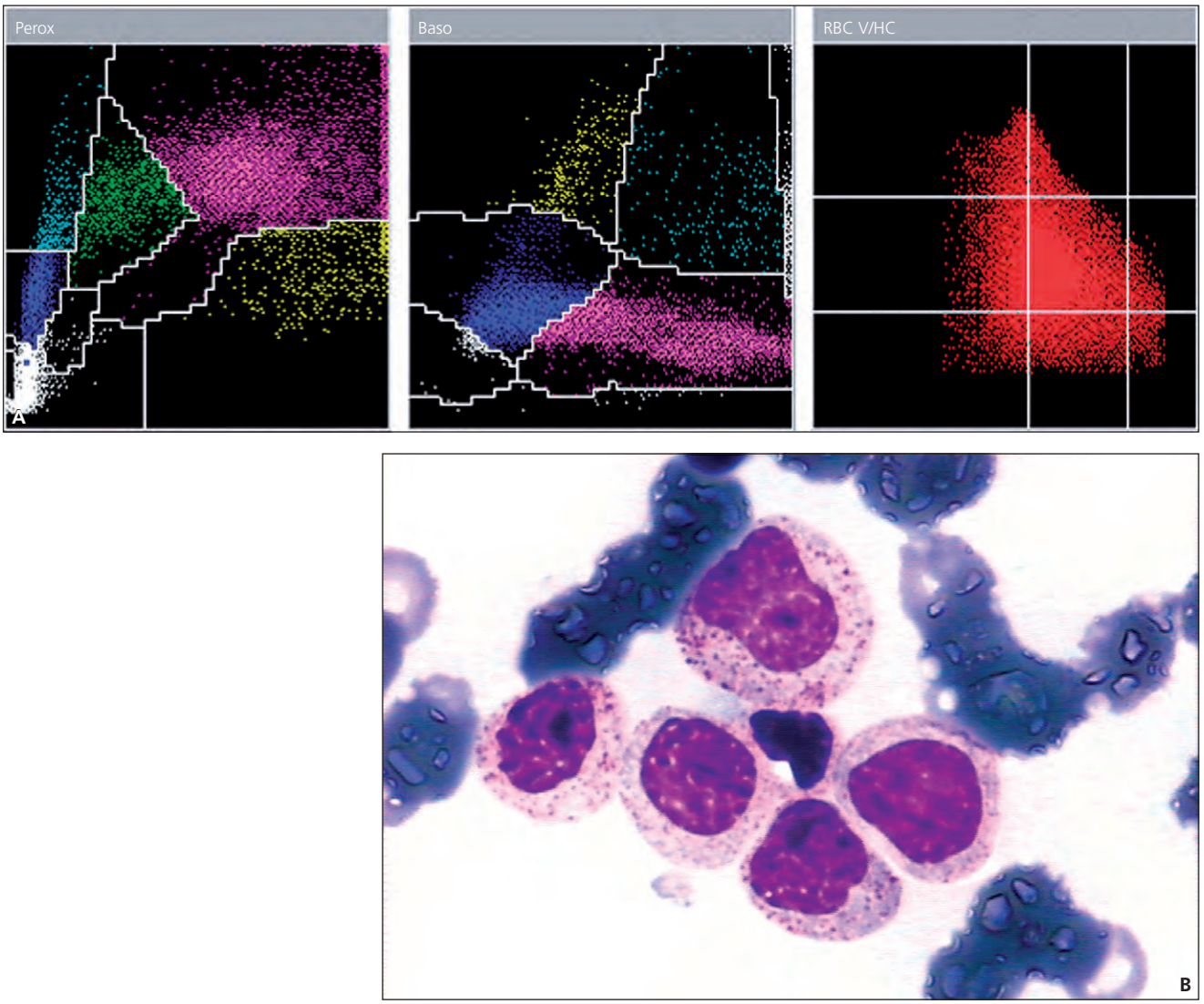



**Fig. 7.26** aCML in a patient at presentation without any previous history of a haematological disorder, hospitalised with acute febrile bronchopneumonia. (A) The full blood count shows a mild leucocytosis (WBC 15.7 × $10^9$/l), mild anaemia (Hb 109 g/l) and moderate thrombocytopenia (platelets 83 × $10^9$/l). The white cell differential count shows normal percentages, apart from a mild absolute neutrophilia (8.8 × $10^9$/l). The monocytes are 6.4% (0.9 × $10^9$/l), eosinophils 3.8% and there is a minimal basophilia (1.5%). The Perox cytogram shows dispersion of the neutrophils with cells in saturation on the right hand edge and with an IG flag; the Baso cytogram appears abnormal, with a cluster of signals in the basophil area and a deformation downwards of the PMN tail. The diagnostic suspicion would be of typical CML, except that it would not be usual to have anaemia and thrombocytopenia with only a slight increase in the leucocyte count. (B) Peripheral blood film. The neutrophils are severely dysplastic with nuclear hyposegmentation of a pseudo-Pelger type and, in some examples, hypogranular basophilic cytoplasm. At the top, a neutrophil metamyelocyte. This morphology does not support a diagnosis of *BCR-ABL1*-positive CML, in which severe dysgranulopoiesis is not present. The pseudobasophilia results from a partial resistance to the lytic effect of the reagent. The increased density of the PMN tail results from the presence of pseudo-Pelger nuclei.

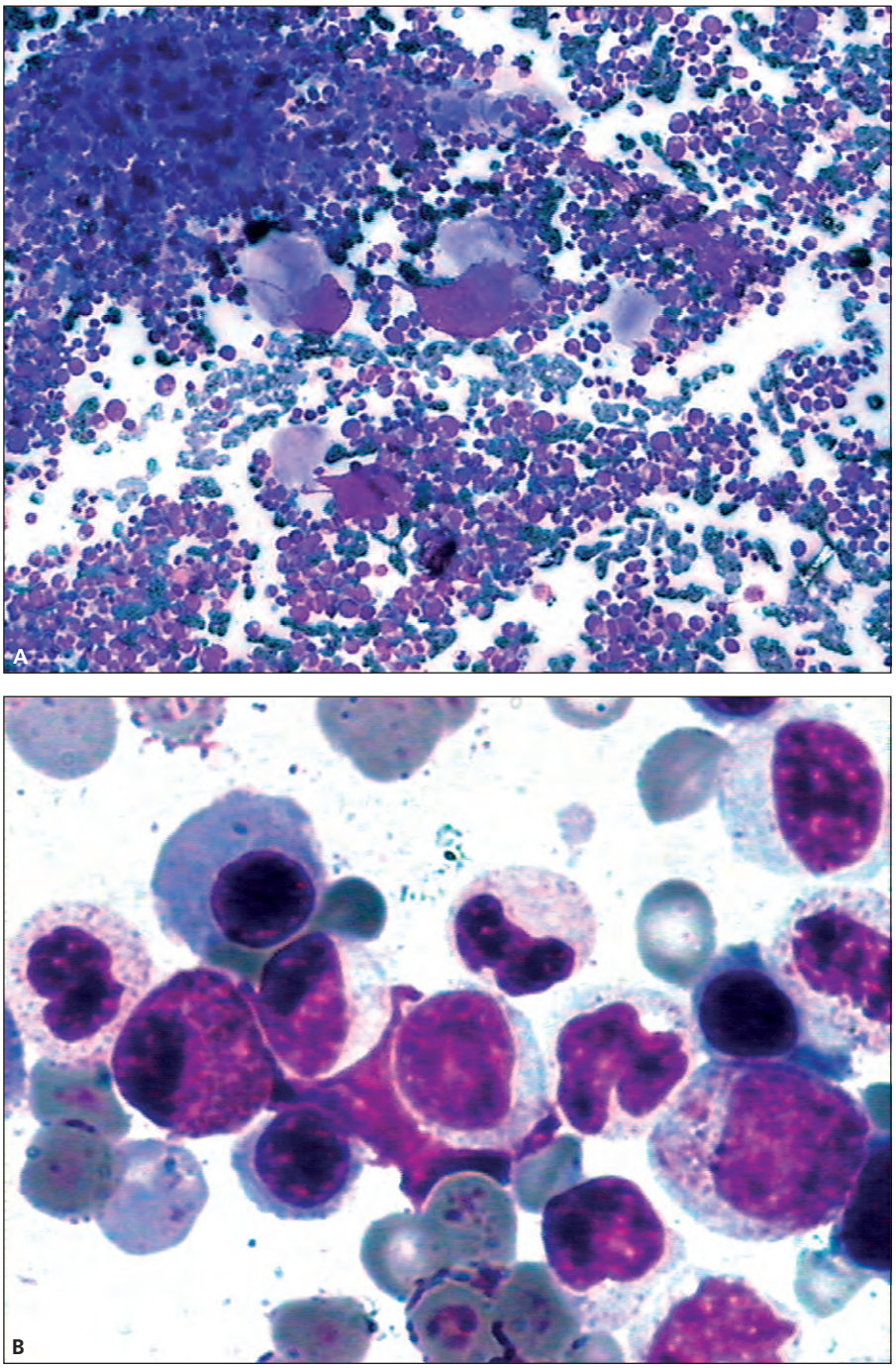



**Fig. 7.27** Same case as Fig. 7.26; bone marrow aspirate. (A) Cellularity is greatly increased with granulocytic hyperplasia and irregular distribution of megakaryocytes, which tend to be clustered. (B) There are a number of neutrophils, a dysplastic myelocyte with granules (to the right) and an agranular myelocyte (top right). Also present are a small mast cell (to the left), some lymphocytes and three polychromatic erythroblasts with some nucleocytoplasmic asynchrony.

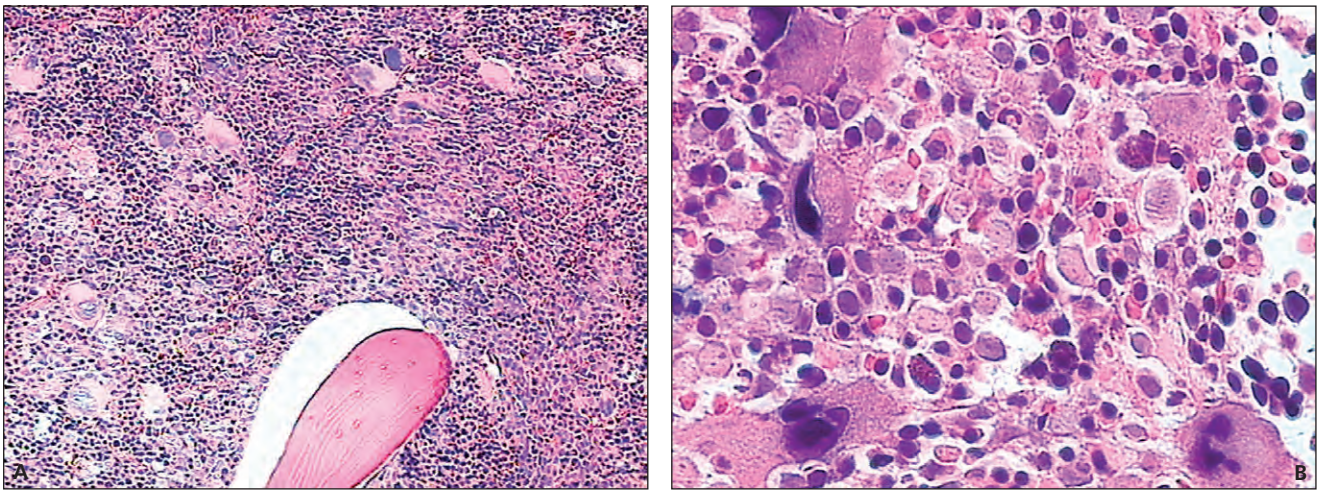



**Fig. 7.28** Same case as Fig. 7.27; histological section of bone marrow biopsy specimen. (A) The bone marrow is very cellular with marked trilineage hyperplasia. (B) The granulocytic series is left shifted with ALIP with blast cells being 5–10%. The erythroid series shows dyserythropoiesis; the megakaryocyte lineage is also hyperplastic with dysmorphism and asynchronous maturation. Cytogenetic and molecular investigations showed that the Philadelphia chromosome and *BCR-ABL1* were absent, whereas t(5;12)(q14;q21) was present with a breakpoint different from that observed in myeloproliferative neoplasms (MPN) with eosinophilia and rearrangement of *PDGFRB*; other anomalies were present in a proportion of cells (ring chromosome 19, isochromosome 8q).

and 19.[46] About a third of patients have a *TET2* mutation[47] and around 13% have alteration of *EZH2*.[48] Cases classified according to the 2008 WHO criteria have been reported to be negative for *JAK2* V617F[49]; however, others have reported cases of aCML with *JAK2* V617F.[50] One of the crucial points to consider with regard to the biological and clinical features of aCML is that in the period preceding the WHO classification, there was a lack of precise genetic and molecular diagnostic features: different entities were grouped together as aCML, including the so-called Philadelphia-negative CML, chronic neutrophilic leukaemia and some forms of CMML.[51] Finally, cases of *BCR-ABL1*-negative CML with t(8;9)(p22;p24), leading to fusion of part of *JAK2* with part of *PCM1*, are sometimes similar to aCML, but are often characterised by eosinophilia and the absence of dysplasia, so that they are assigned to the chronic eosinophilic leukaemia group: it is possible that such patients could be treated with JAK2 inhibitors.[52]

The syndrome of anomalous chromatin condensation, initially described with hyperleucocytosis within the MDS group[53] and then assigned to the MDS/MPN group of disorders,[54,55] is now considered a morphological variant of *BCR-ABL1*-negative aCML, because of the close similarity of the clinical and biological features. The morphological peculiarity of this rare condition is very coarse chromatin condensation in neutrophil granulocytes, with the appearance of large blocks of heterochromatin separated by broad zones of euchromatin (Figs 7.31 and 7.32; see also Fig. 3.12B). This particular appearance is not, however, specific: it has been described also in rare patients with *BCR-ABL1*-positive CML,[56,57] in patients with clinically manifest HIV infection[58] and as a feature of reversible dysplasia secondary to therapy with mycophenolate mofetil.[59]

## Juvenile myelomonocytic leukaemia

This is a rare neoplasm of infancy and childhood with an incidence estimated at one in a million children, with a higher frequency in boys (2:1), occurring under the age of three years in the great majority of cases and always under the age of 15 years. The onset is often acute with constitutional symptoms and hepatosplenomegaly associated with variable lymphadenopathy and a rash, and having an aggressive clinical course.[60] In a manner analogous to other neoplasms in which there is monocytic expansion, there is often extramedullary infiltration by a myelomonocytic population, in sites such as the dermis, the pulmonary alveoli, the spleen and the liver.

From the point of view of taxonomy, this neoplasm, which is now classified as one of the MDS/MPN, was in the past designated juvenile CMML, juvenile atypical myeloid leukaemia or the infantile monosomy 7 syndrome.

Peripheral blood examination, which is the most important diagnostic investigation, shows a moderate leucocytosis (median value of the WBC 25–30 $\times$ $10^9$/l) with an absolute monocytosis; in about 10% of patients, there is massive leucocytosis at onset, with WBC values above 100 $\times$ $10^9$/l. There is generally no granulocytic dysplasia, while it is very common for the blood film to show granulocyte precursors (promyelocytes, myelocytes and metamyelocytes). The percentage of blasts, including

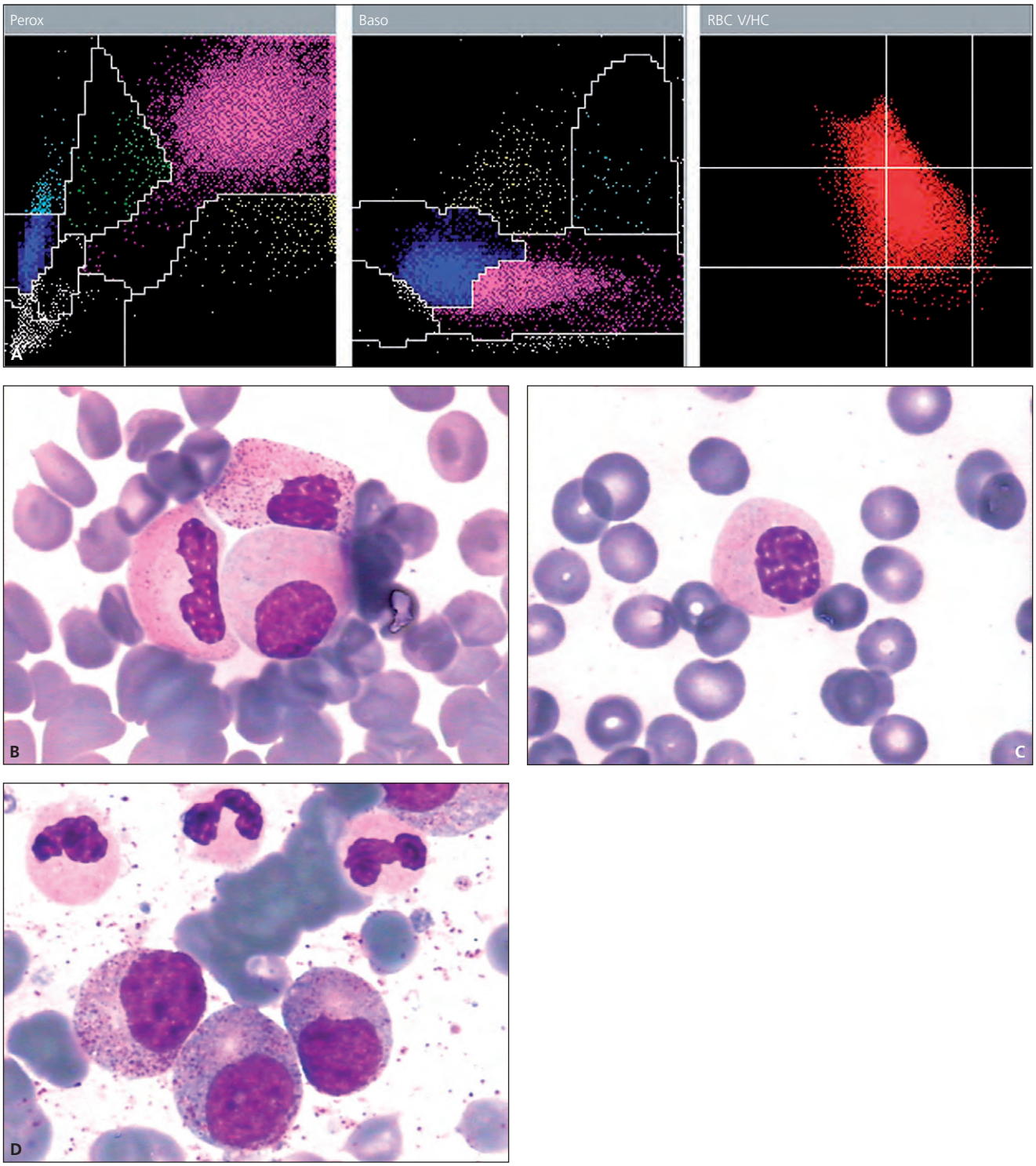



**Fig. 7.29** A different case of aCML at presentation; peripheral blood. (A) Moderate leucocytosis (WBC 20.1 × $10^9$/l), mild anaemia (Hb 114 g/l) and marked thrombocytopenia (27 × $10^9$/l). The leucocyte differential count shows relative (78.5%) and absolute (15.8 × $10^9$/l) neutrophilia with an IG+++ flag. Monocytes are only 0.8%. In the Perox cytogram, the neutrophils show an increase in peroxidase activity (MPXI 9.4) with dispersion to the right and numerous signals in saturation; in the Baso cytogram, the PMN tail is abbreviated so that most of the peroxidase-positive cells are found in the MN cluster and are coloured blue. (B) In the blood film there are numerous neutrophils with marked and heterogeneous dysplasia: nuclei are non-segmented or hyposegmented, cytoplasm is agranular or shows reduced granulation. (C) A typical neutrophil with a non-lobated nucleus, pseudo-Pelger type, with chromatin condensed into pyknotic clumps separated by small empty spaces. (D) At the top, three hypogranular band neutrophils. At the bottom, a neutrophil myelocyte and two promyelocytes with practically normal morphology. As sometimes occurs, the signs of dysgranulopoiesis are greater in the more mature cells.

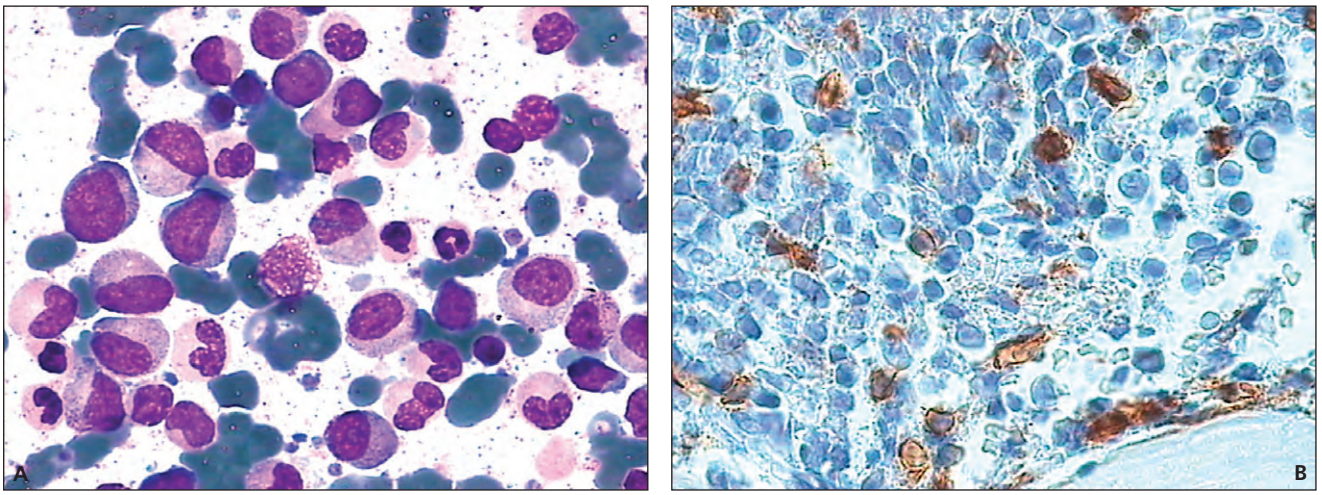



**Fig. 7.30** Same case as Fig. 7.29; bone marrow aspirate. (A) The bone marrow aspirate shows increased cellularity with marked granulocytic hyperplasia and dysplasia. (B) Histological section of trephine biopsy specimen, immunoperoxidase with anti-CD34. Cellularity is preserved and is characterised by left shift of granulocytes and marked dyserythropoiesis; CD34-positive blast cells are not greater than 3–4%. There are rare megakaryocytes. Cytogenetic analysis showed a normal karyotype, 46,XY and RT-PCR confirmed the absence of *BCR-ABL1*.

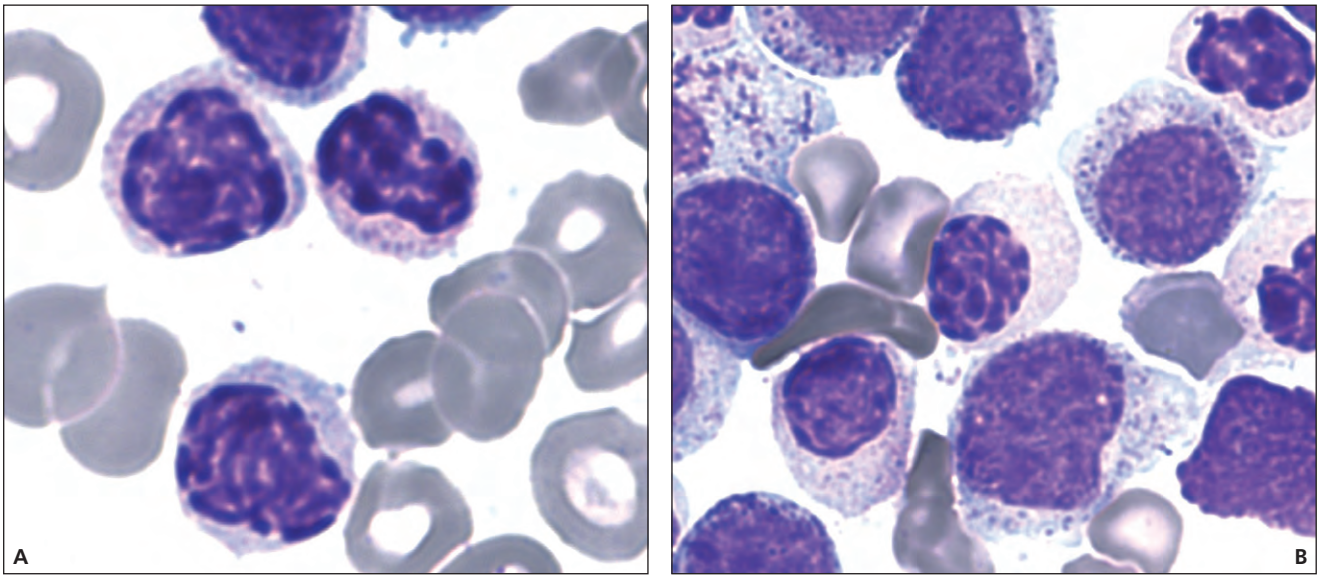



**Fig. 7.31** Syndrome of abnormal chromatin condensation. (A) Peripheral blood film. The neutrophils are dysplastic. Their non-segmented nuclei are composed of hyperchromatic clumps of chromatin separated by fissures, giving a knobbly appearance. There is scanty, basophilic, hypogranular cytoplasm. (B) Bone marrow aspirate. The cellularity is increased with the predominant cells being dysplastic granulocytes at all stages of maturation. There are some anomalies of chromatin condensation, which is much more apparent in the most mature cells (centre and top right).

monoblasts and promonocytes, is usually not above 5%. The presence of anaemia and thrombocytopenia, although moderate, is usual. Macrocytosis and circulating erythroblasts have in general been associated with monosomy 7, which is present in 25% of patients and is also associated with an increased percentage of haemoglobin F. The morphological alterations in the bone marrow are less specific and consist of hypercellularity and expansion of the granulocytic and monocytic series (the latter often proportionally less represented than in the peripheral blood), which show moderate dysplasia (Fig. 7.33). Cytochemical stains for peroxidase and non-specific esterase are useful for demonstrating the presence of the two components. In the marrow also, the proportion of blast cells is not very high, by definition always less than 20%; Auer rods have not been

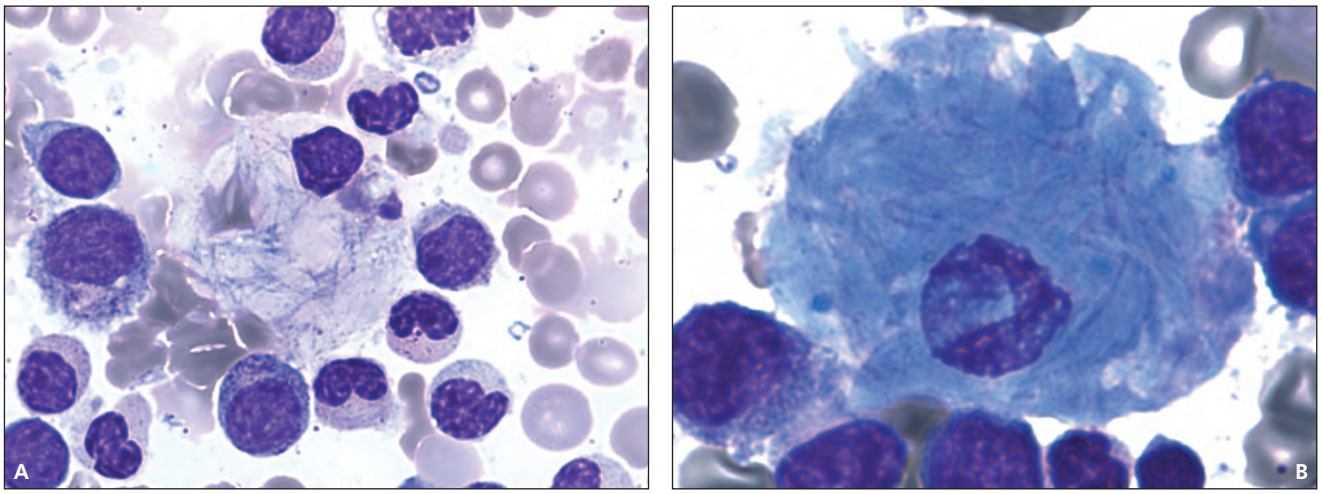



**Fig. 7.32** Syndrome of abnormal chromatin condensation; same case as Fig. 7.31; bone marrow aspirate. (A) Immature granulocytes, among which stand out metamyelocytes and band neutrophils with anomalous chromatin condensation, surrounding a macrophage with a pseudo-Gaucher appearance; the ample pale cytoplasm of the latter contains pale blue fibrils arranged in disorderly bundles (rather than resembling the 'onion skins' of a true Gaucher cell). Top left, a small non-granular blast cell. (B) From the same film as (A), another macrophage, a 'sea-blue histiocyte', which is rich in fibrils that cross over each other in all directions. The presence of these cells is the result of the accelerated catabolism of lipids derived from the cellular membranes of the many dysplastic granulocytes that are dying in the marrow.

described. The erythroid series can also show non-specific dysplastic features. Megakaryocytes are generally reduced in number and sometimes there are dysplastic features such as nuclear hypolobation.

The three cardinal diagnostic criteria of the 2008 WHO classification[61] are:

1. Peripheral monocytosis above $1 \times 10^9$/l;
2. Blasts in the peripheral blood and in the bone marrow less than 20%;
3. Absence of the Philadelphia chromosome or the *BCR-ABL1* fusion gene or both. To these are added two or more of the following criteria:
   - Haemoglobin F increased for age;
   - Presence of granulocyte precursors in the peripheral blood;
   - Leucocytosis above $10 \times 10^9$/l;
   - Abnormal karyotype (particularly monosomy 7);
   - Hypersensitivity of myeloid progenitors to GM-CSF.

A biological characteristic typical of this neoplasm, to the extent that it can be a discriminating diagnostic test in more complex cases is hypersensitivity of bone marrow progenitors *in vitro* to stimulation by GM-CSF (but not to stimulation by IL-3 or G-CSF), resulting from a selective alteration in the regulatory activity of the *RAS* gene. In addition, there is a strong association with type I neurofibromatosis, an autosomal dominant condition characterised by abnormalities of cutaneous pigmentation and a predisposition to benign and malignant neoplasms of the nervous system, which is diagnosed in 11% of patients with JMML. Noonan syndrome, an autosomal dominant condition characterised by reduced stature, abnormal facies, mental retardation and congenital cardiac anomalies, is sometimes also associated with a condition similar to JMML. These two conditions result, respectively, from mutation in the *NF1* gene and the *PTPN11* gene. Other genes that may be mutated in JMML include *KRAS* and *NRAS*,[62] *CBL* and *ASXL1*, these mutations having a central role in pathogenesis.[62]

## Myelodysplastic/myeloproliferative neoplasms, unclassifiable

This category groups haematological neoplasms in which dysplastic features are associated with myeloproliferation, without the diagnostic criteria for any of the specific entities described above being met, and in which there is neither a *BCR-ABL1* fusion gene, nor rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*. The morphological features of the peripheral blood and the bone marrow are very variable, depending on the lineages involved in the proliferation and the dysplasia (Fig. 7.34). The diagnostic criteria of the 2008 WHO classification are given in Table 7.3[63]: it is evident that before making this diagnosis of exclusion, it is necessary to carefully evaluate clinical and laboratory features of these patients. Excluded from this group are haematological neoplasms in evolution and alterations of disease features in patients with a previous diagnosis of a haematological neoplasm; patients who have previously received cytotoxic chemotherapy are also excluded (Figs 7.35, 7.36 and 7.37). Table 7.4 shows in synoptic form the principal criteria used in the differential diagnosis of MDS/MPN.

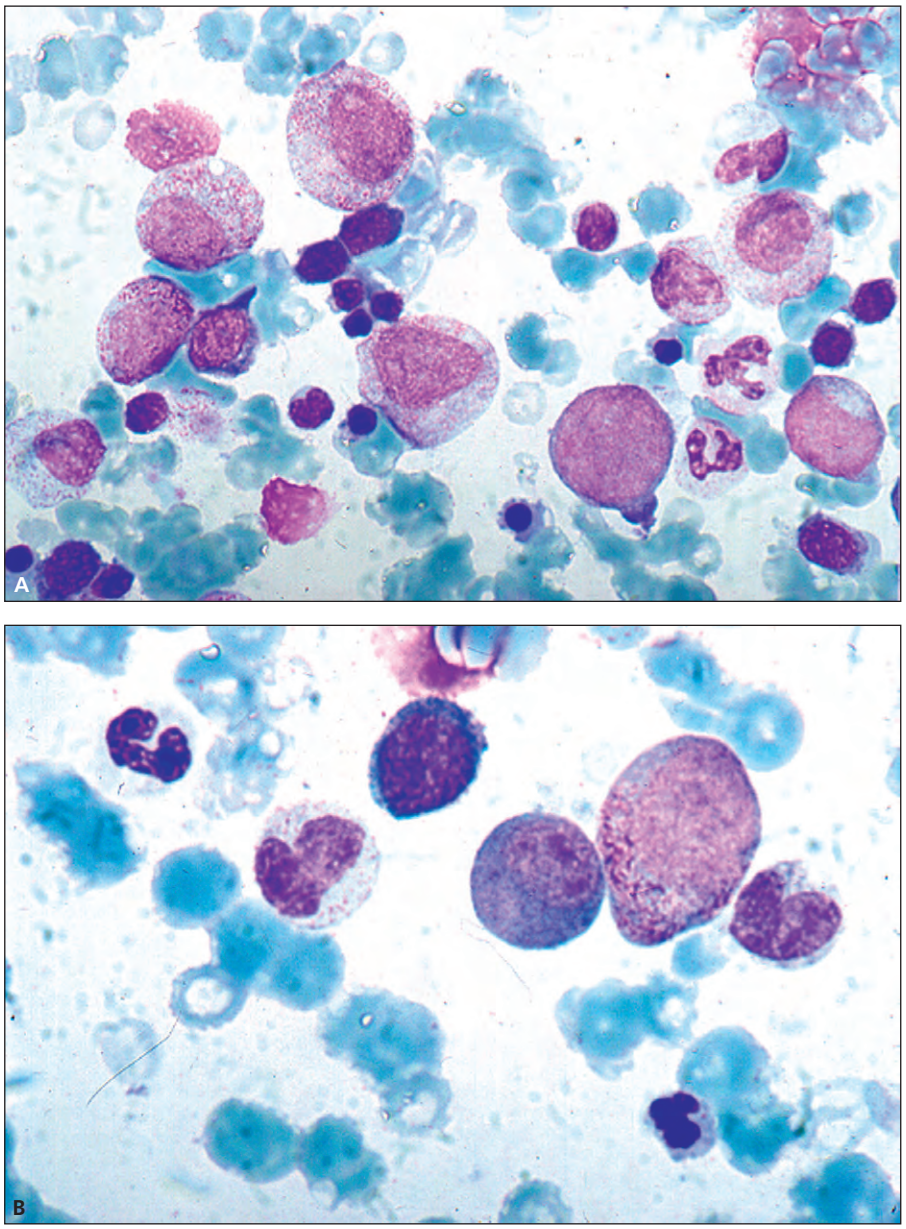



**Fig. 7.33** Juvenile myelomonocytic leukaemia (JMML) in a nine-year-old girl with anaemia, thrombocytopenia and moderate leucocytosis with neutrophilia, with occasional circulating immature granulocytes and erythroblasts; bone marrow aspirate. (A) Cellularity is reduced. Bottom right, a large proerythroblast with cytoplasmic protrusions and a granular blast with a very angulated nucleus with fine chromatin; at the top and in the centre, two neutrophil promyelocytes that appear moderately dysplastic (little evidence of a Golgi zone, disorderly hyperchromatic granules). The other cells are neutrophil myelocytes, metamyelocytes and band forms, and dyserythropoietic erythroblasts. (B) From the left: a dysplastic neutrophil, a hypogranular metamyelocyte, a basophilic erythroblast, an eosinophil myelocyte, a large dysplastic hypergranular promyelocyte, a monocyte and, at the bottom, a polychromatophilic erythroblast with a lobulated nucleus.

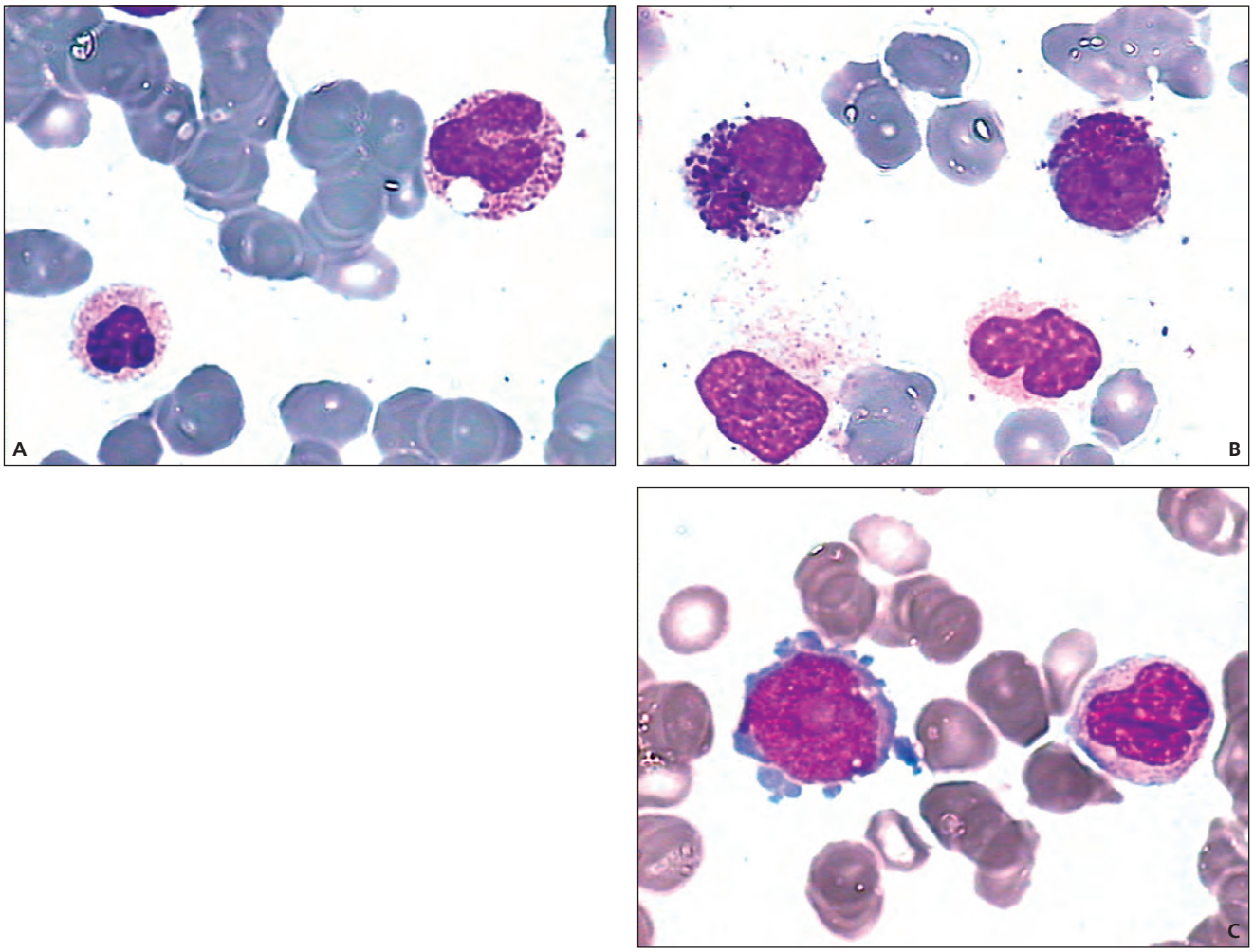



**Fig. 7.34** From the peripheral blood of a patient with MDS/MPN unclassifiable, according to the 2008 WHO criteria, with basophilia. The WBC is $14.5 \times 10^9$/l, with neutrophilia and basophilia, few monocytes and rare blast cells; neutrophils 66%, basophils 11%, lymphocytes 17%, monocytes 4% and blasts 1%. (A) The neutrophils are dysplastic. Two hyposegmented cells, one small with almost pyknotic chromatin, the other hypergranular with a large cytoplasmic vacuole. (B) Two granule-rich basophils, with the granules clustering atypically in only part of the cytoplasm, leaving unobscured large non-lobated nuclei that recall the morphology of a mast cell. (C) An agranular blast cell with a nucleolus in the centre of the nucleus and intensely basophilic cytoplasm, expanding into rounded protrusions. To the right, a hypogranular metamyelocyte.

**Table 7.3** Diagnostic criteria for myelodysplastic/myeloproliferative neoplasms (MDS/MPN), unclassifiable, according to the 2008 WHO classification (reproduced from Swerdlow 2008,[63] with permission of IARC)

- Clinical, laboratory and morphological features characteristic of one of the forms of MDS, with blast cells in the peripheral blood and bone marrow <20%
- Features of myeloproliferation (platelet count at least $450 \times 10^9$/l or leucocyte count at least $13 \times 10^9$/l) with or without splenomegaly
- No history of previous MDS or MPN or of treatment with cytotoxic drugs or growth factors
- Absence of the Philadelphia chromosome or the *BCR-ABL1* fusion gene or both; absence of rearrangement of *PDGFRA*, *PDGFRB* and *FGFR1*
  Absence of isolated del(5q), t(3;3)(q21;q26) and inv(3)(q21q26)
  *or*
- *De novo* presentation with mixed features of myelodysplasia and myeloproliferation which do not satisfy all the criteria necessary for a diagnosis of a specific subtype of MDS, MPN or MDS/MPN

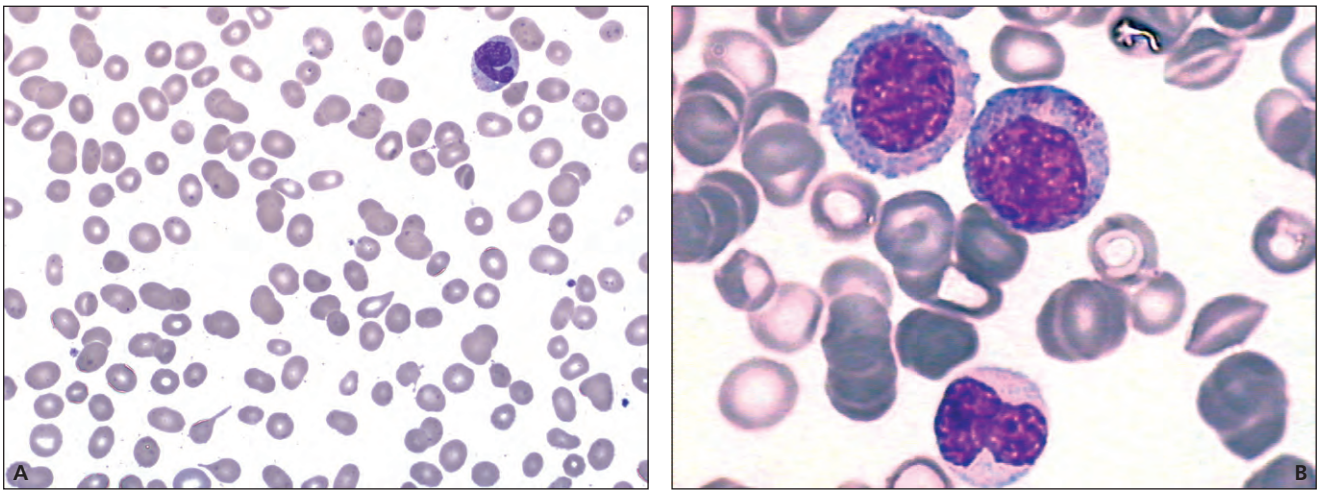



**Fig. 7.35** Picture of pancytopenic myelofibrosis as an atypical evolution of CMML. (A) Peripheral blood leucopenia. There is an atypical monocyte and moderate anisopoikilocytosis with anisochromasia and the presence of a number of dacrocytes. (B) Attempted bone marrow aspiration has yielded scanty medullary blood with no particles and very few nucleated cells; these are mainly dysplastic granulocytes, monocytes and a few erythroblasts. At the top, a myelocyte and an agranular atypical monocyte, with an oval nucleus and heterogeneously condensed chromatin. One notes the difference in the cytoplasmic outline, which is fringed in the monocyte and linear in the myelocyte. At the bottom, a metamyelocyte with pale hypogranular cytoplasm.

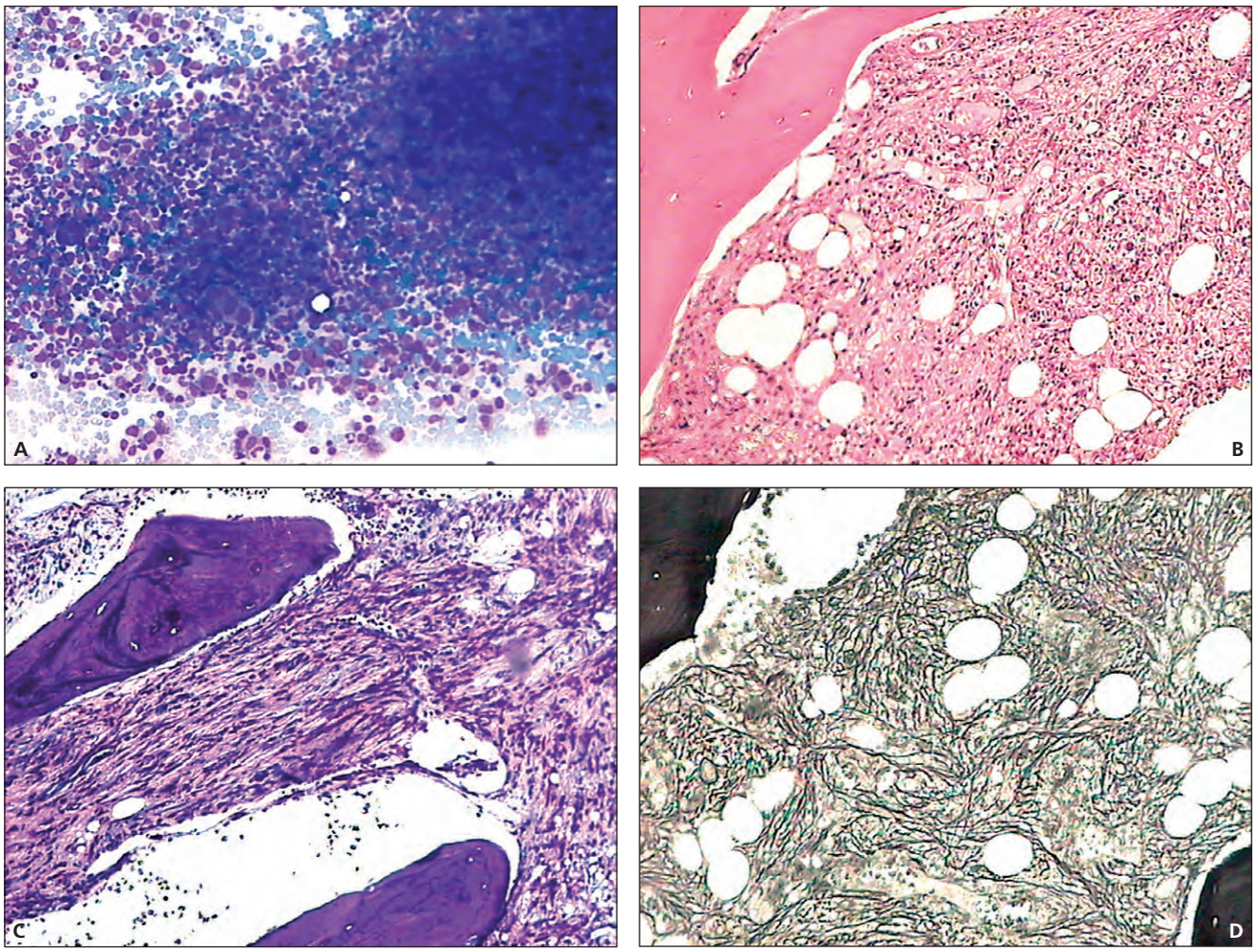



**Fig. 7.36** Bone marrow trephine biopsy in the same patient as Fig. 7.35. (A) Cytological preparation obtained by spreading the core of marrow obtained by needle biopsy. On the right, a fragment with a hypercellular trail. (B and C) Histological sections (both haematoxylin and eosin and panoptic stains) show isolated hypercellular foci in a fibrotic marrow cavity. (D) A silver stain confirms myelofibrotic transformation.

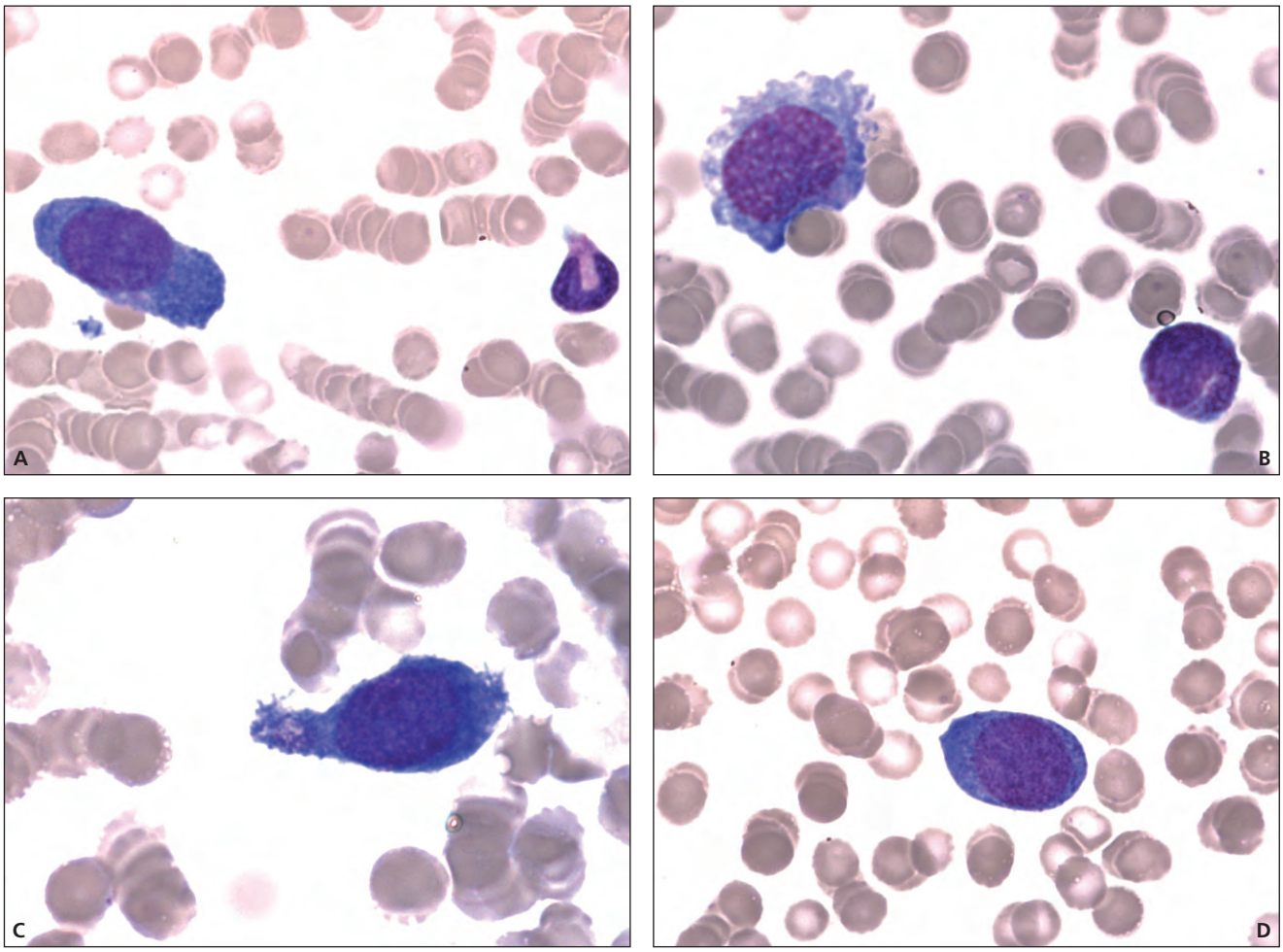



**Fig. 7.37** (A–D) Pancytopenia with the presence in the circulation of cells that are morphologically similar to dendritic cells from a patient with CMML in terminal phase. The blast cells in the peripheral blood film are similar to those in blastic plasmacytoid dendritic cell neoplasm (see Fig. 4.132).

## Refractory anaemia with ring sideroblasts and thrombocytosis

This entity, provisionally included in the MDS/MPN-U group of the 2008 WHO classification,[63] has a frequency of 0.7% of all MDS diagnoses in the Dusseldorf registry.[64] To the diagnostic criteria for the refractory anaemia with ring sideroblasts (RARS) category of MDS (anaemia, dyserythropoiesis, ring sideroblasts at least 15%, bone marrow blasts less than 5%; see Chapter 3) are added thrombocytosis with a platelet count of at least $450 \times 10^9$/l, associated with atypical megakaryocyte morphology with gigantism, pleomorphism and nuclear hyperlobation (cloud-like or cauliflower-shaped nuclei), similar to that described for essential thrombocythaemia and primary myelofibrosis (see Chapter 5). As in RARS, ring sideroblasts are erythroblasts with mitochondria loaded with haemosiderin, which are sited characteristically in a ring around the nucleus and are easily recognisable on a Perls stain.[65]

The diagnosis of RARS-T is revealed by morphology, but must be confirmed by negativity on cytogenetic and molecular genetic analysis for del(5q), t(3;3)(q21;q26) and inv(3)(q21q26). Around 60–80% of patients, however, have *JAK2* V617F or an *MPL* mutation.[66]

In the peripheral blood it is common for there to be anaemia with dimorphism and anisopoikilocytosis, while neither the numerous platelets nor the neutrophils show noteworthy morphological alterations. The blood film shows neither blast cells nor monocytosis nor immature granulocytes. The bone marrow

**Table 7.4** Major diagnostic features for differentiating the various categories of myelodysplastic/myeloproliferative neoplasms (MDS/MPN)

| | Peripheral blood | Bone marrow | Megakaryocytes | Molecular genetics |
|---|---|---|---|---|
| CMML | Persistent monocytosis, $>1 \times 10^9$/l, >10%<br>Subtype 1, blasts + promonocytes <5%<br>Subtype 2, blasts + promonocytes <20%<br>Dysplasia of at least one lineage | Hypercellular, increase of the monocyte series<br>Frequent hyperplasia of neutrophil granulocytes<br>Subtype 1, blasts + promonocytes <10%<br>Subtype 2, blasts + promonocytes <20%<br>Dysplasia of at least one lineage | Prevalence of megakaryocytes of reduced size | Negative for *BCR-ABL1*, *PDGFRA*, *PDGFRB*, *FGFR1*<br>Rarely *JAK2* mutated |
| aCML | Neutrophil leucocytosis<br>Absence of basophilia and monocytosis (<10%)<br>Dysplasia of the granulocyte series and sometimes abnormal chromatin clumping<br>Frequent macro-ovalocytosis | Hypercellular<br>Granulocytic dysplasia, often trilineage dysplasia | Frequent recognition of micromegakaryocytes and hypolobated megakaryocytes | Negative for *JAK2* mutation<br>Negative for *BCR-ABL1* and *PDGFRA*, *PDGFRB* and *FGFR1* rearrangement |
| JMML | Neutrophil leucocytosis<br>Monocytosis<br>Blasts + promonocytes <20%<br>Anaemia with an increase of haemoglobin F<br>Thrombocytopenia | Hypercellular with minor evidence of monocytosis in comparison with the peripheral blood | Reduced | Negative *for BCR-ABL1*<br>Alteration of *PTPN11*, *NRAS*, *KRAS* or *NF1* |
| MDS/MPN-U | Increased counts of cells of at least one lineage<br>Dysplasia of at least one lineage | Hypercellular<br>Dysplasia of at least one lineage | No specific features | Negative for *BCR-ABL1* and *PDGFRA*, *PDGFRB* and *FGFR1* rearrangement<br>Absence of isolated del(5q), t(3;3)(q21;q26), inv(3)(q21q26) |
| MDS/MPN-U with i(17p) | Neutrophil leucocytosis<br>Anaemia<br>Granulocytic dysplasia with nuclear hyposegmentation (pseudo-Pelger) | Hypercellular with granulocytic hyperplasia<br>Frequent trilineage dysplasia | Heterogeneous dysplasia (gigantism and hypolobation) | Presence of isolated i(17p) |
| MDS with del(5q) and *JAK2* mutated | Moderate leucocytosis | Hypercellular, sometimes with granulocytic hyperplasia<br>Less than 15% ring sideroblasts | Increased, predominance of hypolobated and non-lobated forms | *JAK2* V617F |
| RARS-T | Macrocytic anaemia<br>Platelets at least $450 \times 10^9$/l | Hypercellular with inversion of the M : E ratio<br>Sometimes megaloblastosis<br>At least 15% ring sideroblasts | Increased<br>Two morphological variants: essential thrombocythaemia type with gigantism; primary myelofibrosis type with hypolobation | Positive for *JAK2* V617F in 60% of cases Sometimes presence of *MPL* W515K/L |

is cellular (Figs 7.38, 7.39 and 7.40), with erythroid hyperplasia, dyserythropoiesis and possibly a tendency to megaloblastosis. The ring sideroblasts constitute at least 15% of erythroblasts. The granulocytic series is relatively reduced and appears morphologically normal. The megakaryocytes are increased in number and show the morphological features described above; it is not infrequent to see micromegakaryocytes, hypolobated megakaryocytes or both, these being characteristic of MDS.

It is important to note that there is a subgroup of MDS patients belonging to the category of refractory anaemia with isolated del(5q) who also have a *JAK2* V617F mutation with thrombocytosis, hypolobated megakaryocyte nuclei and moderate granulocytic hyperplasia. The distinction from RARS-T is based on the presence of an isolated del(5q) plus *JAK2* V617F. RARS-T has particular characteristics that differ from those of RARS, particularly the frequent presence of mutation of *JAK2* or *MPL*; in comparison to essential thrombocythaemia, it has a worse median survival[67]; in comparison with both these disorders, there is a higher rate of leukaemic transformation.[68] The prompt morphological identification of RARS-T is important since it indicates the need for molecular studies and also because of the significant prognostic implications.

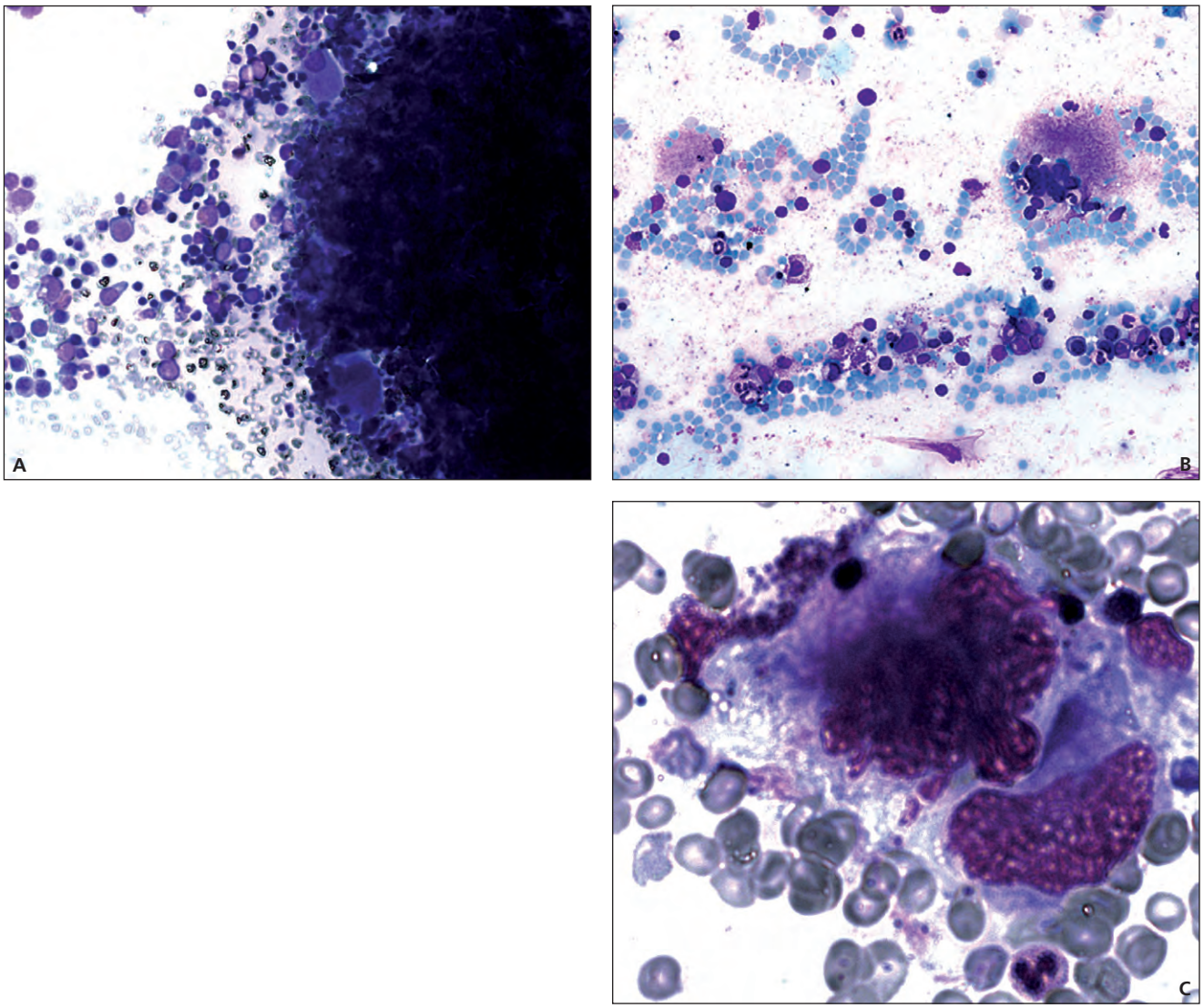



**Fig. 7.38** Refractory anaemia with ring sideroblasts and thrombocytosis (RARS-T): dysmegakaryopoiesis; bone marrow aspirate. (A) Hypercellular fragment. At low power, the majority of cells appear to belong to the erythroid series with preserved maturation: one can see some large cells with intensely basophilic cytoplasm and numerous mature erythroblasts with small round hyperchromatic nuclei. At the edges of the fragment, top and bottom, two megakaryocytes can be recognised. (B) In the better spread area, the megakaryocytes show dysplastic features, both micromegakaryocytes (a micromegakaryocyte with mature cytoplasm is seen on the left) and gigantism and multinuclearity (on the right). (C) A megakaryocyte and a dysmorphic promegakaryocyte with basophilic, not very abundant, cytoplasm. Their large size can best be appreciated by comparison with the mature neutrophil seen bottom right.

It is useful to summarise certain aspects of the differential diagnosis relating to ring sideroblasts, since their detection does not lead automatically to a diagnosis of RARS or RARS-T:

- There are non-neoplastic bone marrow disorders with ring sideroblasts; they are generally transitory conditions, often resulting from the administration of drugs or exposure to toxic substances including alcohol;
- The recognition of ring sideroblasts is not uncommon outside the MDS group of disorders: they are found in 5% of *BCR-ABL1*-negative MPN and occur also in MDS/MPN, including CMML;
- Ring sideroblasts are present in erythropoietic protoporphyria[69], in congenital sideroblastic anaemia and in haemochromatosis: it is interesting that in MDS, particularly RARS, and also RARS-T, an increased frequency of the H63D mutation and particularly the C282Y mutation in the *HFE* gene, responsible for hereditary haemochromatosis, has been recognised.[70,71]

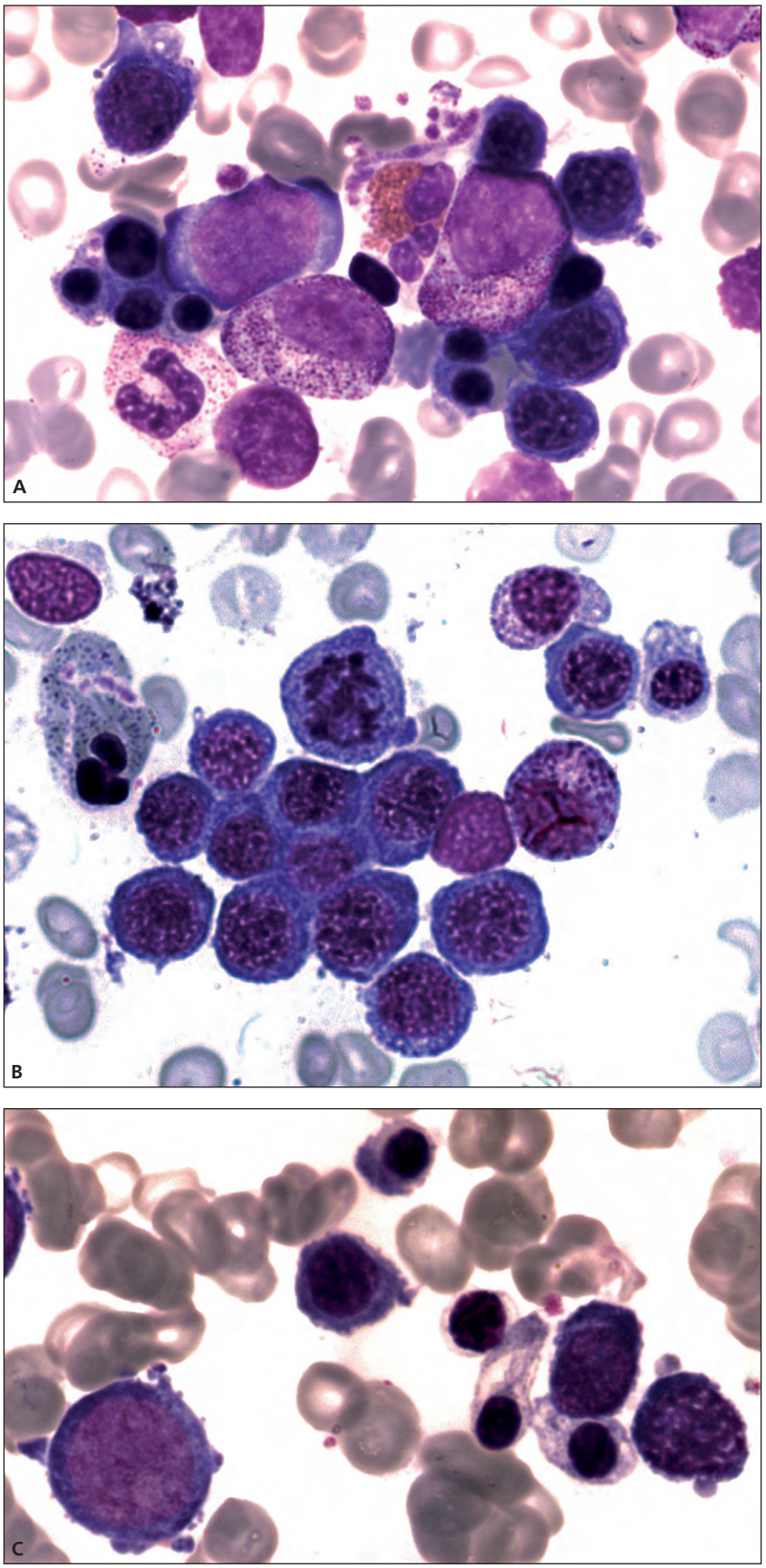



**Fig. 7.39** RARS-T: erythroid hyperplasia and dyserythropoiesis; bone marrow aspirate from the same patient as Fig. 7.38. (A) The erythroid series is clearly hyperplastic: one notes a large proerythroblast with very fine chromatin, several nucleoli and intensely basophilic cytoplasm with plentiful pale perinuclear arcoplasm, plus numerous erythroblasts in a more advanced stage of maturation. In the centre, a trilobed eosinophil between two early neutrophil myelocytes. (B) A monomorphic group of basophilic erythroblasts with asynchronous maturation. At the top, a mitotic figure in an erythroid cell. On the left, an orthochromatic erythroblast with plentiful cytoplasm with very prominent basophilic stippling and a lobulated pyknotic nucleus with some superimposed platelets. On the right, an early neutrophil myelocyte and, higher up, a late neutrophil myelocyte with a small rounded nucleus. (C) On the left, a proerythroblast with numerous cytoplasm protrusions. On the right, two almost orthochromatic erythroblasts, with nuclei now reduced to dark masses of pyknotic chromatin, while the cytoplasm still shows a tinge of basophilia with fine basophilic stippling and large areas that are not haemoglobinised.

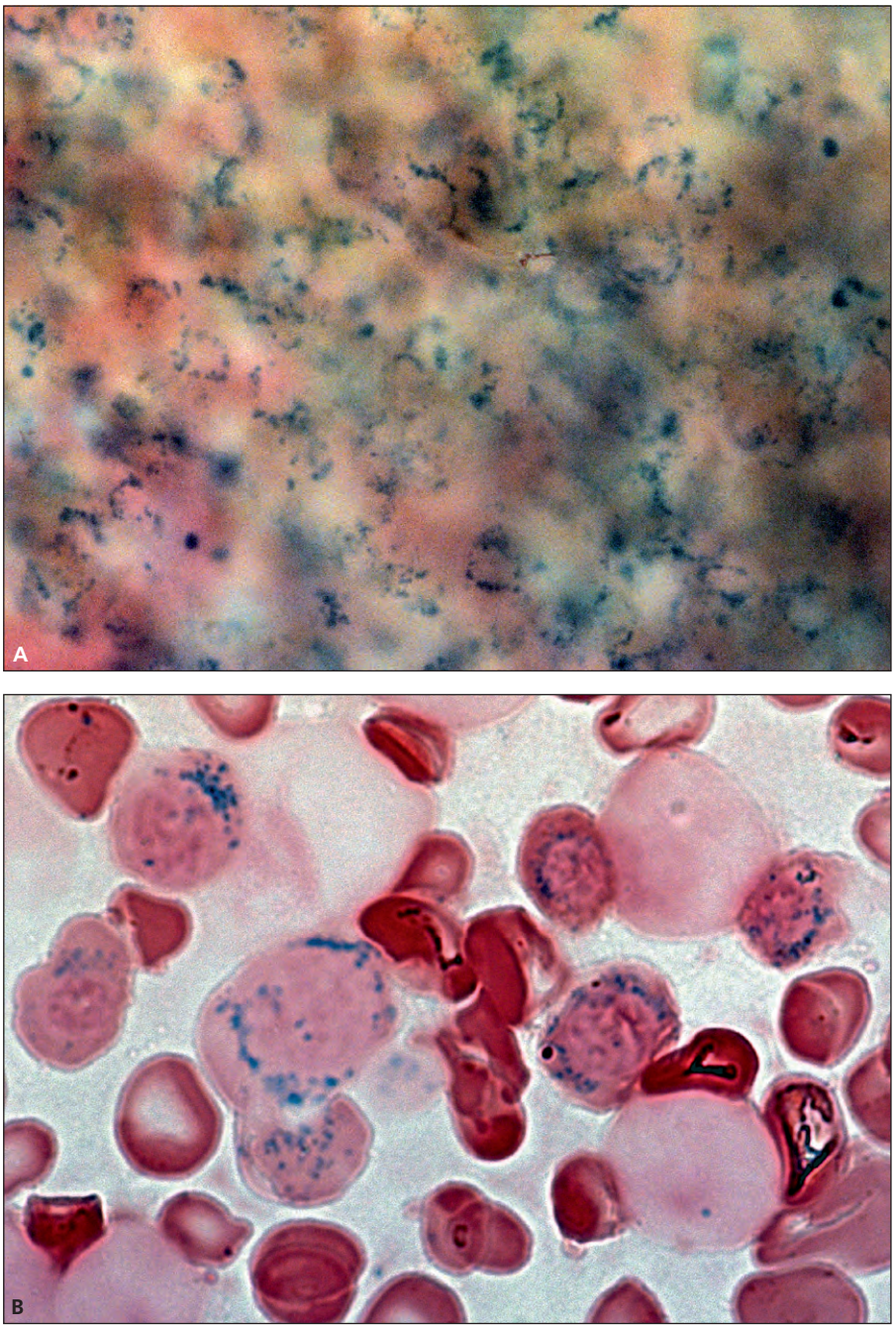
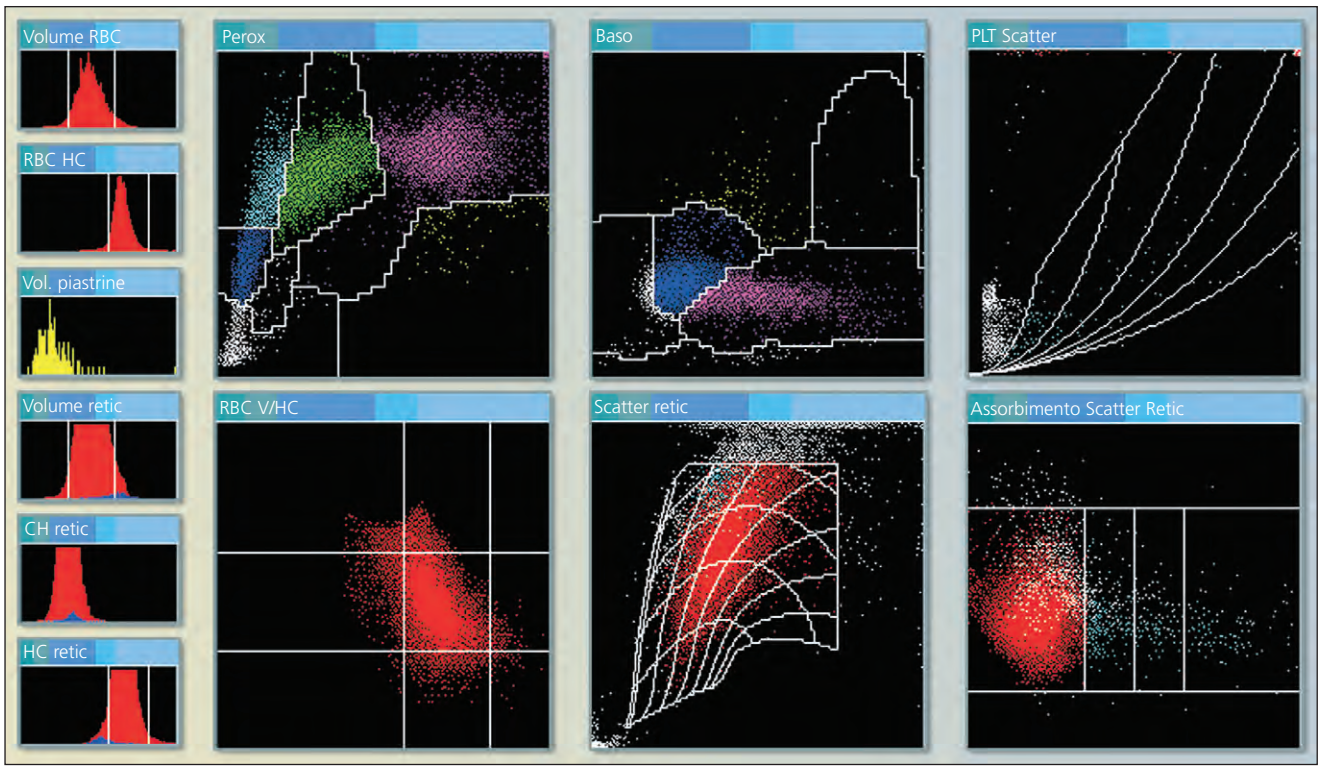



**Fig. 7.40** RARS-T: Perls stain; bone marrow aspirate from the same patient as Fig. 7.38. (A) At medium power, numerous ring sideroblasts stand out in a cellular fragment; they have pale nuclei surrounded by a corona of intensely blue granules. (B) In the thinner part of the film, even though the staining is less intense, it is possible to see cytological details and note that the perinuclear granules are present in erythroblasts that vary in size and are at all stages of maturation.

# REPORT 7.1 Chronic myelomonocytic leukaemia



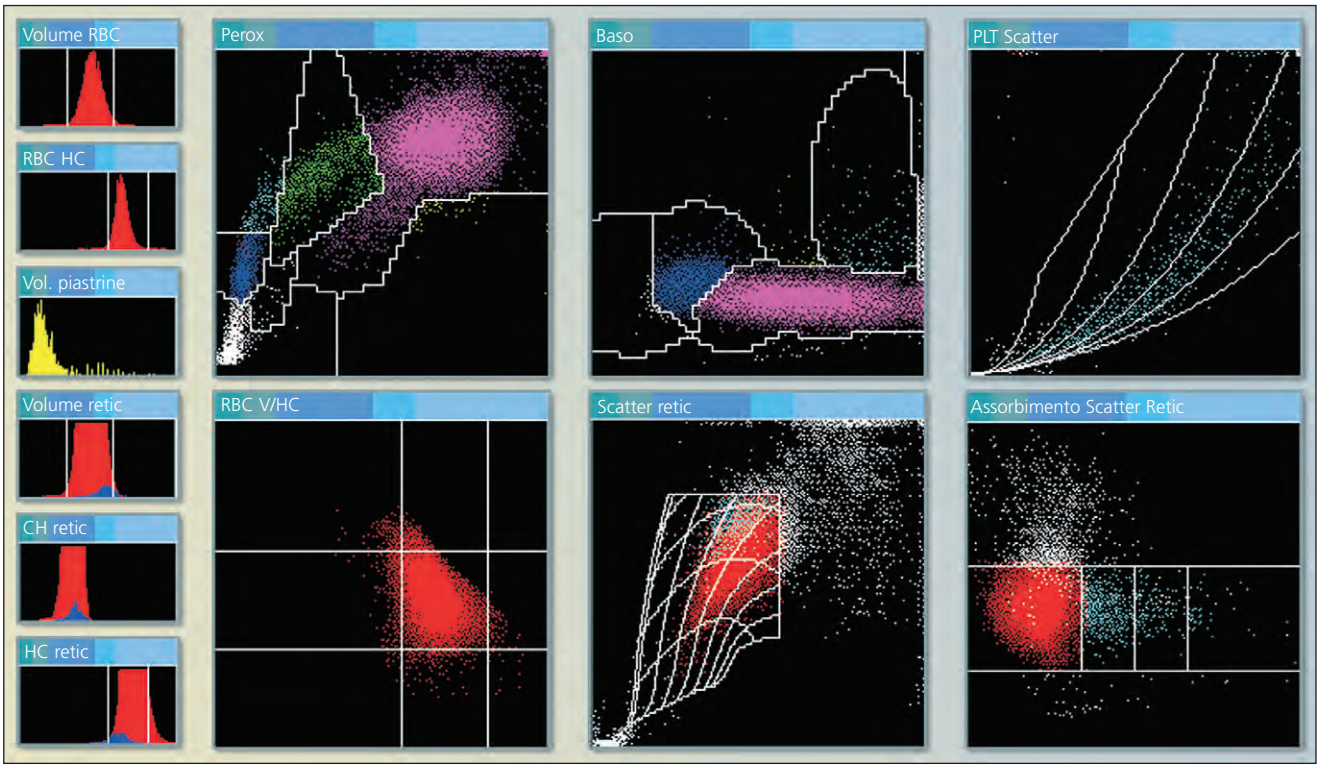

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 92 | g/l | |
| RBC | 3.08 | $\times 10^{12}$/l | * |
| HCT | 0.277 | l/l | * |
| MCV | 90.1 | fl | * |
| MCH | 30.0 | pg | * |
| MCHC | 333 | g/l | * |
| CHCM | 325 | g/l | * |
| RDW | 18.7 | % | * Anisocytosis ++ |
| HDW | 2.80 | g/dl | * |
| HYPO | 3.7 | % | |
| HYPER | 0.5 | % | |
| MICRO | 2.3 | % | |
| MACRO | 4.4 | % | Macrocytosis + |
| RBC fragments | 0.00 | $\times 10^{12}$/l | |
| RBC ghosts | 0.07 | $\times 10^{12}$/l | |
| RET | 1.22 | % | |
| RET | 32.8 | $\times 10^{9}$/l | |
| CHr | 32.8 | pg | |
| IRF (M + H) | 21.4 | % | |
| PLT | 17 | $\times 10^{9}$/l | * PLTORN |
| MPV | 12.8 | fl | * |
| PDW | 54.3 | % | |
| Large platelets | 18 | $\times 10^{9}$/l | |
| MPC | 19.6 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 9.68 | | |
| WBC Perox | 9.65 | | |
| NEUT | 3.79 | 39.2 | |
| LYMPH | 1.35 | 13.9 | |
| MONO | 3.44 | 35.5 | |
| EOS | 0.11 | 1.1 | |
| BASO | 0.12 | 1.2 | |
| LUC | 0.88 | 9.1 | Atyp + |
| NRBC | 0.00 | 0.0 | |
| LI | 1.72 | | * LSH ++ |
| MPXI | 5.3 | | |
| MN | | 72.9 | IG +++ |
| PMN | | 25.6 | B-NV |
| BLASTS | | 3.3 | Blasts + |
| Perox HPX | | 4.7 | |
| Perox SAT | | 2.6 | |

Relative and absolute monocytosis, with increased LUC. In the Perox cytogram the monocytic population appears well circumscribed and falls into the normal area for these cells, with the exception of a partial invasion of the LUC box by some peroxidase-negative cells. The neutrophil cluster shows dispersion towards the right, with the presence of immature granulocytes: their presence is confirmed by an IG flag, which has been activated because of the discrepancy between the count of neutrophils + eosinophils in the Perox channel (40.3) and PMN in the Baso channel (25.6), caused by the presence of peroxidase-positive mononuclear cells, which have also obliterated the MN/PMN valley. The Blast flag results from the count of cells with low chromatin density in the Baso channel associated with a high LUC value, itself responsible for the Atyp flag, which is common in neoplasms involving the monocyte lineage. There is anaemia with anisocytosis and marked thrombocytopenia.

**Morphology.** White cell differential count on blood film: neutrophils 33%, neutrophil myelocytes and metamyelocytes 4%, eosinophils 0.5%, basophils 0.5%, lymphocytes 16%, monocytes 37% in part with atypical morphology, promonocytes 8%, blasts 1%; anisopoikilocytosis; dysmorphic and giant platelets. The monocytosis with only small numbers of circulating blast cells and the dysplastic features of other lineages, with the presence of some immature granulocytes, is compatible with the diagnosis of chronic myelomonocytic leukaemia type 1: this requires confirmation by morphological assessment of the bone marrow and cytogenetic and molecular genetic analysis to exclude abnormalities specifically associated with other, myeloproliferative neoplasms.

## REPORT 7.2 Atypical chronic myeloid leukaemia, *BCR-ABL1* negative




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 137 | g/l | |
| RBC | 4.47 | $\times 10^{12}$/l | |
| HCT | 0.418 | l/l | |
| MCV | 93.7 | fl | |
| MCH | 30.7 | pg | |
| MCHC | 328 | g/l | |
| CHCM | 328 | g/l | |
| RDW | 13.1 | % | |
| HDW | 2.20 | g/dl | |
| HYPO | 1.2 | % | |
| HYPER | 0.,2 | % | |
| MICRO | 0.3 | % | |
| MACRO | 1.3 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 1.74 | % | |
| RET | 77.5 | $\times 10^{9}$/l | |
| CHr | 35.5 | pg | |
| IRF (M + H) | 16.5 | % | |
| PLT | 96 | $\times 10^{9}$/l | |
| MPV | 10.8 | fl | |
| PDW | 47.4 | % | |
| Large platelets | 11 | $\times 10^{9}$/l | |
| MPC | 21.9 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 30.04 | | |
| WBC Perox | 29.99 | | |
| NEUT | 27.28 | 90.8 | |
| LYMPH | 1.54 | 5.1 | |
| MONO | 0.93 | 3.1 | |
| EOS | 0.05 | 0.2 | |
| BASO | 0.04 | 0.1 | |
| LUC | 0.20 | 0.7 | |
| NRBC | 0.00 | | |
| LI | 2.02 | LSH + | |
| MPXI | 2.0 | | |
| MN | | 9.4 | |
| PMN | | 89.0 | |
| BLASTS | | 0.2 | |
| Perox HPX | | 1.8 | |
| Perox SAT | | 0.8 | |

Leucocytosis with marked neutrophilia. The population of neutrophil granulocytes is compact and well positioned in the appropriate zone; from here a trail of signals spread out towards the bottom and the left, similar to those that are observed in specimens kept for more than 24 hours (however, in this case, the blood count was performed a few hours after obtaining the specimen). In addition, the morphology of the MN and PMN in the Baso cytogram does not show qualitative alterations: the preserved length and shape of the PMN tail confirm that the specimen was fresh at the time of analysis. There are no morphological flags with the exception of a left shift, which indicates reduced separation of the MN and PMN, as is seen when neutrophil band forms are present. The platelet count is reduced.

**Morphology and interpretation.** The white cell differential count on the blood film indicated the presence of 89% mature neutrophils, in part hypogranular with defective nuclear segmentation; neutrophil metamyelocytes 2%, lymphocytes 6%, monocytes 3%. It is possible that the trail that deforms the neutrophil cluster in the Perox cytogram results from the presence of dysplastic cells that are susceptible to premature apoptosis.

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CHAPTER 8

# Acute Lymphoblastic and Mixed Phenotype Leukaemia

The third edition of the World Health Organization (WHO) classification of lymphoid neoplasms adopted the principles of the Revised European American Lymphoma (REAL) classification.[1] The diagnostic approach originally suggested by the French–American–British (FAB) group[2,3] was abolished: it had not been found in the years after its introduction to have any real clinical or prognostic value. Lymphoid neoplasms are assigned, in the most recent WHO classification from 2008, to two principal categories: neoplasms derived from B- and T-lineage lymphoid precursors and those derived from mature B, T or NK cells. To the first of these major groups belongs acute lymphoblastic leukaemia (ALL), designated B- or T-lymphoblastic leukaemia/lymphoma,[4] with three principal categories: B-lymphoblastic leukaemia/lymphoma not otherwise specified (B-ALL, NOS), B-lymphoblastic leukaemia/lymphoma with recurrent cytogenetic alterations and T-lymphoblastic leukaemia/lymphoma. One is dealing with neoplasms that derive from a clonal proliferation of lymphoid-committed stem cells, which in the majority of cases appear to be frozen at a specific level of maturation in the T- or B-lymphoid lineage and which express characteristic antigens that permit the determination both of the lineage involved and of the stage of maturation.

The designation leukaemia/lymphoma for this group of neoplasms had already been adopted in the previous WHO classification, in accordance with the principle that neoplasms must be classified on the basis of the biological and molecular characteristics of the neoplastic cells, regardless of the main localisation, which can be, according to conventional usage, leukaemia, i.e. diffuse infiltration of the peripheral blood and the bone marrow, or lymphoma, i.e. confined to lymph nodes with minimal or no involvement of non-lymphoid organs. The terminology 'leukaemia' and 'lymphoma' continues, however, in clinical practice: in the leukaemic form, by definition, the peripheral blood, bone marrow or both must be involved and must contain at least 20% blast cells, although in rare cases the blast count at presentation may be less than this. The form of the disease with a purely leukaemic presentation is most often of B lineage (85%), while T-lineage cases often present with an associated lymphomatous mass involving the mediastinum or other sites.

ALL is the most frequent neoplasm in the paediatric age range: around 75% of cases occur in children under the age of six years. The incidence of ALL in relation to age shows a first peak between the ages of two and four years, and a later gradual increment starting from the sixth decade of life, with a second smaller peak at an advanced age.[5] It is interesting to observe that some cases of ALL in infancy have their origin *in utero*[6]; fewer than 5% of cases have a predisposing cytogenetic or genetic abnormality, such as Down syndrome, Bloom syndrome or ataxia telangiectasia.

ALL develops in the bone marrow and invades the peripheral blood and other extramedullary sites. The infiltration of the haemopoietic parenchyma is often massive at the time of diagnosis and leads to a marked reduction of normal trilineage haemopoiesis. The lack of a preleukaemic phase is generally reflected in the acute onset of clinical symptoms, resulting both from the neoplastic expansion and from the reduced production of mature functioning blood cells: fever, asthenia, increasing malaise and haemorrhagic syndromes. There is often spontaneous bone pain, myalgia and arthralgia. On clinical examination, lymphadenopathy, splenomegaly and hepatomegaly, generally of moderate degree, are present in 50–80% of cases. There is often an increase in plasma uric acid and lactate dehydrogenase (LDH), with a reduction of immunoglobulin concentration in about a half of cases. Around 30% of children with ALL and around 8% of adults present with central nervous system localisation; even if the reference values for mononuclear cells in the cerebrospinal fluid (CSF) are up to 5 cells/μl in the adult and up to 30 cells/μl in the neonate, the recognition of lymphoblasts, even in lower numbers than these, always correlates with an increased risk of relapse (Fig. 8.1). Testicular localisation is rare as the first manifestation of this neoplasm.

The modern diagnostic approach to ALL integrates morphology, immunophenotyping, cytogenetics and molecular genetics. Investigation of the properties of the leukaemic cells is indispensable not only to reach the final diagnosis, but also for monitoring the clinical course of the disease and evaluating the remission status. A correct diagnostic algorithm for these leukaemias starts with a morphological and cytochemical approach, which is limited in general to identifying the presence of a blast population, and continues by determining the lineage involved

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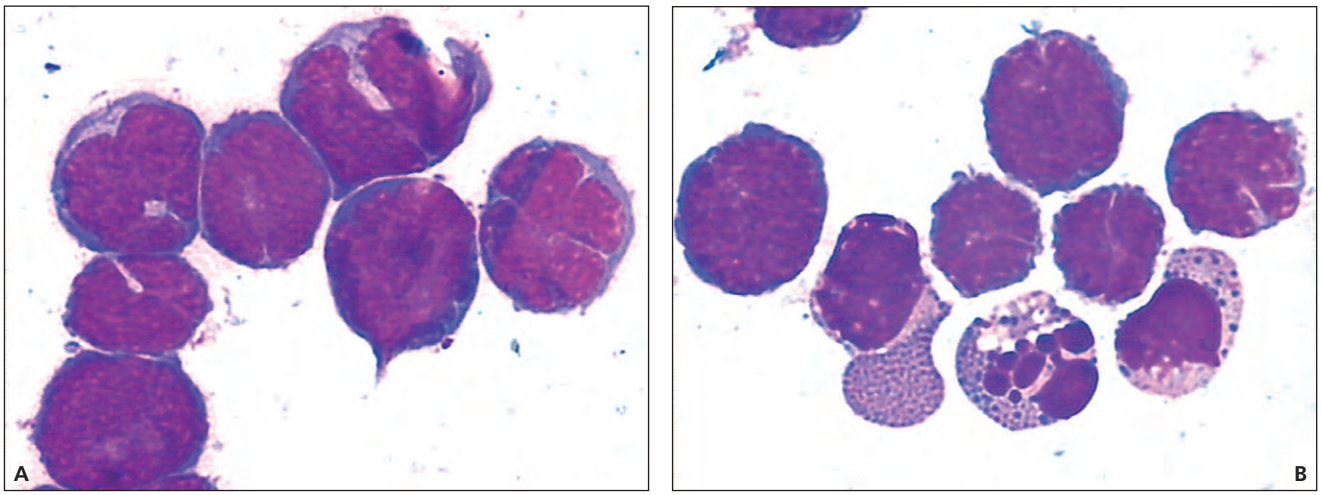



**Fig. 8.1** (A and B) Acute lymphoblastic leukaemia (ALL), B-lineage, common ALL type, CD10 positive, in a four-year-old girl; central nervous system (CNS) localisation. (A) The lymphoblasts in this cytocentrifuge preparation of cerebrospinal fluid (CSF) show a large nucleus that is split and lobulated and scanty hyperbasophilic cytoplasm. (B) Numerous blast cells show apoptosis, resulting from the period they have spent in the CSF: there is chromatin liquefaction, nuclear fragmentation and formation of large necrobiotic masses in the cytoplasm.

and the degree of differentiation, for which flow cytometric immunophenotyping is indispensable. It is also possible to identify immunophenotypic profiles that correlate with specific subtypes of ALL associated with recurrent cytogenetic aberrations. Cytogenetic analysis serves to define the presence of hyperdiploidy (>50 chromosomes) or hypodiploidy (<45 chromosomes), or of alterations that have prognostic significance, such as t(9;22). Finally, molecular genetics can be used to look for gene rearrangements (such as those in immunoglobulin genes or T-cell receptor [TCR] genes), important both for prognostic stratification and in monitoring minimal residual disease.

## Full blood count

At presentation, haematological analysis shows anaemia and thrombocytopenia in more than 80% of patients. In a minority of cases, particularly in the paediatric age range, the platelet count is within normal limits or only slightly reduced; it is very rare to observe a moderate thrombocytosis, with counts above $400 \times 10^9$/l. In some patients, particularly when there is very severe anaemia, there are erythroblasts in the peripheral blood film. The simultaneous presence of blast cells and erythroblasts in the peripheral blood must not be considered secure evidence of erythroleukaemia: erythroblastosis depends on the combined action of an erythropoietic stimulus resulting from anaemia and the damage to the marrow-blood barrier resulting from hypoxia and blastic infiltration. The leucocyte count (WBC) at presentation is variable, with values below $5 \times 10^9$/l in 25% of cases. The finding of leucopenia and neutropenia without circulating blast cells is possible, but less common than in AML. Marked leucocytosis, with a WBC above $15–20 \times 10^9$/l, has an unfavourable prognostic significance, particularly in children. Counts above $100 \times 10^9$/l, more frequent in adults, in T-lineage disease and in infants under the age of one year with conspicuous organomegaly, require an immediate therapeutic intervention because of the high risk of leucostasis.[7] The percentage of lymphoblasts in the circulation correlates in general with the degree of leucocytosis (Figs 8.2, 8.3 and 8.4). In addition to neutropenia, it is fairly common to recognise eosinophilia, even quite marked eosinophilia, sometimes with moderately dysplastic features such as nuclear hyposegmentation or cytoplasmic hypogranularity. Eosinophilia in ALL is usually a reactive phenomenon, independent of the immunophenotype and induced by the release of specific cytokines by the lymphoblasts[8]; it must be carefully controlled because of the possible complication of organ damage, particularly cardiac damage. However, in the presence of eosinophilic infiltration associated with T lymphoblasts, it is necessary to investigate for possible rearrangement of the *FGFR1* gene which, if present, means that the disorder must be assigned to a category that differs in prognosis, clinical course and therapeutic options (see Chapter 6)[9–11] (Fig. 8.5). In the presence of eosinophilic infiltration associated with B-lineage disease, it is useful to investigate for t(5;14).[12]

## Morphology

A morphological assessment represents the first step in the diagnostic pathway, since the majority of cases of lymphoblastic leukaemia/lymphoma present with peripheral blood and bone marrow involvement: Table 8.1 shows morphological criteria that are useful for distinguishing between myeloblasts and lymphoblasts; these can be useful for an orientation with regard to

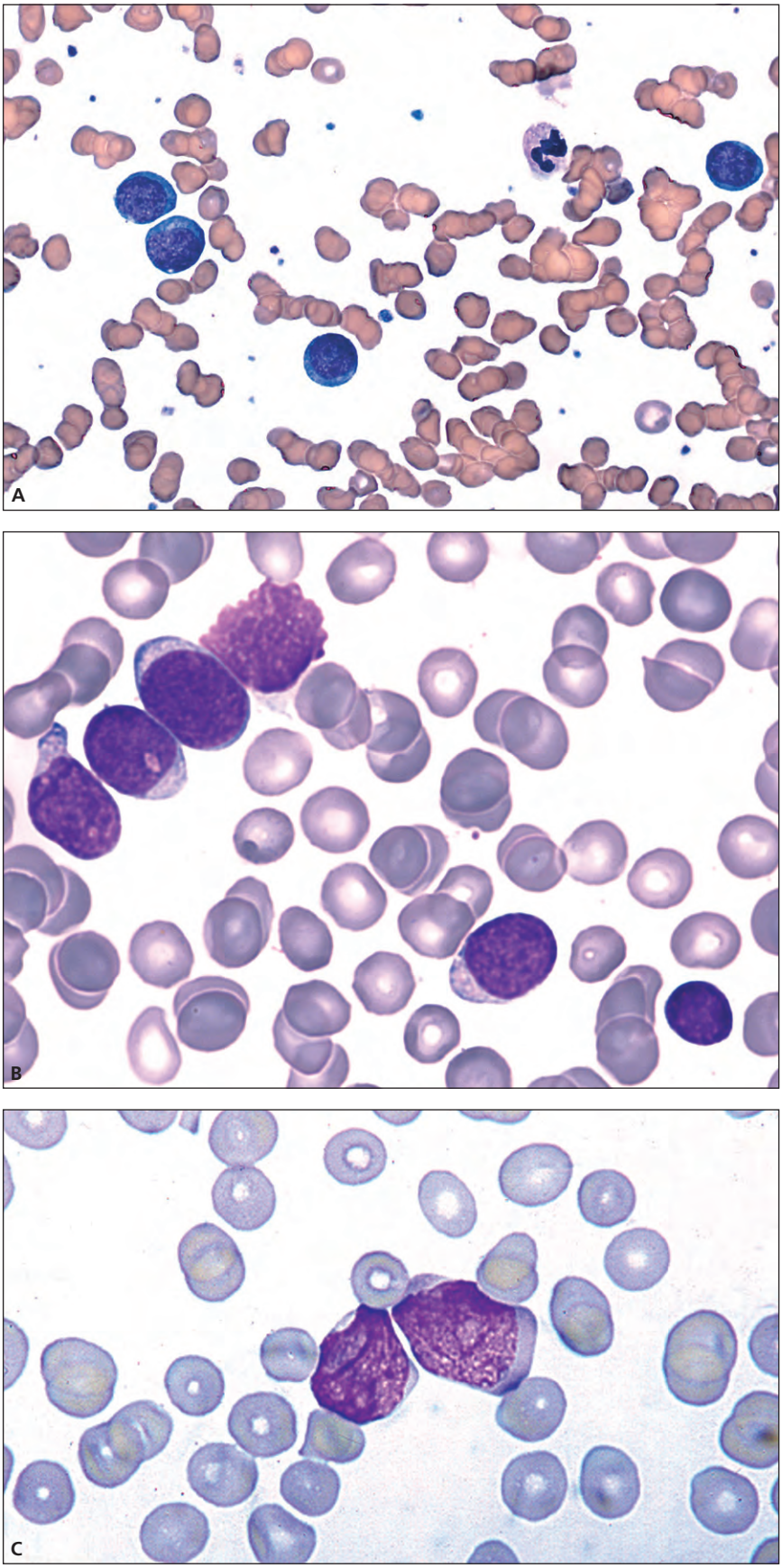



**Fig. 8.2** ALL; peripheral blood films of different patients. (A) Four blast cells with a high nucleocytoplasmic (N:C) ratio. The chromatin is homogeneous but compact; the nucleoli are not visible; the cytoplasm is not at all abundant, basophilic and without granules. The red cells are stacked in rouleaux; bottom right, a polychromatophilic erythrocyte. Numerous platelets and a neutrophil are also present. (B) The morphology of these blast cells suggests their lymphoid origin: they are small with a regular nuclear outline and scanty weakly basophilic cytoplasm; the nucleus is eccentric with compact chromatin, without dark condensed clumps or pale fissures; only a single cell shows a clearly visible nucleolus. This deduction of lymphoid lineage does not, however, have diagnostic value and serves mainly to indicate the need for immunophenotyping. Bottom right, a small hyperchromatic nucleus without cytoplasm; top left, a crushed nucleus. (C) Two blast cells with less regular nuclear outlines, folding and non-homogeneous chromatin condensation and clearly visible nucleoli. The basophilic cytoplasm and the nuclei themselves appear deformed by contact with erythrocytes, indicating a particular structural fragility.

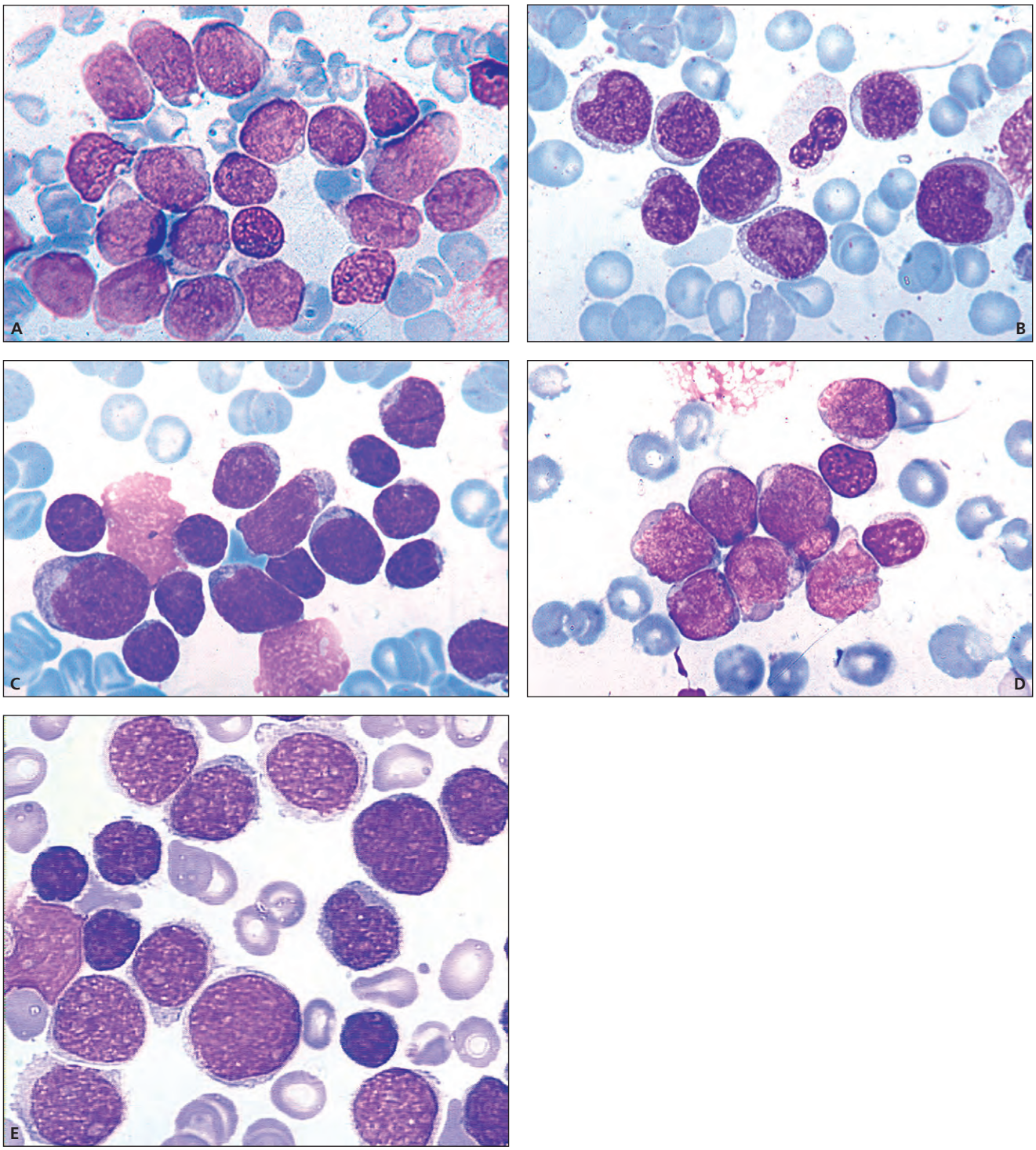



**Fig. 8.3** ALL; peripheral blood films of different patients with very marked leucocytosis. (A) These lymphoblasts are of medium size and uniform in appearance. The cytoplasm is very scanty and weakly basophilic; the nuclei are eccentric and have an outline that is almost always regular, but is sometimes slightly angulated; the chromatin is homogeneous, reticular and tightly woven; nucleoli are visible in only a few of the larger cells. Some cells have a cytoplasmic extension, like the handle of a racquet. (B) Lymphoid blasts that are more heterogeneous in size, some being large. Some cells have indented nuclei; the cytoplasm is light blue and sometimes appears perforated by small, uniformly sized vacuoles. The chromatin is condensed to give a dark, finely granular appearance. The outline of a nucleolus can be seen top left. (C) Cell size is very variable: some cells are large and have chromatin that is dense but structured into numerous small hyperchromatic areas; the smaller of these cells resemble lymphocytes with very compact chromatin and very little cytoplasm that is often almost invisible; nucleoli are absent. Such morphology, with the presence of crushed nuclei, can lead to diagnostic doubt between ALL and a lymphoproliferative disorder of mature B cells. (D) Large uniform lymphoblasts. The chromatin is reticular, homogeneous, with a number of nucleoli, sometimes quite distinct; the nuclear outline is rounded with some irregularity. On the right, a small lymphocyte. (E) Lymphoblasts are often large with a high N:C ratio. In the large cells, the nuclei are round and the chromatin is open, condensed into small clumps, sometimes worm shaped; nucleoli are sometimes clearly visible. A minor proportion of cells have the appearance of small lymphocytes with dark nuclei with chromatin condensed into a mosaic pattern (one of these has a multilobed nucleus), the clonal nature of which can be demonstrated by immunophenotyping.

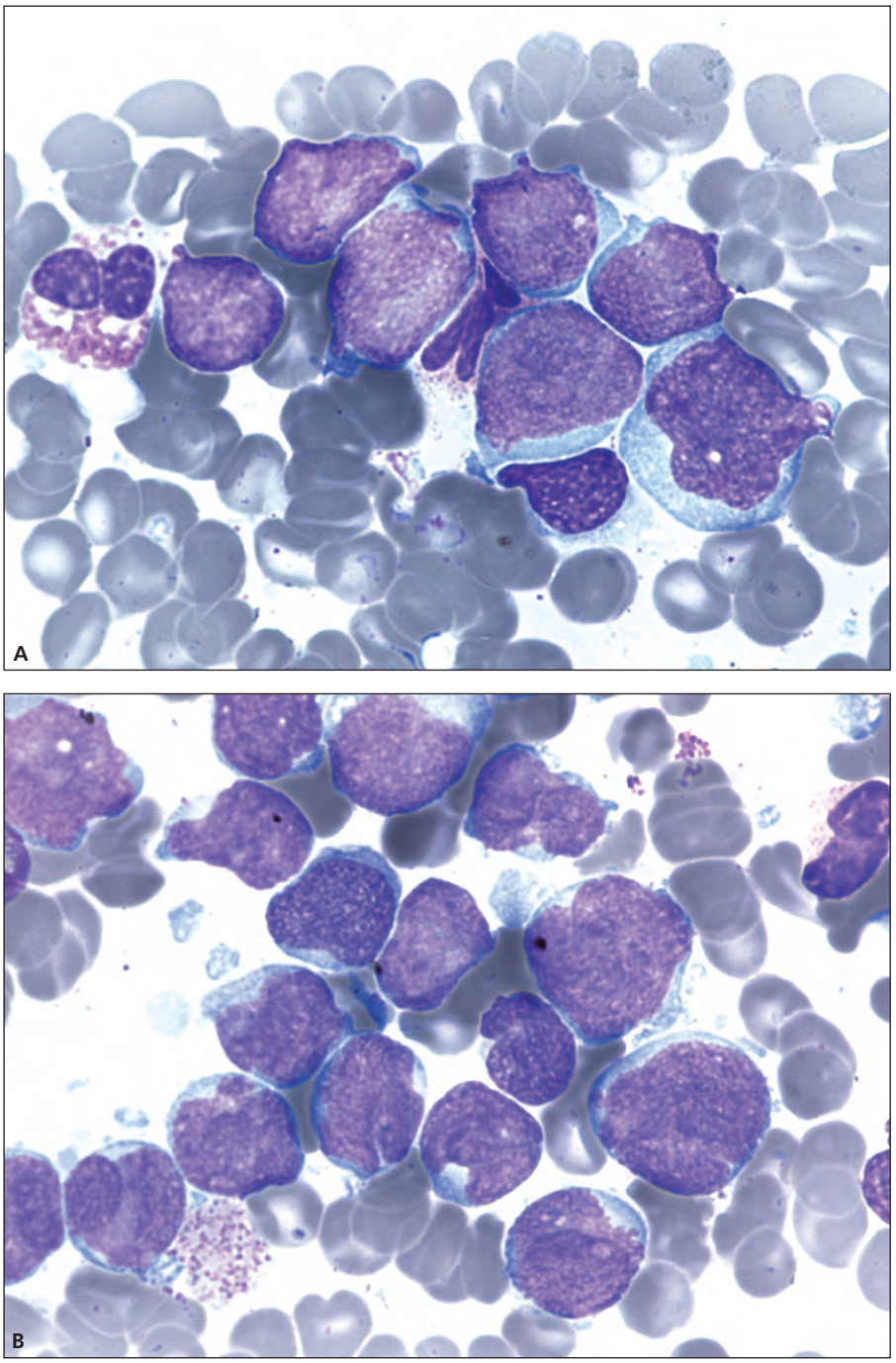



**Fig. 8.4** ALL, B-lineage, common ALL type, with marked morphological heterogeneity (FAB L2). (A) Peripheral blood film. Blasts with heterogeneous morphology, often with large nuclei, the profile of which is sometimes angular or indented with straight stretches in the zone of contact between cells. The chromatin density is quite variable: fine and dispersed in the large cell immediately to the right of the centre (which has a voluminous but not very distinct nucleolus), more compact in the smaller cells. There is a variable amount of basophilic cytoplasm which is pale blue and clean looking. To the left, an eosinophil. (B) Bone marrow aspirate. In the bone marrow the lymphoblasts occupy all the available space. They have a more heterogeneous, pleomorphic appearance, with marked irregularity of the nuclear outline, of the degree of chromatin condensation and of the cytoplasmic extensions. The chromatin, however, has a compactness that is not seen in cells of the monocyte lineage, which otherwise could have similar nuclear morphology. On the right, two large blast cells with pale cytoplasm and very numerous small areas of condensation and evident nuclear notches and grooves. One notes some nuclear fragments detached by the process of clasmacytosis.

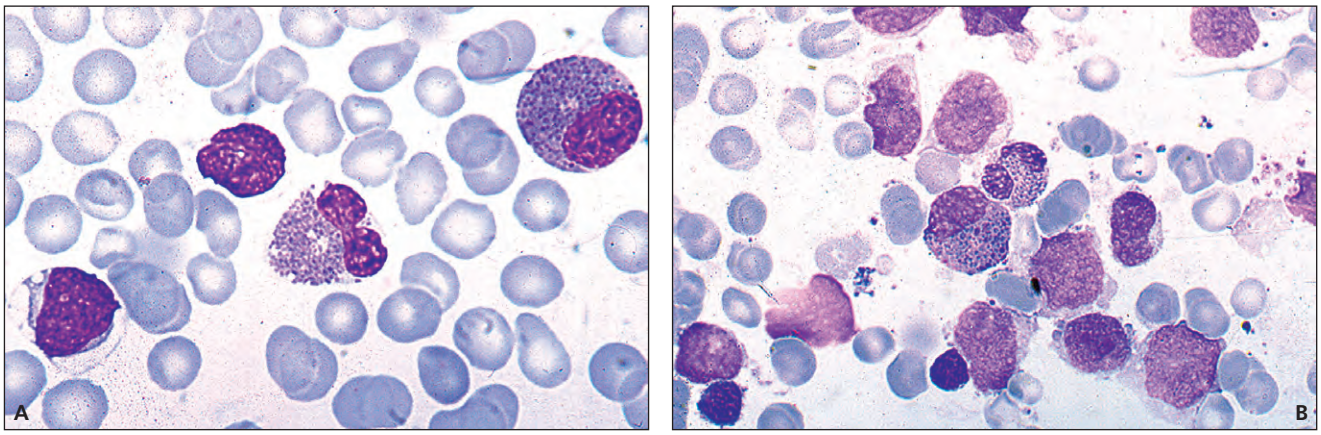



**Fig. 8.5** Eosinophilia in patients with ALL. (A) Peripheral blood film of a patient with common B-ALL shows monomorphic blast cells. On the left, two small blast cells with a high N:C ratio. The nucleus has an angular outline with small notches; the chromatin is heterogeneous with partial condensation into hyperchromic clumps alternating with paler areas; a nucleolus is visible in the cell more to the left. The cytoplasm is very scanty and appears optically empty, with the exception of two weakly basophilic fringes at the sides of the nucleus of the blast cell on the left. In the centre, a mature eosinophil with nuclear lobes still joined by a chromatin band; in the cytoplasm there are several small vacuoles. To the right, an eosinophil myelocyte with an eccentric nucleus and a small Golgi zone: the staining of the granules is the same as that seen in mature eosinophils, but the cytoplasmic background, hidden by the granules, retains a degree of basophilia that gives a greyish tint to the cell. (B) Bone marrow aspirate in ALL associated with eosinophilia. In the centre, a mature eosinophil and an eosinophil myelocyte, which has some reddish proeosinophilic granules mixed with mature specific granules. The blast cells are heterogeneous with regard to size, with an irregular nuclear outline, delicate chromatin, visible nucleoli and moderately plentiful, weakly basophilic cytoplasm.

**Table 8.1** Morphological characteristics of blasts cells that are useful for orientation towards the lineage to which they belong

| | Lymphoblasts | Myeloblasts |
|---|---|---|
| General characteristics | Blast population tends to be homogeneous | Blast population tends to be heterogeneous, with the exception of the undifferentiated form |
| Size | Variable, mainly small | Variable, mainly large |
| Nucleus | Central, mainly round; sometimes indented, particularly in the form in adults<br>Nucleocytoplasmic (N:C) ratio very high in the form that occurs in children<br>N:C ratio lower in the form that occurs in adults | Tending to be eccentric, round, oval or angulated; sometimes convoluted, particularly in the form with a monocytic component<br>N:C ratio high in undifferentiated blast cells and in some megakaryoblasts<br>N:C ratio mainly low in the forms with differentiation |
| Chromatin | Fine, with dispersed foci of condensation<br>Very condensed in small lymphoblasts | Fine, granular, delicately dispersed |
| Nucleoli | Absent in small lymphoblasts<br>Sometimes indistinct | Almost always present, often large and prominent, double or triple |
| Cytoplasm | Scanty, basophilic<br>Sometimes with a single long projection ('hand-mirror cell') | Variable<br>Abundant in monoblasts<br>With protrusions in erythroblasts and megakaryoblasts |
| Granules | Rarely present, azurophilic and always negative for peroxidase, esterases and toluidine blue | Present in forms with differentiation and positive with cytochemical stains:<br>• Peroxidase in the neutrophil and eosinophil lineages<br>• Non-specific esterase in the monocyte lineage<br>• Toluidine blue in the basophil lineage |
| Auer rods | Always absent | Can be present<br>Typically present in the hypergranular promyelocytic form |
| Vacuolation | Can be present | Can be present<br>Almost always present in forms with a monocytic component |

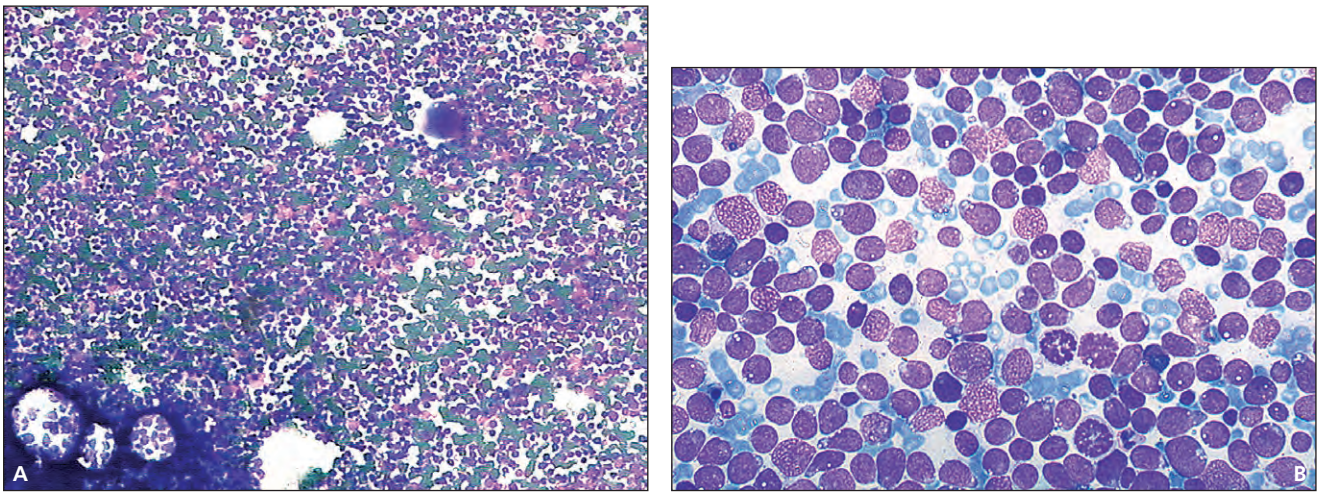



**Fig. 8.6** ALL, B-lineage, common ALL, CD10 positive, negative for cytoplasmic mu (μ) chain; bone marrow aspirate. Infiltration by leukaemic lymphoblasts is often massive and associated with a marked reduction in normal haemopoietic precursors. (A) At low power the film appears hypercellular with monomorphic cytology; the nuclei are dark and overcrowded. At the top, a medium-sized megakaryocyte with basophilic cytoplasm. (B) This microscopic field from a different patient appears completely invaded by a monomorphic population of blast cells with large nuclei and scanty basophilic cytoplasm containing some vacuoles. Cell size is moderately variable, but the characteristics of the nuclei and the cytoplasm appear uniform. In some blast cells the cytoplasm has formed a tail at one side of the cell. In the bottom right quadrant, there are several cells in mitosis. A neutrophil beside a blast cell in prophase is the only representative of the granulocyte lineage; the erythroid lineage is represented only by two small orthochromatic erythroblasts.

lineage, but remembering the limits of morphology in precursor lymphoid neoplasms, for which flow cytometric analysis represents the diagnostic gold standard both for the identification of lineage and for defining the stage of maturation. Morphology of leukaemic cells in the peripheral blood can be significantly different from that of cells in the bone marrow (see, for example, Figs 8.12 and 8.14), the examination of which is always indispensable.

Bone marrow aspirates in ALL are generally hypercellular and almost always show a marked blastic infiltration, often more than 90%, with a clear reduction in the numbers of normal haemopoietic cells (Figs 8.6 and 8.7). Only the megakaryocytic component is in general better preserved than in acute myeloid leukaemia (AML). The blasts of ALL, in the most typical form, are immature, undifferentiated and heterogeneous in size, but generally not particularly large. The nuclei are oval or rounded, tending to be eccentric, not angulated, sometimes notched. The chromatin is in general denser and more heterogeneous than that of non-lymphoid blasts; it can have a granular appearance, but is almost never fine and reticular like that of monoblasts; the nucleoli are barely evident because of a lack of surrounding chromatin enhancement. The cytoplasm is usually scanty or moderate in amount, weakly basophilic, agranular, without a pale paranuclear Golgi zone and is sometimes vacuolated (Figs 8.7 through to 8.14). Before the advent of immunophenotyping, the cytomorphological diagnosis of lymphoblastic leukaemia was in large part a diagnosis of exclusion, based on negativity for peroxidase and the monocytic esterases and on the association of undifferentiated blast cells with a significant proportion of lymphocytes that appeared more mature. Conversely, the absence of morphological signs of granulocytic differentiation (Auer rods, granules or dysplastic neutrophils) or monocytic differentiation (convoluted nuclei, sandy cytoplasm or peripheral monocytosis) and the presence of undifferentiated blast cells favoured a lymphoid lineage. The cytochemical profile of the lymphoblasts, whatever the morphological or immunological subtype, is uniform: they are negative for the cytochemical reactions identifying myeloblasts (peroxidase, chloroacetate esterase and Sudan black B) (Figs 8.8 and 8.9) and for fluoride-sensitive alpha-naphthyl acetate esterase (ANAE). The periodic acid-Schiff (PAS) reaction correlates with the glycogen content and is often positive, with fine or coarse granular positivity or with blocks arranged like a rosary on a negative cytoplasmic background. PAS positivity is less intense in the S phase of the cell cycle and thus is weaker in the form of ALL with a high percentage of cells in S phase, a form more common in adults. The acid phosphatase reaction correlates with the content of lysosomes and is useful for differentiating T-ALL, characterised by a distinct focal paranuclear positivity in blast cells in more than 90% of cases.

Negative morphological features, such as the absence of Auer rods and peroxidase activity, are shared by ALL and certain subtypes of AML with minimal or no granulocytic differentiation. Only immunophenotyping permits a positive diagnosis of ALL and distinguishes it with security not only from AML with minimal differentiation, from erythroleukaemia and from megakaryoblastic leukaemia (FAB subtypes M0, M6 and M7), but also from lymphoproliferative neoplasms which may need to be considered when the lymphoblasts, particularly in the subtype

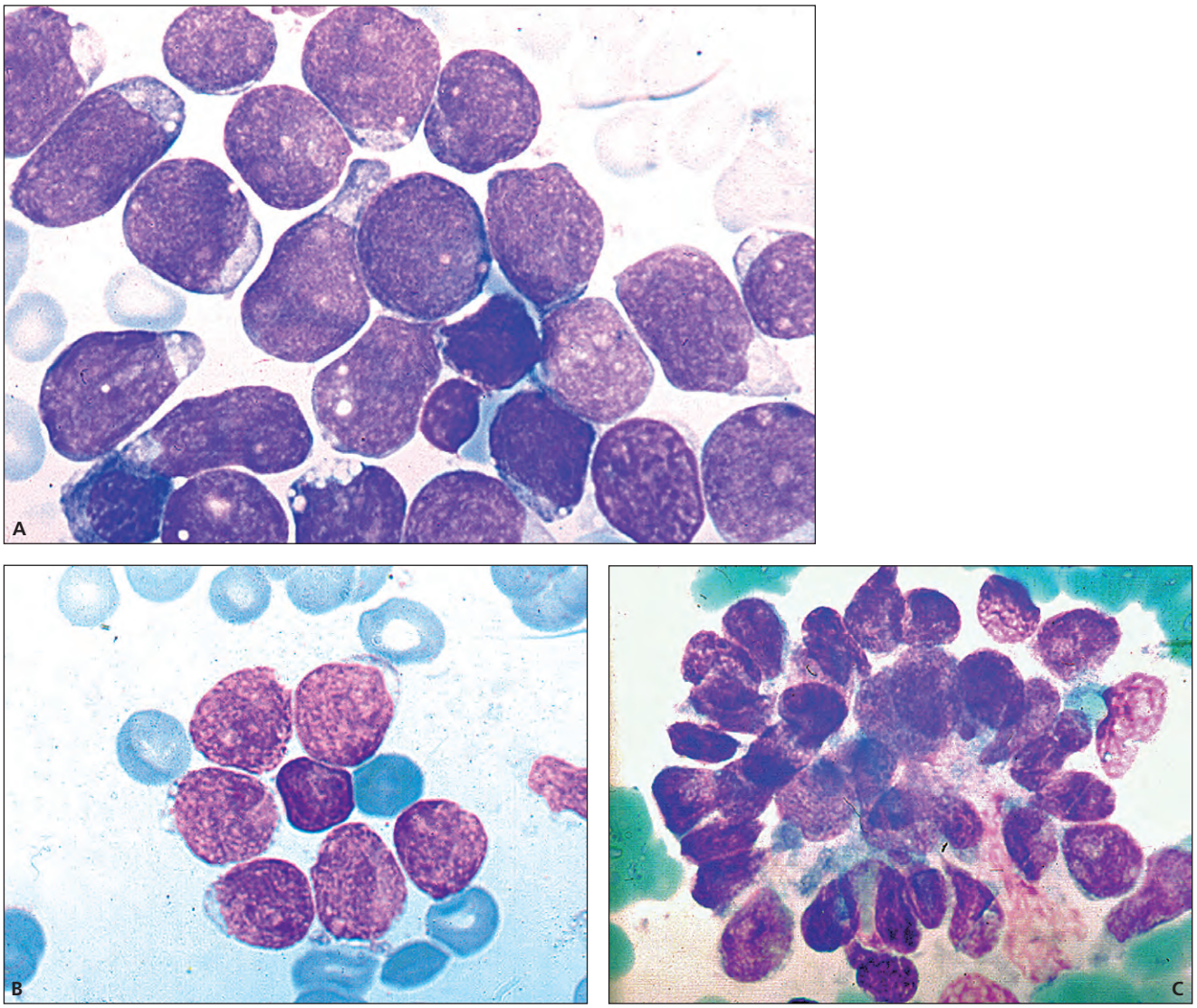



**Fig. 8.7** Lymphoblasts in the bone marrow aspirate of a patient with B-ALL. Common (but not obligatory) characteristics of lymphoid blasts are their small or medium size, scanty cytoplasm, the presence of vacuoles, the oval or round nuclear shape, the partial chromatin condensation and the inconspicuousness of the nucleoli (the picture designated L1 in the FAB classification). This general picture is, however, indistinguishable from some cases of acute myeloid leukaemia (AML) with minimal signs of differentiation or without maturation, since there are no positive diagnostic features. The differential diagnosis is based on immunophenotyping and on the demonstration in AML without maturation of cytochemical positivity for peroxidase. (A) The salient morphological features in this case are the round nuclear outline, the very delicate homogeneous chromatin, the eccentric position of the nucleus in the fairly scanty cytoplasm, the presence of small nucleoli and some vacuoles, and the tendency to a hand-mirror shape. (B) Lymphoblasts of similar appearance to those in (A) adhering by one pole, forming a small rosette, around a bare nucleus with very dense chromatin. (C) A pseudonodular agglomerate of lymphoblasts in pre-B-ALL, with a centripetal disposition of the cytoplasmic tail. This appearance must be differentiated from the similar picture that can be seen with tumour metastases (such as Homer Wright's nodule; see Chapter 11).

with small blast cells, have strongly condensed chromatin. An accurate differential diagnosis, particularly of the forms that occur in infancy, must also include infection, autoimmune disorders, aplastic anaemia, congenital abnormalities and infiltration of the bone marrow by non-haematological neoplasms.[13] In particular, it is necessary to consider the possibility of marrow infiltration by a non-haemopoietic neoplasm with small round cells, such as neuroblastoma, medulloblastoma, Ewing sarcoma or rhabdomyosarcoma, in children and adolescents, and by small cell carcinoma of the lung or other metastasis of undifferentiated carcinoma in adults (see Chapter 11). Improbable, but not to be ignored, is diagnostic confusion between ALL and a reactive picture with atypical lymphocytes, such as infectious mononucleosis or pertussis (Fig. 8.10).

In 1976, the morphological co-operative group, the FAB group, proposed a series of standardised criteria for the

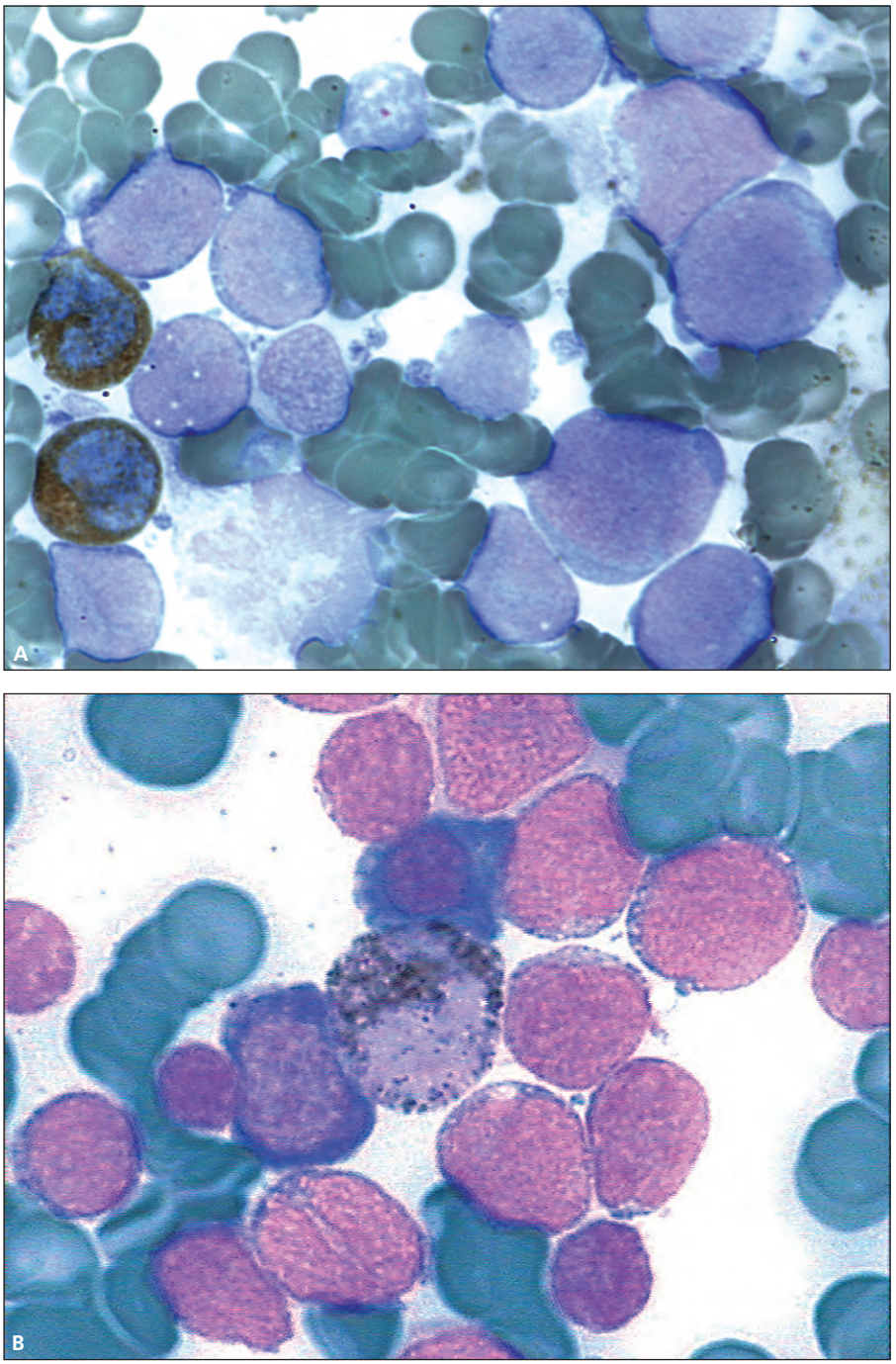



**Fig. 8.8** Cytochemical stains of bone marrow aspirates from two patients with B-ALL. (A) The peroxidase reaction is uniformly negative in all leukaemic cells. On the left, two strongly positive neutrophils, with cytoplasm laden with yellow-gold precipitate. (B) The reaction is positive in a neutrophil and negative in all the lymphoblasts, in a basophilic erythroblast and in a plasma cell; same case as Fig. 8.3E.

cytomorphological diagnosis of ALL and for its subdivision into three categories designated L1, L2 and L3,[2] with a supplementary semiquantitative numerical system based on four criteria.[3] This exclusively morphological and cytochemical approach, now superseded by the 2008 WHO classification, merits being remembered: it both represents a significant step in the development of knowledge and maintains an educational role in cytomorphology, because of the attention paid to a detailed and systematic description of the particular morphological characteristics of the blast cells in ALL, the recognition of which at the microscope leads to a consistent, justifiable provisional diagnosis. The crucial element for the diagnosis of ALL, according to

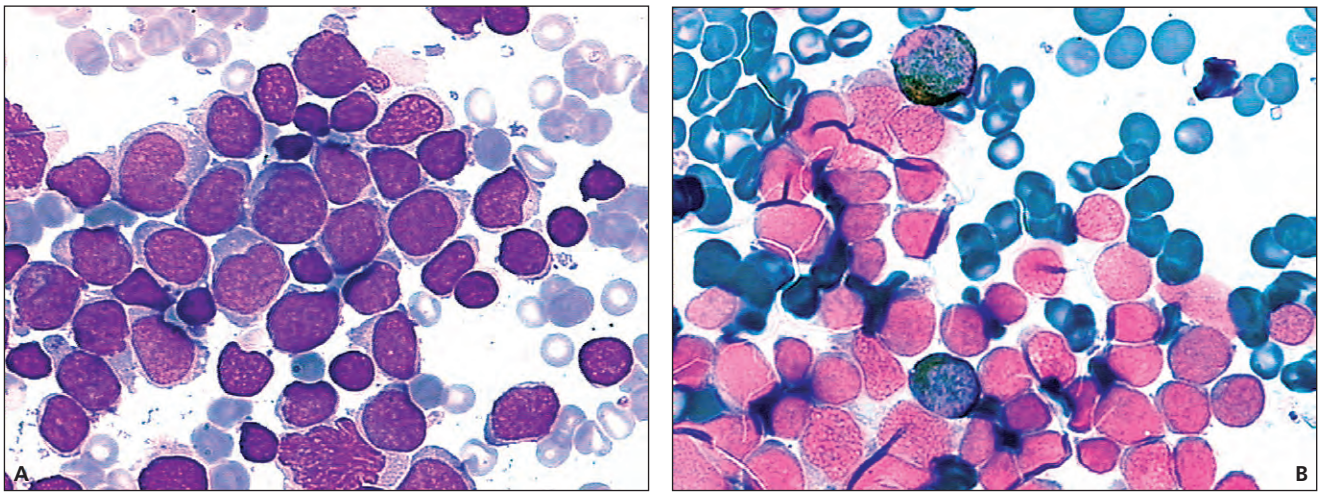



**Fig. 8.9** Pro-B-ALL; bone marrow aspirate. (A) Massive infiltration by blast cells that are heterogeneous with regard to size, with rounded nuclei, compact chromatin without nucleoli and scanty cytoplasm. There are several cells with the morphology of mature lymphocytes. (B) Peroxidase cytochemistry. The blast population is uniformly negative. Only two surviving neutrophil precursors retain their usual intense cytoplasmic positivity.

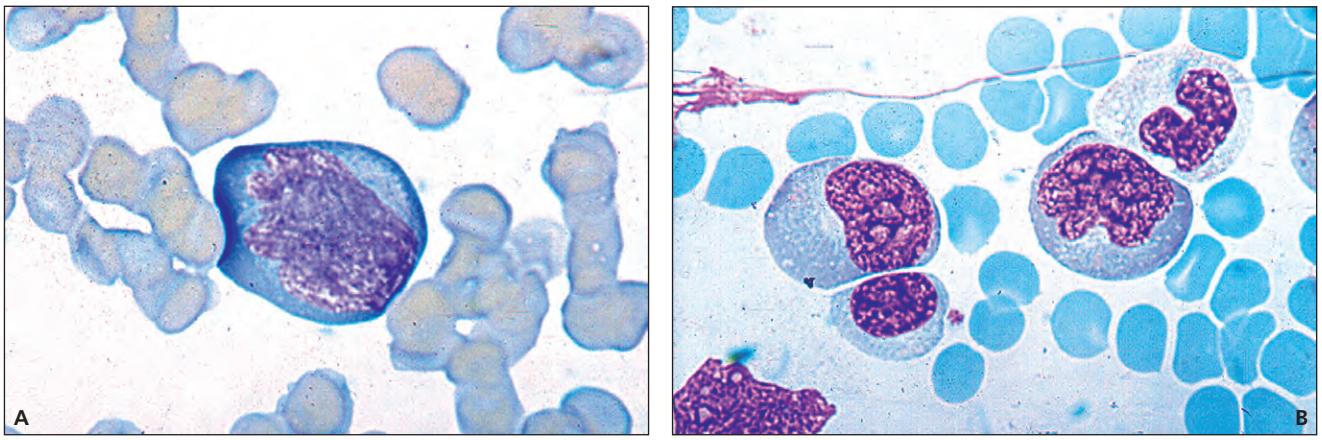



**Fig. 8.10** Non-neoplastic conditions that can simulate ALL. (A) Large immunoblast (Downey cell type III) in the peripheral blood of a patient with varicella. The nuclear outline is irregular and the chromatin is condensed into longish, coarse blocks separated by pale fissures. The cytoplasm is abundant and intensely basophilic, with a large pale zone that almost completely surrounds the nucleus. Although the morphology is very typical, it is not possible to identify secure criteria to distinguish these activated cells from leukaemic blast cells: great importance must be given to the cytological context (the presence of activated lymphocytes such as lymphoplasmacytoid cells and the absence of cytopenia) and the clinical context. (B) Diagnostic doubt can be difficult to resolve by morphology alone in certain cases of infectious mononucleosis that present with significant generalised lymphadenopathy, thrombocytopenia and leucocytosis, with the presence in the blood film of numerous atypical nucleolated immature-appearing cells. In the early phase of infection, serology may be negative and the morphological alterations often precede the appearance of specific antibodies. This image is from the blood film of a boy with infectious mononucleosis at onset and still serologically negative: between a small lymphocyte with plentiful cytoplasm and an eccentric nucleus (bottom) and a neutrophil band form with hypogranular cytoplasm, there are two large blast cells with heterogeneous chromatin and various nucleoli; there is very plentiful cytoplasm with a paranuclear Golgi zone; the cytoplasm shows peripheral basophilia. Determining the immunophenotype, which is that of a CD8-positive T cell, can clarify the diagnosis.

the FAB group, was cytochemical negativity for peroxidase: the percentage of peroxidase-positive blasts had to be less than 3%. Starting from this basis, the criteria for subclassification took into consideration the morphological characteristics of the leukaemic cells and the degree of heterogeneity or homogeneity of the entire blastic population (Fig. 8.11). The FAB L1 subtype was comprised of cases in which the blast cells were small and homogeneous, with a diameter less than double that of a small lymphocyte. The nucleocytoplasmic (N:C) ratio was high. The nucleus was round with a regular outline; only rarely was it indented or folded. The chromatin was homogeneous, even though in the smaller cells there was a tendency to condensation into small dark clumps. The nucleoli were not visible or were small and inconspicuous; often there was only one nucleolus,

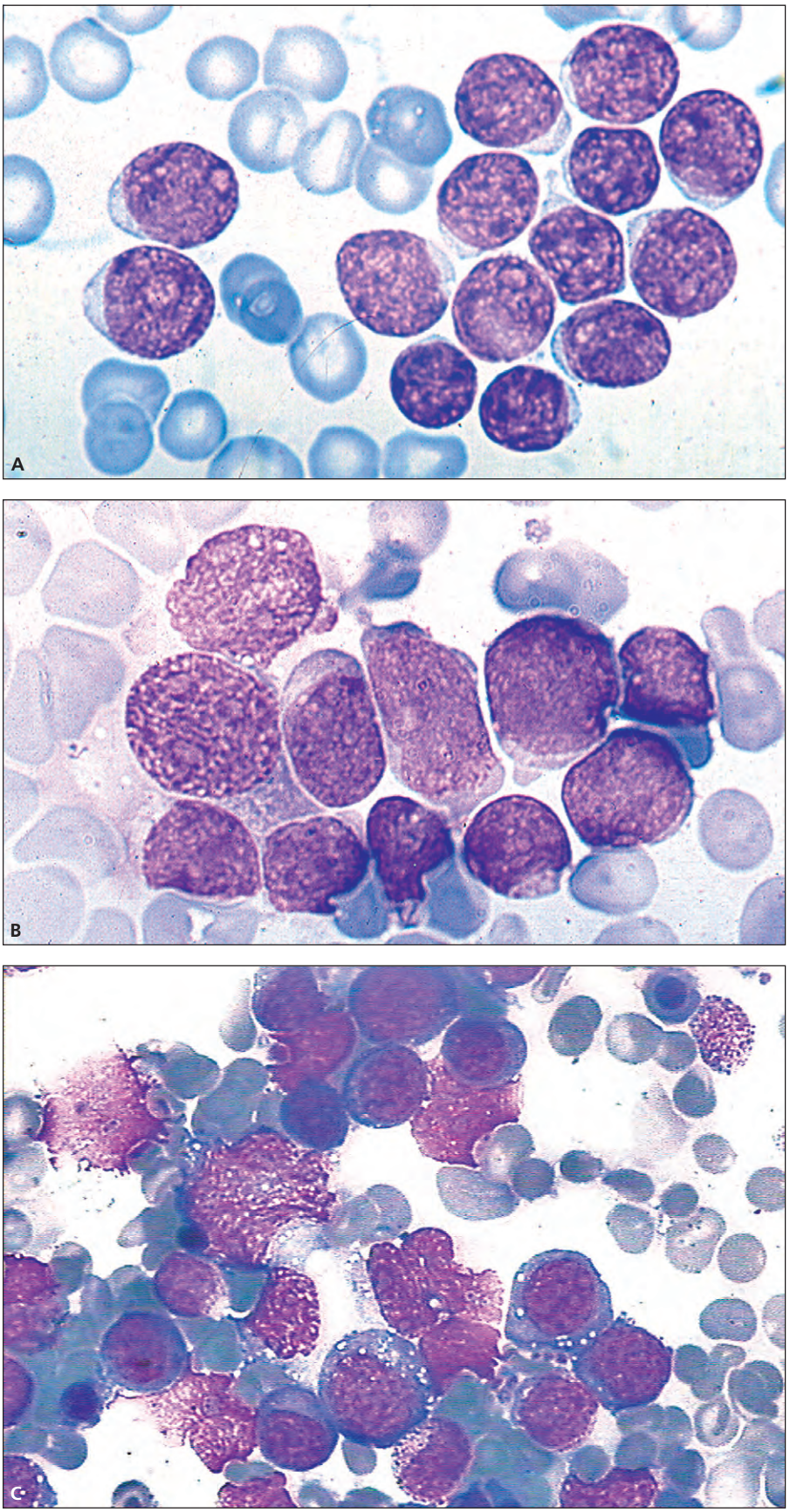



**Fig. 8.11** Characteristic cytological features of the three types of ALL defined by the FAB group in 1976; the concordance between different observers was always unsatisfactory, partly because the cells of ALL are sensitive to the effects of mechanical force applied in making the film and also to the effects of drying. (A) Lymphoblasts with FAB L1 morphology. There is great uniformity: there are small to medium-sized cells; the chromatin appears dense and homogeneous, the nuclear outline is rounded and nucleoli are visible in less than half of the cells. (B) Lymphoblasts with FAB L2 morphology. One notes a double blast population, with large cells with fine chromatin and nucleoli that are fairly well seen, and small lymphoblasts with denser chromatin. The nuclear outline is fairly irregular. (C) Lymphoma cells with FAB L3 morphology. These cells have plentiful vacuolated basophilic cytoplasm and the immunophenotype of a mature B cell; they are no longer considered lymphoblasts but are classified with neoplasms of mature B cells (leukaemic variant of Burkitt lymphoma).

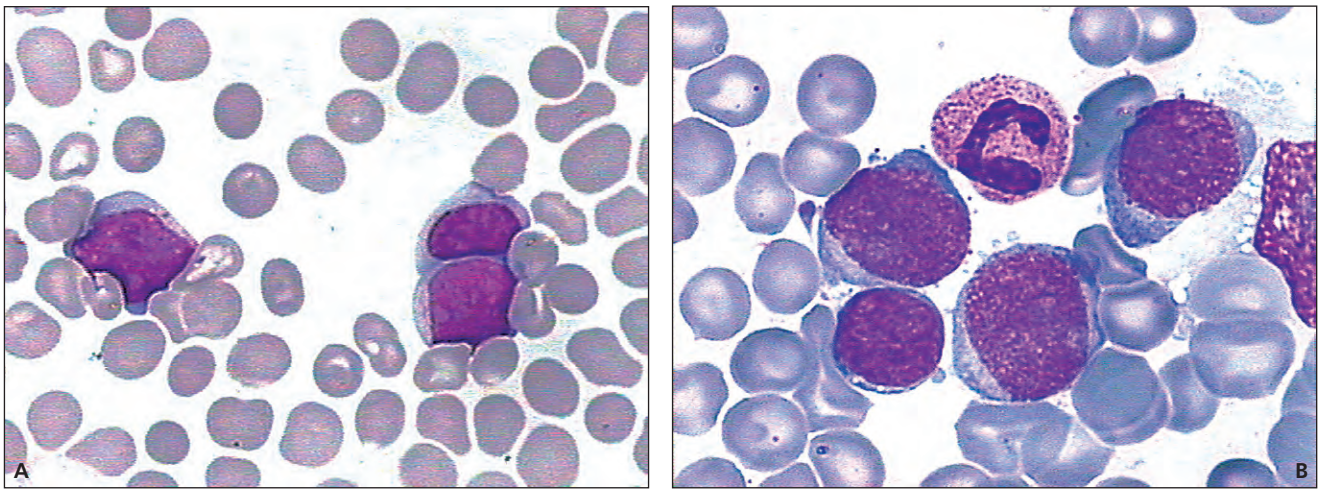



**Fig. 8.12** B-ALL, common ALL type, in an adolescent male presenting with fever for the previous week, splenomegaly and asymmetric tonsilar enlargement. Full blood count (FBC) showed: haemoglobin concentration (Hb) 85 g/l; WBC 6.3 × $10^9$/l; platelets 36 × $10^9$/l. (A) The differential white cell count on the blood film showed 33% lymphocytes, rare neutrophils (2%), a few erythroblasts and 65% medium-sized, fairly uniform blast cells with a high N:C ratio. The nuclei are oval or sometimes angulated, particularly because of pressure from the surrounding erythrocytes. The chromatin is fine and homogeneous and the nucleoli are clearly evident. The cytoplasm shows irregular basophilia. (B) The bone marrow aspirate shows hypercellularity, with more than 90% blast cells with morphology that is fairly different from that of the blood film. The chromatin appears more granular, the nuclei are round and the cytoplasm is more basophilic than in the circulating blast cells. One of the four blast cells is as small as a lymphocyte, but has homogeneous chromatin, without clumping, and a small nucleolus. Flow cytometery showed these cells to be positive for CD19, CD20, CD10, CD5 and HLA-DR, and negative for CD34, myeloperoxidase (MPO), cytoplasmic μ chain and immunoglobulin.

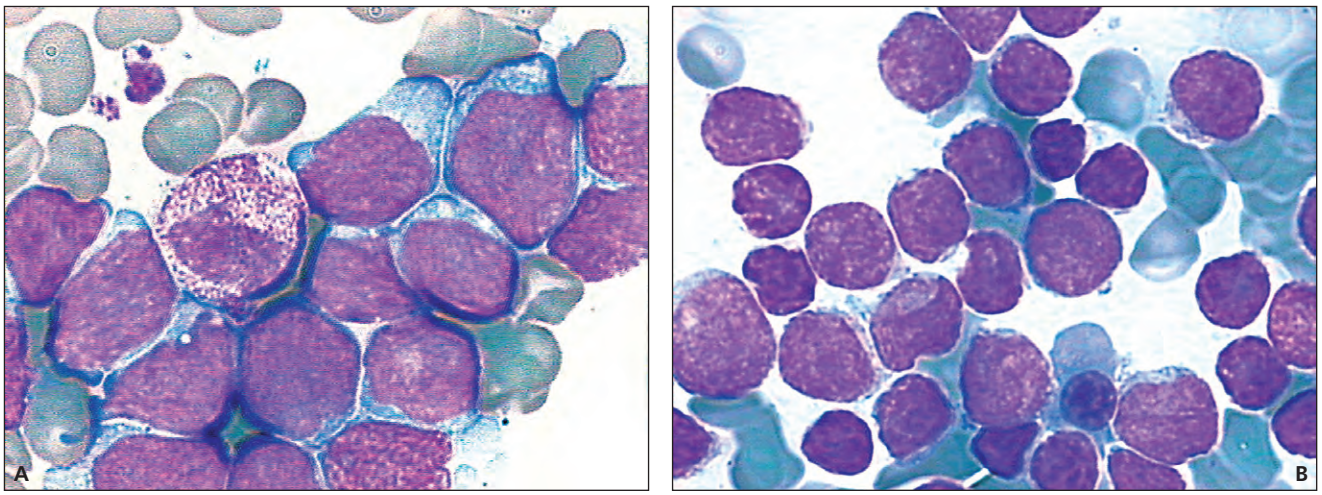



**Fig. 8.13** Examples of characteristic morphological pictures in another two cases of B-ALL. (A) Fairly large blast cells, with dense homogeneous chromatin, barely visible nucleoli and a variable quantity of weakly basophilic cytoplasm. (B) Massive infiltration by lymphoblasts with a very high N:C ratio because of the virtual absence of cytoplasm and round nuclei with homogeneous compact chromatin; the large cell shows nuclear folding. There are also numerous cells with the morphology of lymphocytes and, at the bottom, a polychromatic erythroblast with clumped chromatin.

which appeared incomplete and was not clearly differentiated from the surrounding chromatin. The cytoplasm was scanty and weakly basophilic. Rarely, it was more basophilic with some vacuoles. This homogeneous repetitive appearance was more frequent among cases in the paediatric age range (around 60–70% of paediatric ALL in comparison with 10–15% of cases in adults) and was characterised by a favourable prognosis in terms of complete remission rate and remission duration, but not overall survival. The FAB L2 category included cases with blasts that were heterogeneous in size, with diameters that were often greater than double that of a small lymphocyte, a variable N:C ratio and an irregular nucleus variously indented, cleaved

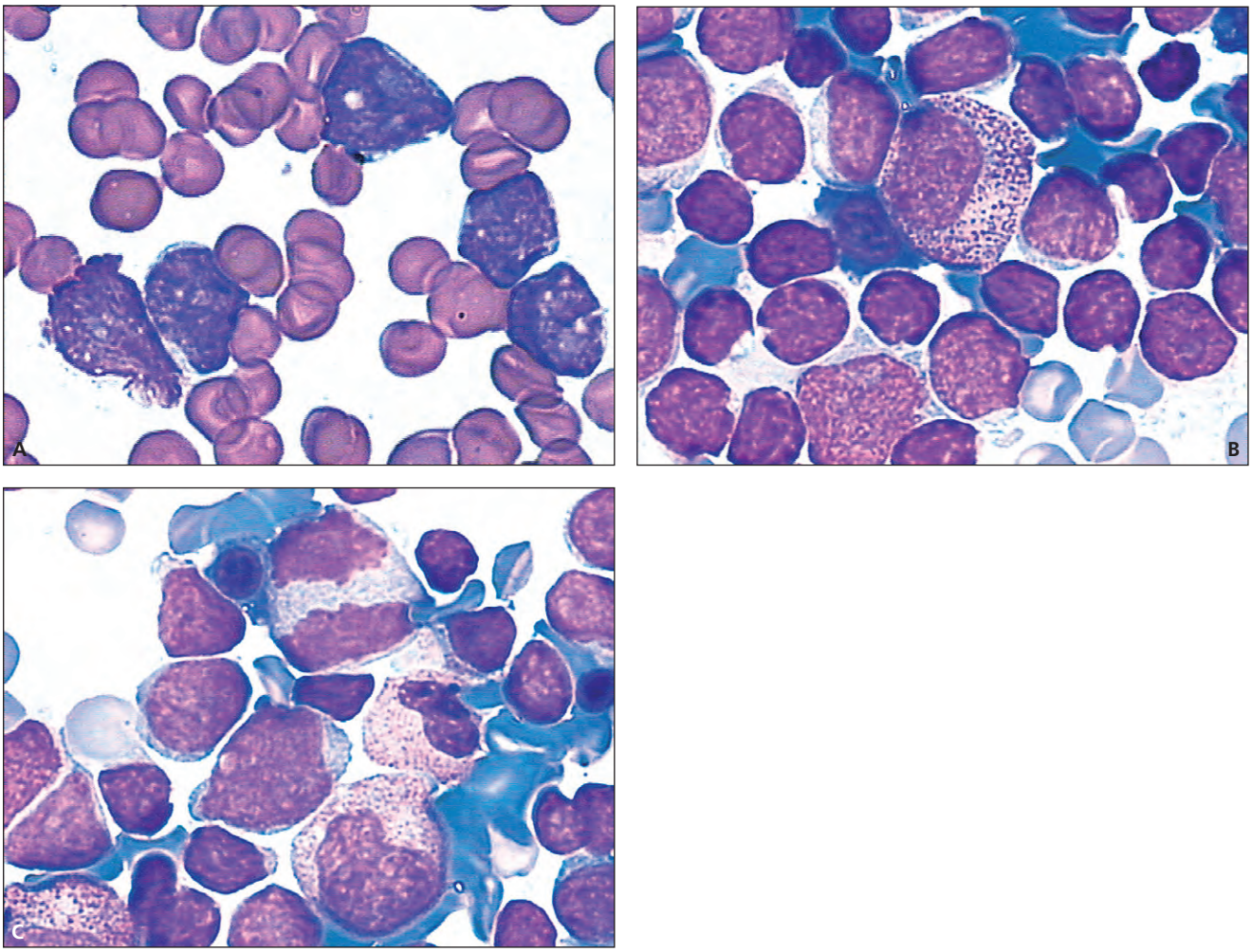



**Fig. 8.14** Pre-B-ALL (with cytoplasmic μ chain). (A) In the peripheral blood film the lymphoblasts have compact chromatin with some residues of nucleoli, a very irregular nuclear outline and very scanty cytoplasm. (B) In the bone marrow aspirate the majority of the cells resemble lymphocytes, being small with compact coarsely clumped chromatin and round nuclei with small notches. In the centre, a neutrophil myelocyte. (C) Bone marrow aspirate. Some lymphoblasts are larger and their pale blue cytoplasm is more abundant. At the top, a mitotic figure in anaphase, with the chromosomes already gathered at the two poles of the cell.

or folded. The chromatin pattern was not homogeneous, varying from one cell to another, from finely dispersed to coarsely condensed. Nucleoli were almost always visible and varied in number and size. The cytoplasm was also variable, but more abundant and basophilic than in L1 ALL. This subtype, characterised thus by heterogeneity of morphological features, was more common in adults and in Philadelphia (Ph)-positive cases. The FAB L3 category, characterised by large homogeneous blast cells with basophilic, often vacuolated cytoplasm was included in the 2008 WHO classification within the group of mature B-cell lymphomas, as a leukaemic variant of Burkitt lymphoma (see Chapter 9), with the provision that the term B-ALL should not be used to indicate Burkitt leukaemia/lymphoma, although historically this had been the case.[14]

A morphological variant of ALL is that designated 'hand-mirror leukaemia', because of the similarity of the shape of the blast cells to a hand-mirror or a tennis racquet.[15] The more ample part of the cytoplasm is occupied by the nucleus; from this extends a thinner projection in the form of a tail or a handle, similar to the uropods that can be observed in granulocytes, monocytes and lymphocytes of healthy subjects when these cells are moving (Figs 8.15, 8.16 and 8.17). By electron microscopy, uropods contain numerous mitochondria and centrioles, still attached to residual fibres of the mitotic spindle. At the distal extremity of a uropod are small spicular projections that favour intercellular contact.[16] Typically, these cells are more numerous in the bone marrow than in the peripheral blood. Observations reported in the literature have shown a correlation with immunophenotype, which is more often B than T,[17] but not with clinical features or prognosis. Some authors have reported a female predominance and a relatively indolent clinical course, but without complete remission.[18]

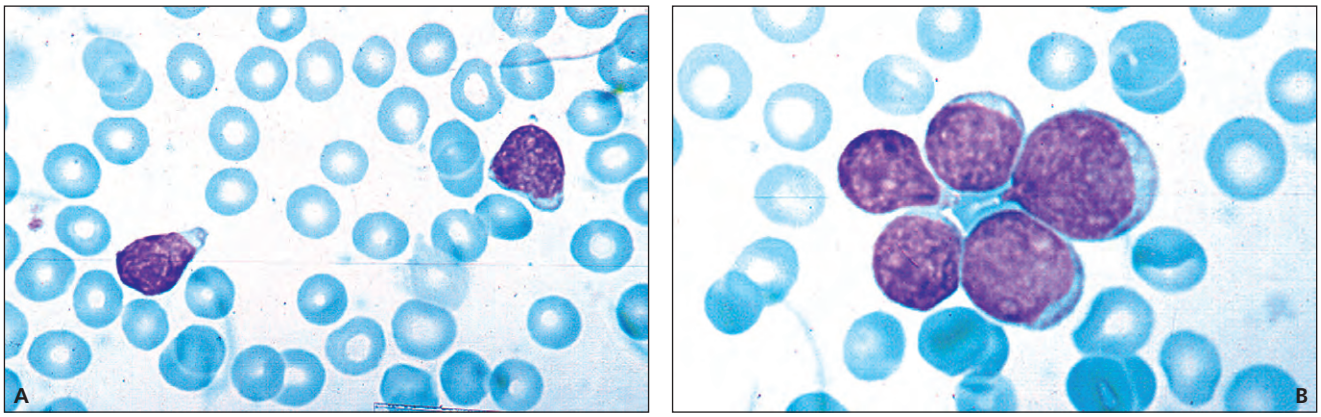



**Fig. 8.15** B-ALL, a case with hand-mirror morphology. (A) Peripheral blood film. Two small lymphoblasts, barely larger than the red cells, with scanty cytoplasm all gathered together into a uropod that extends for a short distance from one pole of the cell. The nuclei have a regular outline, with condensed chromatin and no visible nucleoli (FAB L1). The immunophenotype was common ALL, with aberrant expression of CD11c and CD13. (B) Bone marrow aspirate. The lymphoblasts are forming small rosettes, resulting from reciprocal contact between the tips of the uropods. The chromatin condensation is consistently homogeneous, but the degree of condensation varies inversely with the size of the cells.

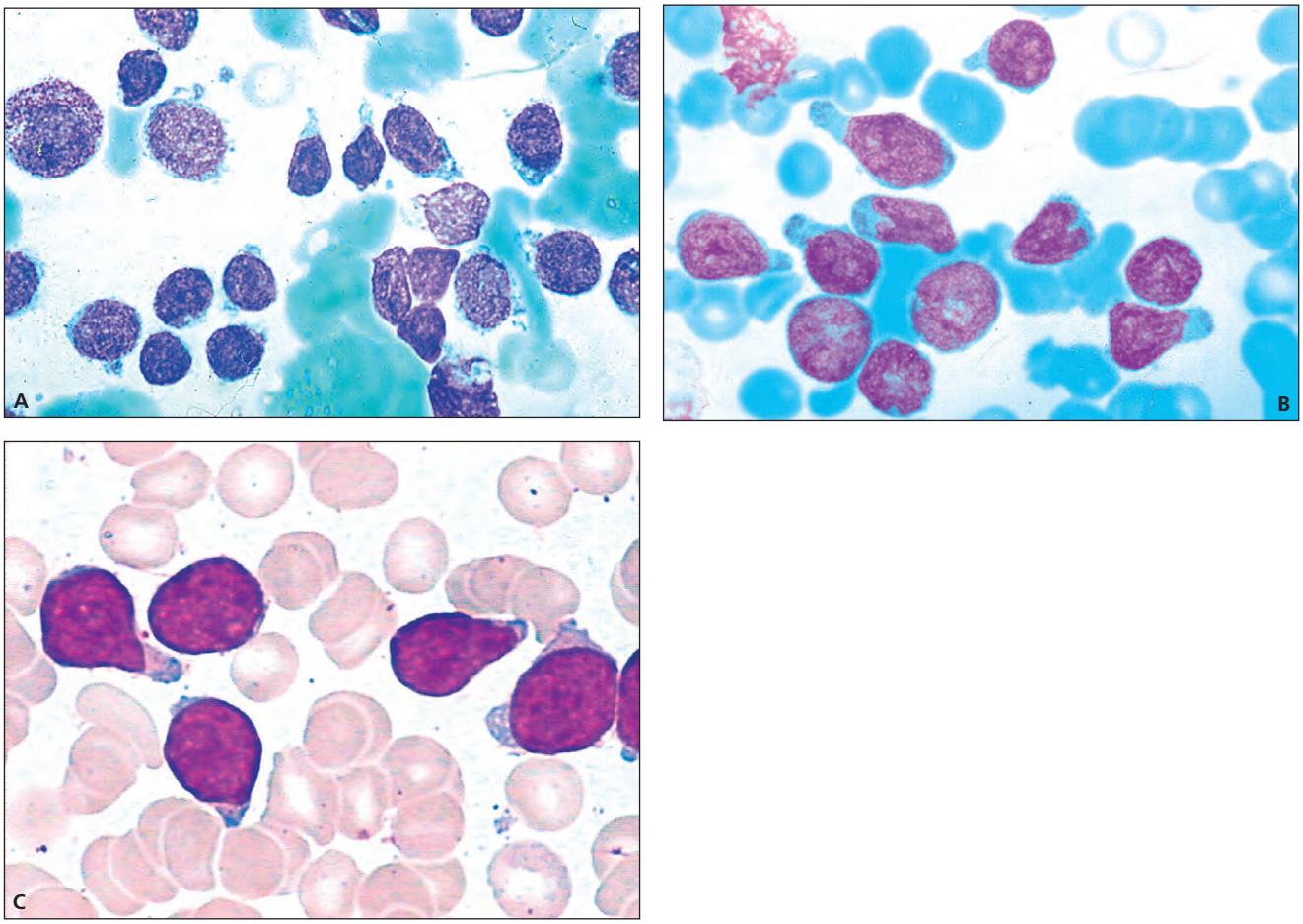



**Fig. 8.16** B-ALL of hand-mirror type; bone marrow aspirate. (A) The morphology of the small bone marrow blasts is typically 'hand mirror' (racquet shaped), particularly in the smaller elongated cells, visible at the centre of the image. In almost all cells there is a small polar cytoplasmic extension, corresponding to a uropod. The characteristics of these lymphoblasts are typical of a form of FAB L1 ALL (small, regularly shaped nucleus without nucleoli, high N:C ratio). (B) Another case with a pro-B phenotype (CD19 positive, CD10 and surface membrane immunoglobulin negative). The blast population is diffusely infiltrating the marrow and half of the cells have this typical racquet shape. (C) Other small racquet-shaped B lymphoblasts, not much bigger than the surrounding erythrocytes, with cytoplasm hardly visible except in the uropod.

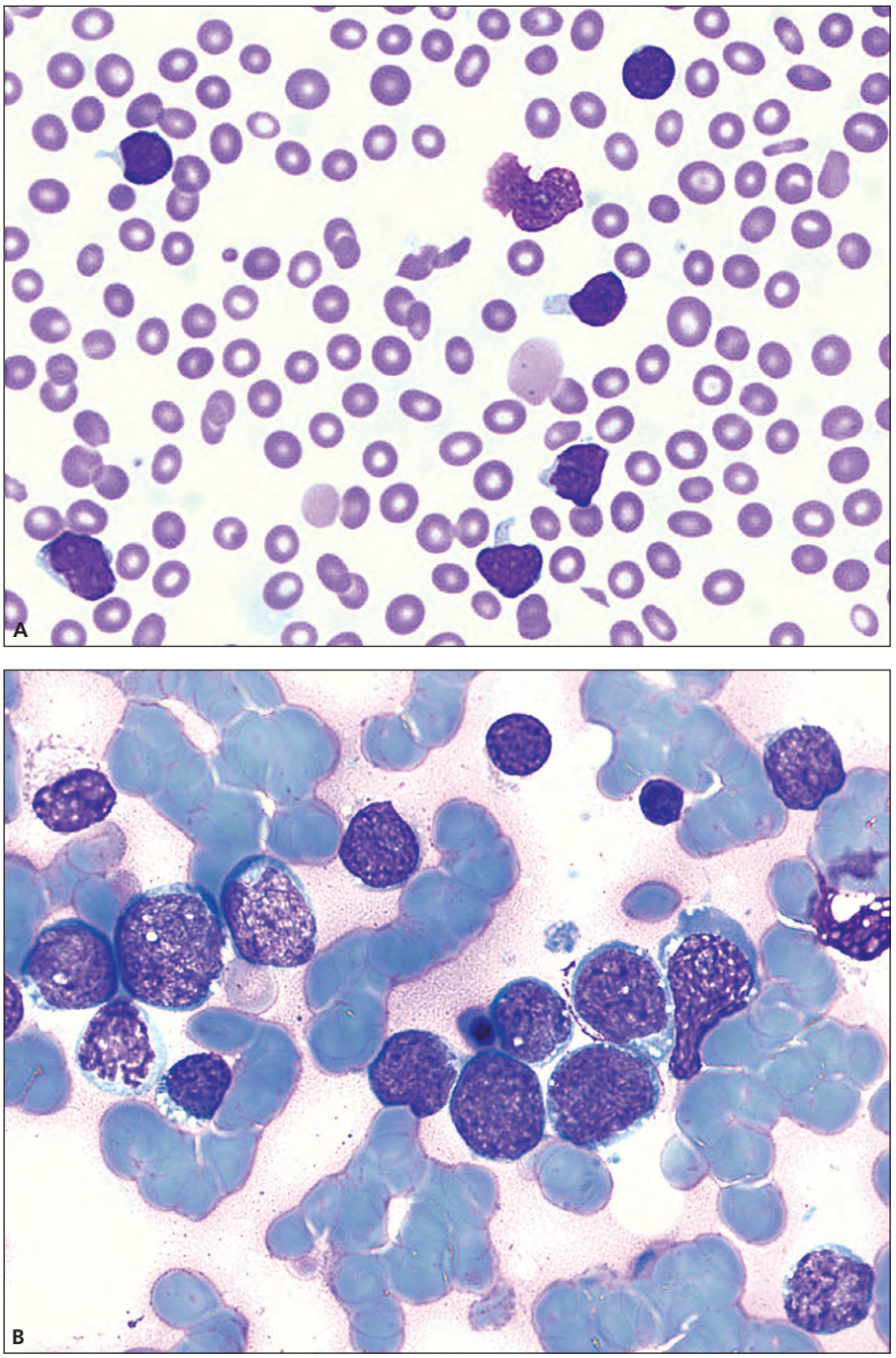



**Fig. 8.17** Pre-B-ALL, hand-mirror type. (A) Small lymphoblasts with small cytoplasmic tails in the peripheral blood film. (B) In the bone marrow this feature is a good deal less evident: the blasts are heterogeneous as regards to size, with granular chromatin and intensely basophilic cytoplasm.

In around 10% of cases, the cytoplasm of lymphoblasts contains a variable number of large azurophilic granules, varying in size and shape,[19–21] which are negative for peroxidase and variably positive for PAS (Figs 8.18 and 8.19); Sudan black B is sometimes weakly positive. By electron microscopy the granules have the appearance of giant lysosomes similar to those observed in the granulocytes of patients with Chédiak-Higashi syndrome.[22] The immunophenotype is lymphoid. This morphological feature does not have prognostic or therapeutic implications: it is, however, important to keep it in mind in the differential diagnosis of AML. The rare cases with a B-lineage, common ALL phenotype, but with the presence of granules showing weak peroxidase positivity, must, however, be classified as mixed phenotype acute leukaemia.[23]

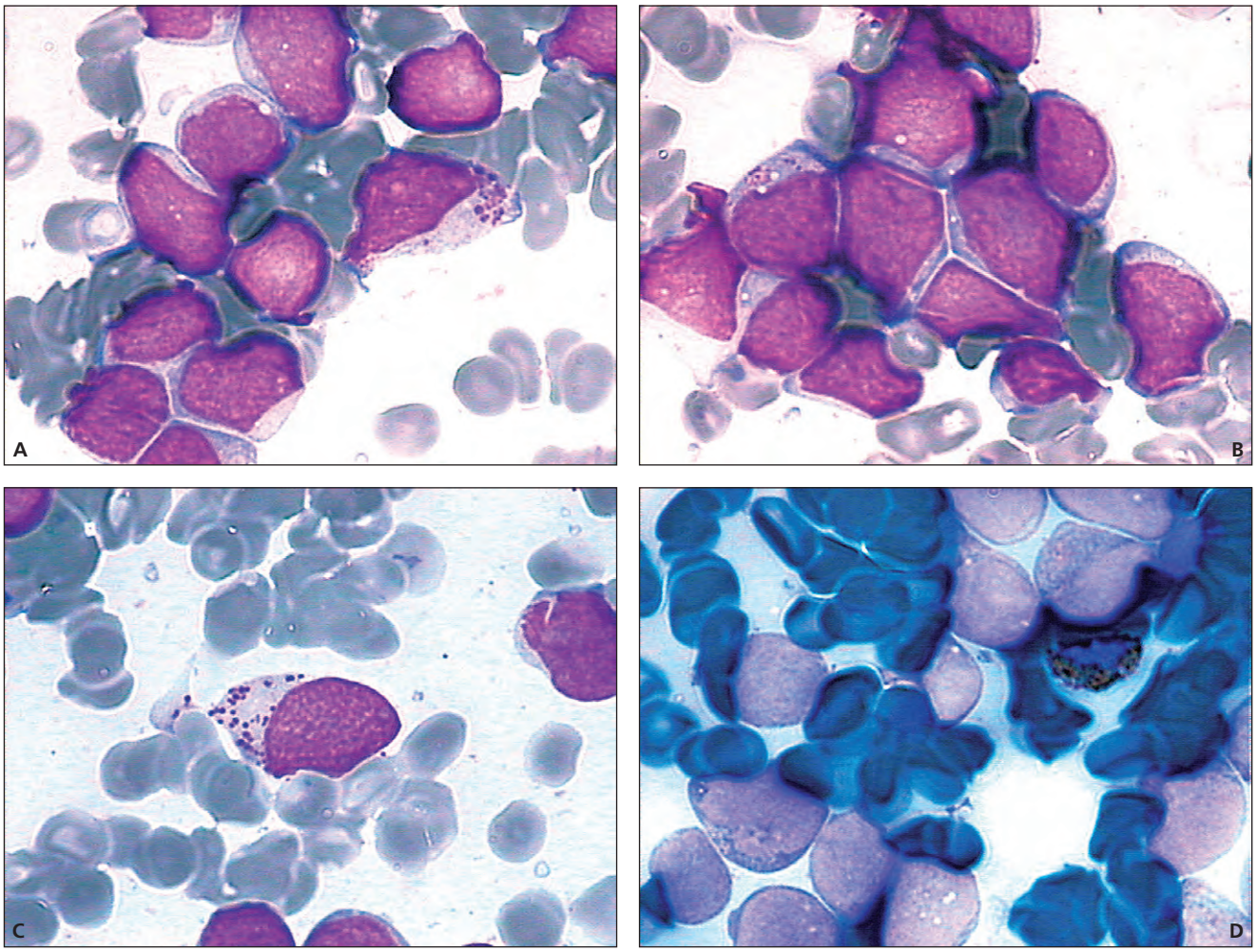



**Fig. 8.18** B-ALL, common ALL subtype, with cytoplasmic granules. (A) The blast cells have homogeneous dense chromatin; one of these has fairly large dark granules clustered at one end of the cell. (B) A granular blast, on the left. (C) In this cell, with a fairly spread out nucleus, the plentiful and pale cytoplasm contains 30 or so large dark granules, which are quite distinct; the presence of nucleoli, in addition to the general context, excludes these being mature large granular lymphocytes. (D) The granular blast cells (bottom left) are peroxidase negative; a small neutrophil (top right) is positive.

From the morphological point of view, there are no reproducible criteria to distinguish between B- and T-lineage ALL. It can also be difficult to distinguish B-lineage lymphoblasts from normal B-lineage lymphoid precursors, known as haematogones, which are observed in the peripheral blood in various conditions, including primary myelofibrosis and, in children, in the recovery phase following chemotherapy (Fig. 8.20).[24] Haematogones typically have an even higher N:C ratio than lymphoblasts, with more homogeneous chromatin and a complete absence of visible nucleoli. They can also express the CD10 antigen, but can be distinguished from blast cells of B-ALL by other immunophenotypic features, being characterised by regular, orderly acquisition and loss of B-lineage antigens; they can also be distinguished from mature lymphocytes by their weak expression of CD45 and, sometimes, by the expression of CD34.[25,26]

## Immunophenotypic analysis

In contrast to the relative morphological uniformity, ALL shows a notable immunophenotypic heterogeneity. Investigation of antigen expression by cells of the leukaemic clone is fundamental, not only for distinguishing ALL from AML with minimal differentiation, but also for identifying both the lineage from which the clone originated, B or T, and the stage of maturation or differentiation at which the cells are arrested.[27–32] None of this can be deduced from the morphological features. ALL cells express antigens of immature lymphoid progenitors: terminal deoxynucleotidyl transferase (TdT), in particular, is expressed in all ALL, both B and T; HLA-DR is expressed in all B-ALL and in very immature T-ALL; CD34 is expressed in the blast cells of a little more than half of patients.

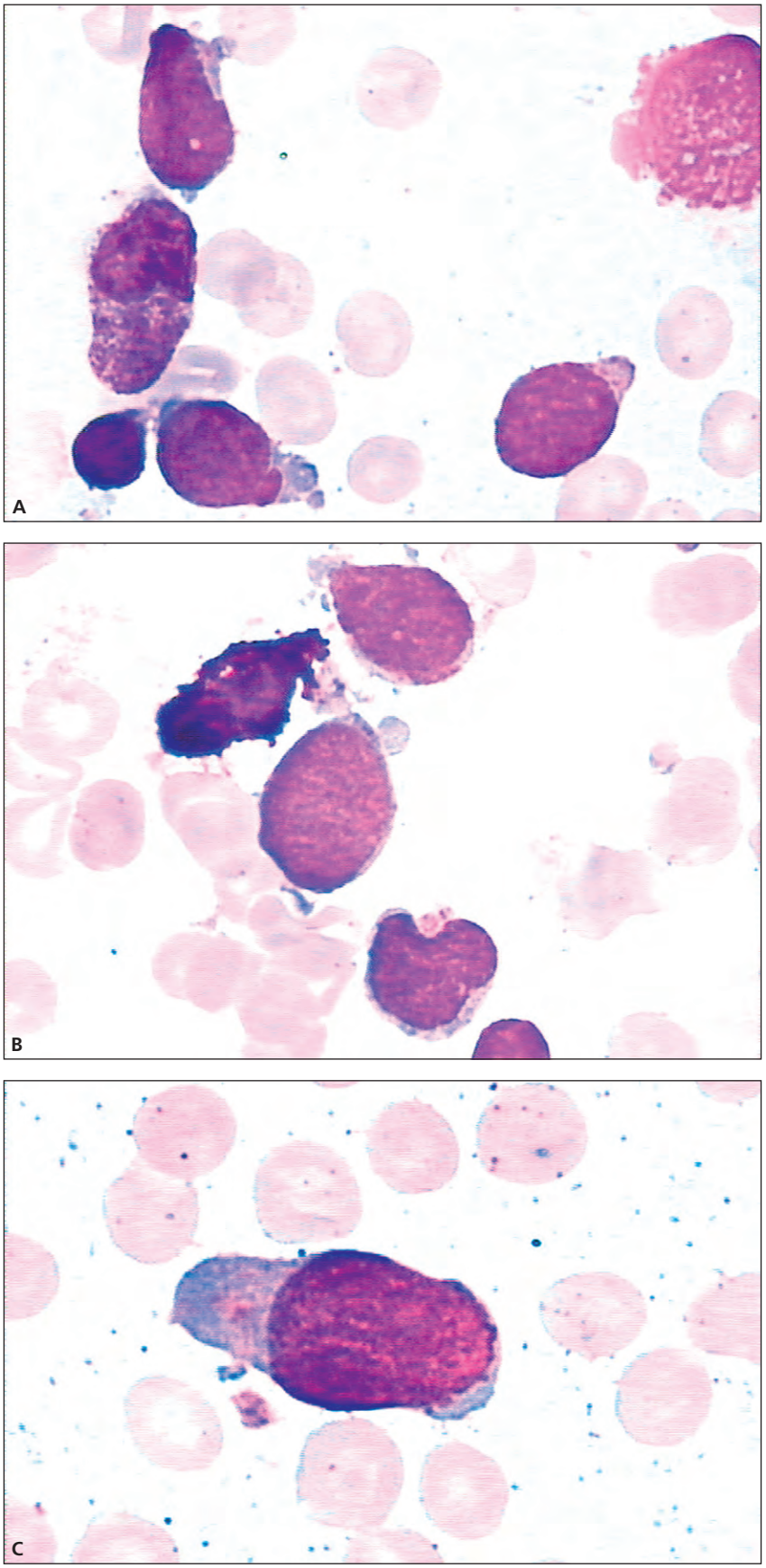



**Fig. 8.19** T-ALL, hand-mirror morphology and cytoplasmic granules. (A) An elongated neutrophil myelocyte and three racquet-shaped small T lymphoblasts: the one on the right contains some azurophilic granules within the uropod. (B) Adjacent to the lymphoblasts, a small fusiform mast cell. (C), Lymphoblast with fine azurophilic granules, which stand out from the blue-grey cytoplasm.

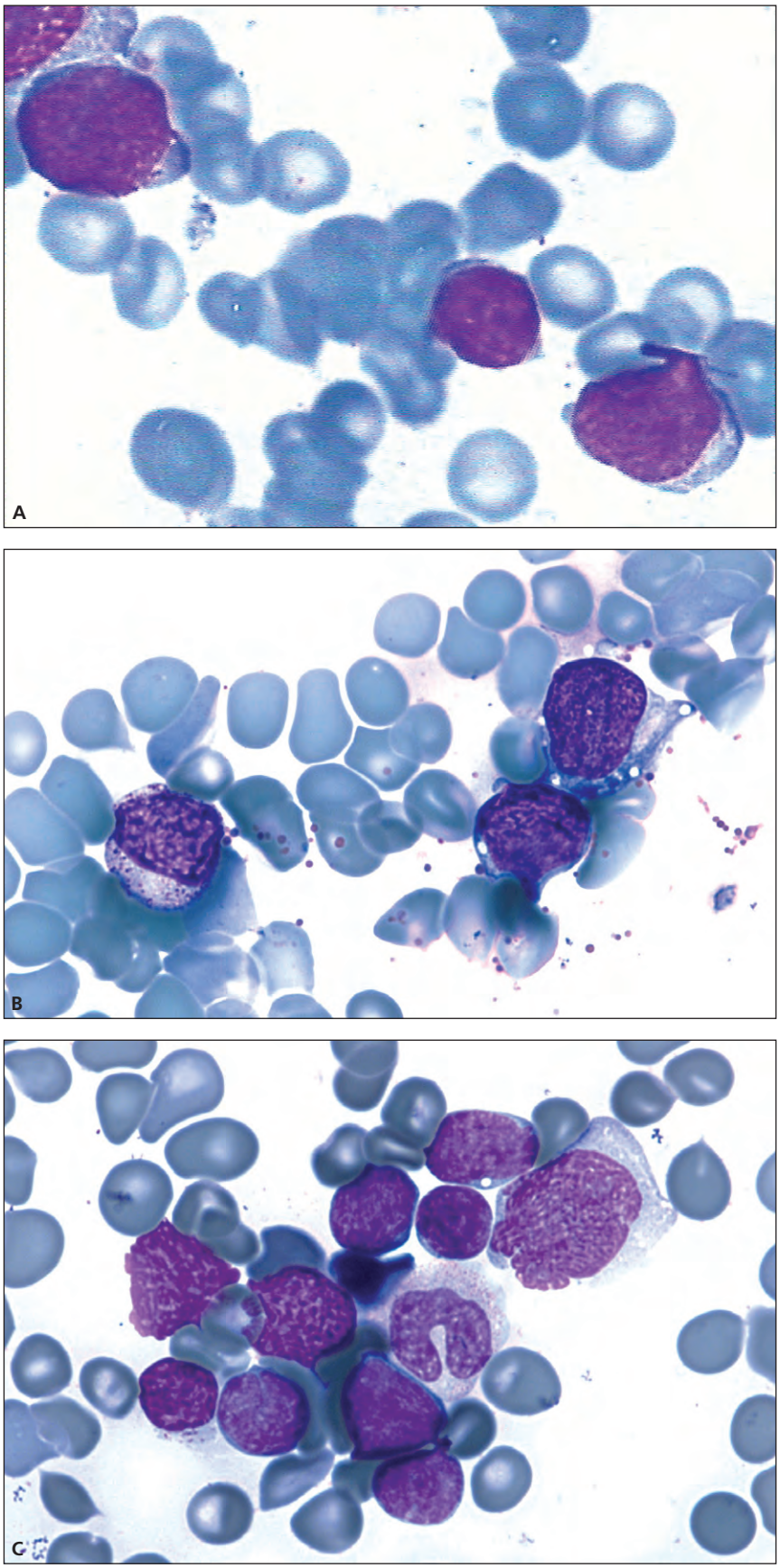



**Fig. 8.20** Haematogones. These immature precursors of B lymphocytes can appear in patients with ALL in the recovery phase following chemotherapy-induced aplasia. (A) Bone marrow aspirate. Three immature cells similar to lymphoblasts, with a B immunophenotype, in a patient with T-ALL. Following haemopoietic recovery, the patient showed a complete remission with disappearance of the leukaemic population. (B and C) Haematogones in the bone marrow aspirate of a boy with B-ALL during the course of treatment.

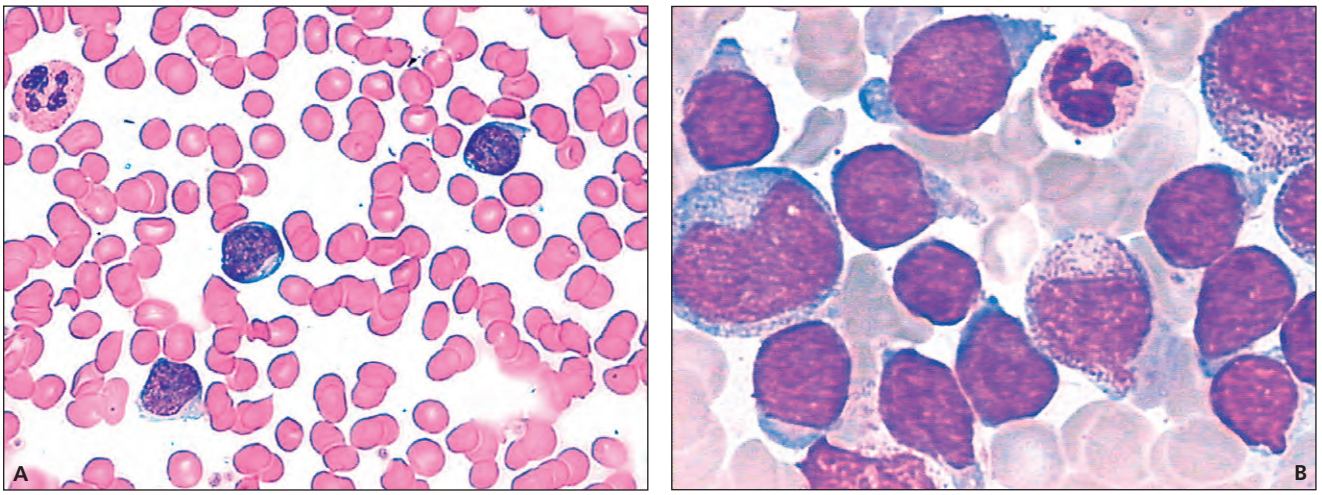



**Fig. 8.21** T-ALL at presentation in a patient hospitalised with bronchopneumonia. The WBC is increased with neutrophilia and lymphocytosis ($23.2 \times 10^9$/l); there is no anaemia or thrombocytopenia. (A) Peripheral blood film. Blasts constitute 49% of cells; they are small to medium sized with dense chromatin and scanty cytoplasm. One notes some dacrocytes and a schistocyte. (B) Bone marrow aspirate. Marked infiltration by small blast cells, with round compact nuclei and a tendency for the scanty cytoplasm to form a small tail. The granulocyte series is still well represented. The lymphoblasts were positive for CD7 and cytoplasmic CD3.

ALL originating in progenitors of B lineage represents 75–80% of ALL: B lymphoblasts are positive in almost all cases for CD19, CD22 and CD79a. On the basis of expression of other antigens, it is possible to identify some main phenotypes that correspond to various stages of maturation at which the leukaemic cells are arrested[28]:

- Early precursor B-ALL (BI or pro-B): there is expression of CD19, CD79a, cytoplasmic CD22 and nuclear TdT; negativity for CD10 associated with positivity for NG2 (chondroitin sulphate glycosaminoglycan), CD15 and CD56 is recognised in cases with rearrangement of the *MLL* gene;
- Common B-ALL (BII, intermediate B): this is the most frequent phenotype, representing about 50% of all cases of ALL; the leukaemic clone expresses CD10 (known in the past as CALLA, common ALL antigen) and TdT, in around 70–80% of cases CD34 and in around 50% of cases CD20. Hyperdiploid cases are generally negative for CD34;
- Pre-B-ALL (about 20% of cases of ALL), in which the blast cells, in addition to CD10, TdT, and lineage-specific antigens express the mu (μ) heavy chain of IgM in the cytoplasm, but not on the cell surface. CD34 is positive in around 70% of cases, while expression of CD20 is variable;
- Mature B-ALL, which is rare (4–8% of ALL): the majority of blasts express μ heavy chains on the surface membrane together with either kappa (κ) or lambda (λ) light chains; they are CD10 and CD20 positive but do not express CD34. This form of ALL is characterised by specific clinical, cytogenetic and morphological characteristics (FAB L3 morphology and thus classified as Burkitt lymphoma according to the 2008 WHO classification). Only a small percentage of cases in this category express CD34, variously associated with TdT, and showing FAB L1 or L2 morphology.

ALL of T lineage represents around 20–25% of cases of ALL; the morphological features, according to the 2008 WHO classification, are not characteristic even if sometimes the small size of the blast cells and their cytoplasmic basophilia do suggest T lineage (Figs 8.21, 8.22 and 8.23). T lymphoblasts are positive for CD2, CD5, CD7, TdT and cytoplasmic CD3. TCR is variably expressed and can also be negative at the level of the cytoplasmic membrane. On the basis of the immunophenotype, which shows the degree of maturation, a division into three principal subtypes can be made:

- Early T-ALL (about 20%), in which the lymphoblasts express an early thymic phenotype (TdT, CD7 and cytoplasmic CD3) and are negative for membrane CD3, CD4 and CD8;
- Common T-ALL (80%), which adds membrane expression of CD1a, CD4 and CD8 (but not CD3) to the phenotype of early T-ALL;
- Mature T-ALL, rare, with blast cells positive for CD3, CD4 or CD8, but negative for CD1a.

Lineage attribution can also be made by demonstrating rearrangement of genes encoding various immunoglobulin chains or the chains of the TCR.

The immunophenotype has relevant clinical and therapeutic implications. In B-lineage cases, early precursor B-ALL have a worse prognosis than common or pre-B cases; the shortest survival is seen in the mature B-cell cases. In T-lineage cases, the most unfavourable course is associated with the most immature phenotype. In general, T-ALL is characterised by a greater frequency and severity of clinical signs associated with a significant

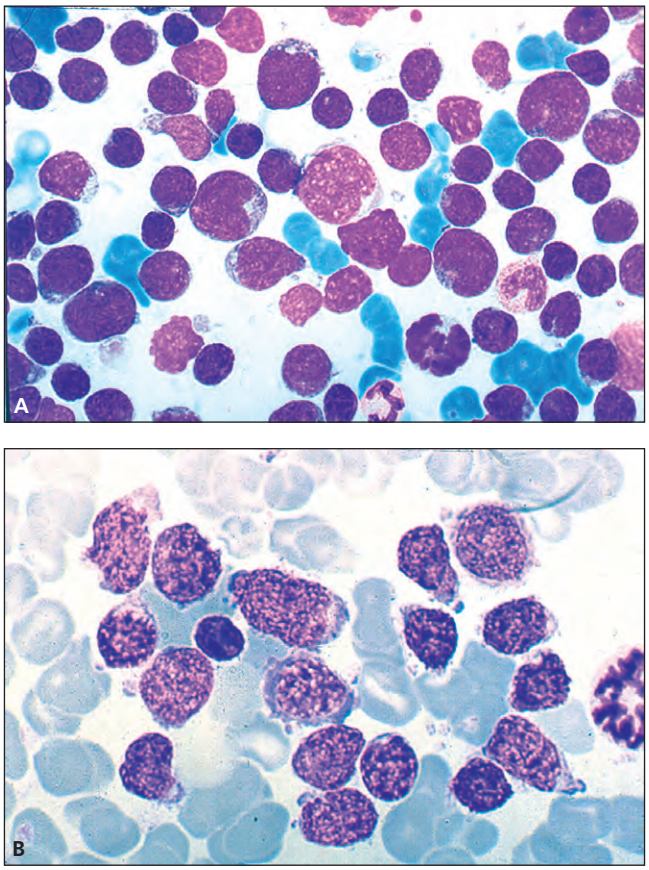



**Fig. 8.22** Another two cases of T-ALL; bone marrow aspirates. (A) T-ALL with intermediate maturity (positive for CD2, cytoplasmic CD3, CD5, CD4 and CD8). The blastic population, which is massively infiltrating the marrow, shows some variation in cell size: there are large cells with a regular or notched nuclear outline, granular chromatin and scanty basophilic cytoplasm, together with small cells with denser chromatin, round nuclei and almost invisible cytoplasm. Nucleoli are sometimes evident. Some cells are vacuolated. At the bottom to the right of the centre, a disordered mitotic figure. (B) T-ALL of pre-T type (positive for TdT and CD7, negative for membrane CD3, CD2, CD4 and CD8). The blast cells have a high N:C ratio, absent or barely visible nucleoli and variable chromatin condensation. The cytoplasm is pale and very scanty. In the centre of the image, a basophilic erythroblast with chromatin in large clumps. On the right, a partially visible mitotic figure.

tumour mass: the leucocyte count is elevated, often to above $100 \times 10^9$/l; serum LDH reaches concentrations above 2200 IU/l; in half of cases there is a mediastinal mass; organomegaly is more prominent and meningeal involvement is more common than in B-lineage cases.[33] In adult ALL the median age is lower in T-lineage cases.

## Cytogenetic and molecular analysis

Cytogenetic analysis in ALL often shows either hyperdiploidy or a pseudodiploid karyotype with structural alterations.[34–37] The hyperdiploid cases, in which the total number of chromosomes is greater than 50 (and in general <66), in the absence of other genetic or cytogenetic alterations, represent a quarter of cases of ALL in early childhood and are associated with a good prognosis. The hypodiploid form constitutes 5% of cases and is associated with an unfavourable prognosis.

Since the introduction of more refined methods of molecular and genetic analysis, cases with no detectable genetic abnormality are rare. In ALL, as in AML, recurrent genetic abnormalities have been identified.[37–40] The WHO classification identifies as entities the recurrent cytogenetic abnormalities that are characterised by specific clinical, biological or prognostic features.

In the B-ALL group, the most frequent aberrations are translocations that lead to fusion genes and the production of anomalous chimaeric proteins. In other cases, the genetic alterations affect the regulation of genes that encode transcription factors or growth factors. The most typical example of the first mechanism is t(9;22)(q34;q11.2), which is manifest on cytogenetic analysis by the presence of the Ph chromosome and on DNA analysis by the presence of the hybrid *BCR-ABL1* gene. As in chronic myeloid leukaemia (CML), in this translocation part of the *ABL1* oncogene, the cellular homologue of a retroviral oncogene, is moved from its normal position on chromosome 9 to the region designated the breakpoint cluster region (BCR) on chromosome 22: this leads to the formation of the chimaeric *BCR-ABL1* gene, which very actively encodes a chimaeric protein endowed with tyrosine kinase activity; this is implicated in the generation of abnormal intracellular signals that activate cellular proliferation and induce leukaemogenesis. One of two types of protein with molecular weights of 190 or 210 kDa respectively can be observed in Ph-positive ALL (the p210 protein being characteristic of CML).

The other genetic alterations in B-ALL, among which rearrangement of the *MLL* gene is particularly common and important, will be described below. T-ALL almost always has clonal rearrangement of TCR genes; karyotypic alterations are observed in about two thirds of cases.[41,42] The most common translocations involve the loci for TCR genes on chromosomes 7 and 14: for the alpha and delta chains 14q11.2, for the beta chain 7q34 and for the gamma chain 7p14–15. The most frequent translocation is t(10;14), which in children is associated with a favourable prognosis. Cases with a normal karyotype, however, have a better prognosis.[43]

## WHO classification of acute lymphoblastic leukaemia

Table 8.2 shows the 2008 WHO classification of ALL.

### B-lymphoblastic leukaemia/lymphoma, not otherwise specified

This is the commonest form in children, in whom presentation is mainly with infiltration by small homogeneous blast cells,

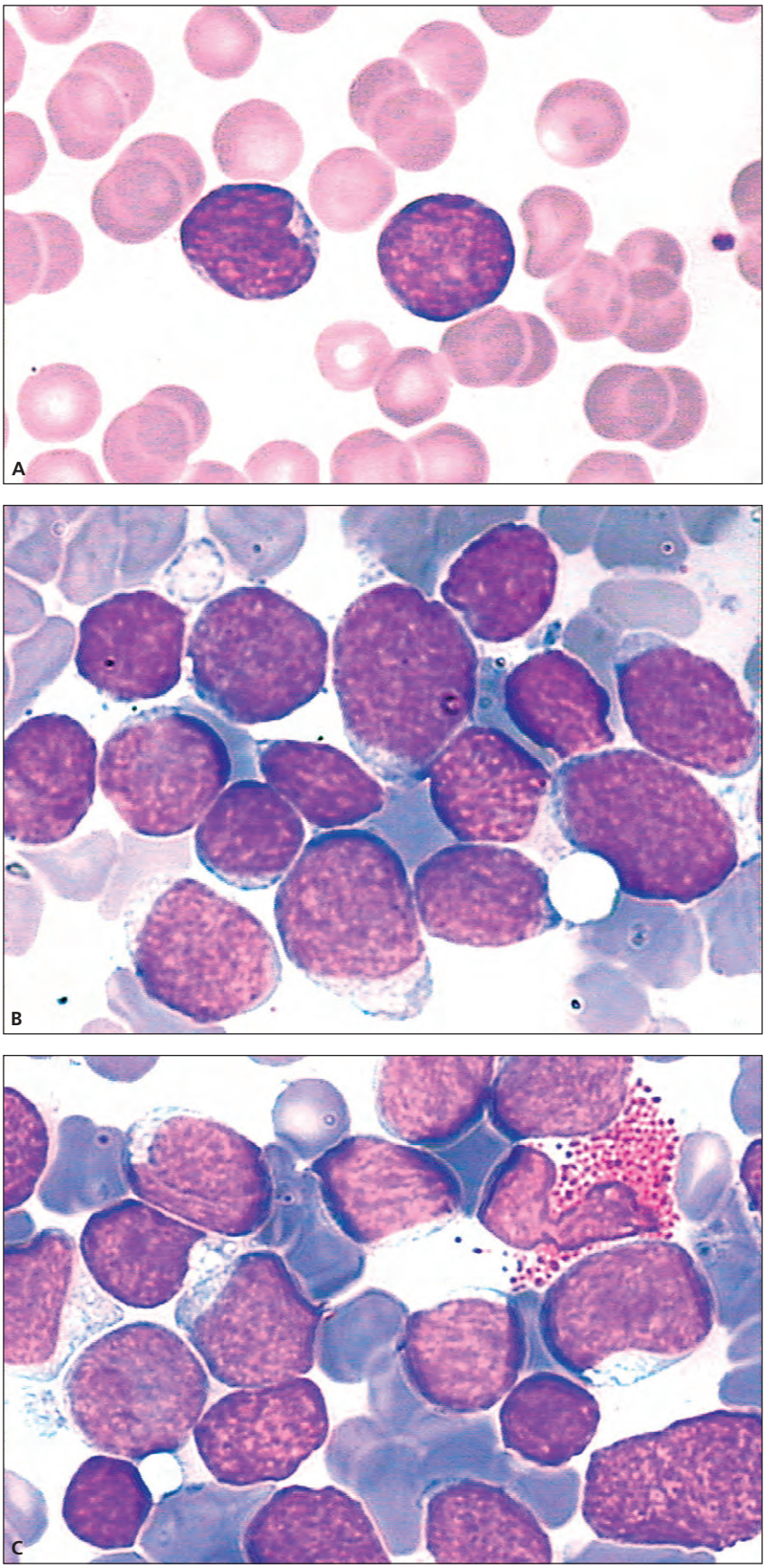



**Fig. 8.23** T-ALL with associated bone marrow eosinophilia (3%). (A) Peripheral blood film. Small blasts with compact chromatin and round nuclei with notches or grooves; the cytoplasm is very scanty. (B) Dense blastic infiltration in the bone marrow aspirate: the nuclei vary in size but the cytoplasm is always poorly represented. (C) In the context of a blastic population one observes, top right, a mature eosinophil.

with indistinct nucleoli and a high N:C ratio (the morphology is illustrated in Figs 8.1 through to 8.19, with the exception of 8.13). One can also, however, recognise cases with more heterogeneous morphology, dispersed nuclear chromatin and more ample, weakly basophilic cytoplasm, sometimes with vacuoles or the hand-mirror type of pseudopodia. Peroxidase-negative azurophilic granules are present in 10% of cases. The immunophenotype is B, with the possible alternatives described above. CD45 may be absent, while sometimes there is expression of the myeloid antigens, CD13 and CD33, the presence of which does not exclude a diagnosis of B-ALL. The detection of myeloperoxidase (MPO) antigen in the cytoplasm, however, is incompatible with such a diagnosis and indicates a mixed phenotype acute leukaemia.

**Table 8.2** 2008 WHO classification of lymphoblastic leukaemias derived from immature lymphoid precursors

**B-lymphoblastic leukaemia/lymphoma**
- B-lymphoblastic leukaemia/lymphoma, not otherwise specified (NOS)
- B-lymphoblastic leukaemia/lymphoma with recurrent cytogenetic alterations
  - B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2)
  - B-lymphoblastic leukaemia/lymphoma with t(v;11q23), (*MLL* rearrangement)
  - B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22), *TEL-AML1* (*ETV6-RUNX1*)
  - B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
  - B-lymphoblastic leukaemia/lymphoma with hypodiploidy
  - B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31;q32), (*IL3-IGH*)
  - B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;q13.3), *E2A-PBX1* (*TCF3-PBX1*)

**T-lymphoblastic leukaemia/lymphoma**

## B-lymphoblastic leukaemia/lymphoma with recurrent cytogenetic alterations

The entities included in this category are distinguished by association with a chromosomal anomaly, which is often a balanced translocation, and specific clinical and immunophenotypic features, which have prognostic implications.[44] For all these forms, on the other hand, the 2008 WHO classification repeatedly specifies that there are no morphological or cytochemical features that distinguish any of these cases from other cases of ALL.

## B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2)

This form, for which the genotype has already been described, has a higher incidence in adults (20–30% of ALL) and in older children (in comparison with 3–5% of ALL under the age of 15 years) (Figs 8.24, 8.25 and 8.26, Report 8.1). There is often a common ALL immunophenotype (expression of CD10, CD19 and TdT) and it is not rare for there to be associated expression of the myeloid antigens CD13 and CD33. Very rarely this translocation has been described in T-ALL. The leucocyte count is

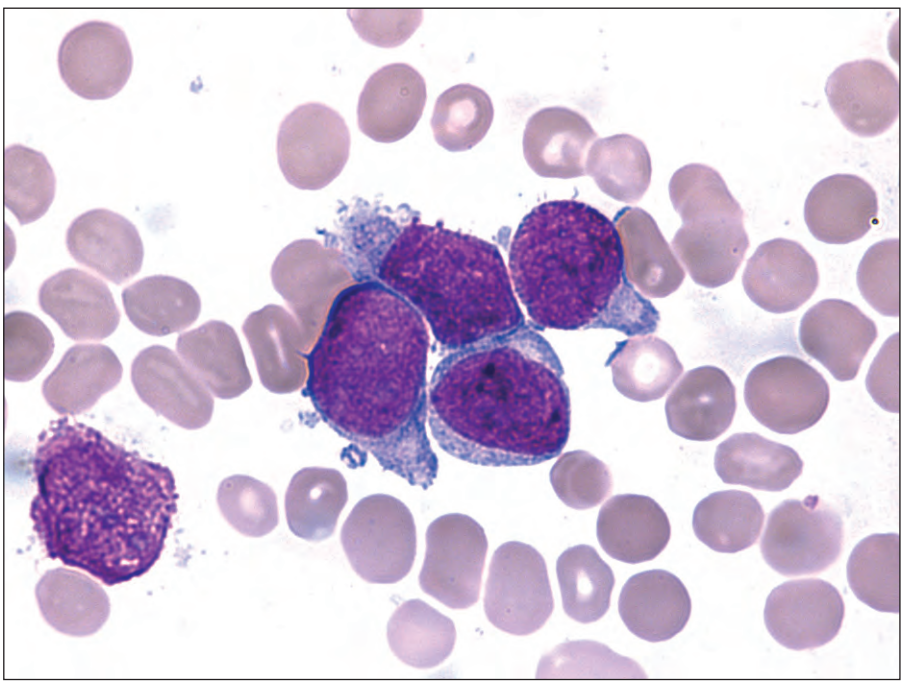


**Fig. 8.24** B-ALL with t(9;22); peripheral blood film. These blast cells are relatively large in relation to the adjacent erythrocytes. The cytoplasm is moderately basophilic and varies from scanty to moderately abundant with sometimes either a single projection giving a hand-mirror shape, or multiple short filamentous extensions; the nuclear chromatin is homogeneous with a granular structure; nucleoli are ill-defined.

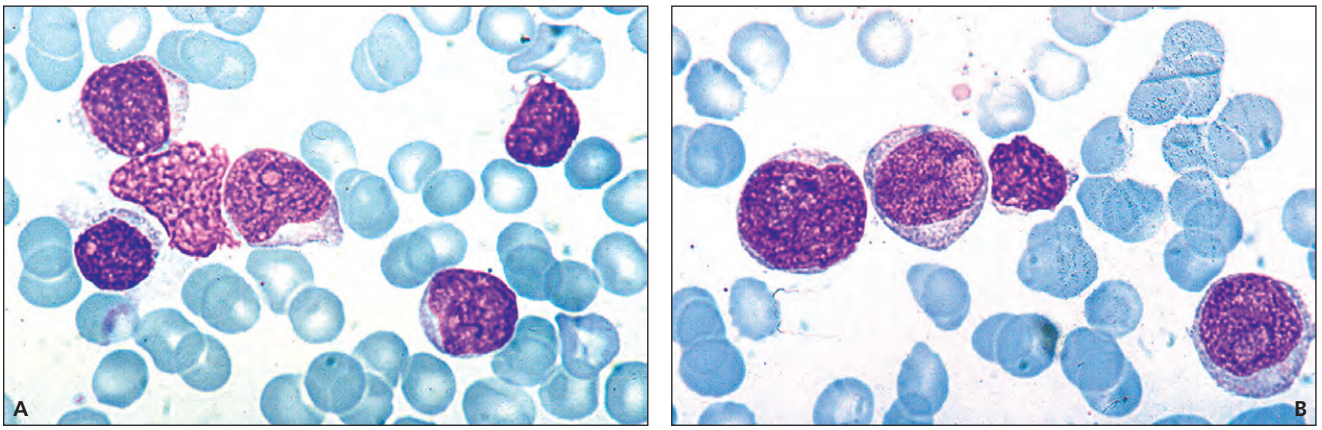



**Fig. 8.25** B-ALL, common ALL subtype with t(9;22); bone marrow aspirate. (A) The morphology is heterogeneous with variably sized cells, well-represented cytoplasm, granular chromatin and frequent nucleoli. The cytoplasm is weakly basophilic with frequent vacuoles. (B) The appearance of the larger blast cells is not distinguishable from that of undifferentiated AML. The chromatin is fine, the nucleoli prominent and the cytoplasm weakly basophilic with a fairly extensive pale paranuclear Golgi zone. The immunophenotype was of common B-ALL with aberrant expression of one myeloid antigen (positive for CD19, CD10, CD34 and CD13).

often elevated, indicating a high risk case. A lymphomatous presentation is rare. The prognosis was grave until the introduction of treatment with imatinib, in association with high dose chemotherapy, which has noticeably improved the survival in these patients.

### B-lymphoblastic leukaemia/lymphoma with t(v;11q23) and rearrangement of *MLL*

The *MLL* gene, situated at band 11q23, is involved in translocations with various partner genes in this type of disorder (e.g. the *AFF1(AF4)* gene on chromosome 4 or the *MLLT1*(*ENL*) gene on chromosome 19). These translocations can occur *in utero* and be clinically manifest a short time after birth or, in the majority of cases, within the first year of life. The leucocyte count is usually above $100 \times 10^9$/l and there is a high frequency of neurological involvement. The immunophenotype is B without expression of CD10.[45] There is often present, together with the lymphoblasts, a proportion of blast cells that are morphologically and cytochemically typical of monoblasts, this being confirmed on immunophenotyping and leading to classification as mixed phenotype acute leukaemia.

### B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22)

This aberration leads to the fusion of part of *ETV6* (*TEL*) with part of *RUNX1* (*AML1*); it is observed in around a quarter of cases of B-ALL with a maximum incidence at the age of 3–4 years. It can actually occur *in utero*, this representing a very early event but one that is not in itself sufficient for the process of leukaemogenesis.[46,47] It is associated with a relatively good prognosis and a common B immunophenotype (CD19 and CD10 positive); it is also associated with positivity for CD34 and, often, for CD13.

### B-lymphoblastic leukaemia/lymphoma with hyperdiploidy

Frequent particularly in children (25% of cases of B ALL) (Figs 8.27 and 8.28), these cases are characterised by a particularly good prognosis, with cure in more than 90% of cases.

### B-lymphoblastic leukaemia/lymphoma with hypodiploidy

This subtype affects children and adults equally. The prognosis is unfavourable.

### B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31;q32)

This translocation causes fusion between the *IGH* gene on chromosome 14 and the *IL3* gene on chromosome 5, with resultant hyperexpression of the latter. It is typically associated with a variable degree of eosinophilia, both in the peripheral blood and the bone marrow, which can be the cause of organ damage.[48,49] The eosinophils are part of a reactive expansion and not part of the leukaemic clone.

### B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3)

Around 6% of cases of B-ALL are associated with this translocation; the immunophenotype is usually pre-B. The prognosis was previously adverse, but has improved with current management.

### T-lymphoblastic leukaemia/lymphoma

The most typical clinical profile of T-ALL is of a very high WBC and a tumoral mass in the mediastinum or other tissues.[50] The morphology has some distinctive aspects in comparison with B-ALL. The cells are heterogeneous in size with morphology

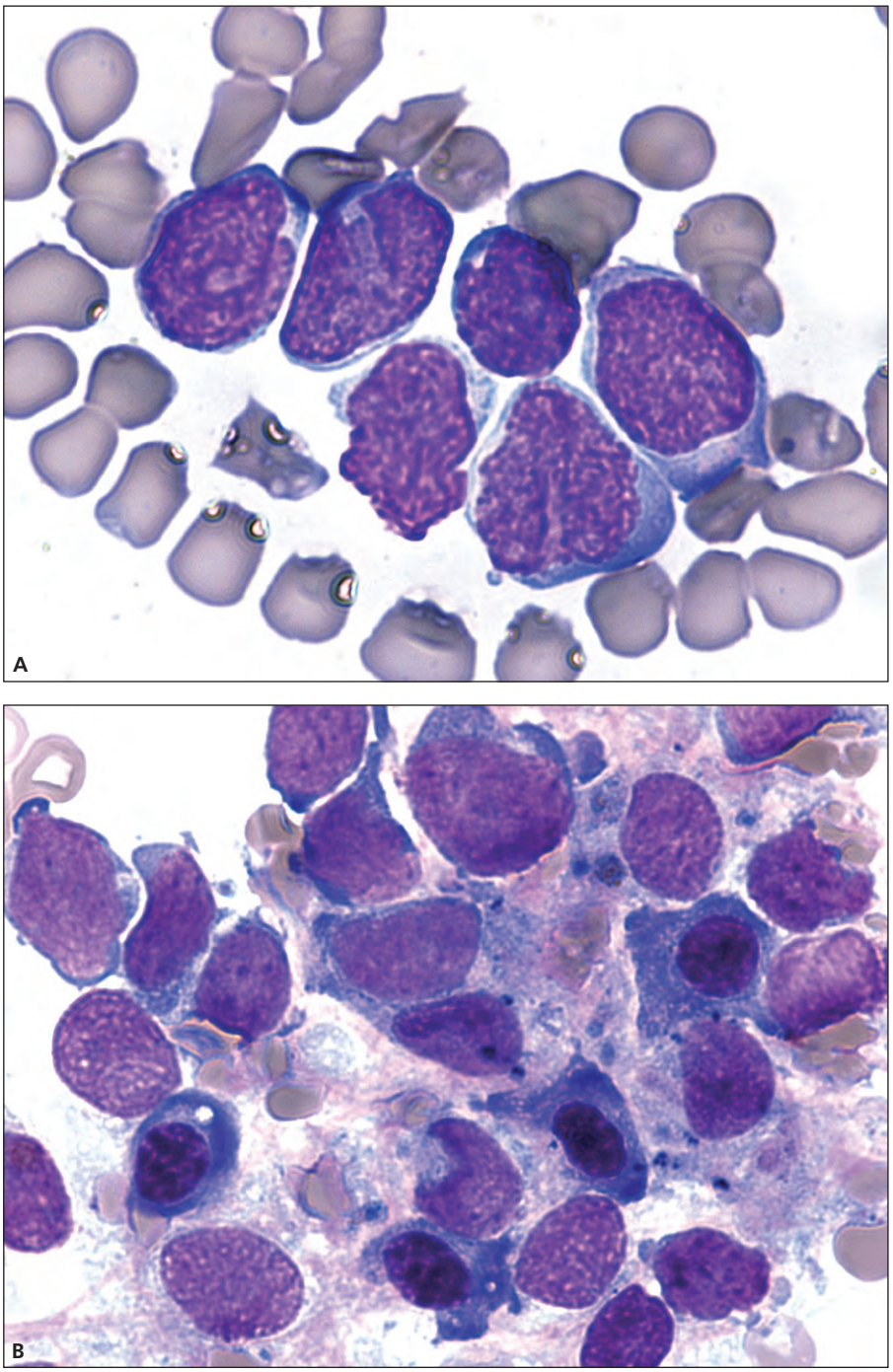



**Fig. 8.26** Pro-B-ALL with t(9;22). (A) Peripheral blood film. The lymphoblasts are fairly large with a variable amount of intensely basophilic cytoplasm. Some cells have an irregular nuclear outline with deep grooves, indentations and folds. The chromatin is condensed into elongated, sometimes vermiform, masses of variable size. Nucleoli are visible, but not always distinct. (B) Bone marrow aspirate. Numerous blasts of variable size, appearing less irregular than those in the circulation. The cells with a small eccentric nucleus, chromatin in blocks and intensely basophilic cytoplasm are small plasma cells.

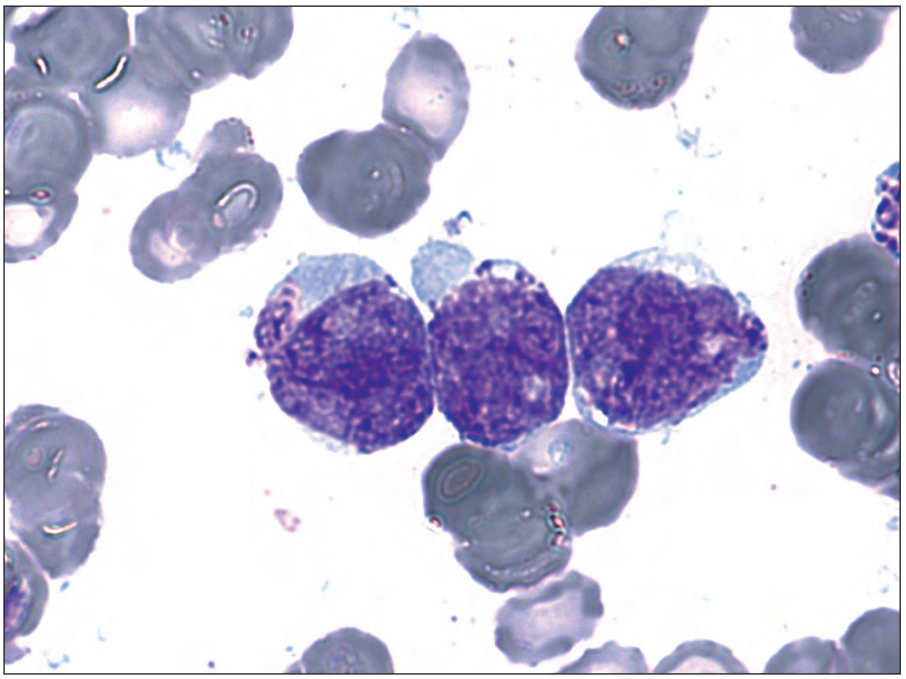


**Fig. 8.27** B-ALL, common ALL subtype with hyperdiploidy; peripheral blood film. These lymphoblasts are characterised by their medium size and a moderate amount of basophilic cytoplasm with some vacuoles. The nuclei have irregularly condensed chromatin and a very irregular outline, the continuity of which is interrupted by nuclear protrusions.

varying from small blasts with a high N:C ratio and compact chromatin without nucleoli to larger blasts with more ample cytoplasm and nuclei with dispersed chromatin and clearly visible nucleoli (see Figs 8.21, 8.22 and 8.23, Report 8.2). There is often focal positivity for acid phosphatase.

As mentioned above, in cases associated with eosinophilia it is necessary, before making a diagnosis of T-ALL, to exclude the presence of rearrangement of *FGR1*, which is associated with a specific myeloproliferative neoplasm with eosinophilia, previously known as the 8p11 stem cell syndrome (see Chapter 6).

## Ambiguous lineage acute leukaemias, including mixed phenotype acute leukaemia

It is not rare for leukaemic cells to express single markers that are aberrant for the lineage in question, as happens, for example, with CD7 in AML or either CD13 or CD33 in some cases of B-ALL.[51] In these cases, the minimal deviation of the phenotype is not sufficient to alter the diagnosis or classification of a leukaemia (Fig. 8.29). However, in a small proportion of cases, estimated to be less than 5% of all acute leukaemias, both in children and adults, there is co-expression of markers that are very specific for a lineage and generally absent from other lineages, this truly representing leukaemic transformation of a very early haemopoietic stem cell that has the potential to differentiate along different pathways. From the taxonomic point of view, these forms have been called mixed or hybrid, when characterised by the co-expression by blast cells of markers of at least two clearly distinct lineages, or as bilineage or trilineage leukaemia when there are present in the circulation two or three populations of blast cells that differ in nature.[52–59]

The diagnosis of this type of leukaemia is based on immunophenotyping and on the value that specific markers have for lineage assignment,[60] according to the scoring system proposed by the European Group for Immunological Characterization of Leukemias (EGIL).[30] These criteria were included in the third edition of the WHO classification.[61] For example, the expression of peroxidase, either at the cytochemical or immunophenotypic or ultrastructural level, or the presence of Auer rods, is sufficient in itself for attribution to the myeloid lineage. In the same way, the presence of the CD22 antigen or of cytoplasmic μ chain indicates the B-lymphocyte nature, while cytoplasmic CD3 is specific for the T lineage.

The 2008 WHO classification proposed a new definition for a condition now designated mixed phenotype acute leukaemia (MPAL) (Table 8.3); one of the main purposes of the new definition was to provide very strict criteria in order to exclude from the MPAL group cases of acute leukaemia with aberrant marker expression and, in addition, lymphoid blast crisis of CML and therapy-related acute leukaemia even when these were associated with a mixed phenotype.[62,63] The diagnostic criteria are based on the identification of very specific lineage markers by

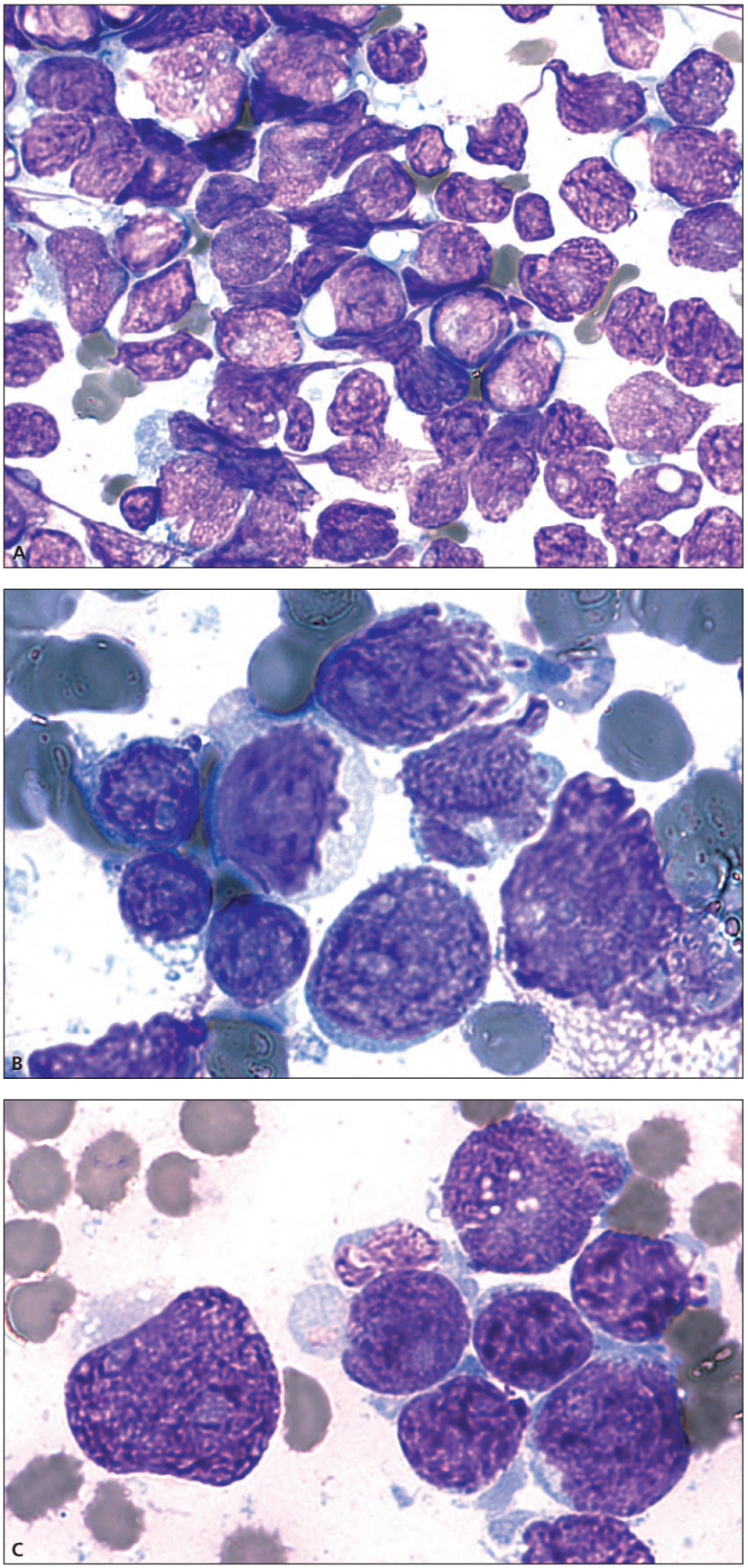



**Fig. 8.28** B-ALL with hyperdiploidy; bone marrow aspirate, same case as Fig. 8.27. (A) Massive blastic infiltration of the bone marrow; many torn nuclei have formed violet filaments during the preparation of the film. (B) The nuclei of many of these cells have not resumed their normal form after their most recent mitosis; some chromosomes appear to be in part still identifiable and separate from the main chromatin mass, as is well seen in the blast cell at the top and that at bottom left. (C) The size and morphology of the lymphoblasts is very variable; on the left is a cell with a giant, spread out nucleus with worm-like chromatin, two lilac nucleoli and a small halo of cytoplasm; on the right, between two large blast cells, a small lymphoblast with very compact chromatin with a nucleus that appears to be budding.

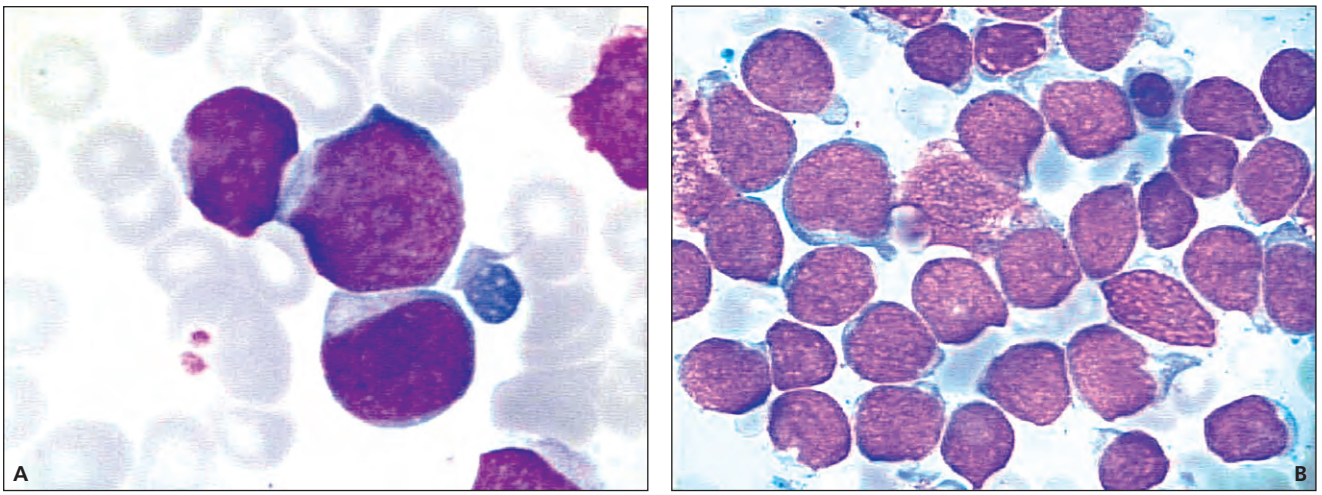



**Fig. 8.29** B-ALL, common ALL subtype, with expression of an aberrant myeloid antigen. FBC showed: WBC 16.3 × $10^9$/l; Hb 131 g/l; platelets 182 × $10^9$/l. (A) Peripheral blood film. The blasts are heterogeneous with regard to size, they have rounded nuclei with compact chromatin and the scanty cytoplasm is basophilic. The immunophenotype showed expression of TdT, CD34, CD19, CD22, CD10 and CD33, whereas MPO was not expressed. (B) Bone marrow aspirate. The blastic population infiltrating the bone marrow is morphologically fairly uniform with round nuclei and scanty cytoplasm. The only morphological feature that is not uniform is the degree of chromatin condensation, which is almost reticular in many cells but more compact in others, such as that in the top right corner adjacent to an orthochromatic erythroblast.

**Table 8.3** 2008 WHO classification of acute leukaemia of ambiguous lineage including mixed phenotype acute leukaemia (MPAL)

- Acute undifferentiated leukaemia
- Mixed phenotype acute leukaemia (MPAL):
  - Mixed phenotype acute leukaemia with t(9;22)(q34;q11.2) or *BCR-ABL1*
  - Mixed phenotype acute leukaemia with t(v;11q23) with *MLL* rearrangement
  - Mixed phenotype acute leukaemia, B/myeloid, NOS
  - Mixed phenotype acute leukaemia, T/myeloid, NOS
  - Mixed phenotype acute leukaemia, NOS: rare form T/B
- Lymphoblastic leukaemia T/NK cell

flow cytometric methods, cytochemistry or morphology. The markers proposed are:

- For the granulocyte lineage, MPO, by any technique;
- For the monocyte lineage, at least two lineage markers chosen from non-specific esterase, CD11c, CD14, CD64 and lysozyme;
- For the T lineage, the expression of cytoplasmic or membrane CD3 (the latter a very rare occurrence since MPAL is an immature leukaemia);
- For the B lineage, the strong or weak expression of CD19 associated respectively with at least one or at least two markers chosen from CD79a, cytoplasmic CD22 and CD10 (i.e. weak expression requires the presence of at least two other antigens).

In the form associated with t(9;22) or the presence of *BCR-ABL1*, a previous diagnosis of CML must be definitely excluded, as must straightforward ALL with t(9;22); in the cases with *MLL* rearrangement, it is necessary to exclude cases better regarded as AML or ALL with this rearrangement; the diagnoses of MPAL, NOS, B/myeloid, T/myeloid and B/T are very rare (Fig. 8.30).

Included in this definition are the leukaemias which, following therapy or in relapse, show a change of lineage (lineage switch).

The morphological characteristics of this rare form of acute leukaemia have recently been analysed in a multicentric study in which 100 cases of MPAL meeting the 2008 WHO criteria were re-evaluated.[64] Out of 90 cases in whom morphology was reviewed, 39 (43%) patients had lymphoblastic morphology; two of these, one T/myeloid and one T/B, showed paranuclear acid phosphatase activity, as is characteristic of T-ALL. Thirty-eight cases (42%) had myeloid morphology, mainly granulocytic with little maturation (FAB M1) and monoblastic (FAB M5), and only rarely well-differentiated granulocytic (FAB M2) or myelomonocytic (FAB M4): in no case did the leukaemic cells have the morphology of promyelocytes, erythroblasts or megakaryoblasts. In 13 cases, regarded morphologically as acute undifferentiated leukaemia, there were two distinct populations of large and small blast cells. Characteristically, no case had dysplastic features. A single case with mixed T/myeloid phenotype with Auer rods in mature granulocytes has recently been decribed.[65] Figs 8.31 and 8.32 illustrate another patient presenting with mixed phenotype acute leukaemia, B/myeloid, in which the blast cells contained peroxidase-positive Auer rods, this thus being a second case of MPAL with Auer rods.

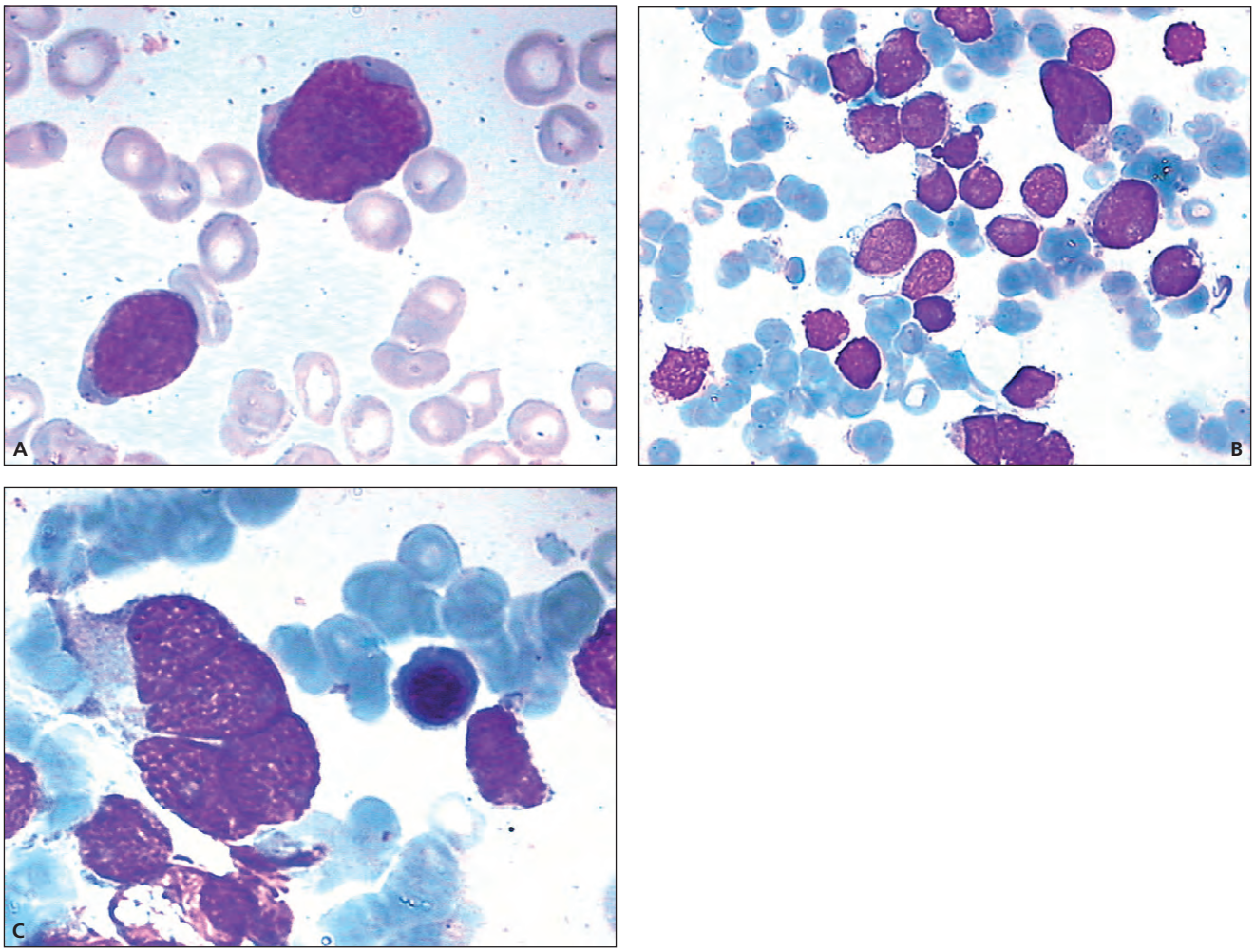



**Fig. 8.30** Mixed phenotype acute leukaemia (MPAL), pre-B and T. The lymphoblasts were positive for cytoplasmic CD3, CD7, CD10, CD19 and CD79a. (A) In the peripheral blood film, there are two blast cells that differ in size, with basophilic, not very abundant, cytoplasm. The chromatin is irregularly condensed; definite nucleoli are not visible and the nuclear outline is very irregular, spilling over, with indentations and interruptions in the continuity of the outline, particularly in the larger cell. (B) The bone marrow aspirate is very cellular. The blast cells are mainly small with condensed chromatin and scanty cytoplasm, sometimes expanding into semivillous projections. In the more spread out cells, a nucleolus is apparent. Top right, two larger blast cells with hyperdiploid nuclei. (C) In the marrow there are several giant multinucleated lymphoblasts.

The 2008 WHO classification identified several conditions of ambiguous or unusual lineage that are distinct from MPAL (Table 8.3). It proposed new criteria for the diagnosis of those forms of leukaemia in which the blasts do not have any features indicating differentiation towards a specific lineage, the rare acute undifferentiated leukaemia (AUL); this must be distinguished from AML with minimal evidence of differentiation. Similarly, lymphoblastic leukaemia/lymphoma of NK cell precursors was identified as a separate group, which must be distinguished from plasmacytoid dendritic cell neoplasm.

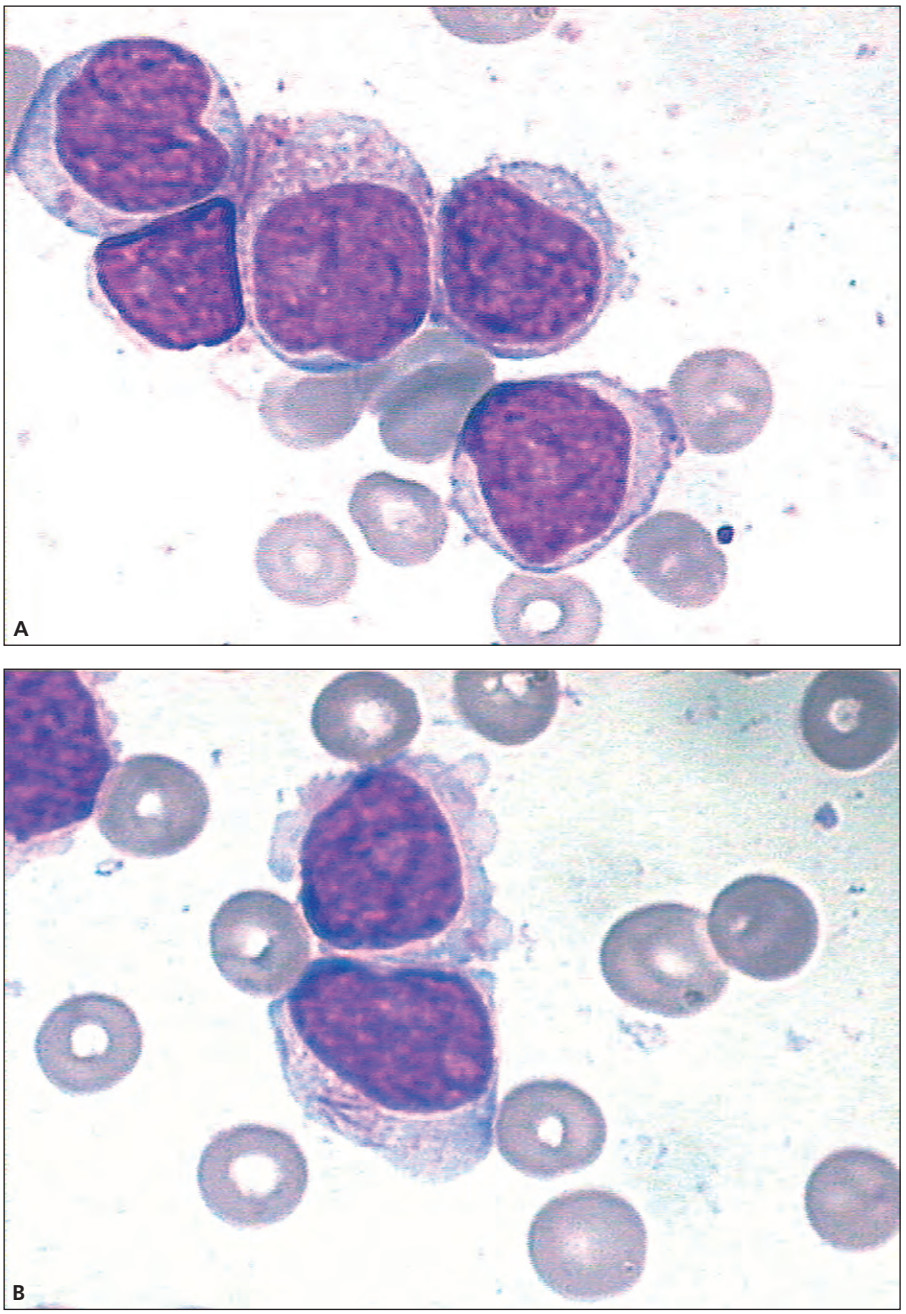



**Fig. 8.31** Mixed phenotype acute leukaemia, pre-B and myeloid, with Auer rods; peripheral blood film. (A) The circulating blast cells were negative for cytoplasmic and membrane CD3, and positive for TdT, CD34, CD33, MPO, CD19 and CD22. They are medium sized to large; some nuclei have fine chromatin and in others it is condensed into small clumps; the nuclear outline shows notches, angulation and short straight sections. The cytoplasm is moderately abundant and pale blue, sometimes with small vacuoles. The larger cells show azurophilic granules and some thin Auer rods superimposed on each other. (B) In these cells the chromatin is condensed into indistinct clumps that also surround the nucleoli; at the bottom, the ample pale cytoplasm contains more Auer rods with similar features.

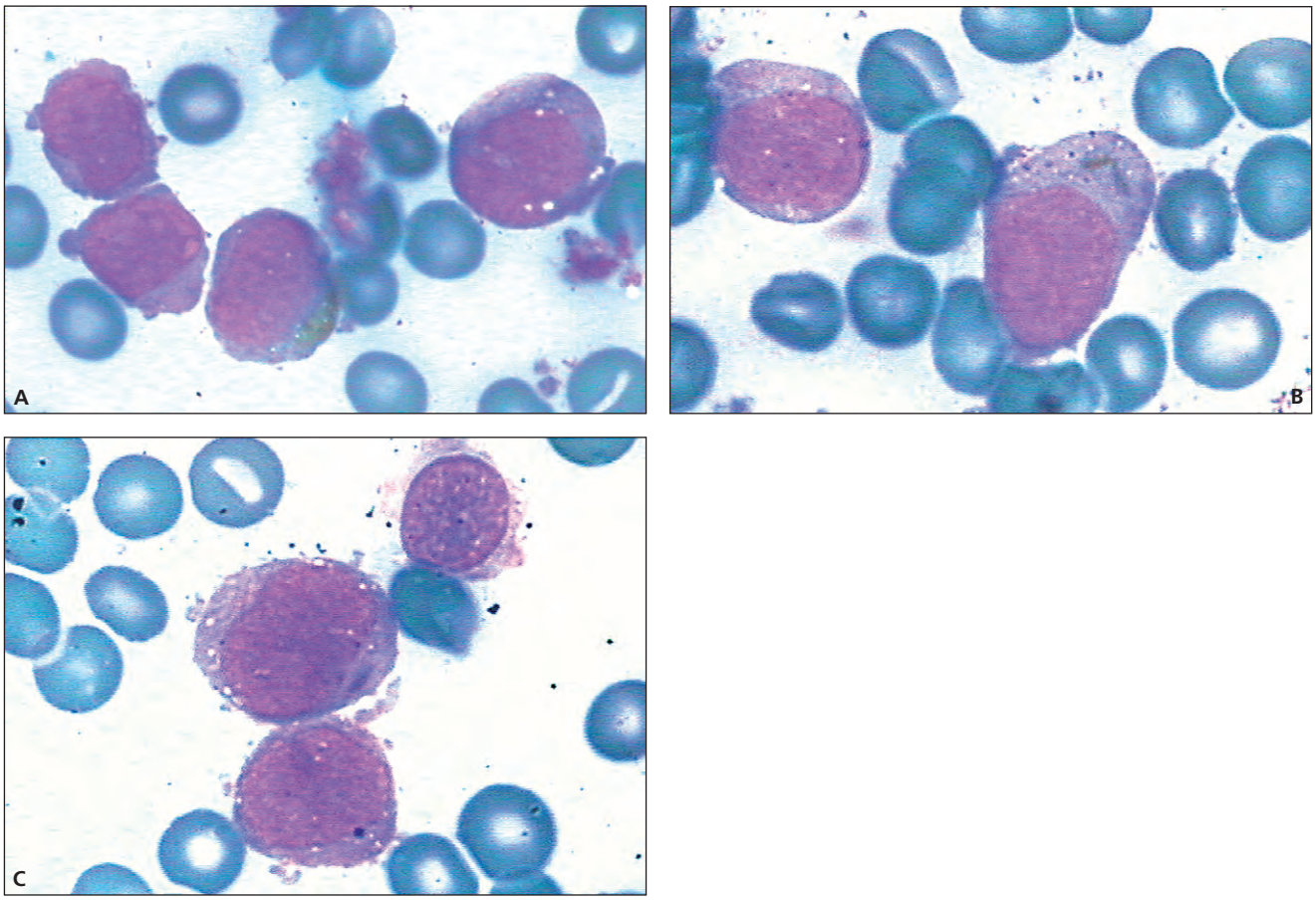
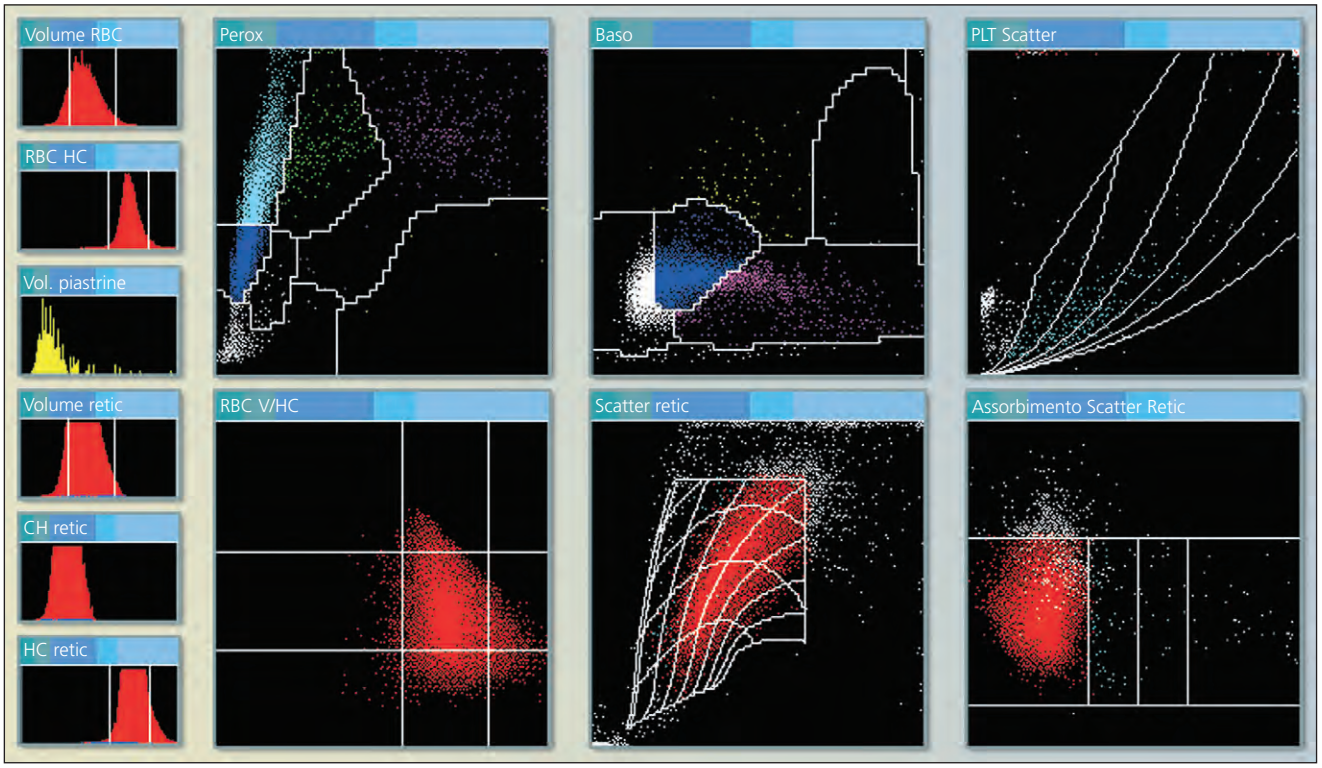



**Fig. 8.32** Peroxidase reaction. (A) Weak cytoplasmic positivity in some blast cells. (B and C) The double and single Auer rods are clearly stained and on a background of basophilic cytoplasm.

## REPORT 8.1 Acute lymphoblastic leukaemia with recurrent genetic alteration, t(9;22), with pro-B immunophenotype



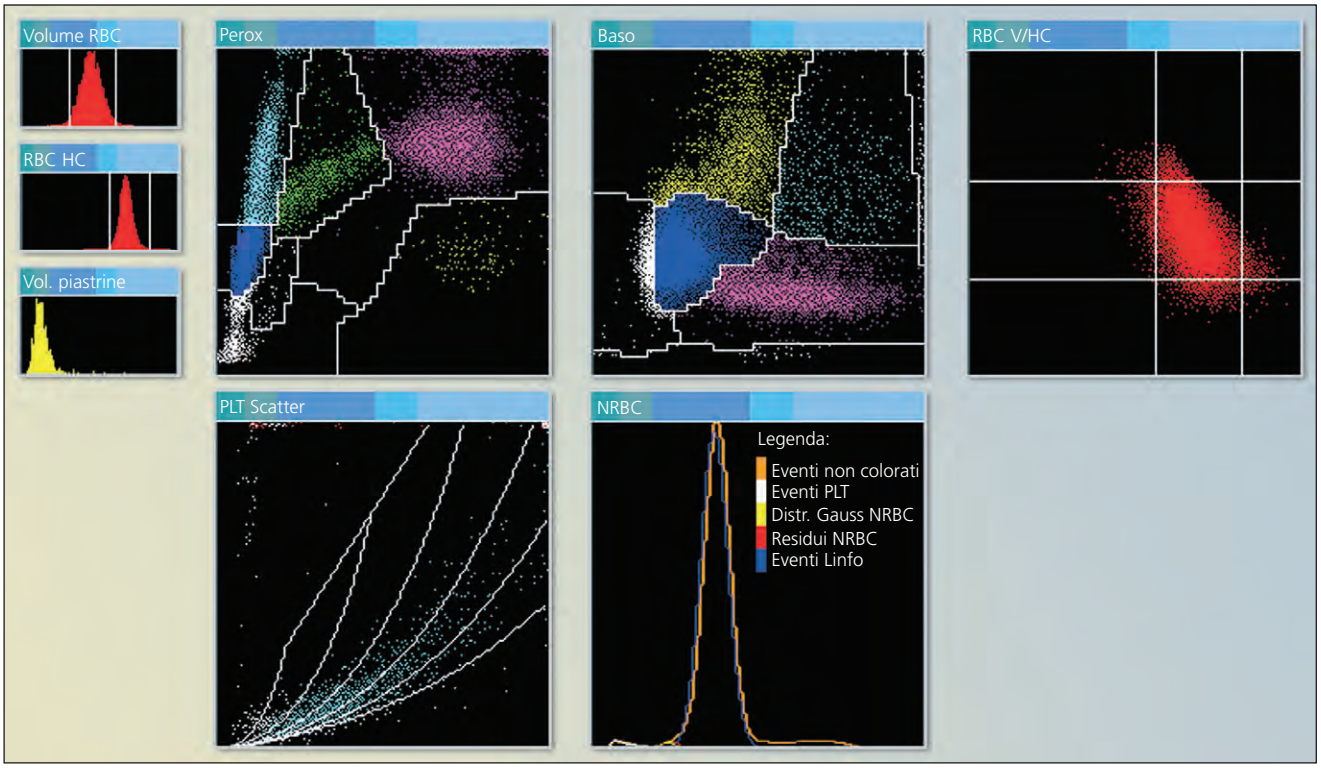

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 67 | g/l | |
| RBC | 2.37 | $\times 10^{12}$/l | |
| HCT | 0.191 | l/l | |
| MCV | 80.8 | fl | |
| MCH | 28.3 | pg | |
| MCHC | 350 | g/l | |
| CHCM | 354 | g/l | |
| RDW | 22.6 | % | Anisocytosis +++ |
| HDW | 3.14 | g/dl | |
| HYPO | 0.8 | % | |
| HYPER | 4.2 | % | Hyperchromia + |
| MICRO | 11.9 | % | Microcytosis +++ |
| MACRO | 2.0 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.04 | $\times 10^{12}$/l | |
| RET | 0.37 | % | |
| RET | 8.8 | $\times 10^{9}$/l | |
| CHr | 29.6 | pg | |
| IRF (M + H) | 21.4 | % | |
| PLT | 38 | $\times 10^{9}$/l | |
| MPV | 12.4 | fl | |
| PDW | 49.5 | % | |
| Large platelets | 3 | $\times 10^{9}$/l | |
| MPC | 21.9 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 17.25 | | |
| WBC Perox | 16.95 | | |
| NEUT | 0.44 | 2.5 | |
| LYMPH | 9.79 | 56.7 | |
| MONO | 0.27 | 1.5 | |
| EOS | 0.01 | 0.1 | |
| BASO | 0.10 | 0.6 | |
| LUC | 6.65 | 38.6 | Atyp +++ |
| NRBC | 0.00 | 0.0 | |
| LI | 1.65 | | |
| MPXI | 5.9 | | |
| MN | | 93.0 | |
| PMN | | 6.3 | |
| BLASTS | | 31.1 | Blasts +++ |
| Perox HPX | | 0.3 | |
| Perox SAT | | 0.1 | |

Leucocytosis with anaemia, thrombocytopenia and severe neutropenia. The Perox cytogram shows only a large population of variably sized peroxidase-negative cells, which form a fine fusiform cluster at the upper limit of the Y axis, with an increased LUC percentage which has led to an Atyp flag; a few scattered neutrophil signals are visible. In the Baso cytogram, almost all the signals are in the MN head, which is deformed downwards and to the left, invading considerably the zone of cells with low chromatin density, thus activating the Blast flag at its maximum level. The PANDA morphology is P0a, D1. Despite the elevated leucocyte count there is no pseudobasophilia. The almost identical values for MCHC and CHCM attest to the lack of interference from turbidity on the measurement of haemoglobin concentration.

**Morphology.** The leukaemic cells are lymphoblasts that are very heterogeneous with regard to size, with an indented nucleus and ample, weakly basophilic, agranular cytoplasm, mixed with 20% of lymphoid cells that appear more mature. The diagnosis is confirmed by immunophenotyping and molecular analysis.

## REPORT 8.2 Acute lymphoblastic leukaemia with T immunophenotype




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 152 | g/l | |
| RBC | 5.16 | $\times 10^{12}$/l | |
| HCT | 0.461 | l/l | |
| MCV | 89.2 | fl | |
| MCH | 29.5 | pg | |
| MCHC | 330 | g/l | |
| CHCM | 336 | g/l | |
| RDW | 15.3 | % | |
| HDW | 2.31 | g/dl | |
| HYPO | 0.9 | % | |
| HYPER | 0.5 | % | |
| MICRO | 1.5 | % | |
| MACRO | 1.1 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 0.8 | % | |
| RET | 41.3 | $\times 10^{9}$/l | |
| CHr | 31.4 | pg | |
| IRF (M + H) | 13.8 | % | |
| PLT | 134 | $\times 10^{9}$/l | |
| MPV | 7.9 | fl | |
| PDW | 43.8 | % | |
| Large platelets | 1 | $\times 10^{9}$/l | |
| MPC | 26.5 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 74.56 | | WBC-CE |
| WBC Perox | 64.72 | | |
| NEUT | 7.23 | | * |
| LYMPH | 58.38 | | * |
| MONO | 1.35 | | * |
| EOS | 0.20 | | * |
| BASO | 2.70 | | |
| LUC | 4.70 | | * Atyp + |
| NRBC | 0.00 | | |
| LI | 1.78 | | |
| MPXI | 7.1 | | |
| MN | | 86.4 | |
| PMN | | 8.8 | |
| BLASTS | | 2.9 | Blasts + |
| Perox HPX | | 0.15 | |
| Perox SAT | | 0.1 | |

Leucocytosis is associated in this patient with a normal haemoglobin concentration and a platelet count that is only slightly reduced. Both mature neutrophils and monocytes are numerous and are well represented in the Perox cytogram; here one observes, in addition, a tight cluster of small peroxidase-negative cells in the lymphocyte box, which continues upwards to a sharp peak in the LUC box. In the Baso cytogram the leukaemic population invades the blast area only partially, but has nevertheless triggered the blast flag; pseudobasophilia is evident, as a result of invasion of the basophil area by partially lysed blast cells, which differentiates this case from that illustrated in Report 8.1. The WBC-CE sample/system flag results from the fact that the leucocyte count in the Perox channel is underestimated in comparison with that in the Baso channel, the latter being the count that is reported: the reason is that the lower left threshold that separates the lymphocytes from noise is too high, so that a proportion of smaller lymphoid cells have been included in the Noise box.

**Morphology.** The peripheral blood film shows a population of small to medium-sized blast cells with a high N:C ratio, scanty basophilic cytoplasm and often an irregular nucleus, with fairly condensed chromatin and nucleoli that are barely visible. Immunophenotyping shows the expression of TdT, cytoplasmic CD3 and CD7.

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CHAPTER 9

# Neoplasms of Mature B, T and NK Cells

Neoplasms that result from clonal aberrations of lymphoid cells are numerous and heterogeneous, as might be anticipated from the very diverse cytological composition of lymphoid tissues. In normal primary and secondary lymphoid organs, there is a physiological co-existence of highly specialised end cells, such as plasma cells, and others, such as long-living T lymphocytes, which are reversibly arrested in early G1 phase of the cell cycle and are capable of proliferating and differentiating following an appropriate stimulus. Primitive lymphoid progenitors originate from multipotent haemopoietic stem cells; from these there are two distinct pathways of lymphoid differentiation, B and T, within which can be recognised numerous levels of maturation. Natural killer (NK) cells are nosographically quite close to T cells, with which they share various immunophenotypic and functional properties.

From various points of view, lymphoid neoplasms tend to recapitulate, in their heterogeneity, the various stages of differentiation of normal T and B cells. However, this rule is not absolute: hairy cell leukaemia (HCL), for example, has not yet been shown to correspond to a known stage of B-cell differentiation. The techniques available for characterising cells, particularly immunophenotypic and molecular analysis, permit a precise definition of the molecular and antigenic alterations that occur during lymphoid differentiation and make possible the characterisation of neoplastic clonal lymphoid populations with regard to cell lineage and stage of maturation, which largely determine their biological behaviours. The acute or chronic clinical evolution is often, but not always, correlated with the level of maturation of the neoplastic clone, while the pattern of organ involvement depends on the distribution of the clonal cells in various anatomical sites. In the leukaemic form, derived generally from precursors that can localise also in the bone marrow, there is involvement of the peripheral blood and bone marrow, whereas in the lymphomatous form the primary site is generally the lymph nodes and lymphoid tissues of other organs.

## Lymphoproliferative or neoplastic conditions of mature B and T/NK cells: definition and classification

This chapter on lymphoproliferative disorders includes a large spectrum of neoplasms, generally with a chronic clinical course, characterised by the clonal proliferation of T or B lymphocytes at various stages of differentiation, which involve simultaneously the lymphoid system and the haemopoietic organs. Within the B or T lineage one can identify specific subpopulations, the clonal expansion of which produces clinicopathological pictures that are readily distinguishable on the basis of peripheral blood and bone marrow morphology, histology of lymph nodes, spleen and other lymphoid organs, immunophenotype and, increasingly often, the results of cytogenetic and molecular analysis. Various non-Hodgkin lymphomas of B or T derivation can also be classified as 'leukaemia' since they are characterised from disease onset or during progression by the presence of neoplastic cells in the peripheral blood and the bone marrow.

The classification of lymphoproliferative disorders must be based on an integration of the results of all analytical methods and the clinical picture. In 1989, the French–American–British (FAB) co-operative morphology group proposed a classification of 'chronic (mature) B and T lymphoid leukaemias', which identified, on the basis of an effective correlation of cytomorphology with the membrane phenotype, various categories characterised by clinical and epidemiological features.[1] The FAB proposals had the merit of unifying different terminologies. They also underlined the necessity for an integrated morphological and immunophenotypic approach in order to increase the accuracy and reproducibility of diagnosis in the face of clinicopathological conditions that differed in prognosis and in sensitivity to various specific forms of treatment. Firstly, they outlined concepts, which remain valid, with regard to cytological features of neoplastic lymphoid cells, identifying features to be assessed: cell size, nuclear shape and regularity of nuclear outline, chromatin characteristics and the presence and nature of nucleoli, and amount, staining characteristics and shape of the cytoplasm. Size was evaluated in practice by comparing the diameter of the lymphoid cells with that of erythrocytes in the blood film: a cell was defined as large if the diameter was more than double the diameter of a normal erythrocyte. Peripheral blood analysis is of great importance in the diagnosis of lymphoproliferative disorders: the blood count permits an evaluation of the degree of lymphocytosis and of manifestations of bone marrow insufficiency, which vary between different diagnostic categories. Furthermore, in a thin, clean and well-spread peripheral blood film, cellular morphology is often clearer and better defined than in bone marrow films. Histological evaluation of a bone

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marrow biopsy can provide complementary information that is important for diagnosis, e.g. in HCL, and also permits the recognition of the topography of neoplastic infiltration, which correlates with prognosis. In leukaemic lymphomas, it is often indispensable to complement the initial diagnostic approach with investigation of lymph nodes, spleen and other sites of infiltration.

Morphology alone is able to provide a diagnosis in only a minority of cases and one must not neglect immunophenotyping with monoclonal antibodies, using either immunocytochemistry or flow cytometry.[2,3] In many cases, the association of a particular morphological picture with a specific antigenic profile is such that an unequivocal diagnosis can be made. In the lymphoproliferative disorders, the immunophenotype is that of a mature cell: there is no expression, for example, of terminal deoxynucleotidyl transferase (TdT), a typical marker of acute lymphoblastic leukaemia (ALL), or of CD34, the marker of haemopoietic progenitors. Certain basic immunological markers are expressed in the majority of cells of a certain derivation and thus permit the attribution of a leukaemic population to the B or T lineage: other monoclonal antibodies are used in a basic flow cytometry panel with the aim of recognising surface membrane immunoglobulin (SmIg), either kappa (κ) or lambda (λ), the antigens CD10, CD19, CD20, CD45 and CD103, with CD5, for the B lineage, and the antigens CD2, CD3, CD4, CD5, CD7, CD8, CD45 and CD56 for the T lineage. Numerous other markers are used in secondary panels for the identification of specific disorders (e.g. CD11c, CD25, CD30, CD79a, CD79b and FMC7).[4] The presence of SmIg with expression restricted to a single light chain type, κ or λ, is the most important criterion establishing the monoclonal nature of a B-cell population: the intensity of SmIg expression on flow cytometry is useful for distinguishing chronic lymphocytic leukaemia (CLL), which typically shows weak expression, from other conditions, such as prolymphocytic leukaemia (PLL), in which the intensity of expression is equivalent to, or greater than, that of normal lymphocytes. For the T lineage, the clonal nature of a lymphoid population is demonstrated by investigation for rearrangement of the genes that encode the chains of the T-cell receptor (TCR); nowadays demonstration of T-cell clonality can also be achieved with flow cytometric methods, which can distinguish the early phase of a lymphoproliferative disorder from a reactive lymphocytosis. The HLA-DR antigen is present in all cells of B lineage, but is not lineage specific, being widely expressed also in haemopoietic progenitors, leukaemic blast cells and activated T lymphocytes. Flow cytometric immunophenotyping with multiple markers is important for demonstrating expression of a marker that would be unusual for the lineage from which the population is derived: this permits, for example, the identification of co-expression of CD5, CD23 and CD19 in the B lymphocytes of CLL, or that of CD11c (a monocyte marker) and CD19 (a B-lineage marker) in HCL. In other cases, the expression of particular antigens has a prognostic value, such as the expression of CD38 in B-CLL, or provides therapeutic indications, such as the expression of CD20, the therapeutic target of rituximab.

Although morphological and immunophenotypic investigation is sufficient to classify the majority of lymphoid neoplasms, there are not yet specific markers for specific diseases and the diagnosis must always be made by recognising the characteristic cytomorphological features in combination with cytofluorometric and immunohistochemical immunophenotyping using a wide panel of antibodies. Furthermore, investigation of cytogenetic and molecular alterations, by means of cytogenetic analysis and molecular techniques such as the polymerase chain reaction (PCR) and fluorescence *in situ* hybridisation (FISH) has led to the recognition of recurrent specific aberrations, particularly in B-lineage neoplasms. Molecular techniques also permit investigation for rearrangement of the immunoglobulin and TCR genes. The first anomalies identified in lymphoproliferative disorders were t(14;18)(q32;q21), found in follicular lymphoma, and t(8;14)(q24;q32), found in Burkitt lymphoma. The accurate morphological, immunophenotypic and clinical description of specific entities has recently permitted the identification of their molecular pathogenesis: for example, in mantle cell lymphoma (MCL) a clear description of the disorder has permitted the identification of the critical role of the *CCND1* gene in its pathogenesis, with this subsequently leading to further diagnostic precision with the use of a monoclonal antibody reactive with cyclin D1 in histological sections.[5]

The contributions of these diagnostic methods to the solid base provided by histological investigation, led in 1994, after a long period of work by a consensus group, to the publication of the Revised European–American classification of Lymphoid neoplasms, the acronym of which, REAL, was also used to underline that the aim was the identification of 'real' neoplastic entities, not based solely on theoretical suppositions.[6] The 2008 World Health Organization (WHO) classification, in the section dealing with lymphoid neoplasms, represents a further development of the REAL classification. It is based on the distinction between mature and immature neoplasms of haemopoietic and lymphoid tissues, with recognition of the lineage and the degree of maturation of the neoplastic population, which is often the counterpart of a particular stage of maturation of normal lymphoid cells.[7] The conditions previously known as lymphoproliferative disorders thus became, in this new classification, neoplasms of mature or peripheral B or T/NK cells. According to this scheme, normal B-cell ontogeny starts with a progenitor which is a lymphoblast, from which ALL originates. These cells start the process of maturation with rearrangement of the *VDJ* genes at the immunoglobulin locus, which leads to the formation of virgin (naïve) B cells, which express the CD5 antigen and SmIg M, circulate in the peripheral blood and localise in the primary lymphoid follicles and in the mantle zone of lymphoid follicles: the majority of MCL develop from these cells. An encounter with antigen leads to the proliferation and maturation of lymphoid cells: lymphocytes sensitised in this way migrate towards the centre of the primary follicle and

fill the spaces in the network of follicular dendritic cells, forming the germinal centre and becoming centroblasts, which express the CD10 antigen and the BCL2 and BCL6 proteins. Here the rearrangement of the V region of the immunoglobulin gene (*IGV*) occurs. Genetic lesions occurring in these proliferating cells in the germinal centre lead to the development of aggressive lymphomas, such as diffuse large B-cell lymphoma and Burkitt lymphoma. Normally, however, the further maturation of these cells leads to the formation of centrocytes, among which only those with a high affinity for antigen are spared from apoptosis. Follicular lymphomas are neoplasms of germinal centre cells, composed of a variable proportion of centrocytes and centroblasts, which have escaped apoptosis, usually because of genetic damage resulting from t(14;18), which impairs the suppression of the anti-apoptotic protein, BCL2. The final stages of B-cell maturation give rise, on the one hand, to plasma cells capable of antibody production, which are localised mainly in the bone marrow and from which multiple myeloma develops, and on the other hand, to memory B lymphocytes, which circulate in the blood and localise in the marginal zone of follicles in lymph nodes, the spleen and lymphoid tissues associated with mucous membranes (mucosa-associated lymphoid tissues [MALT]), which give origin to marginal zone lymphomas.

An aetiological role of infective agents is being recognised in an increasing number of cases of lymphoid neoplasms; these agents include the Epstein–Barr virus (EBV), which is implicated not only in all cases of endemic Burkitt lymphoma and in 15–30% of sporadic and HIV-associated cases, but also in many lymphomas in immunosuppressed patients and in some T/NK-cell lymphomas.[8] Human herpesvirus 8 (HHV8) has been associated with various B-cell lymphomas and lymphoproliferative disorders. The human T-cell lymphotropic virus (HTLV-1) is the causative agent of adult T-cell leukaemia/lymphoma. The hepatitis C virus (HCV) is associated with B-cell lymphoma, not as a direct result of infection, but due to the protracted immune response that follows infection. Certain bacteria, either directly or because of the immune activation that they cause, favour the development of MALT-type lymphoma.

Table 9.1 lists the categories of peripheral lymphoma included in the 2008 WHO classification. In this chapter we shall deal in detail with the lymphomas that involve the peripheral blood and bone marrow at presentation or during successive phases of the disease, with a particular emphasis on cytomorphology.

**Table 9.1** 2008 WHO classification of lymphoid neoplasms of mature B and T/NK cells (adapted from Swerdlow 2008[118], reproduced with permission from IARC)

**Neoplasms of mature B cells**
- Chronic lymphocytic leukaemia/small lymphocytic lymphoma
- B-cell prolymphocytic leukaemia
- Splenic marginal zone lymphoma
- Hairy cell leukaemia
- Splenic lymphoma/leukaemia, unclassifiable (including hairy cell leukaemia variant)
- Lymphoplasmacytic lymphoma (including Waldenström macroglobulinaemia)
- Heavy chain diseases
- Plasma cell neoplasms (see Chapter 10)
- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
- Follicular lymphoma
- Primary cutaneous follicle centre lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma, not otherwise specified
- T-cell/histiocyte-rich large B-cell lymphoma
- Primary diffuse large B-cell lymphoma of the central nervous system
- Primary cutaneous diffuse large B-cell lymphoma (leg type)
- EBV-positive diffuse large B-cell lymphoma of the elderly
- Diffuse large B-cell lymphoma associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK-positive large B-cell lymphoma
- Plasmablastic lymphoma
- Large B-cell lymphoma developing in HHV8-associated multicentric Castleman disease
- Primary effusion lymphoma
- Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

**Hodgkin lymphoma**

**T/NK cell neoplasms**
- T-cell prolymphocytic leukaemia
- T-cell large granular lymphocytic leukaemia
- Chronic lymphoproliferative disorder of NK cells
- Aggressive NK cell leukaemia
- EBV-positive T-cell lymphoproliferative disorders of childhood
- Adult T-cell leukaemia/lymphoma
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-associated T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides
- Sézary syndrome
- Primary cutaneous CD30-positive lymphoproliferative disorders
- Primary cutaneous gamma–delta T-cell lymphoma
- Peripheral T-cell lymphoma, not otherwise specified
- Angioimmunoblastic T-cell lymphoma
- Anaplastic large cell lymphoma, ALK positive
- Anaplastic large cell lymphoma, ALK negative

## Mature B-cell neoplasms

These are the most common clonal disorders of B lymphocytes, representing around 4% of all tumours, with an incidence above 25 per 100 000 per year in western countries. The neoplastic cells are B cells that are analogous to one of the various stages of differentiation and that involve peripheral lymphoid tissues; they express immunophenotypic markers of mature B cells such as CD19 and SmIg (Table 9.2).

**Table 9.2** Lymphoproliferative disorders of mature B cells: differential diagnosis in the forms that show frequent and relevant involvement of the bone marrow

| | Bone marrow involvement | Leucocyte count ($\times 10^9$/l) | Morphology | Immunophenotype* | Recurrent genetic anomalies |
|---|---|---|---|---|---|
| Chronic lymphocytic leukaemia (CLL) | 100% | From 5 to >100 | Monomorphic: small mature lymphocytes, chromatin in a mosaic pattern; some cases with variation in size, smear cells, prolymphocytes <10% | Weak SmIg and CD20, CD5+, CD23+, CD79b−, FMC7− | del(13q), trisomy 12, del(11q), del(17p) |
| Mixed cell type of CLL (includes CLL/PL) | 100% | On average higher than CLL | Prolymphocytes 10–55%; morphological heterogeneity | Intermediate between CLL and PLL; CD5+, CD22 and FMC7 variable | Similar to CLL |
| Prolymphocytic leukaemia | 100% | Always elevated, >100 in two thirds of cases | Prolymphocytes >55% | CD79b+, FMC7+, CD5− and CD23− (85% of cases) | del(17p), del (13)(q14) |
| Hairy cell leukaemia | 100% | <5 (60% of cases), pancytopenia | Typical hairy cells, dry tap, TRAP positive | CD25+, CD11c+, CD103+, CD123+, FMC7+ | *BRAF2* mutation |
| Splenic marginal zone lymphoma (SMZL) | 100% | Variable, up to 40 | Lymphocytes with short polar villi, plasmacytoid features | B markers, CD5 rarely positive, CD10− | del(7q) |
| Splenic red pulp lymphoma | 100% | Moderate lymphocytosis | Villous lymphocytes similar to SMZL | B markers | Complex abnormalities |
| Hairy cell leukaemia variant | 100% | From 40 to 110 | Villous cytoplasm and prolymphocytic nucleus (nucleolus) | Similar to LPL, CD11c+, CD103+ | No specific abnormality |
| Follicular lymphoma | 30–60% | From normal to >200 | Small cleft cells, very scanty cytoplasm, sometimes also larger cells | CD10+, FMC7+, CD5− | t(14;18)(q32;q21), rearrangement of *BCL2* |
| Mantle cell lymphoma | Frequent | Moderate variable leucocytosis (<20%) | Polymorphism: prolymphocytoid cells, small lymphocytes, blast cells | CD5+, CD10− , CD23− | t(11;14)(q13;q22), *CCND1* |
| Lymphoplasmacytic lymphoma (LPL), including Waldenström macroglobulinaemia | Not common, 100% in Waldenström macroglobulinaemia | Variable | Plasmacytoid lymphocytes, plasma cells | CD5−, CD10−, CD25+, CD38+, CD71+, | *MYD88* L265P |
| Gamma heavy chain disease | Frequent | Small numbers of lymphoplasmacytoid cells | Plasmacytoid lymphocytes | B markers, CD138 | None known |
| Mu heavy chain disease | 100% | Similar to CLL | Similar to CLL | CD5-, CD10- | None known |
| Diffuse large B-cell lymphoma | Occasional | Rare leucocytosis | Large cells with polymorphic nuclei | Variable | No specific abnormality |
| Burkitt lymphoma | 15% | Variable | Basophilic vacuolated blast cells | Mature B phenotype | t(8;14)(q24;q32), less often t(2;8) or t(8;22) |

*The antigens typical of mature B cells are always present (except in lymphoblastic lymphoma): CD19, CD20, CD24, CD79a, SmIg with clonality (light chain restriction) and HLA-DR.

## Chronic lymphocytic leukaemia

This condition, CLL or B-CLL, is a clonal B-cell proliferation, typically of small monomorphic lymphocytes, which are cytologically mature, but are immature from the biological point of view and with regard to their immunological competence (Figs 9.1 through to 9.17).[9] In 96–98% of cases the lineage is B cell (and T-cell cases should be regarded as a different disorder), representing the leukaemic counterpart of a particular subpopulation of B lymphocytes, characterised, in part, by the weak expression of SmIg with light chain restriction and co-expression

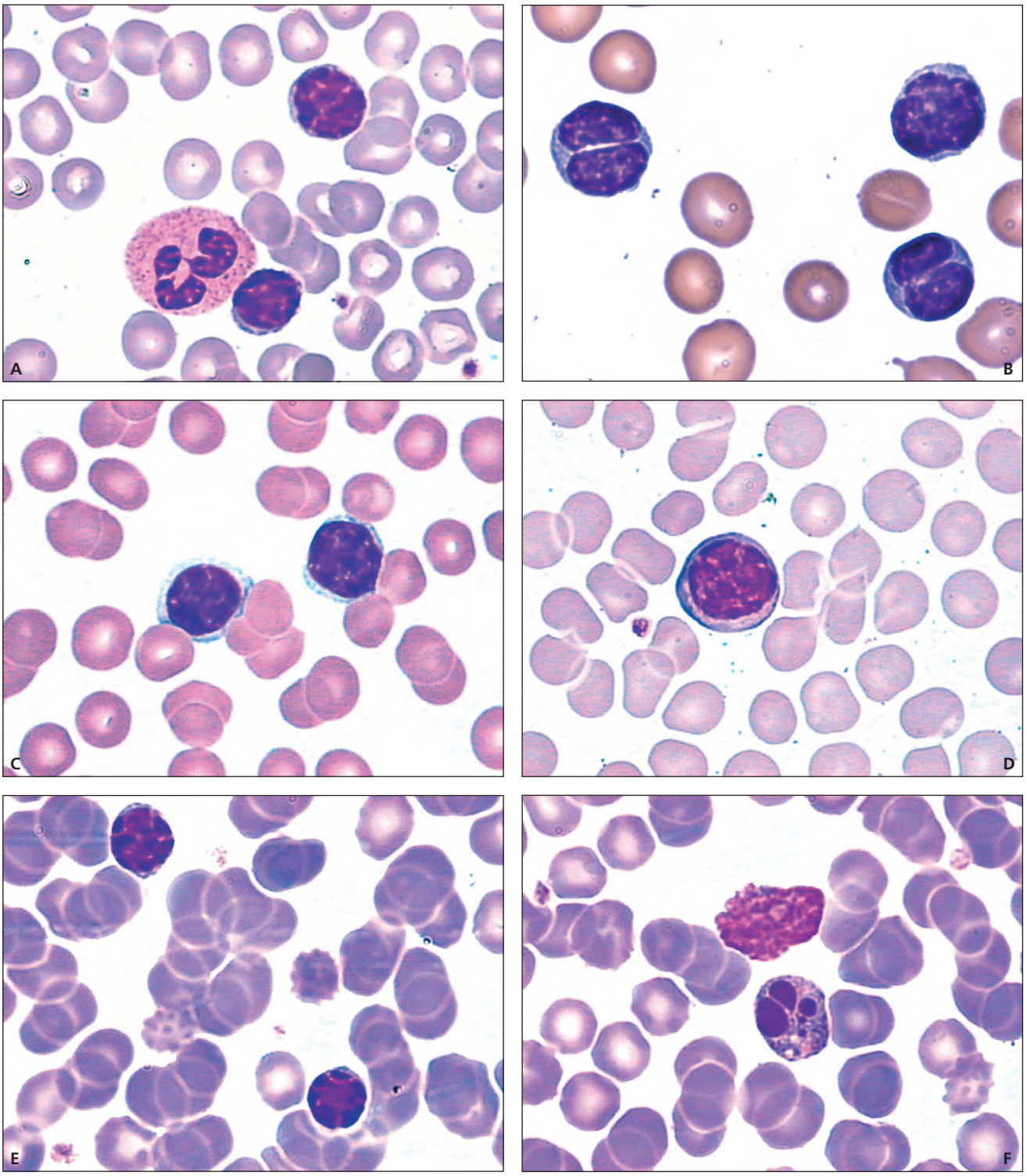



**Fig. 9.1** Typical features of lymphocytes in lymphocytosis of various origins; different peripheral blood films. (A) Monoclonal B lymphocytosis. Two lymphocytes with regular features; the upper one, with just visible cytoplasm, has chromatin condensed into blocks, as in chronic lymphocytic leukaemia (CLL). (B) Two lymphocytes with bilobed nuclei, in the shape of a saddlebag; the third nucleus has an indented nuclear prolife. The cytoplasm is more basophilic than usual. Two of these three lymphocytes have deep nuclear clefts. (C) B-lineage CLL. Two small lymphocytes with round nuclei and coarsely clumped chromatin. This chromatin pattern is typical. The nuclear outline is rounded. The cytoplasm is better represented and forms a thin rim around almost the entire nucleus; it has an empty watery appearance. (D) B-lineage CLL. The slightly eccentric nucleus appears as if split into dark blocks, with the distinctive appearance of a football. The cytoplasm is more basophilic and shows a pale paranuclear half-moon. (E) From a different patient with B-CLL: the nuclear morphology (round outline, chromatin in clumps separated by fissures) again is very typical. (F) At the top, a smear cell (a small Gumprecht's shadow); at the bottom, a still intact lymphocyte with the nucleus fragmented into lobules of liquefied structureless chromatin, as a result of apoptosis.

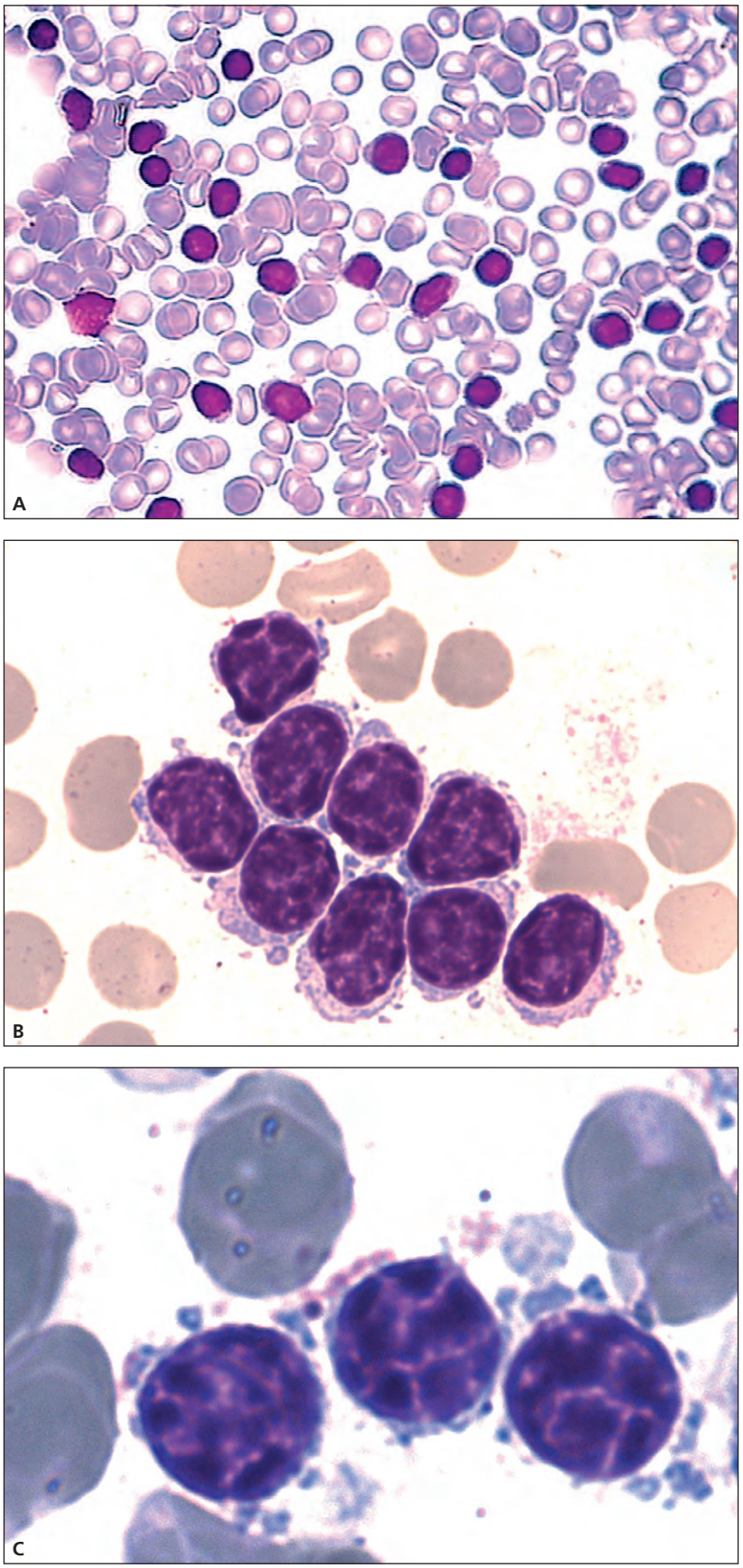



**Fig. 9.2** B-lineage CLL; peripheral blood film. (A) At low power, numerous small lymphocytes with clumped dark rounded nuclei, the chromatin appearing compact and homogeneous; in some cells it is possible to see a very thin half-moon of cytoplasm. The diameter is only a little greater than that of the red cells. On the left, a crushed nucleus (a smear cell). (B and C) At higher power the mosaic structure of the nuclei is more evident; there are large pentagonal blocks of very condensed chromatin, aubergine coloured, separated by thin pale fissures. This appearance is very characteristic of the lymphocytes of B-CLL, even though it is not seen in all cells, or in all patients. The cytoplasm is very scanty and, in a manner that is not frequent in this condition, is tending to form small vesicular protrusions.

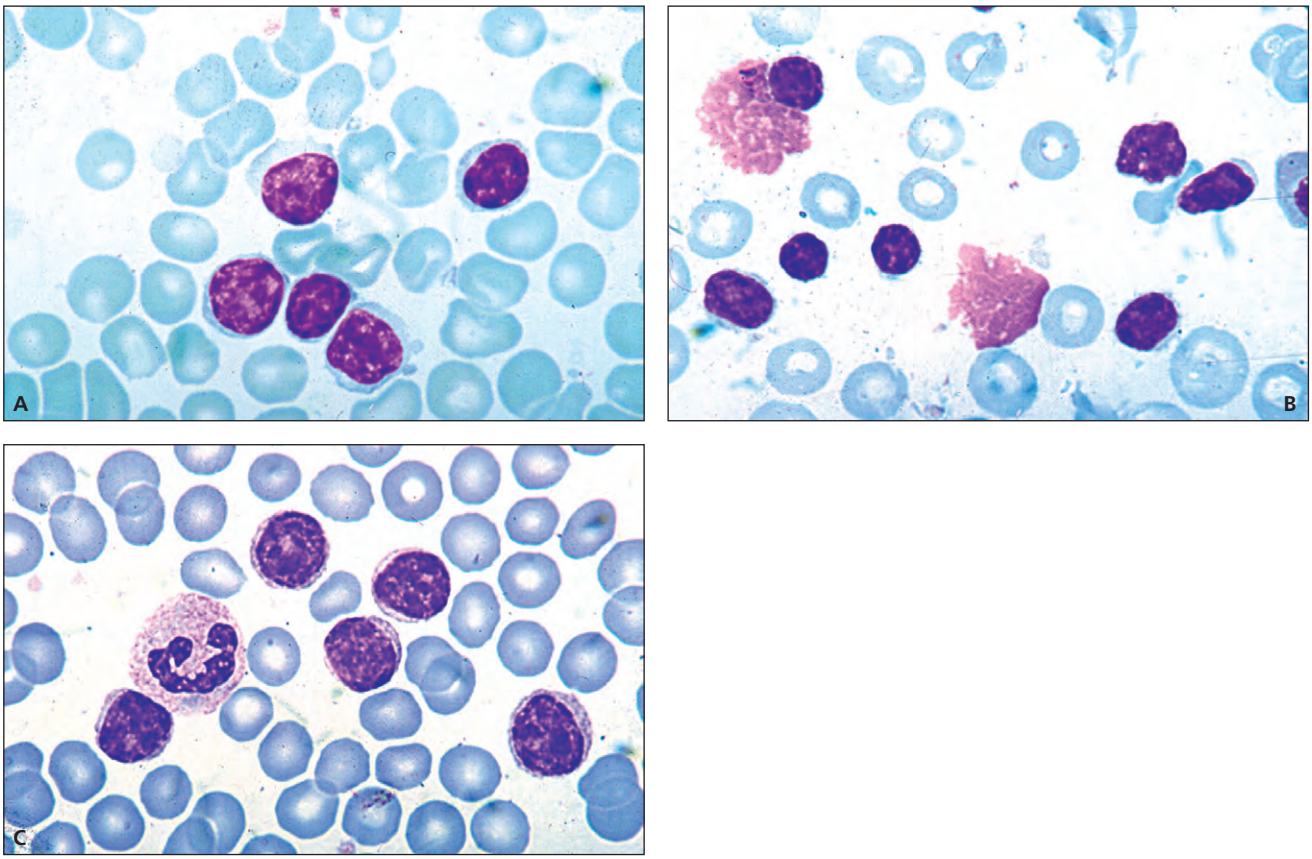



**Fig. 9.3** Typical morphology of the lymphocytes of B-CLL; from various peripheral blood films. (A) Small CD19- and CD5-positive lymphocytes, with weak surface membrane immunoglobulin (SmIg). The cytoplasm is scanty, pale blue, limpid, without inclusions or granules. The nuclei are rounded and eccentric, with chromatin condensed into large dark clumps. The most typical nuclear morphology, with blocks separated by fissures, is seen in the smaller lymphocyte low down in the centre. (B) The leukaemic cells are very small: some cells have a diameter less than that of the adjacent red cells. The chromatin is very dense, even if the typical appearance of blocks is present in only some of the nuclei. Nucleoli are not seen. The cytoplasm is almost invisible. A picture such as this has analogies with the leukaemic form of follicular lymphoma, from which it can be differentiated by the absence of nuclear notches and grooves. There are two violet smudges corresponding to disintegrated nuclei (smear cells or Gumprecht's shadows), which are frequent in these cases because of the mechanical fragility of the cells when subjected to spreading on a glass slide. (C) Adjacent to a neutrophil granulocyte there are five lymphocytes that are a little larger than the red cells. The nucleus, which occupies almost all the available space, has a rounded or oval shape: there is typical condensation of chromatin into large blocks separated by thin pale fissures; at the centre of some nuclei, there is a pale area, suggesting the faint outline of a nucleolus. The nucleus is generally eccentric, touching the cell membrane and leaving free only a very thin crescent of pale basophilic cytoplasm.

of the CD5 antigen. Lymphocytes of this type are present at a very low concentration in normal peripheral blood; their origin is still controversial, but they may correspond to B lymphocytes recognised in the marginal zone of lymphoid follicles, in which the variable region of the *IGH* locus is sometimes unmutated and sometimes already mutated as a result of antigen exposure.[10,11] CLL is the most frequent leukaemia of adults with an incidence in western countries of 4.2 per 100 000 per year, representing 30% of all leukaemia diagnoses and 60–70% of lymphoproliferative neoplasms. It is rare in Japanese and Asiatic populations: this geographical diversity is very likely indicative of genetic factors, since it is maintained in successive generations of these ethnic groups following migration to the west. It is a disease of adults of an advanced age: 90% of cases are diagnosed in individuals above the age of 55 years with a median age of 72 years; it is more common in men with a male : female ratio of 2–3 : 1, which tends to decrease with increasing age. Less than 10% of cases occur in adults under the age of 40 years; it is extremely rare in children. More than any other haematological neoplasm, it shows a genetic influence with multiple diagnoses in family members in 5–10% of cases; the relatives of patients with CLL have a more than two-fold increase in the risk of this disorder and also have an increased incidence of monoclonal B-cell lymphocytosis.

In around 20% of patients affected by CLL, the diagnosis is an incidental one in an asymptomatic individual. In other cases there is a symmetrical enlargement of lymph nodes, these being mobile and painless, particularly in the supraclavicular, axillary

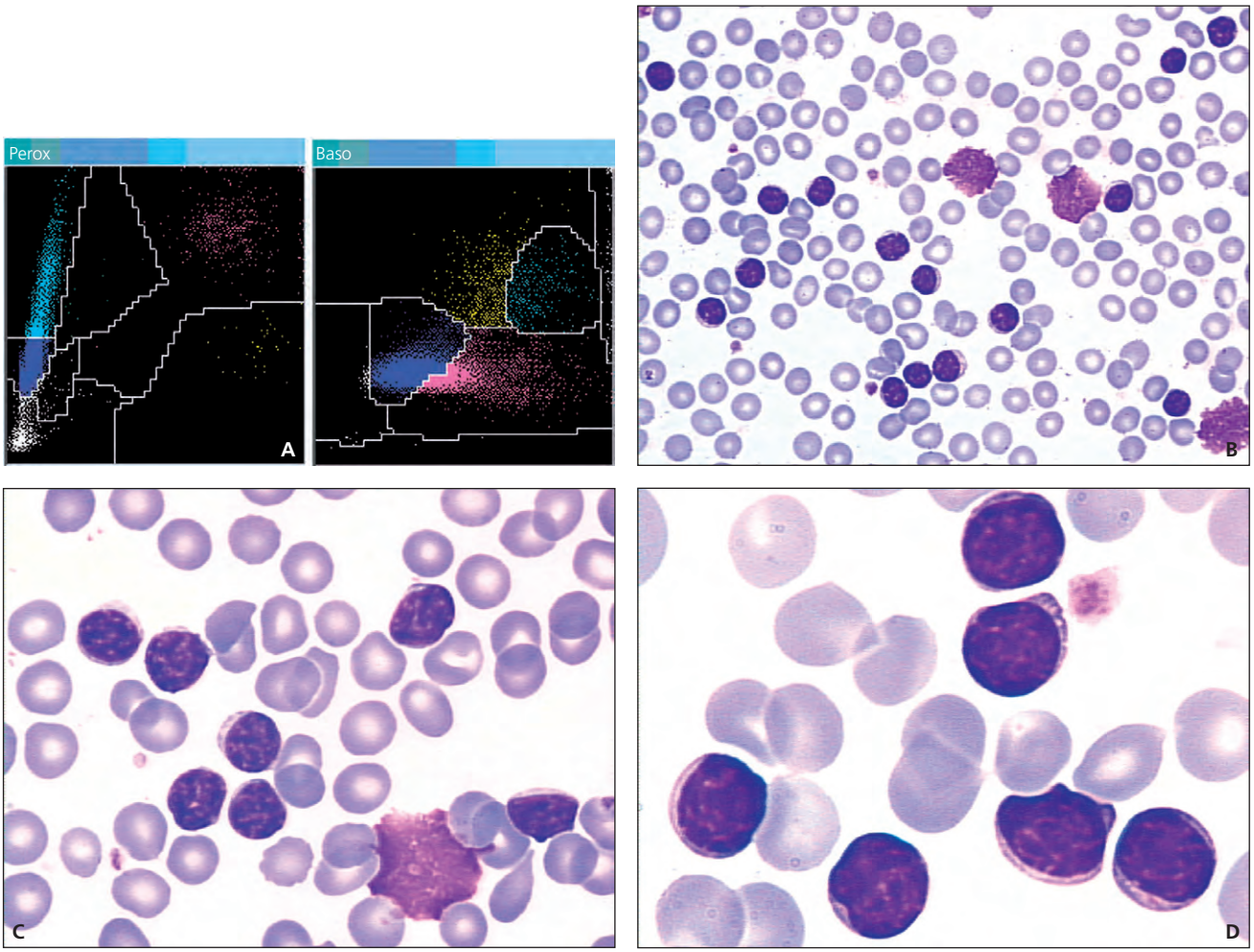



**Fig. 9.4** A typical case of B-CLL at presentation; automated instrument printout and peripheral blood film. (A) In the ADVIA cytograms the small lymphocytes of CLL produce a very recognisable picture: in the Perox channel they form a fusiform cluster of variable length of peroxidase-negative cells, which thins out and comes to a point towards the top of the LUC box, without losing continuity or invading the monocyte box; in the Baso channel, the nuclei with compact chromatin form an elongated oval cluster of MN which do not penetrate the blast area but overflow into the PMN and also, because of co-incidence, into the upper zone, causing pseudobasophilia. (B) Numerous small lymphocytes with eccentric nuclei, dense chromatin and the usual half-moon of pale cytoplasm. The nuclear outline is round without any notches. Three smear cells. (C and D) All the features described above are shown in these small cells, which confirm the monomorphism that characterises most cases of CLL.

and inguinal regions. In around a half of cases there is splenomegaly and, less often, hepatomegaly.

The diagnosis of CLL must be based on minimal diagnostic criteria which, according to the International Workshop on CLL,[12] updated more recently,[13,14] are represented by:

- An increase of small mature lymphocytes in the peripheral blood, with a concentration of at least $5 \times 10^9$/l, for a period of at least three months, without any apparent cause (see Fig. 9.1A);
- Flow cytometric demonstration of monoclonality (weak expression of SmIg with light chain restriction) and the presence of CD5 antigen;
- Typical morphology in the peripheral blood film: small mature lymphocytes with a thin rim of cytoplasm and nuclei with irregularly clumped chromatin, without visible nucleoli. These cells can be admixed with a proportion of larger or atypical cleft cells or with prolymphocytes, the latter being less than 55%. The presence of smear cells (also known as smudge cells and as Gumprecht's shadows) is recognised as the final characteristic feature.

The criterion of a bone marrow infiltrate representing at least 30% of all nucleated cells no longer needs to be met. In addition, if the peripheral blood lymphocyte count does not reach $5 \times 10^9$/l, but there is nevertheless cytopenia as a result of bone marrow lymphoid infiltration, the diagnosis of CLL can be made.

In patients who have lymphadenopathy with histological sections typical of CLL, but with a lymphocyte count less than

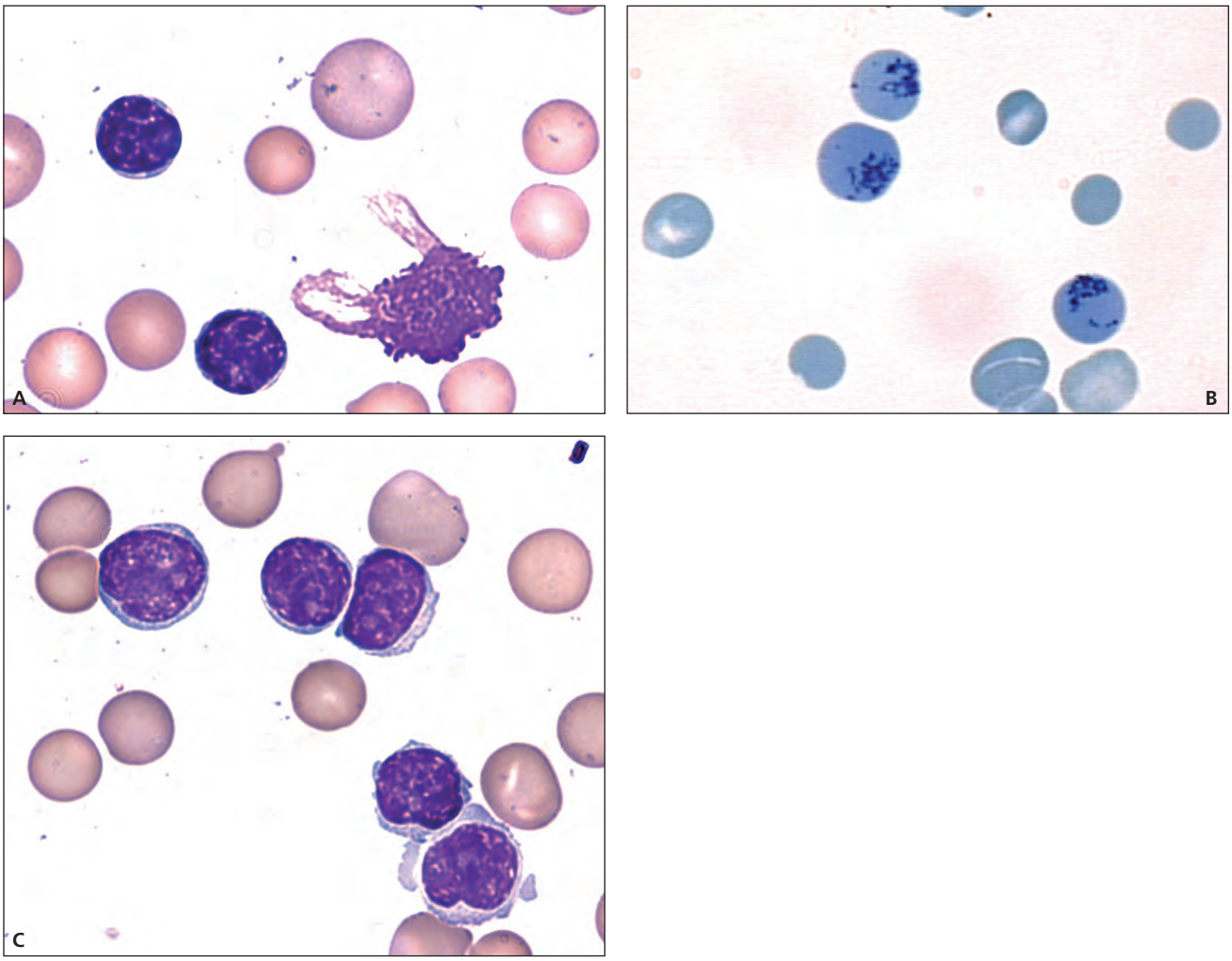



**Fig. 9.5** Complicating autoimmune haemolytic anaemia in a patient with B-CLL (see Report 9.1). (A) Peripheral blood film; two lymphocytes with round nuclei with very scanty cytoplasm, a crushed nucleus and, at the top, a spherocyte indicating haemolysis, and a large polychromatic macrocyte indicating erythroid regeneration. (B) Reticulocytosis with some cells having abundant reticulum (elevated immature fraction) and some microspherocytes. The appearance of anaemia with reticulocytosis should always suggest the development of autoimmune haemolytic anaemia, which often complicates the course of these patients, and is not in itself, if transitory or controllable with therapy, an unfavourable prognostic feature. (C) The nuclei in this patient sometimes have small notches and pale traces of nucleoli; the chromatin clumps are not clearly separated from each other, but give the tactile sensation of a nodular surface. The cytoplasm is better represented than in other cases and is pale blue and not homogeneous, with a paler paranuclear area. At the top, a dacrocyte. At the bottom, fragments of detached cytoplasm. The cells were positive for CD19 and had trisomy 12.

$5 \times 10^9$/l and no cytopenia attributable to bone marrow infiltration, the diagnosis is small lymphocytic lymphoma; the immunophenotype is identical to that of CLL. In the 2008 WHO classification, CLL and small lymphocytic lymphoma are considered a single entity with different clinical expressions[15]: the lymphoma is distinguishable from CLL only on the basis of the predominating lymph node or tissue localisation (see Fig. 9.12). The neoplastic lymphoid population has characteristics analogous to those of CLL with regard to morphology (small lymphocytes with round nuclei and condensed chromatin, no visible nucleoli and scanty cytoplasm), immunophenotype (B-lineage antigens plus CD5 and weak SmIg) and cytogenetics (frequent trisomy 12).[16,17] From the practical point of view then, cases in which the disorder is manifest initially with bone marrow infiltration and peripheral lymphocytosis are classified as CLL, while an extramedullary localisation leads to classification as small lymphocytic lymphoma. There are, however, intermediate pictures in which there is bone marrow infiltration without peripheral blood involvement, with also the possibility of conversion from one form to the other during the course of the disease.

The presence of clonal B lymphocytosis not reaching $5 \times 10^9$/l in adults without organomegaly on clinical or radiological examination and without cytopenia or systemic symptoms

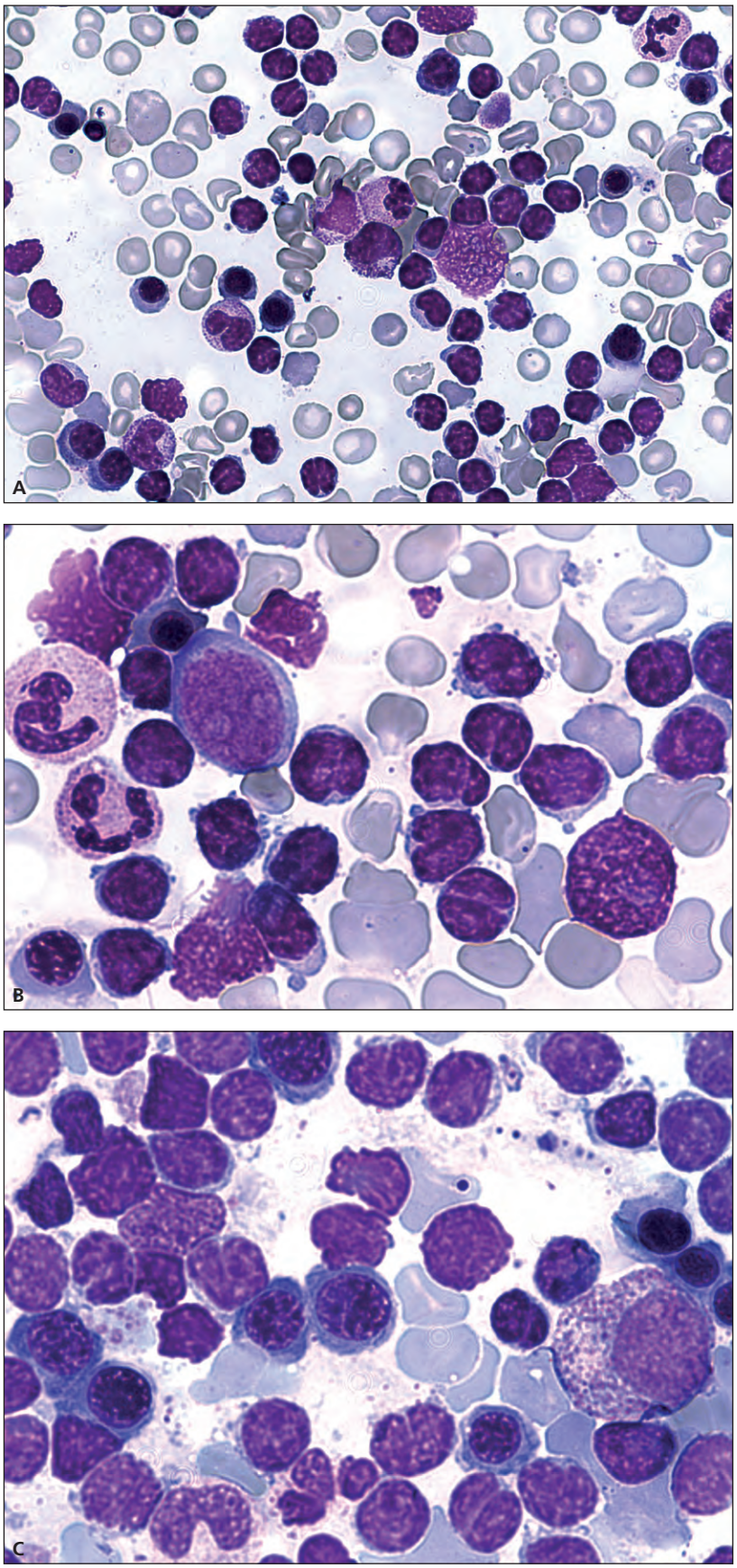



**Fig. 9.6** The same case of B-CLL as Fig. 9.5; bone marrow aspirate. (A) The marrow is very cellular and in this field shows mainly small lymphocytes with morphological features analogous to those of the circulating lymphocytes: there is a higher frequency of nuclei with nipple-like protrusions, notches or a central sulcus (e.g. adjacent to the inferior border, immediately to the left of centre): the cytological differential diagnosis with follicular (centrocytic) lymphoma is easier with the aid of immunophenotyping. In the centre, three cells of granulocyte lineage. There are also numerous erythroblasts (two small basophilic erythroblasts bottom left, with polychromatic and orthochromatic erythroblasts widely distributed), recognisable from the staining of the cytoplasm, the perfect roundness of the nuclear outline and the total loss of chromatin structure, reduced to a blackish mass in the most mature cells. (B) Numerous lymphocytes, one of which, bottom right, has a coffee-bean shaped nucleus, adjacent to a large crushed nucleus; in the bottom left corner, a polychromatic erythroblast; higher up, a large proerythroblast with two nucleoli and a small polychromatic erythroblast. (C) In this field there is a relatively greater abundance of basophilic, polychromatic and orthochromatic erythroblasts, indicating erythroid hyperplasia secondary to the autoimmune haemolytic anaemia. Images of this type are useful for comparing the cytological details of erythroblasts (round central nucleus) and mature lymphocytes (nucleus often notched, eccentric, with a different chromatin structure). To the right, a late neutrophil promyelocyte, with residual cytoplasmic basophilia and an eccentric nucleus with a nucleolus.

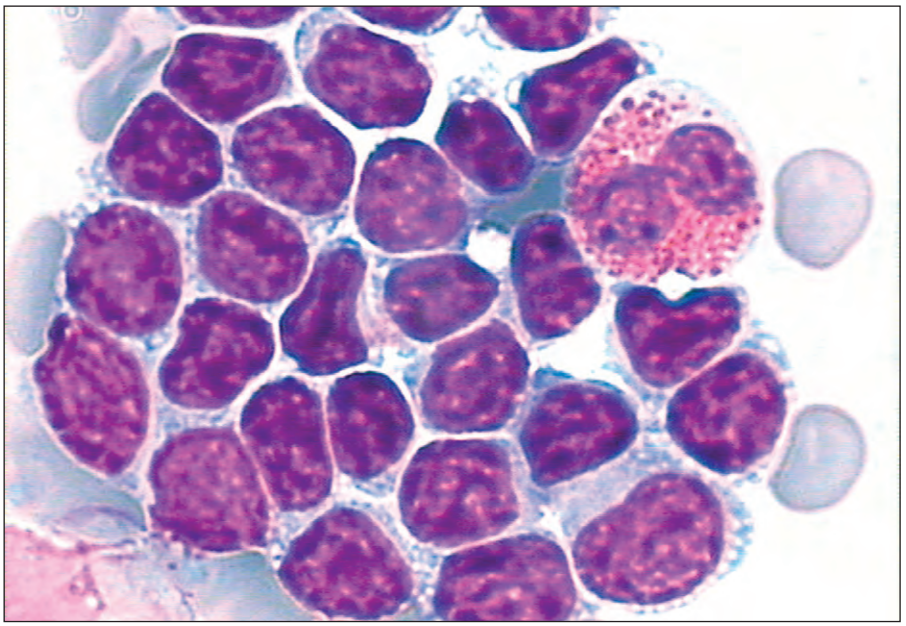


**Fig. 9.7** Small group of lymphocytes with characteristic morphology in the peripheral blood film of a patient with B-CLL. When they are numerous, the lymphocytes tend to mass together on the edges and in the tail of the film in a pseudonodular formation, which is not present *in vivo*. The nuclei are rounded with the chromatin condensed into clumps. The cytoplasm is very scanty, pale blue and agranular. Top right, an eosinophil.

defines a common condition designated monoclonal B lymphocytosis (see Fig. 9.1A). According to recent studies, 3% of normal adults have a small clonal B-lymphocyte population in the peripheral blood with the phenotypic features of CLL and carrying the same chromosomal abnormalities[18]; the incidence climbs to more than 10% in HCV carriers and in the relatives of patients with CLL.[19,20] In the majority of cases, the lymphocytes are positive for CD19, CD5, CD23 and SmIg, with a kappa : lambda or lambda : kappa ratio greater than 3 : 1. The risk of progression to overt CLL in these subjects is 1–2% per year: a situation analogous to monoclonal gammopathy of undetermined significance in relation to multiple myeloma. In the absence of criteria predictive of progression to CLL, only monitoring by a full blood count (FBC) and blood film every 6–12 months is required. In the absence of cytopenia or a rapidly increased leucocytosis, a bone marrow aspirate is not necessary; it would show only an increase of lymphocytes to 10–20%. It is controversial at present whether individuals with monoclonal B lymphocytosis should be used as blood donors. A persistent polyclonal B lymphocytosis has also been described, with a strong female predominance, occurring in heavy smokers,[21] in association with binucleated B lymphocytes in the blood film[22] and with an isochromosome of the long arm of chromosome 3, +i(3)(q10)[23,24] (see Fig. 9.1B).

## Blood count

The total leucocyte count can vary between 5–10 and more than $100 \times 10^9$/l. Very high values at presentation are not frequent, with the most prevalent cases having a count between 30 and $50 \times 10^9$/l. The differential count shows a marked relative and absolute lymphocytosis. Neutrophils are reduced as a percentage: their absolute count, however, is usually normal or, in some cases, moderately increased; neutropenia is unusual at presentation. Anaemia and thrombocytopenia are usually moderate or absent at the time of diagnosis. Their presence is an important unfavourable feature, which correlates with the extent of the disease and, more than the degree of lymphocytosis, influences prognosis and survival, the exception being when cytopenia is the result of autoantibodies (see Report 9.1). The most widely used systems for staging CLL are still those of Binet[25] and of Rai,[26] which are based on the presence and severity of cytopenia (anaemia and thrombocytopenia) and on the extent of lymphadenopathy. The Binet system, which is the staging system most used in Europe, recognises three prognostic categories:

- Stage A: haemoglobin concentration (Hb) not below 100 g/l and platelets not below $100 \times 10^9$/l, with involvement of fewer than three lymph node areas or lymphoid organs (neck, axilla, groin, spleen and liver);
- Stage B: absence of anaemia and thrombocytopenia, with involvement of three or more lymph node areas;
- Stage C: presence of anaemia, thrombocytopenia or both, defined according to the above criteria.

The Rai system has five stages and takes account also of the degree of lymphocytosis.

The cytopenias that influence prognosis are those that result from bone marrow insufficiency. It is also necessary to exclude

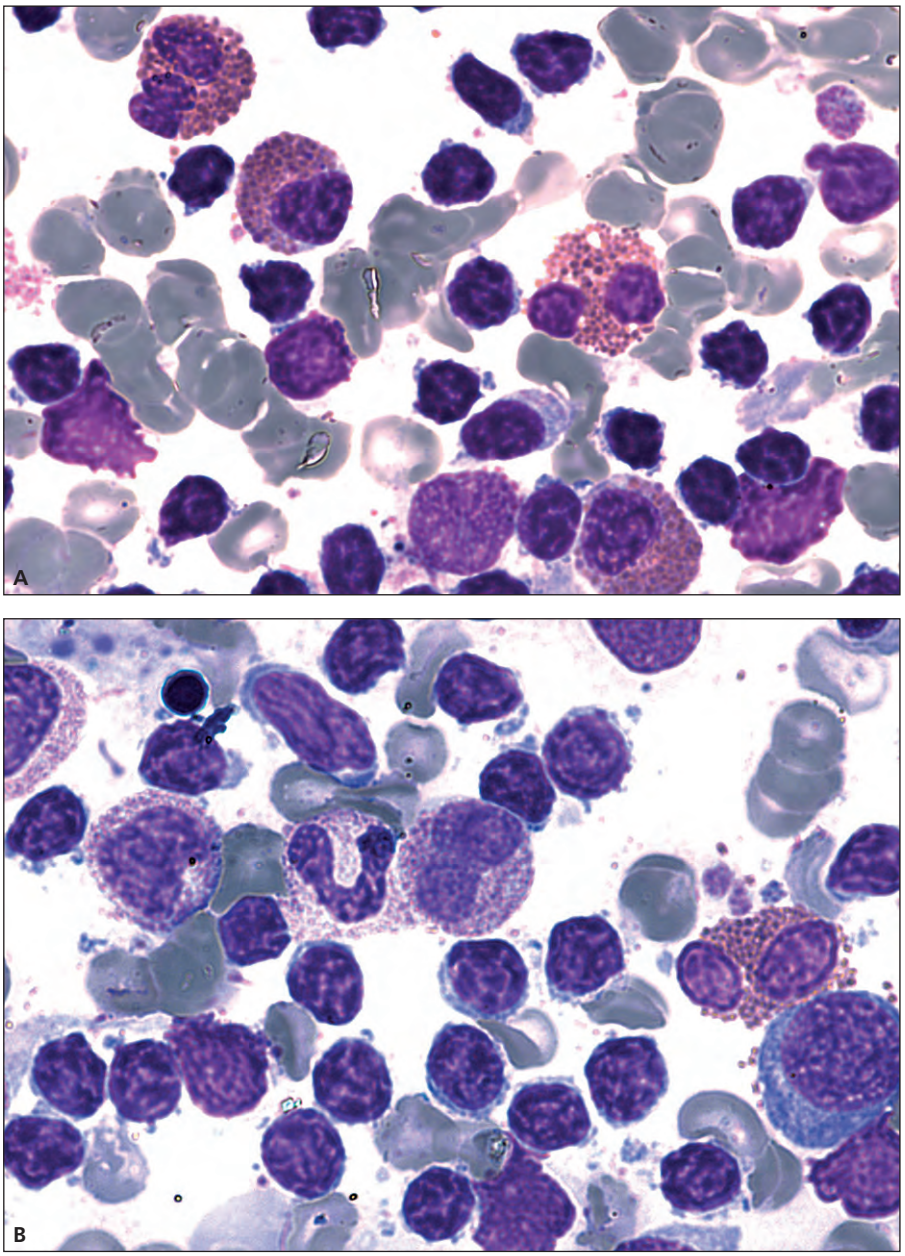
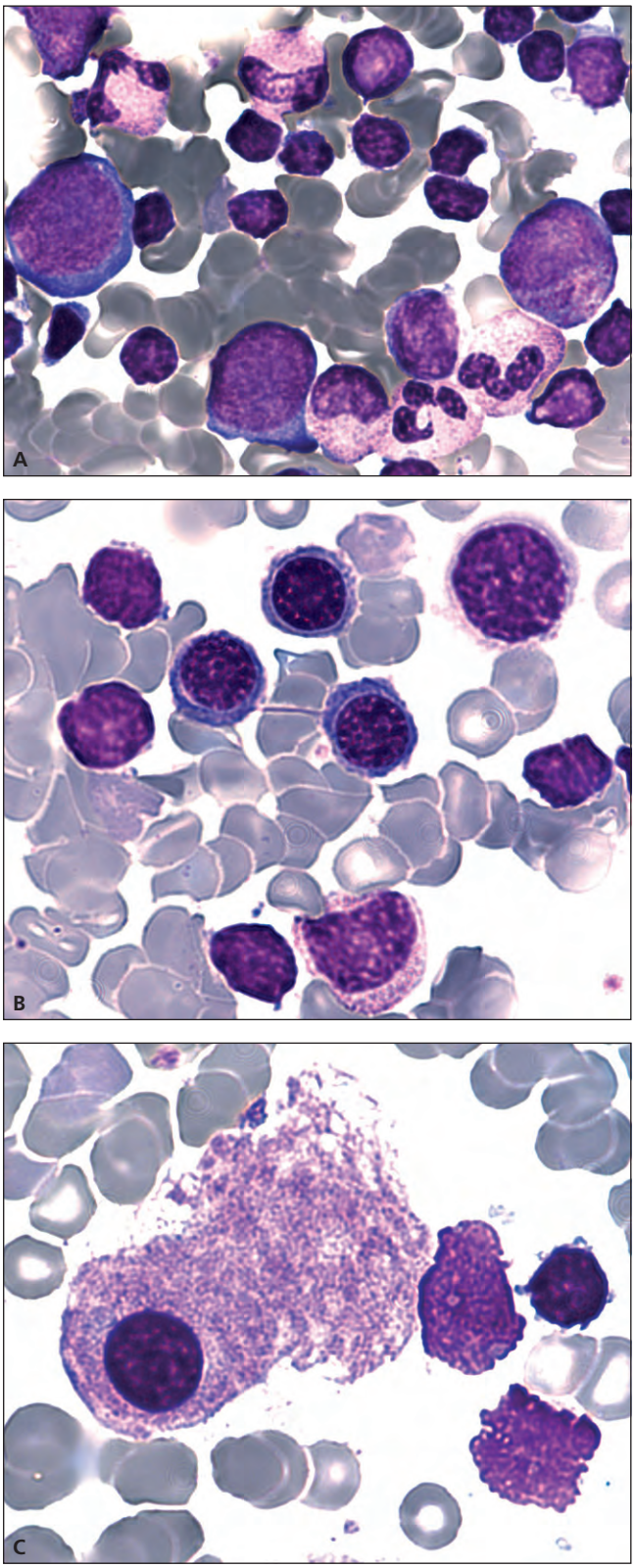



**Fig. 9.8** Bone marrow aspirate; same case as Fig. 9.2. (A) The bone marrow shows conspicuous infiltration by small lymphocytes with extremely scanty cytoplasm and nuclei having a mosaic chromatin pattern. There are also two eosinophils with segmented nuclei and two eosinophil myelocytes. (B) The lymphocyte population is monomorphic: all the cells are the same, without any features of immaturity. Top left, a small pyknotic erythroblast nucleus and three cells of neutrophil lineage. On the right, a bilobed eosinophil and a proerythroblast.

possible causes of anaemia other than CLL, such as a haematinic deficiency anaemia or blood loss. The recognition of reticulocytosis as a sign of haemolysis raises the suspicion of an autoimmune haemolytic anaemia, confirmed by a positive Coombs test; this occurs in 10–30% of patients with CLL and is usually due to IgG warm antibodies. Much rarer is pure red cell aplasia, which has an immunological basis and is associated with a marked anaemia without reticulocytosis. In the case of thrombocytopenia, it is necessary to exclude splenic sequestration and the presence of antiplatelet antibodies as the cause. Autoimmune phenomena that complicate the clinical course of CLL are the result of a numerical and functional deficit of T and NK cells.

## Peripheral blood morphology

The circulating lymphocytes are small and mature. Their size can be slightly less, the same as or larger than that of normal lymphocytes, but their diameter is not more than twice that of




a red cell. The analysis of the volumetric histograms produced by automated impedance-based analysers has confirmed the small size of these cells (median volume 211.5 fl) in comparison with that of prolymphocytes (between 280 and 350 fl).[27] The lymphocytes of CLL are round or slightly oval (see Figs 9.1 through to 9.5). They have a high nucleocytoplasmic (N:C) ratio: the cytoplasm is usually scanty, weakly basophilic, agranular and with a regular outline: in typical cases, it is 'limpid and clear like a mountain stream'. The nucleus is usually round and eccentric, so that the cytoplasm is visible only on one side of the cell. The nuclear outline is regular: sometimes there is a small notch in the area where cytoplasm is more abundant. The appearance of the chromatin is very characteristic, being markedly condensed but in a heterogeneous manner so that there are dark clumps or blocks separated by pale slits, creating a 'mosaic' pattern, similar to that of a football.[9] The distinction between euchromatin and heterochromatin, in other words, is clearer than in normal lymphocytes. The nucleoli are not visible by light microscopy, but can be visualised by electron microscopy. The lymphocyte population in an individual patient is characterised, in typical cases, by a monotonous population of cells, which would be unusual in healthy subjects or in reactive lymphocytosis.

The lymphocytes of CLL are very sensitive to mechanical insults: characteristic, although not pathognomonic, is the presence in the blood film of damaged cells – flattened, spread out, fragmented – classically known as Gumprecht's shadows, after the author who in 1896 described 'the disintegration of leucocytes in leukaemic blood' (e.g. see Figs 9.3B, 9.4A and 9.10D). Gumprecht's shadows or smear cells are caused by the mechanical compression and stretching that occurs when a blood film is spread: they are not found when a preparation is made by delicate cytocentrifugation or when fresh cells are examined by phase microscopy. Their high frequency in CLL is considered to be an artefact related to the fragility of the cells; however, their numbers correlate with other biological characteristics: their percentage is lower in cases that are positive for CD38 and ZAP70 (poor prognosis indicators); patients with more than

**Fig. 9.9** Features of multilineage dysplasia in the bone marrow aspirate of another patient with B-CLL after four years of chemotherapy. (A) Numerous lymphocytes, appearing to have virtually no cytoplasm, with the chromatin condensed into small button-like clumps. Bottom left, two proerythroblasts (the lower one has its cytoplasm distorted by contact with the adjacent cells); to the right, a promyelocyte with intensely basophilic cytoplasm. (B) Four small lymphocytes, one of which, on the right, has the nucleus crossed by a groove, thus resembling a centrocyte in follicular lymphoma. At the top, two polychromatic erythroblasts (the one further to the right has been abnormally spread out during spreading of the film) with asynchronous maturation; two basophilic erythroblasts joined by a thick internuclear chromatin bridge. (C) A small megakaryocyte with a spherical, non-lobated nucleus; a lymphocyte and the remains of two torn nuclei, analogous to Gumprecht's shadows (smear cells) in the peripheral blood.

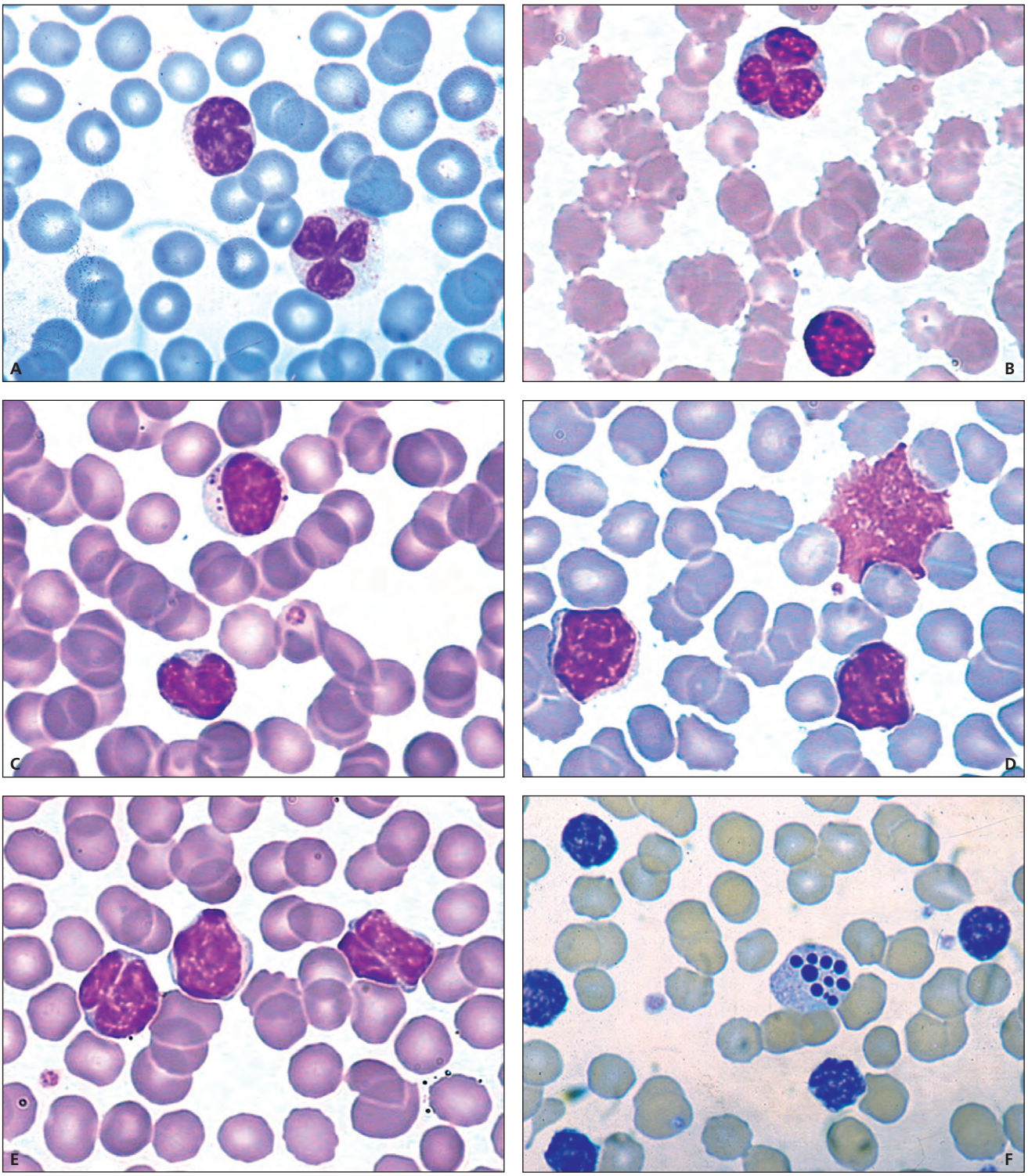



**Fig. 9.10** Nuclear atypia, without any diagnostic or prognostic significance, in small lymphocytes in a number of cases of B-CLL. (A) Top, a small lymphocyte with an indentation in the nuclear profile. At the bottom, a large granular lymphocyte with a trilobed nucleus (similar to the cells of adult T-cell leukaemia/lymphoma which, however, do not usually have granules). (B) Two small leukaemic B lymphocytes; the one at the top also has a trilobed nucleus. (C) The lymphocyte at the bottom has a nucleus that is indented on both sides, appearing almost bilobed. The one at the top is a typical large granular lymphocyte with large dark cytoplasmic granules. (D) In these lymphocytes, the nuclear outline is less regular, forming several angulations, and the chromatin mass is less clearly divided, with a smoother appearance. At the top, a typical smear cell. (F) The nucleus of one of these cells, probably also a small lymphocyte, is fragmented into separate rounded masses as a result of apoptosis.

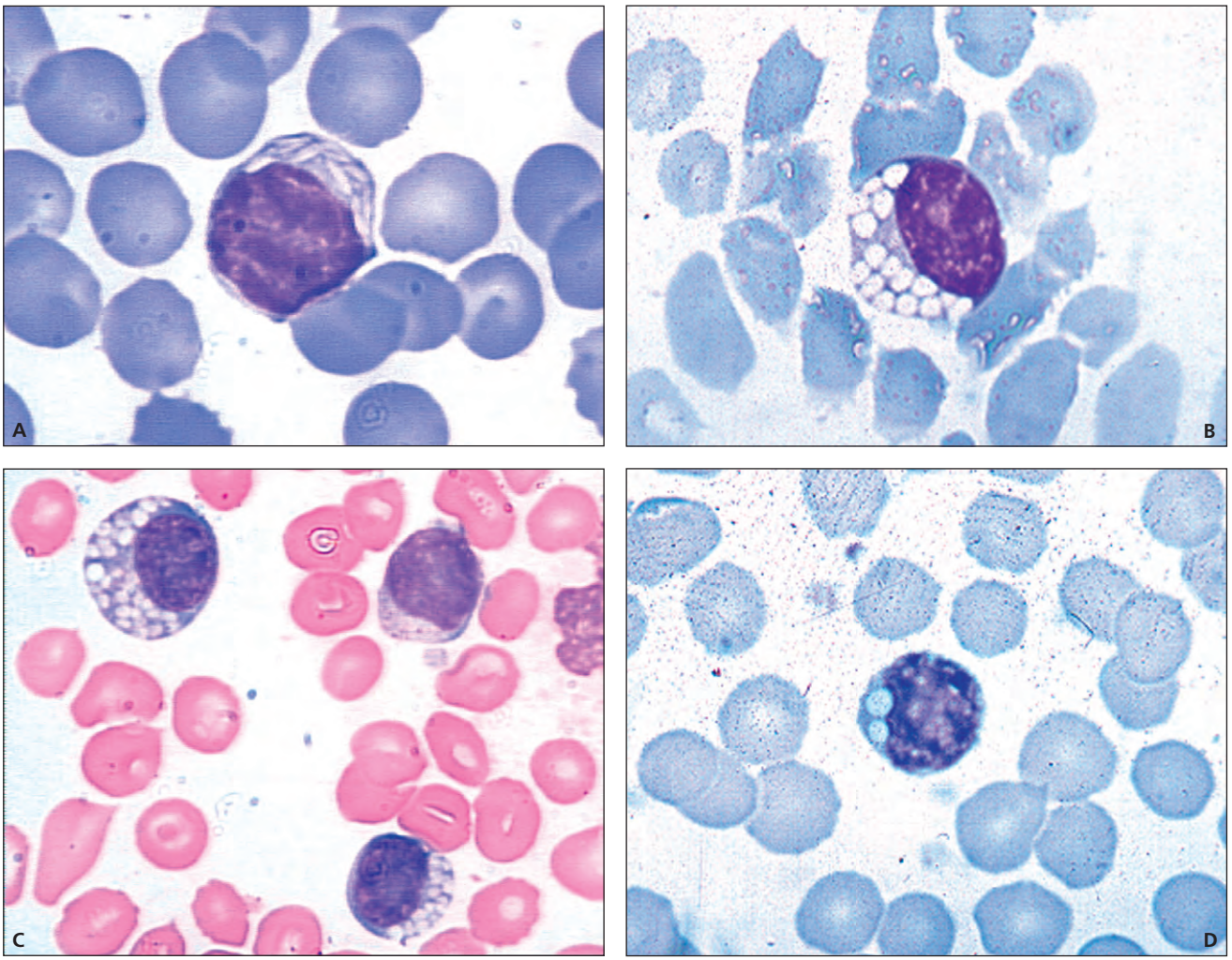



**Fig. 9.11** Cytoplasmic inclusions, without any particular diagnostic or prognostic significance, in several cases of B-CLL. (A) Formation of short, thick colourless crystals, probably representing immunoglobulin. (B) The cytoplasm is almost completely perforated by vacuoles, which all appear to be the same size. (C) Prominent cytoplasmic vacuolation in two lymphocytes in another patient. (D) In the cytoplasm of this cell there are solid spherules staining pale blue, similar to the more numerous inclusions representing immunoglobulin that are seen in the cytoplasm of Mott cells.

30% smear cells have a 10-year survival of 80%, in comparison with 50% in those with a lower number.[28,29] A correlation has been found between the number of smear cells and the proportion of lymphocytes with a very dense nucleus measured on the H*1 system.[30]

One can observe occasional cells with an unusual or bizarre appearance (see Fig. 9.10); these can suggest another lymphoproliferative disorder but in fact lack significance. In rare patients with CLL, the cytoplasm of some of the small lymphocytes shows crystals, vacuoles or small drops of amorphous or refractile material[31,32] (see Fig. 9.11). On electron microscopy these are found to be localised to the cisternae of the rough endoplasmic reticulum; they correspond to immunoglobulin light chains or entire IgM or IgA molecules, often different from that expressed on the surface membrane or found in the serum or urine as a monoclonal band.

Cytochemistry does not make any essential contribution to the diagnosis of CLL. The periodic acid-Schiff (PAS) reaction can show granular positivity particularly in cases with vacuolated cytoplasm. Alpha-naphthyl acetate esterase (ANAE) and alpha-naphthyl butyrate esterase (ANBE) can show finely granular positivity or a weak diffuse reaction. Other enzymatic stains that give a reaction to some extent characteristic of CLL are acid phosphatase, 5′-nucleotidase and beta-glucuronidase.

### Morphological heterogeneity

The monomorphism of the small lymphocytes with chromatin in blocks is typical of CLL, but it is neither specific nor universally

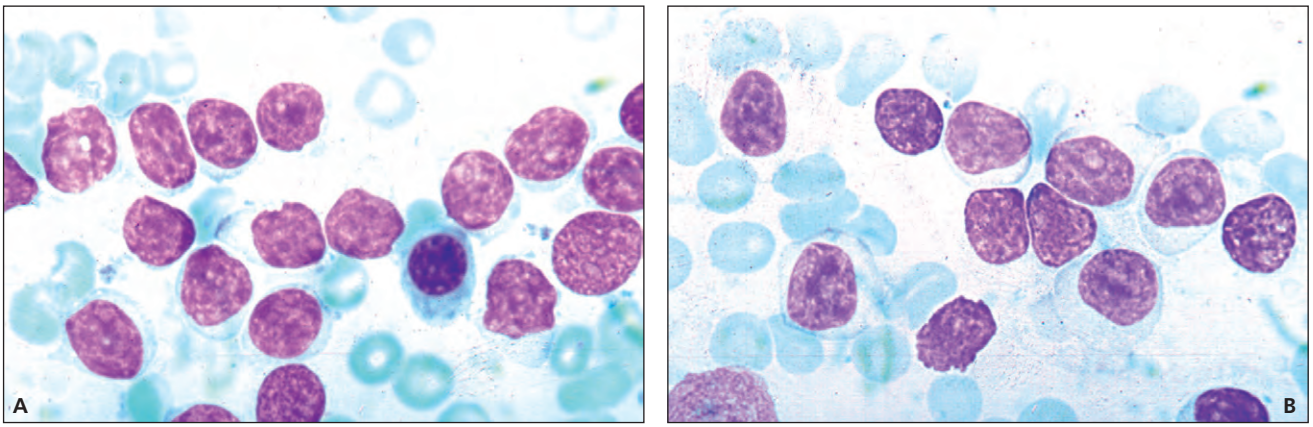



**Fig. 9.12** B-cell non-Hodgkin lymphoma (B-NHL), small lymphocytic lymphoma; bone marrow localisation. The peripheral blood shows anaemia, neutropenia and mild thrombocytopenia. (A) The bone marrow is heavily infiltrated by a uniform population of lymphoid cells with a mature oval or rounded nucleus, with chromatin condensed into heterogeneous clumps, rare nucleoli with indistinct outlines and scanty pale cytoplasm. (B) The lymphoma cells show marked similarity to the mixed cell type of B-CLL, with some nucleolated prolymphocytoid cells, but they remain confined to the bone marrow without peripheral blood invasion.

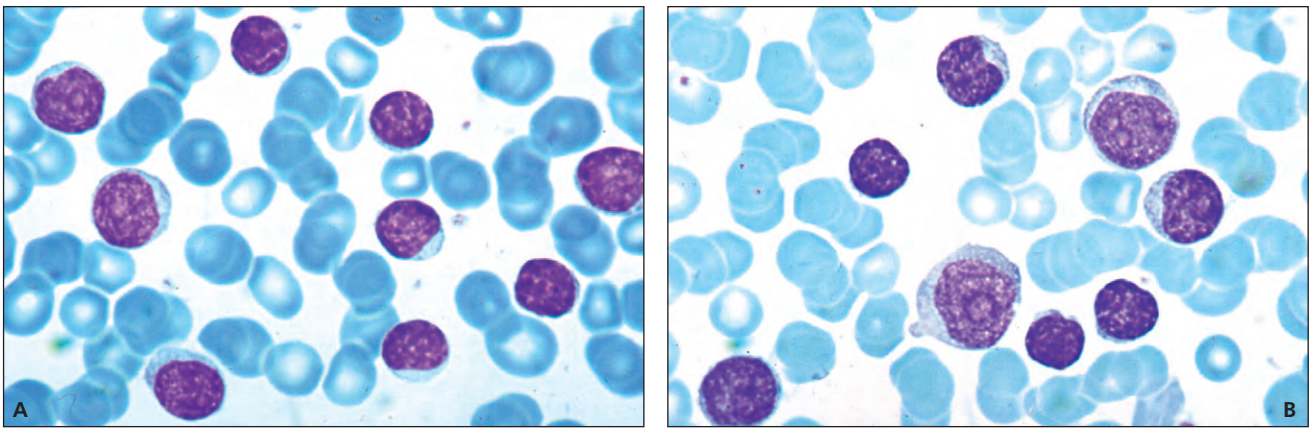



**Fig. 9.13** B-CLL, mixed cell type, CLL/PL; peripheral blood film. (A) Six lymphocytes, on the right, have the monomorphic morphology of CLL; on the left, larger cells with more plentiful cytoplasm and less compact chromatin, with small, in part incomplete, nucleoli. (B) The large cell, at the bottom, is a typical prolymphocyte, with a large central nucleolus surrounded by a rim of condensed chromatin. The cell higher up on the right appears almost blastic with granular cytoplasm and two nucleoli. The other cells are small mature lymphocytes.

present: in about a half of patient there are a variable proportion of cells with less of a mosaic pattern and with moderately plentiful cytoplasm, resembling reactive lymphocytes. This morphological variation does not have any adverse prognostic significance.[33,34] In some cases, a minority of cells have a more irregular nuclear outline, with notches or folds, and there can be a small percentage of prolymphocytes, characterised by larger size, more abundant cytoplasm and nuclei with less dense chromatin and a single prominent nucleolus. The percentage of typical prolymphocytes, as described here, is less than 10% in typical CLL. Cases of CLL with more heterogeneous morphology were included in the FAB document of 1989 in a category regarded as intermediate between CLL and B-lineage PLL, designated CLL, mixed cell type[1]; the 2008 WHO classification did not retain this group as a separate category, but described such cases as atypical CLL and underlined the tendency to a more aggressive clinical course (correlating with the number of prolymphocytes) and the characteristic association with cytogenetic abnormalities, particularly trisomy 12.[15] According to the original description, this form has two possible variants: in the first, the peripheral blood lymphocytes are dimorphic, with a mixture of typical small lymphocytes and prolymphocytes, the latter between 10% and 55% of lymphocytes (designated CLL/PL); in the second, the morphological picture is even more heterogeneous with lymphocytes of variable size and generally with a lower percentage of prolymphocytes. The prolymphocytes in CLL/PL

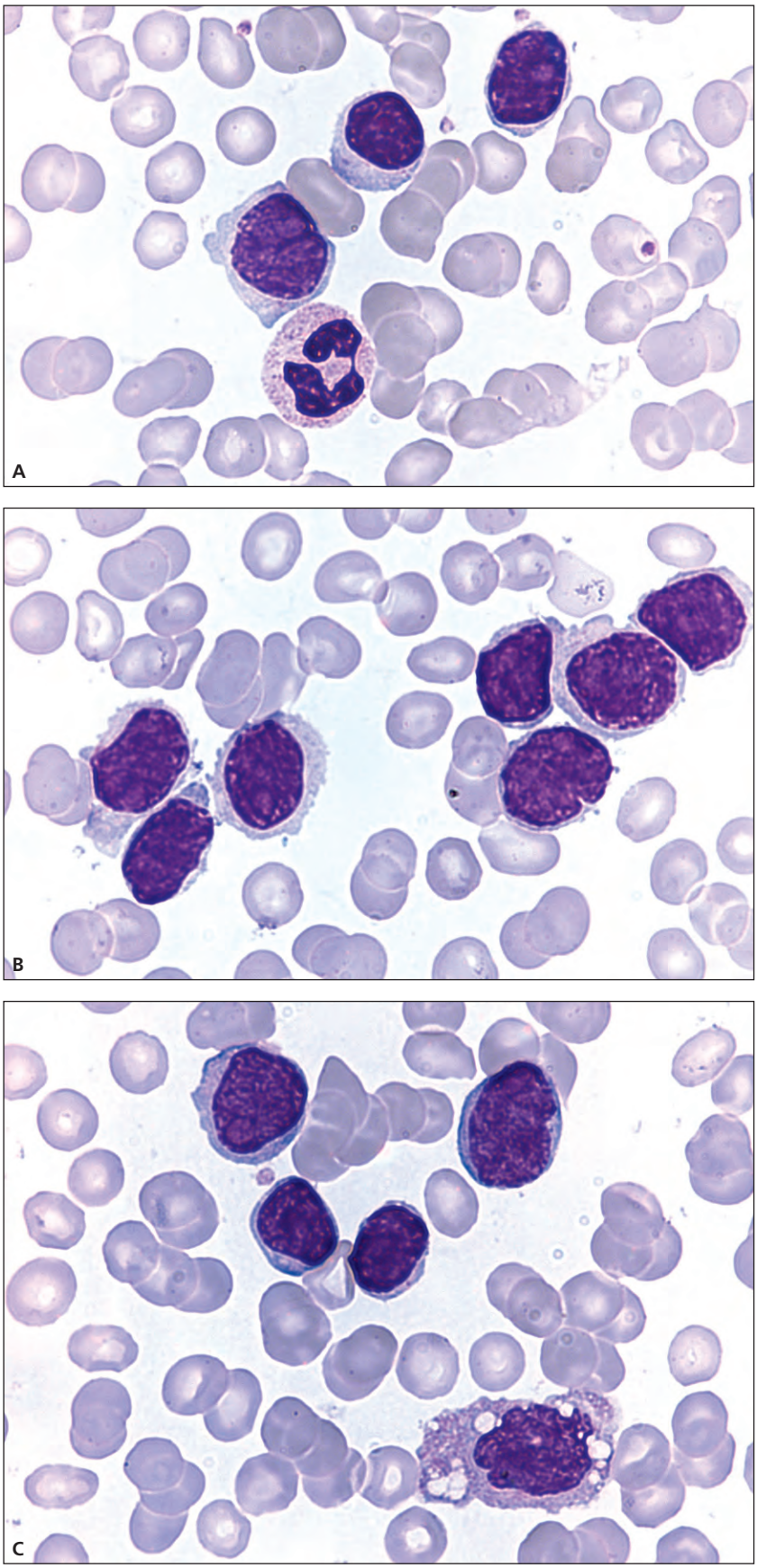



**Fig. 9.14** Heterogeneity of size and morphology in a case of B-CLL, mixed cell type, at diagnosis; peripheral blood film. (A) On the left, above a segmented neutrophil, a large prolymphocytoid cell with plentiful cytoplasm, a nucleolus and open, paler chromatin; a small lymphocyte with an almost plasmacytoid appearance with plentiful cytoplasm and peripheral basophilia; another prolymphocytoid cell with hardly any cytoplasm. (B) The nuclei are rounded but with some angulation and in one cell there is a notch; the outlines of several nucleoli are apparent; the chromatin shows a variable degree of condensation, without a mosaic appearance. The cytoplasm is more plentiful than in typical cases. (C) Two large atypical lymphocytes, two small mature lymphocytes and, at the bottom, a monocyte.

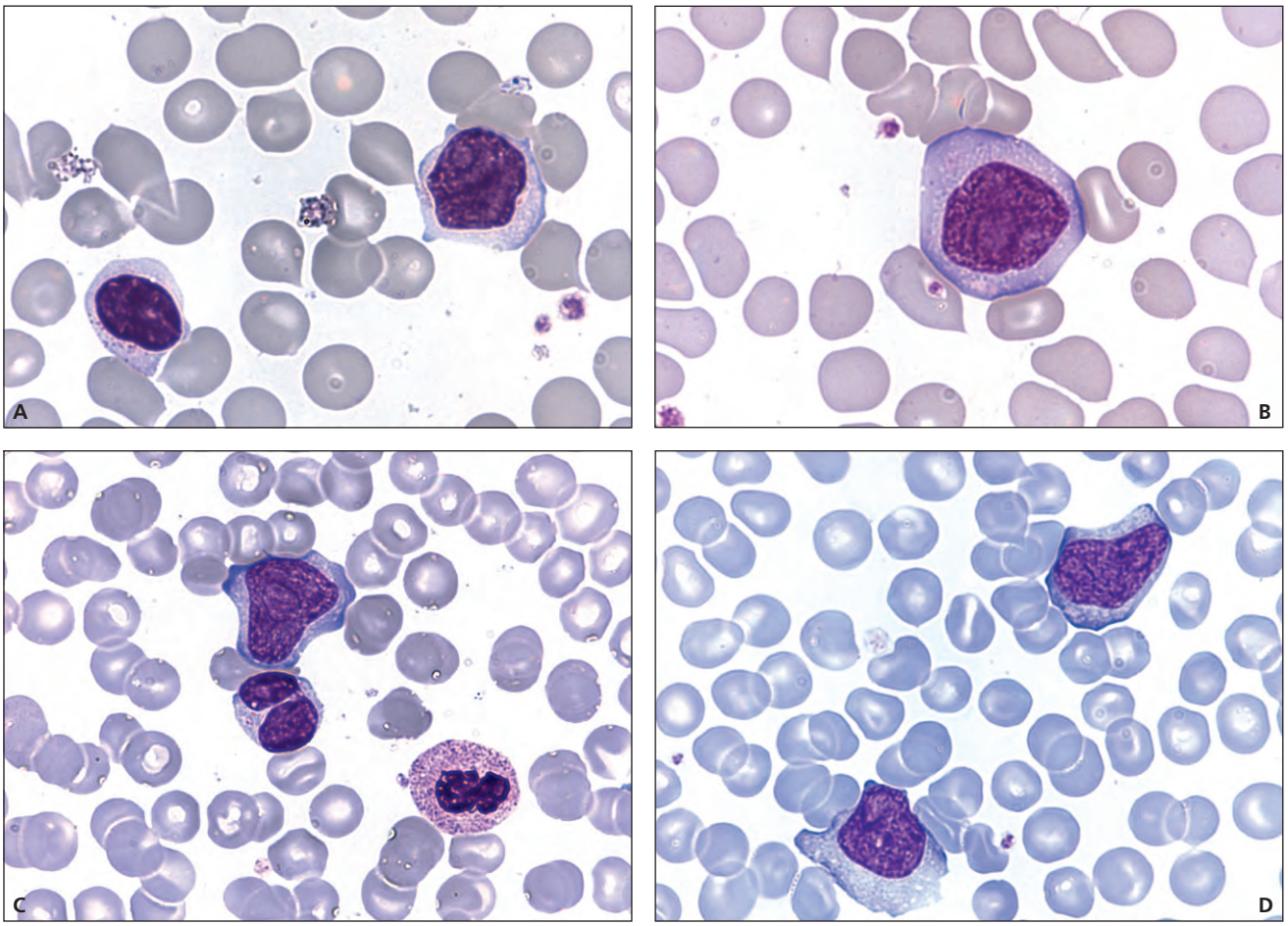



**Fig. 9.15** B-CLL, mixed cell type, with marked cytological atypia and immaturity, in a different patient under treatment; peripheral blood film. (A) The lymphocyte on the left is a large granular lymphocyte, with plentiful, slightly basophilic, granular cytoplasm; the large cell, to the right, is a prolymphocyte. (B) Large prolymphocytoid cell with a notched, angulated nucleus, with chromatin that is open or only slightly condensed and with a large pale central nucleolus; the pale cytoplasm is very plentiful with peripheral basophilia. (C) At the top, a large lymphoid cell with an immature nucleolated nucleus and intensely basophilic cytoplasm. Beneath it, a granular lymphocyte with a bilobed nucleus. On the right, a non-segmented neutrophil. (D) Another two cells with immature characteristics. A morphological evolution of this type requires a careful evaluation of the risk of progression or of transformation to Richter syndrome.

are generally less characteristic than those of true PLL (see Figs 9.13 through to 9.17), having less prominent nucleoli, a more variable amount of cytoplasm and variation in size. There may be a low percentage of larger lymphoid cells, morphologically not uniform, from large lymphocytes with abundant cytoplasm tending to basophilia and a heterogeneous chromatin structure, to true immunoblasts with ample basophilic cytoplasm, dispersed chromatin and large nucleoli. There is still uncertainty and discordance between classifications as to the clinical, therapeutic and prognostic significance of these morphologically heterogeneous variants in comparison with that of cases with a typical monomorphic picture. The majority of cases presenting with CLL, mixed cell type, have a stable clinical course, the same as that of classical CLL. In many instances, one is dealing with a morphologically distinct entity but with clinical similarity, having these characteristics from the beginning and not as a result of evolution or transformation of the disease. The term 'prolymphocytoid transformation' can, however, be applied when the percentage of prolymphocytes increases fairly rapidly in association with clinical deterioration.

The morphological variability is reflected in the diversity shown by measurements of cell size with impedance technology.[27] The percentage of cells classified as large unstained cells (LUC) and as blasts on automated methods based on light scatter and automated cytochemistry correlates with the presence of prolymphocytes and immature cells in the blood film and also with clinical evolution.[35]

## Bone marrow morphology

Guidelines on the diagnosis of CLL do not require examination of the bone marrow, but this is strongly recommended prior to

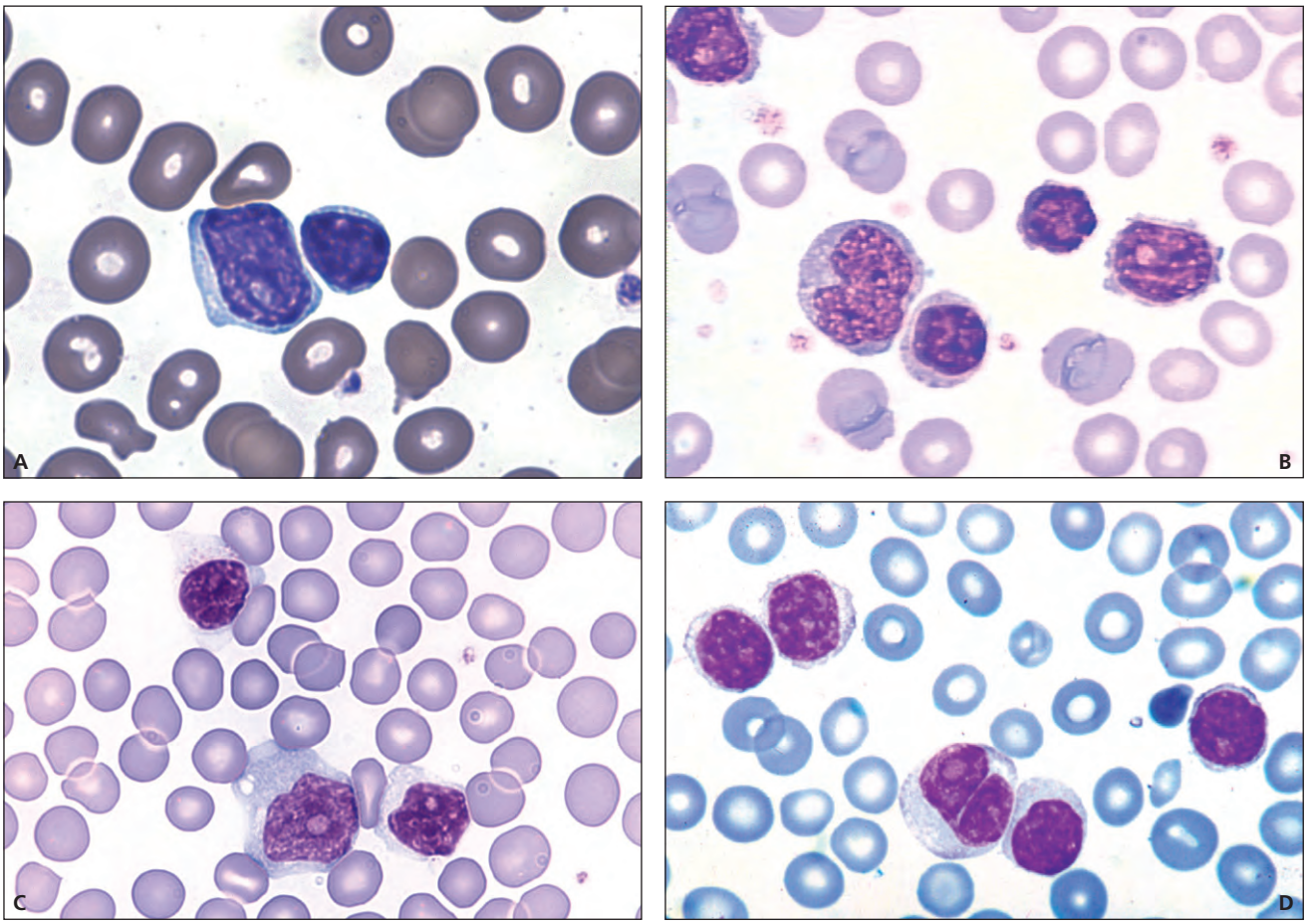



**Fig. 9.16** Other examples of morphological heterogeneity in patients with B-CLL, mixed cell type; peripheral blood film. (A) On the left, a large prolymphocytoid cell, with compact chromatin; on the right, a small lymphocyte with a diameter only a little greater than that of the erythrocytes. (B) On the left: a large polymorphic cell with a convoluted nucleus and worm-like chromatin; two lymphocytes with a mosaic chromatin pattern and different amounts of cytoplasm; a lymphocyte with a prolymphocytoid nucleus and scanty cytoplasm. (C) At the top, a large granular lymphocyte. At the bottom, an enormous prolymphocytoid cell with partially condensed chromatin and a smaller lymphocyte with clumped chromatin and plentiful pale cytoplasm. (D) Between four lymphocytes with compact nuclei (on the left with artefactual formation of villi), a large prolymphocytoid lymphocyte with a bilobed nucleus, like a saddlebag.

commencing immunosuppressive therapy or when it is necessary to ascertain the cause of cytopenia.[13] On a bone marrow aspirate, the marrow appears hypercellular because of infiltration by lymphoid cells morphologically similar to those in the peripheral blood (see Figs 9.5 through to 9.9), comprising at least 30% of all nucleated cells in the bone marrow (or >50% of all non-erythroid nucleated cells in cases in which there is erythroid hyperplasia as a result of haemolytic anaemia). When the peripheral blood shows a proportion of heterogeneous lymphocytes and prolymphocytes, they are also present in the marrow. At diagnosis, normal trilineage haemopoiesis may be well preserved, but more often it is reduced: an accurate quantitative and qualitative evaluation of the haemopoietic precursors is important both for determining prognosis and for subsequent monitoring. There can be an increase of mast cells, while plasma cells are often reduced.

The picture observed when small lymphocytic lymphoma infiltrates the marrow is indistinguishable from that of CLL (see Fig. 9.12).

Even when there is moderate lymphoid infiltration, it is not infrequent for erythroid or megakaryocytic hyperplasia to be observed, as a compensatory mechanism when there is associated autoimmune cytopenia or hypersplenism. Rarity or absence of erythroid precursors, associated with a significant anaemia that is sometimes macrocytic, reticulocytopenia and high serum iron suggests a diagnosis of acquired pure red cell aplasia, a dramatic and sudden event that has a grave prognosis.[36]

Histological examination of the bone marrow is indispensable in CLL before starting treatment, since it permits an accurate assessment of the haemopoietic capacity of the bone marrow and the degree of infiltration, which correlates with the stage and with prognosis. In histological sections, the haemopoietic

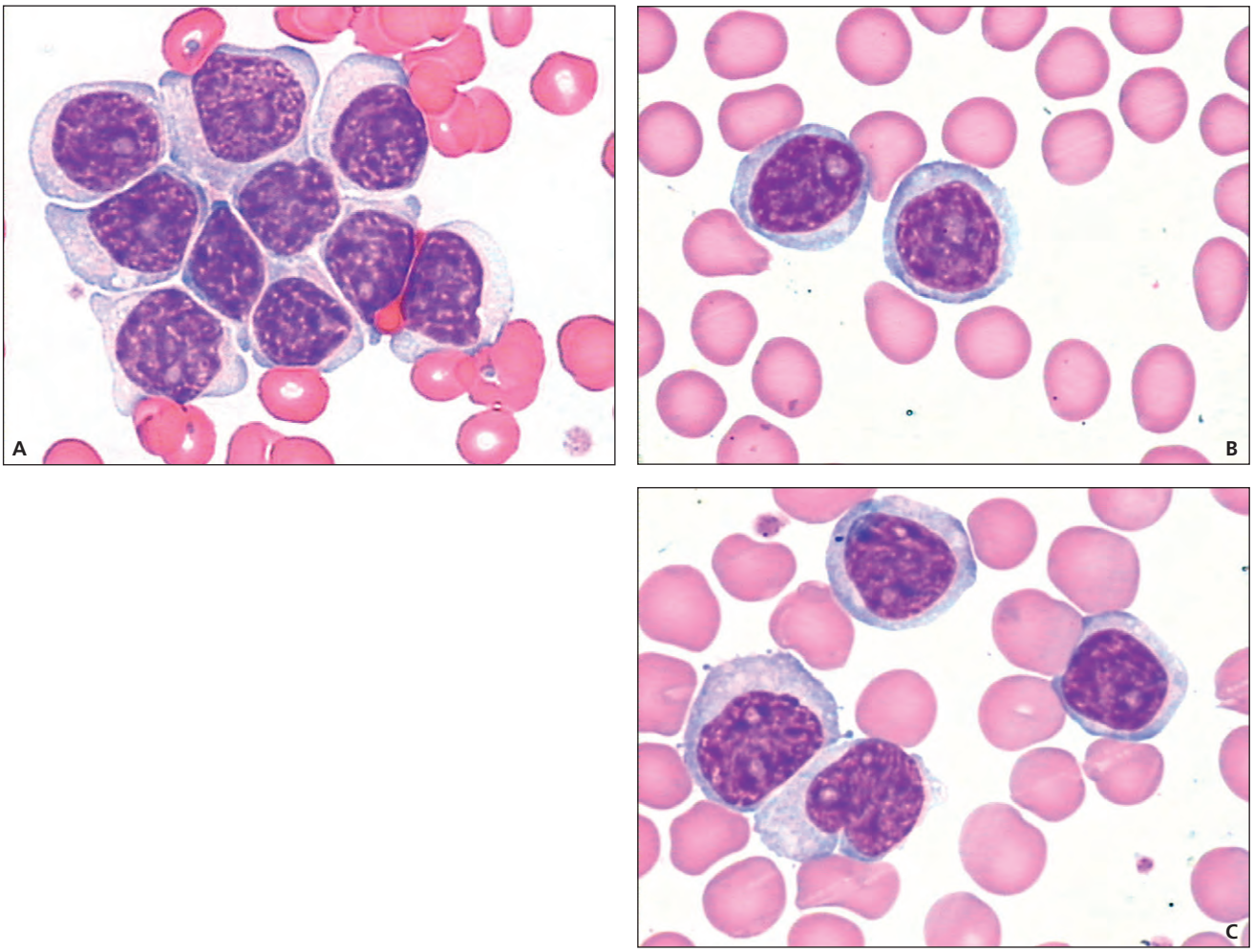



**Fig. 9.17** Two cases of B-CLL, mixed cell type, with a high percentage of prolymphocytes; peripheral blood film. (A) The cells that form this agglomerate show various intermediate stages between small lymphocytes with chromatin in blocks and large prolymphocytoid cells with more or less central nucleoli, more dispersed chromatin clumps and plentiful pale cytoplasm with basophilia that is accentuated beneath the cellular membrane. (B) Large basophilic prolymphocytoid lymphocytes in a patient at presentation, with a leucocyte count of 49.7 × $10^9$/l, 63% lymphocytes and 31% prolymphocytes. The haemoglobin concentration (Hb) was 151 g/l and the platelet count 210 × $10^9$/l. (C) The morphology of these cells is similar to that of prolymphocytes, because of the plentiful basophilic cytoplasm, but the nucleoli are small and not very prominent. Morphologically this is a case on the borderline between CLL/PL and genuine prolymphocytic leukaemia. The immunophenotype, CD19 and CD5 positive with weak SmIg, was typical of CLL.

parenchyma can appear hypercellular, with a homogeneous lymphoid infiltrate: in addition to small lymphoid cells with dense chromatin, no visible nucleoli and scanty cytoplasm, there are a variable number of prolymphocytes (larger cells with less dense chromatin and a prominent nucleolus) and immunoblasts (large cells with plentiful basophilic cytoplasm and a large nucleus with a very large nucleolus), which have the same cytological features as lymphoid cells that have been exposed to mitogens. Various patterns of infiltration have been described[37,38]: nodular, analogous to that in small lymphocytic lymphoma, seen in about 10% of cases of CLL, particularly in patients with early stage disease; interstitial with preservation of the normal medullary architecture and with zones of haemopoiesis; mixed nodular and interstitial; diffuse, with almost complete replacement of the haemopoietic marrow and the adipose component by leukaemic cells. Diffuse infiltration is present at diagnosis in about a quarter of cases and is associated with an adverse prognosis.

Lymph node biopsy is not a routine investigation in the diagnosis of CLL, but as with other sites suspected of infiltration, can be useful in individual cases, as in suspected CLL with atypical morphology and immunophenotype or for the evaluation of suspected lymphomatous evolution.

## Immunophenotype

The antigenic profile of CLL is very characteristic and is diagnostically useful. Expression of the B-lineage antigens CD19, CD20 and CD23, in association with the CD5 antigen, is typical

and fundamental for the diagnosis; weak expression of CD20, CD79b and SmIg is also typical. There is restriction to a single light chain type, while cytoplasmic immunoglobulin is absent. FMC7 and CD22 are negative. In a small proportion of patients with CLL, there is expression of CD25 and of certain myeloid antigens, such as CD13 or CD33. In other rare cases, otherwise similar to classical CLL, there is no expression of CD5.[39] The immunophenotype of CLL, mixed cell type, is variable, with characteristics intermediate between those of typical CLL and those of PLL; CD5, for example, is generally positive but the intensity of expression of SmIg is greater, closer to that of prolymphocytes, and FMC7 may be positive. The expression of CD38 and the ZAP70 protein is associated with a more unfavourable prognosis (see below).

## Genetic analysis

The immunoglobulin heavy chain variable region genes, *IGVH*, are non-mutated (>98% homology with the germ-line configuration) in 40–50% of cases, and mutated in the remaining patients (somatic hypermutation). These two states, recognisable by nucleotide sequencing, differ also in other clinical and biological characteristics: in particular, the expression of membrane CD38 and cytoplasmic zeta-associated protein 70 (ZAP70) is seen in most patients with non-mutated genes, such cases being characterised by a shorter survival.[40]

Cytogenetic analysis at presentation, little done in the past because of the difficulty in obtaining metaphase cells for analysis, is useful particularly for helping to define prognosis.[9] The investigation of aberrant genes by interphase FISH is indicated at diagnosis and permits genetic lesions to be recognised in more than 80% of cases. The most common chromosomal abnormality in CLL is deletion of part of the long arm of chromosome 13, del(13q). The most frequent other aberrations in order are trisomy 12, del(11q) and del(6q), and deletion of part of the short arm of chromosome 17, del(17p). The recognition of these anomalies has prognostic and therapeutic implications: cases with del(11q) and del(17p) may have massive lymphadenopathy, shorter survival and resistance to chemotherapy. Cases with trisomy 12 are distinguished by atypical or mixed morphology (such as CLL/PL), with a variable percentage of large lymphocytes with nuclear irregularity and less dense chromatin (see Fig. 9.5). Cases with del(13q) and those without identifiable genetic abnormalities have an indolent course and a more favourable prognosis.

## Differential diagnosis

CLL has to be distinguished from benign and reactive lymphocytosis, from other lymphoproliferative disorders, from non-Hodgkin lymphoma and from some cases of ALL with blast cells having very dense chromatin and the cytoplasm being scanty. Marked reactive lymphocytosis is associated with viral infection, pertussis, trauma, surgery and the administration of certain drugs. These are characterised by cytological heterogeneity and by the morphological features of activation (see Tables 1.6 and 1.7). In general, one is dealing with polyclonal T-cell proliferation. Benign lymphoid nodules can be observed in bone marrow sections (more rarely in aspirates) of mainly elderly patients, sometime with autoimmune conditions of abnormal reactive states: these are well-circumscribed and non-confluent nodules of polyclonal T and B cells.

The differential diagnosis in relation to other neoplasms of mature lymphoid cells concerns mainly cases with cytological characteristics that recall mature or atypical lymphocytes, such as the leukaemic form of follicular lymphoma and some MCL. The immunophenotypic characteristics generally exclude these diagnoses; the pathological cells of lymphoplasmacytic lymphoma (LPL) often express CD5 and typically express high levels of CD20 and SmIg; those of follicular lymphoma express CD10 and not CD5; those of MCL express CD5 but not CD23.

## Disease evolution

In the majority of patients with CLL, the haematological picture is fairly stable for many years and the clinical course is mainly determined by infective and autoimmune complications. A transformation of the leukaemic clone with morphological and biological alteration is seen in about a third of patients, there being two main forms of transformation: prolymphocytic transformation and Richter syndrome.

In prolymphocytoid transformation (Fig. 9.18) there is a gradual and progressive increase in the percentage of prolymphocytes in the peripheral blood and in the bone marrow, with a trend towards marked leucocytosis, anaemia, thrombocytopenia, increasing lymphadenopathy and splenomegaly and resistance to conventional therapeutic approaches.[41] In some cases there is an increase not only in prolymphocytes but also in heterogeneous atypical lymphocytes, sometimes appearing frankly blastic. The immunophenotype may be partially altered, with a partial loss of CD5, acquisition of FMC7 and increased expression of SmIg. Prolymphocytoid transformation occurs in 10–20% of patients. This worsens the prognosis with a median survival of around one year.

Richter syndrome corresponds to transformation of CLL (rarely it occurs also in Waldenström macroglobulinaemia) into a diffuse large B-cell lymphoma; this occurs in 2–8% of patients.[42–44] The new neoplastic clone usually originates in a lymph node or other extra-haemopoietic lymphoid tissue, often, but not always, arising from the pre-existing neoplastic clone. The clinical picture is characterised by the acute or subacute development of significant clinical features such as fever, severe malaise, weight loss, significant organomegaly and refractoriness to treatment, with survival being less than a year. The neoplastic cellular population is very heterogeneous, pleomorphic, similar to that in large cell lymphoma (Figs 9.19 and 9.20). Bone marrow infiltration by the large atypical lymphoma cells is frequent, whereas their appearance in the peripheral blood is variable and not always marked. Sometimes the expression is frankly leukaemic, with massive blood and marrow infiltration by large blastic cells with plentiful intensely basophilic

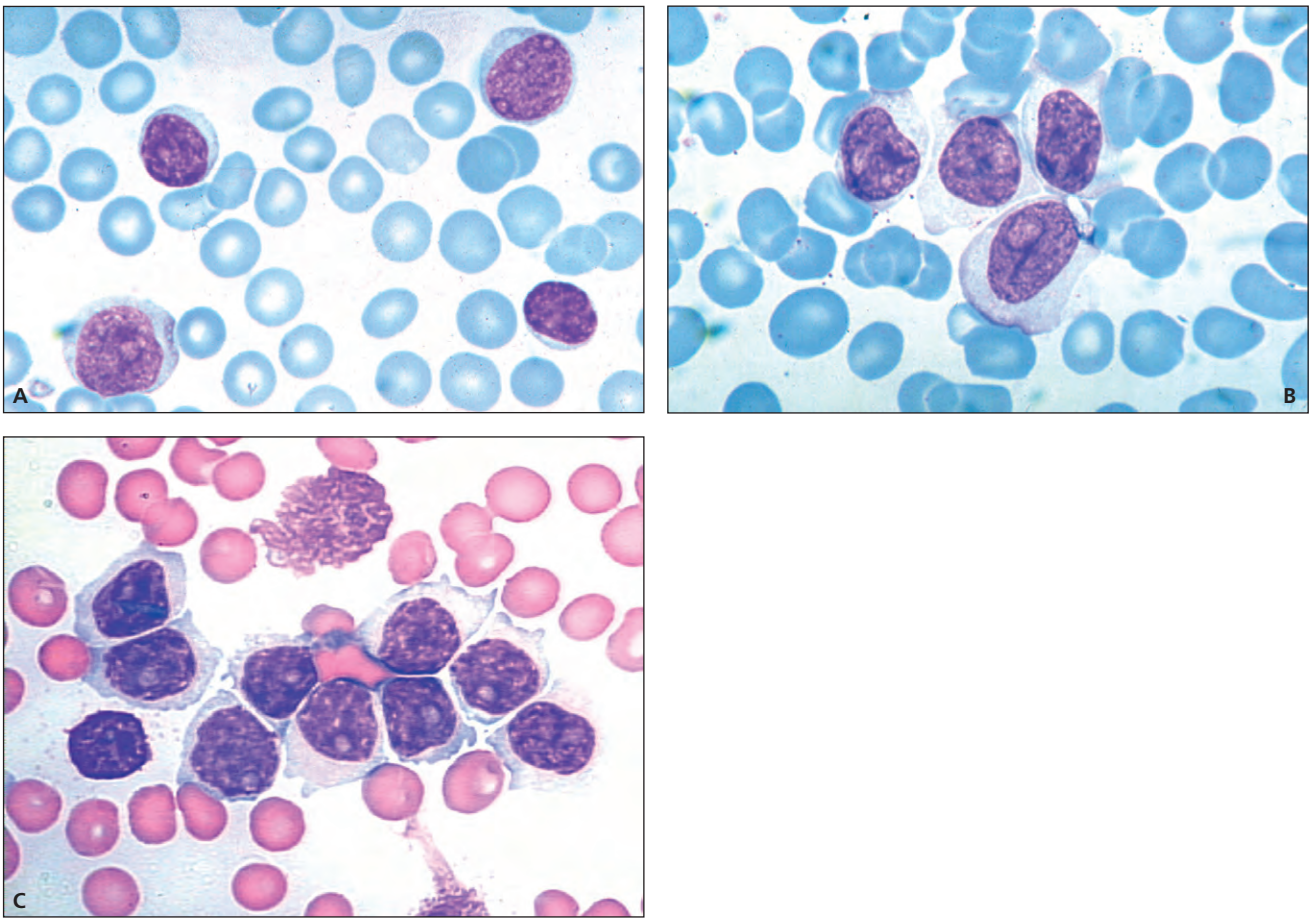



**Fig. 9.18** Three examples of prolymphocytoid transformation of B-CLL. (A) Two small lymphocytes and two immature cells: the immature cell at bottom left has prolymphocyte morphology with a large prominent nucleolus, even if the chromatin is not very condensed; the other, in the top right corner, has a more frankly blastic appearance, with granular chromatin and three small nucleoli, two of which stand out because of the presence of a thin rim of condensed chromatin around them. (B) Three medium-sized lymphocytes with pale chromatin and a nucleocytoplasmic (N:C) ratio that is lower than normal; at the bottom, a large prolymphocytoid cell with a central fold in the chromatin, which appears finely granular but thicker around an enormous central nucleolus. (C) A patient with mixed cell type of B-CLL followed for about two years without specific treatment, with the following blood count results: Hb 107 g/l; leucocyte count 52.7 $\times$ $10^9$/l; platelets 253 $\times$ $10^9$/l. All these cells, which are tending to adhere to each other in the blood film, have the appearance of prolymphocytes with plentiful cytoplasm and prominent nucleoli.

cytoplasm; the nucleus is conspicuous and irregular, with delicately dispersed chromatin and one or two easily visible nucleoli in a central position. It is not rare to see multinucleated, highly anaplastic cells. The immunophenotype in Richter syndrome is variable: loss of CD5 expression is common as is conservation of SmIg with the same light chain type as the previous CLL in the cases arising from the pre-existing neoplastic clone.

Very rarely (1–2%) CLL evolves into Hodgkin lymphoma or, even more rarely, into ALL, Burkitt lymphoma, multiple myeloma or plasma cell leukaemia. The still rarer appearance, described in the literature, of an acute myeloid leukaemia (AML) during the course of CLL must not be considered a transformation of the CLL, but rather a therapy-related leukaemia or an incidental occurrence in cases when it occurs without any treatment having been given.

## Prolymphocytic leukaemia

Described by Galton in 1974,[45] PLL (or B-PLL) was initially considered a rare variant of CLL. It is now identified as a distinct entity with biological, clinical, morphological and immunophenotypic features that differ from those of CLL, to which it appears related because of the existence of the mixed form, CLL/PL.[46–48] In 70–80% of cases, there is a clonal proliferation of B cells. The T-cell form, which is rarer, has different and characteristic features, which will be described below; it should be regarded as a separate entity. Distinctive characteristics of PLL are a very high white blood cell count (WBC) and prominent splenomegaly in the absence of lymphadenopathy. These can appear during the advanced stage of the disease. The highest incidence of PLL is in the seventh or eighth decade of life, with a slight male predominance. It represents less than 10% of new

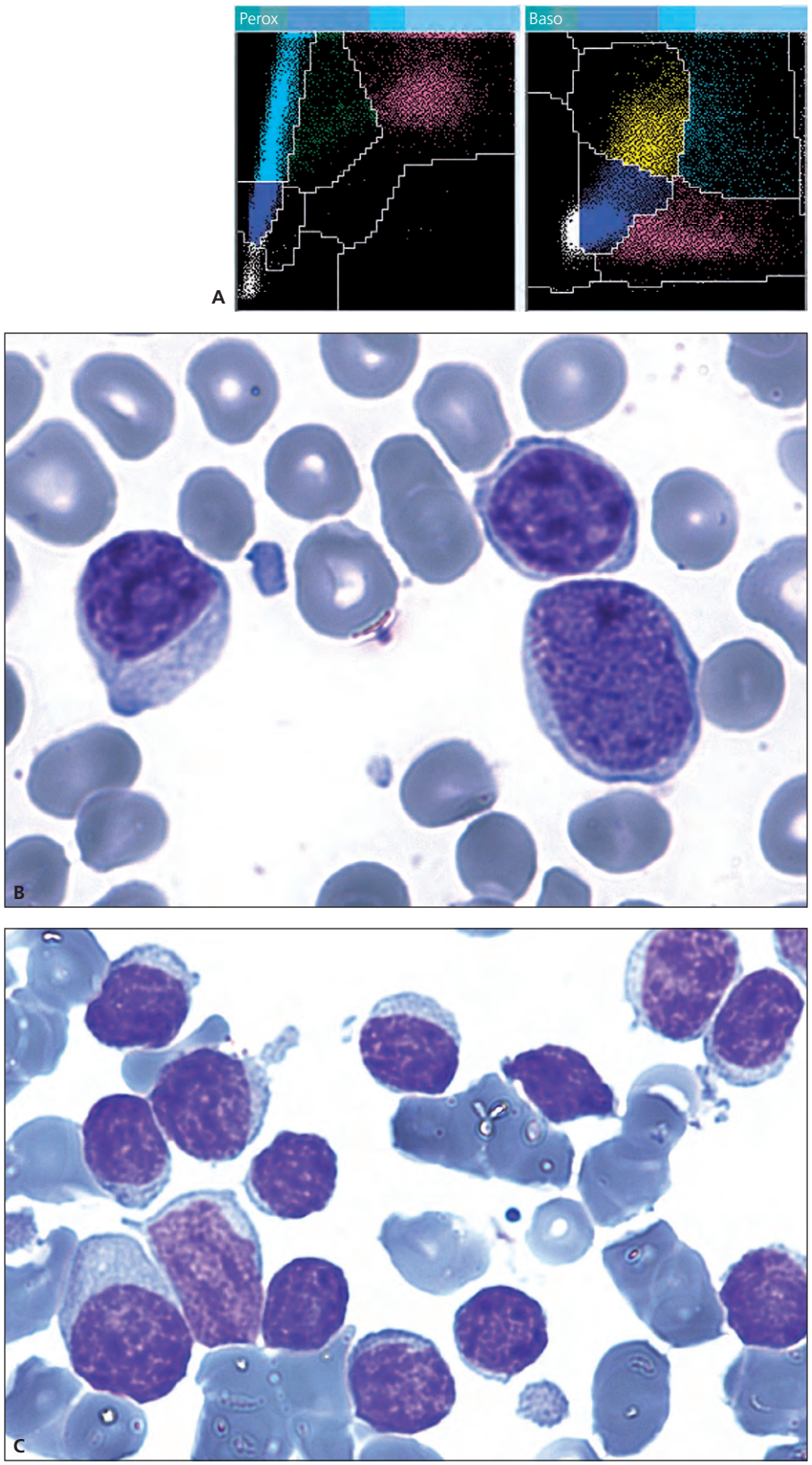



**Fig. 9.19** Evolution of B-CLL into diffuse large B-cell lymphoma (Richter syndrome). (A) Massive leucocytosis: the peroxidase-negative cells form a short spindle shape, like an inclined cypress tree, which is rising into the LUC box; in the Baso channel the invasion of the area of cells with low nuclear density is conspicuous (8.7% of signals, with flags Blasts ++ and Atyp +++); there is pseudobasophilia. The leucocyte count is 223.4 × $10^9$/l. (B) In the peripheral blood film the leukaemia-lymphoma population appears very heterogeneous, with prolymphocytoid cells (on the left), lymphocytes of various sizes with a mosaic chromatin pattern (top right) and lymphoma cells with a blastic appearance (bottom right). (C) The majority of these lymphoid cells have nuclei with coarse chromatin clumps; bottom left, a larger lymphocyte with a round nucleus and an atypical cell with an angulated nuclear outline and granular chromatin.

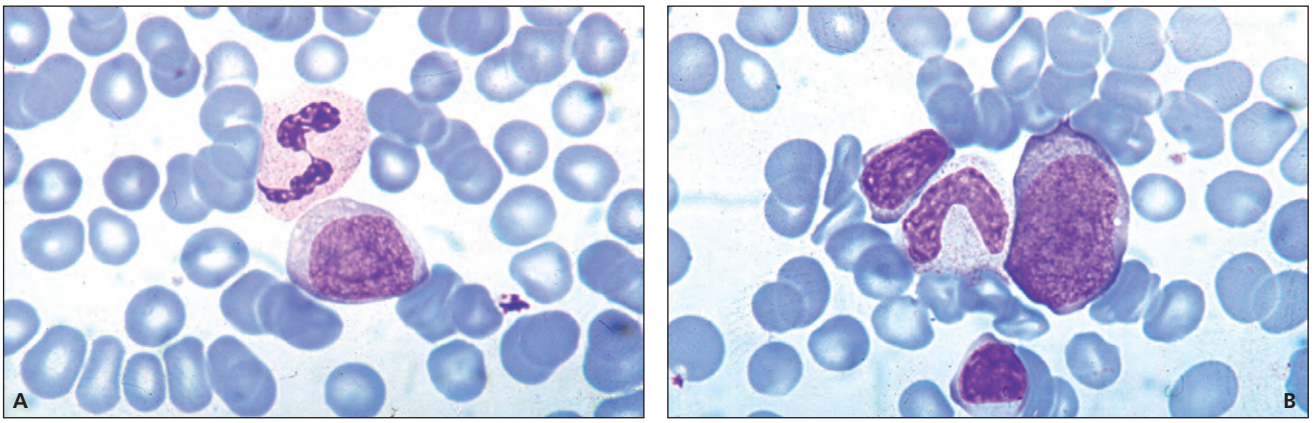



**Fig. 9.20** Another case of B-CLL evolved into diffuse large B-cell lymphoma (Richter syndrome); peripheral blood film. (A) Blastic cell of similar size to a neutrophil granulocyte. The nucleus is large and almost square; the chromatin network is delicate, reticular with a loose weave, similar to that of a myeloid blast cell; there is a large central nucleolus. The cytoplasm is plentiful and basophilic and appears heterogeneous because of the presence of fine indistinct vacuoles. (B) This large immature cell has the morphological characteristics of an immunoblast, with partial condensation of the nuclear chromatin and a definite basophilia that is more intense at the periphery. The two small lymphocytes also have cytoplasm that is more basophilic than in usual in CLL. The fourth cell is a neutrophil band form.

diagnoses of lymphoproliferative disorder. The clinical course is more aggressive than that of CLL and the therapeutic response is generally modest; the median survival is around three years.

### Blood count

The leucocyte count is always elevated and exceeds $100 \times 10^9$/l in two thirds of patients at diagnosis.[27,49,50] Because of the massive bone marrow infiltration, there is co-existing trilineage haemopoietic insufficiency with anaemia, thrombocytopenia and neutropenia. The median Hb at diagnosis is 90 g/l; thrombocytopenia is more moderate with median values around $100 \times 10^9$/l. With progression of the disease, normal haemopoiesis is further reduced, causing severe peripheral cytopenia.

### Peripheral blood morphology

The leukaemic population is characteristic and monomorphic, usually with more than 90% prolymphocytes. These cells must be more than 55%.[1,51] Prolymphocytes are larger than normal lymphocytes and the cells of CLL (Figs 9.21 and 9.22). The nucleus is round with an outline that is usually smooth and regular; in about 20% of cases, some nuclei show an indentation. Chromatin is condensed into heterogeneous clumps; in a central position, a single vesicular nucleolus stands out, paler than the surrounding chromatin, sometimes surrounded by a border of chromatin condensation. The presence of the nucleolus may suggest, erroneously, that these are blast cells and thus lead to a misdiagnosis of acute leukaemia: the dense structure of the chromatin is, however, fairly easy to recognise and permits an accurate differential diagnosis in relation to more immature neoplastic lymphoid cells. The cytoplasm is clearly more plentiful than that of lymphocytes and is moderately basophilic and agranular. The N:C ratio is lower than that of CLL lymphocytes. The clear and uniform cellular outline is an important morphological criterion in making a distinction from hairy cell leukaemia variant (HCLV). There can be moderate heterogeneity with regard to size, with smaller cells in which it is difficult to identify the nucleolus, together with large basophilic immunoblast-like cells. The diagnosis of PLL is based initially on peripheral blood examination with identification of the particular characteristics of prolymphocytes: it is indispensable to have a blood film that is free of artefacts and that examination of the film is done in an area in which the cells are fairly uniformly spread so that the cellular structure can be better appreciated. With preparations that are too thick, the characteristic condensation of the chromatin is accentuated, the nucleolus disappears and the cells contract so that the features appear more similar to those of CLL. When the film is too thin, the nuclear morphology is altered and the chromatin appears more immature, making confusion with blast cells more likely.

From the point of view of cytochemistry, PLL cells are only weakly positive for ANAE and for acid phosphatase: this can be useful in the differential diagnosis with T-PLL, in which the neoplastic cells are strongly positive for both these reactions. Some cases express tartrate-resistant acid phosphatase (TRAP): this reduces the specificity of this reaction for HCL.

### Bone marrow morphology

Bone marrow involvement is massive and a constant feature. Sometimes the morphology of the bone marrow prolymphocytes appears less typical in comparison with those in the peripheral blood. With progression of the disorder, the haemopoietic marrow is totally replaced by a uniform carpet of prolymphocytes.

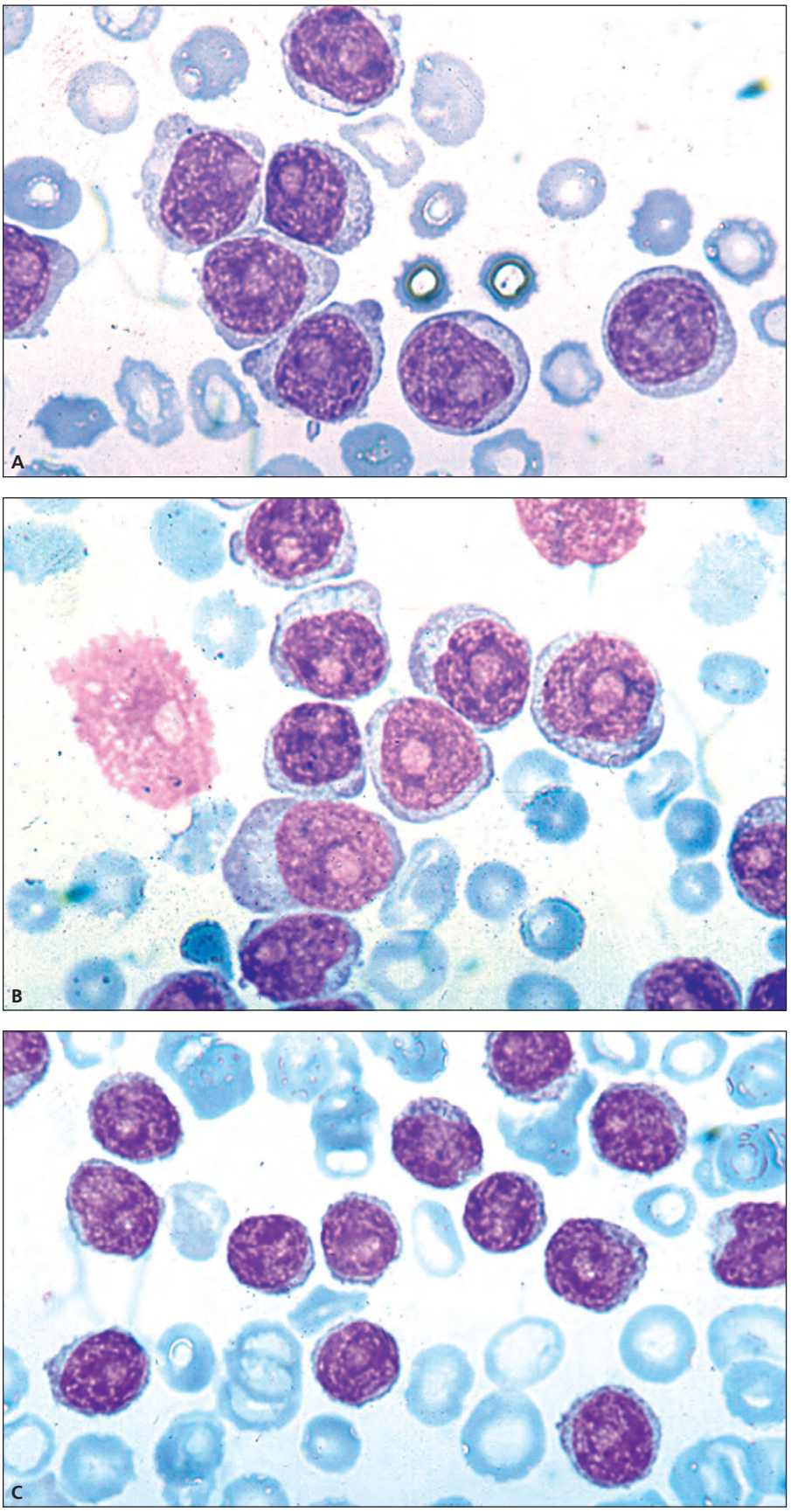
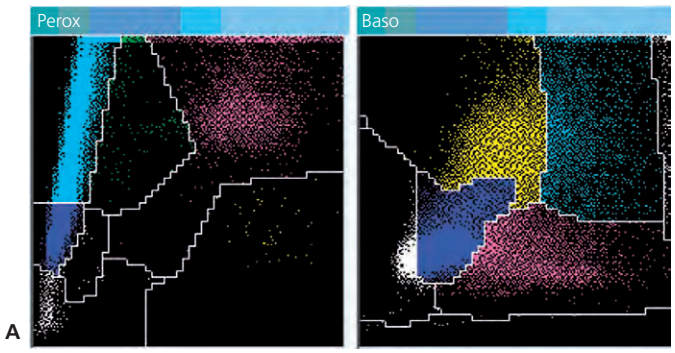
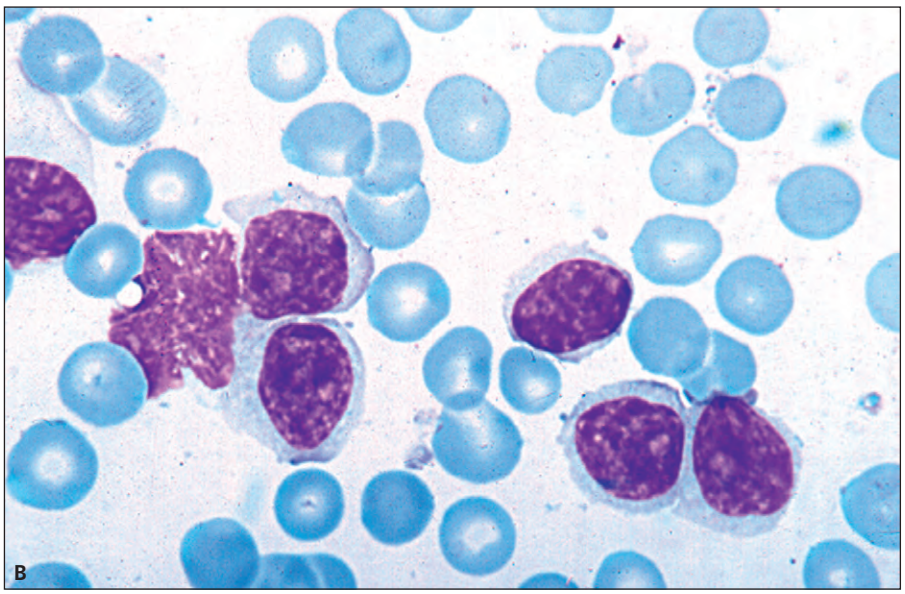



**Fig. 9.21** B-lineage prolymphocytic leukaemia (B-PLL); peripheral blood film. (A) The leukaemic population in the peripheral blood and bone marrow is very homogeneous both with regard to the morphology and the immunophenotype (CD19+, CD5−, CD22+ and SmIg+). These are medium-sized or large cells with an N:C ratio that is clearly lower than that of CLL, because of the presence of a plentiful halo of cytoplasm characterised also by more intense basophilia. The nucleus is large, central or slightly eccentric, with slight irregularity of its outline; the chromatin is condensed into smaller and less numerous clumps, between which are thin pale spaces. The nucleolus is always single, pale, easily visible in almost all cells and often eccentric. (B) The morphology of the prolymphocytes is greatly influenced by the area of the film in which they are observed. In fields close to the tail, where they are quite spread out, as in this image, the nuclear chromatin appears more granular and immature, creating the possibility of confusion with an acute leukaemia. (C) In an area of the same film, a little more towards the centre, in which the distribution of the cells still appears optimal, without superimposition, the lesser degree of distension of the chromatin network means that the chromatin appears less open, more condensed, so that the nucleoli are barely evident. In a field such as this, it can be difficult to make the correct morphological diagnosis of PLL.



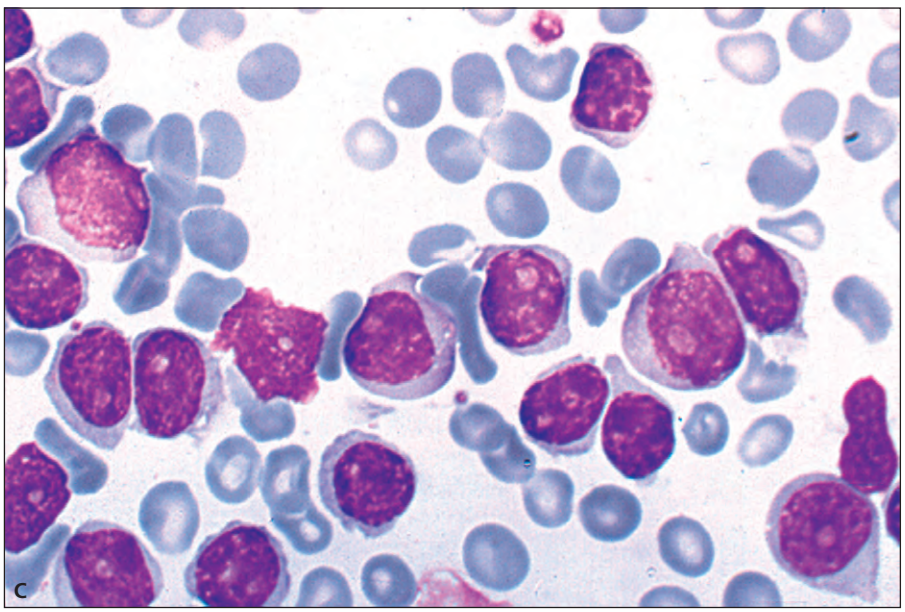




**Fig. 9.22** Automated instrument output and peripheral blood films from two different cases of B-PLL. (A) Extreme leucocytosis (WBC 360.5 × $10^9$/l), frequent in this condition. In the Perox cytogram the peroxidase-negative population is compact, staying within clear limits and not invading the monocyte area. In the Baso channel, the nuclei of the mononuclear cells are invading the blast area and the upper zone, with pseudobasophilia because of co-incidence. The Hb has been slightly overestimated because of turbidity with a value of 77 g/l (MCHC 342); the calculated Hb is 71 g/l (CHCM 321). (B) In comparison with the case in Fig. 9.21, the morphology of the prolymphocytes is less homogeneous. In addition to the larger typical forms, there are prolymphocytes with a less prominent nucleolus and a minority of small lymphocytes with condensed chromatin. In cases such as this, the distinction from CLL/PL is based, rather than on an imprecise count of the percentage of prolymphocytes, on the immunophenotypic characteristics (CD5+, CD22+ and SmIg strong) and on the clinical features (large spleen without lymphadenopathy). (C) In this second case, the population of prolymphocytes is more uniform, but the nuclear chromatin is condensed in a fairly homogeneous manner and the nucleoli, even if typical with regard to number and position, are poorly defined.

In histological sections, the pattern of infiltration is most often mixed or diffuse. The increase in reticulin fibres is more marked than in CLL. The marrow is hypercellular: the prolymphocytes are medium sized or large, with a round nucleus, condensed chromatin and a prominent nucleolus.

Splenic infiltration involves both the red and the white pulp: the white pulp appearance is characteristic, there being large proliferating nodules with typical prolymphocytes massed at the centre, surrounded at the periphery by large cells that are less densely packed, giving a typical appearance of two zones. The lymph nodes, usually studied only when a splenectomy is performed or at autopsy, are also diffusely infiltrated with total destruction of the normal architecture, sometimes with a pseudonodular appearance.

### Immunophenotype

The prolymphocytes of PLL express the main B-lineage markers (CD19, CD20, CD22 and CD24); SmIg, usually IgM, is strongly expressed, causing typically intense fluorescence. In contrast to CLL, the expression of CD22, CD11c and FMC7 is very strong, while CD5 and CD23 are expressed in only 20% of cases. There is no expression of CD25. These immunophenotypic characteristics suggest that PLL is derived from a B-lymphoid cell at a more advanced stage of maturation than is the case in CLL or is characterised by a particular state of activation that precedes maturation into a plasma cell.[52]

### Genetic analysis

Variable and complex chromosomal abnormalities have been described, with the frequent occurrence of del(17p), identifiable by FISH, and mutation of the *TP53* gene. The previously described cases with t(11;14) with breakpoints close to the *IGH* locus are now considered to be a leukaemic and splenomegalic variant of MCL.[51]

## Hairy cell leukaemia

Initially described under the designation leukaemic reticuloendotheliosis, HCL has been much discussed both with regard to its appropriate classification and to the cell of origin. Clinically, it is manifest with splenomegaly, cytopenia and the presence in the circulation of characteristic mononuclear cells with cytoplasmic prolongations that give the disease its name of 'hairy cell leukaemia'. Immunological and genotypic studies have shown that this condition results from the clonal proliferation of a subpopulation of mature memory B cells strongly activated by cytokines and multiple stimuli from the microenvironment.[53–56] The proliferation and accumulation of hairy cells occurs in the bone marrow and in the spleen, as a result of interaction between cell surface receptor molecules and adhesion molecules, and the accessory cells and stroma of these organs. The state of activation makes them particularly sensitive to therapy with interferon and nucleoside analogues, which give a high rate of prolonged complete remission. Individuals above the age of 30 years are affected, with a median age a little over 50 years, thus younger than in the majority of lymphoproliferative disorders. There is a male predominance with a male : female ratio of 5 : 1. The clinical course, before effective therapy was available, was indolent and marked by infections, autoimmune complications and progressive haemopoietic insufficiency. The median survival was four years, but varied from a few months to many years, relating particularly to whether diagnosis was early and whether effective therapeutic interventions were made.

### Blood count

The typical peripheral blood picture of HCL at presentation is pancytopenia. Leucopenia is almost always present at diagnosis: the leucocyte count is less than $5 \times 10^9$/l in 60% of cases. In addition to neutropenia, with an absolute neutrophil count of less than $1 \times 10^9$/l in around 80% of patients, there is a marked reduction in circulating monocytes, which is one of the most specific signs of HCL. The percentage of circulating hairy cells is very variable: sometimes they are absent; sometimes, in more typical cases, they are scanty and therefore more easily identified on buffy coat preparations; in other cases they are numerous, causing an increase, usually moderate, in the leucocyte count. Leucocyte counts above $10 \times 10^9$/l are present in about 20% of cases. Anaemia is moderate, normocytic and normochromic. The platelet count is almost always less than $100 \times 10^9$/l.

### Peripheral blood morphology

Hairy cells are larger than both normal lymphocytes and CLL cells with a diameter between 10 and 20 μm (Figs 9.23 through to 9.28). The nucleus is central or slightly eccentric, but almost never in contact with the cytoplasmic membrane; the shape is heterogeneous, the most typical being oval, bean shaped or kidney shaped with an indentation of variable size corresponding to the centre of the cell in the area where the cytoplasm is most abundant. Some cells are binucleate or have two half-nuclei side by side, appearing like a coffee bean. The chromatin is a homogeneous network with partial condensation into small dispersed clumps, which give a spongy appearance. The nucleoli are generally not visible; in some particularly spread out cells, one can see a small, indistinct nucleolus with a blurred outline. The N : C ratio is low because of the consistent presence of ample cytoplasm, which is pale and weakly basophilic, and can appear blotchy because of the presence of ill-defined areas where the basophilia is either more or less intense; the cytoplasm can contain small peroxidase-negative azurophilic granules, small vacuoles or, rarely, rod-shaped inclusions corresponding to the ribosome-lamellar complex that is detected on ultrastructural examination. The distinctive feature of hairy cells is the appearance of the cytoplasmic periphery, which is always ill defined, irregular or fringed, because of the presence of villous or digitiform projections of variable length. The villi of HCL are usually numerous and occupy the entire circumference of the cell. The appearance of these cells is very characteristic and permits a distinction to be made from occasional lymphocytes with cytoplasmic projections that result from the spreading of the film or

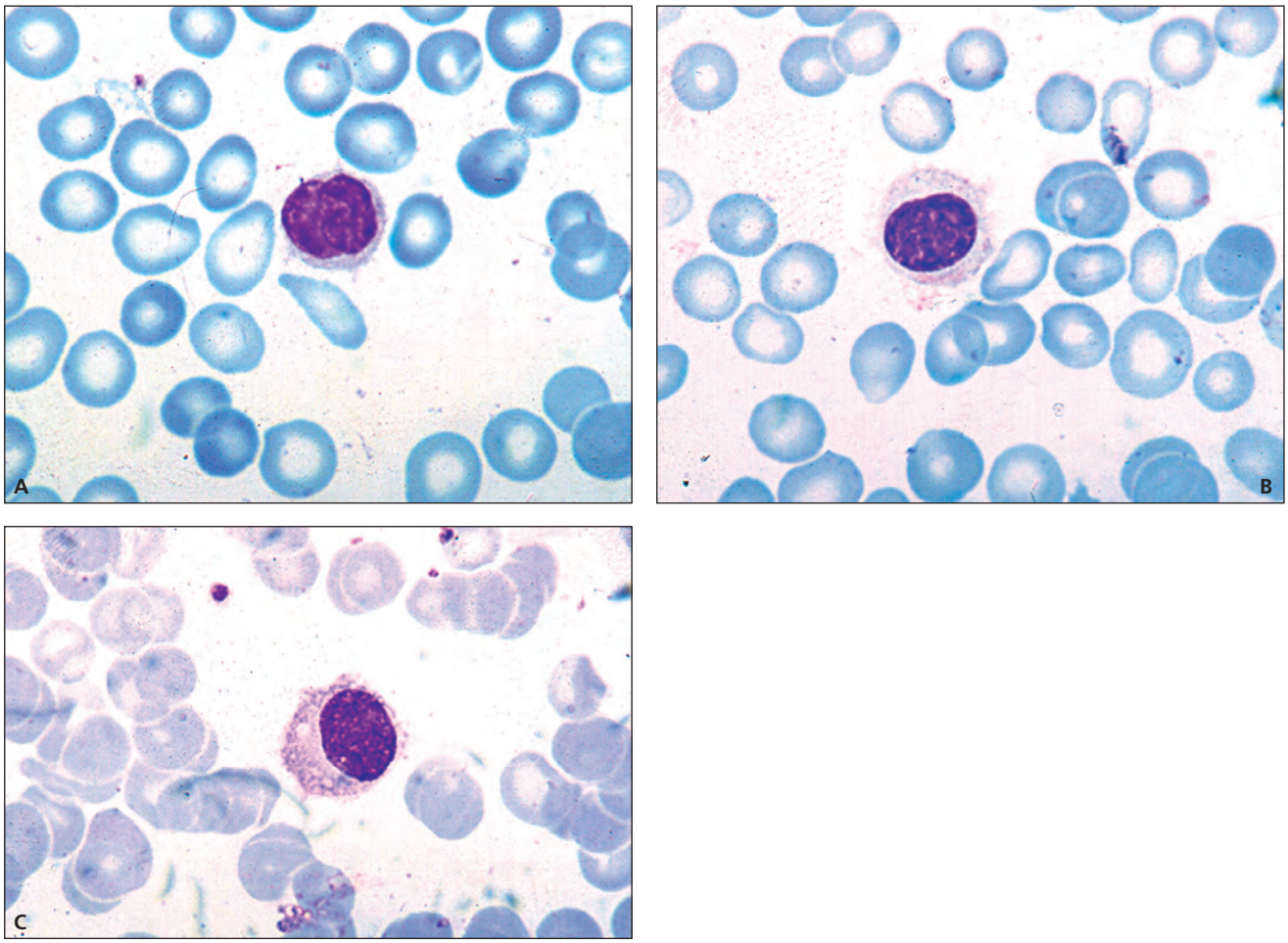



**Fig. 9.23** Hairy cell leukaemia (HCL); peripheral blood film. The leukopenia characteristic of this lymphoproliferative disorder means that, in the majority of cases, only isolated hairy cells are present in the blood film. (A) Typical morphology which permits the recognition not only of the villosity of the cytoplasm but also the low N:C ratio, resulting from the fairly abundant cytoplasm, and the morphology of the nucleus: the latter in this oval cell is formed of two superimposed lobes, as one can see by following the nuclear outline, which from the left continues into a central curve. The villi, fairly short and thin, are visible around the entire circumference of the cell. (B) The nucleus is bean shaped and central; the chromatin is condensed into small clumps and nucleoli are not visible. The cytoplasm is plentiful and pale; villous tufts spread out in all directions. (C) This hairy cell is medium sized with an oval eccentric nucleus with homogeneous granular chromatin. The cytoplasm is plentiful and not homogeneous, greyish with small vacuoles and an indefinite outline because of the fine cytoplasmic projections and the associated shorter and thicker villi.

from the effect of drying (Fig. 9.29): to make the distinction, it is important to take note not only of the cell outline, but also the nuclear shape and the chromatin structure, which in hairy cells is very different from the heterogeneous condensed chromatin of normal or reactive lymphocytes. The cytoplasmic projections, even the more delicate ones, are more easily seen by phase contrast microscopy of fresh unstained preparations (Fig. 9.27). Rare atypical forms of HCL have been described, characterised by the presence of cells that are fusiform or have multilobated nuclei or immature, blastic chromatin.

Electron microscopy can be used when there is diagnostic doubt since hairy cells, in addition to showing clearly the presence of villi, possess a typical cytoplasmic organelle: the ribosome-lamellar complex, visible only occasionally by light microscopy as a rod-shaped inclusion that is paler in the centre. Lysosomes, cytoplasmic vesicles and saccules of the Golgi apparatus of the hairy cell are rich in isoenzyme V of acid phosphatase, which shows unique resistance to tartrate inhibition. The cytochemical demonstration of TRAP activity is one of the classical methods for confirming the diagnosis of HCL[57] (Fig. 9.27). The degree of positivity is, however, very variable: intense and diffuse in the majority of cases, but sometimes weaker and in less than 5% of cases totally negative. TRAP positivity is not, however, absolutely specific for HCL, being observed sometimes also in B-PLL, Waldenström macroglobulinaemia and in mast cells.

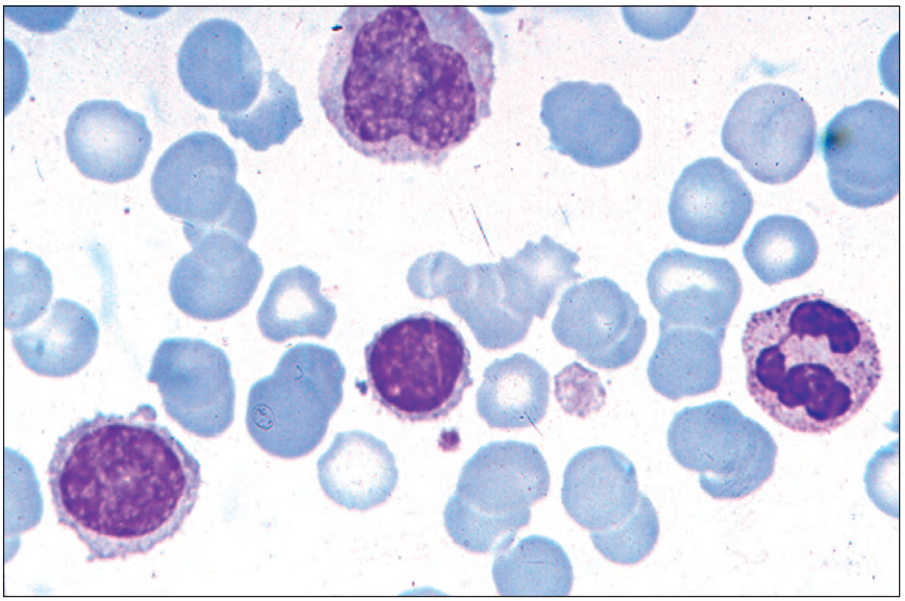


**Fig. 9.24** Villous lymphocytes in the peripheral blood of a normal subject. The one in the bottom left corner in particular resembles a hairy cell because of the abundance of the cytoplasm, which completely surrounds the nucleus. The morphological differential diagnosis is not always easy with isolated cells and must be based, not on the appearance of a single cell, but on the cytological and clinical context. Often normal lymphocytes that appear villous are found only in certain parts of the film, which must always be examined in its entirety. The cell at the top is a monocyte, a cell that is rarely observed in the company of hairy cells.

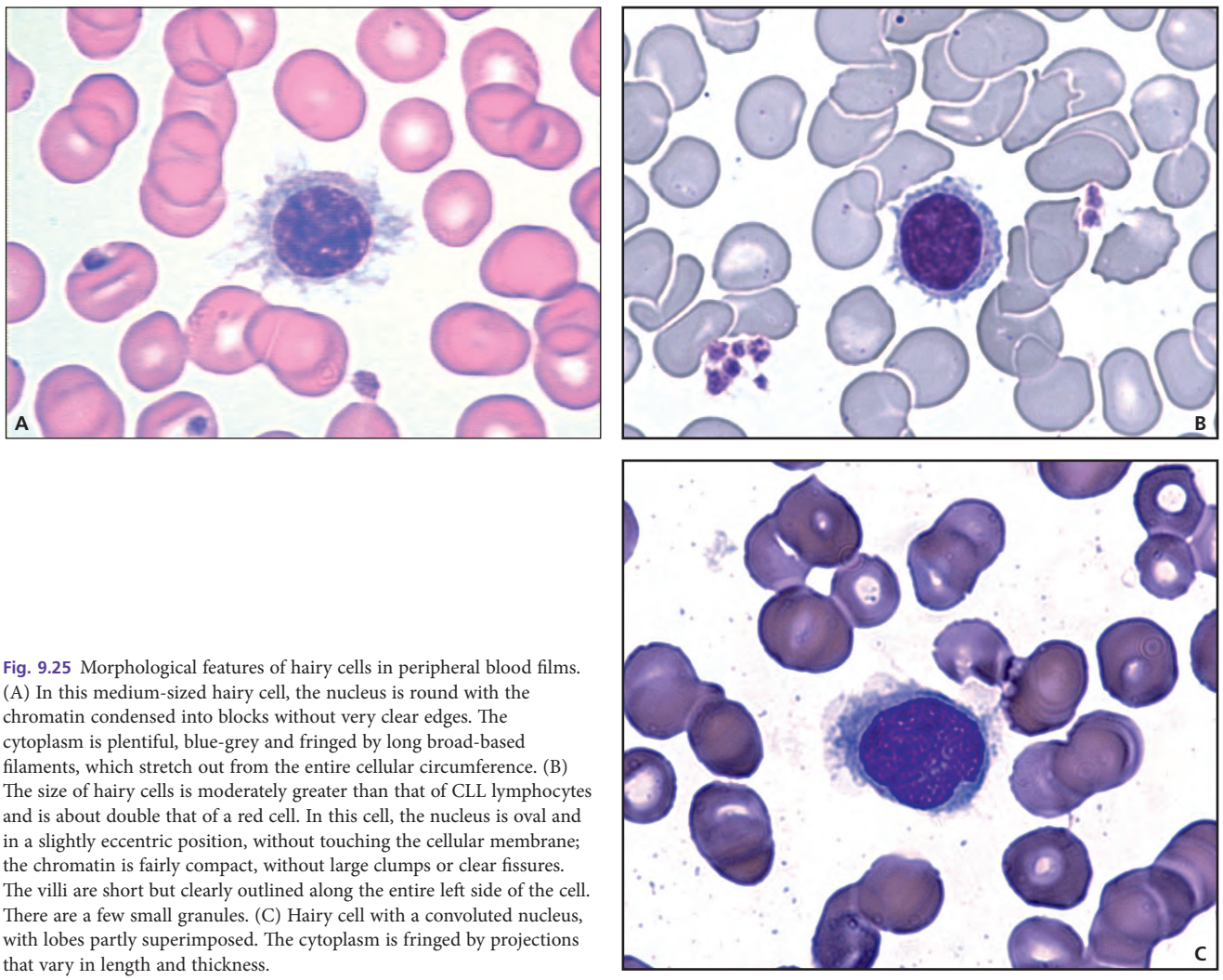



**Fig. 9.25** Morphological features of hairy cells in peripheral blood films. (A) In this medium-sized hairy cell, the nucleus is round with the chromatin condensed into blocks without very clear edges. The cytoplasm is plentiful, blue-grey and fringed by long broad-based filaments, which stretch out from the entire cellular circumference. (B) The size of hairy cells is moderately greater than that of CLL lymphocytes and is about double that of a red cell. In this cell, the nucleus is oval and in a slightly eccentric position, without touching the cellular membrane; the chromatin is fairly compact, without large clumps or clear fissures. The villi are short but clearly outlined along the entire left side of the cell. There are a few small granules. (C) Hairy cell with a convoluted nucleus, with lobes partly superimposed. The cytoplasm is fringed by projections that vary in length and thickness.

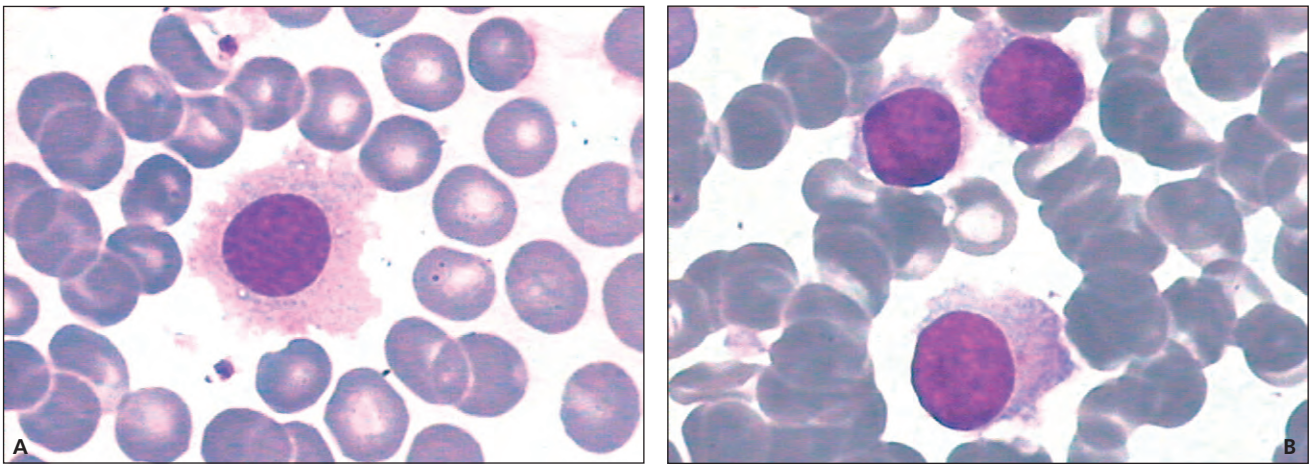



**Fig. 9.26** Morphological features of hairy cells in peripheral blood films. (A) The cytoplasm is very plentiful and rose-coloured with short villi giving a frayed appearance. The nucleus is central and round, with homogeneously condensed chromatin. (B) Ovoid hairy cell with an eccentric nucleus: although the nucleus is at one side of the cell, the morphology is otherwise identical to that of the preceding cell; the villi are less numerous and short.

### Bone marrow morphology

In around 50% of cases, attempted bone marrow aspiration does not yield evaluable material. This is the result of reticulin fibrosis, with the reticulin fibres entrapping the cellular elements; furthermore, these cells are reciprocally adhesive by means of their cytoplasmic projections. Often it is possible to aspirate a few drops of hypocellular aparticulate blood in which there are rare haemopoietic precursors and some mast cells, but mainly hairy cells (Figs 9.28 and 9.30). In bone marrow preparations, possibly as a technical artefact related to the aspiration, which tears apart the interdigitations between hairy cells when dragging them from the fibrous matrix, the typical cytoplasmic projections are less evident.

Histological examination of bone marrow sections can be important for the diagnosis of HCL.[58,59] In the majority of cases there is diffuse infiltration or poorly defined areas of infiltration by hairy cells. The nuclei of the hairy cells are typically spaced apart, surrounded by a halo corresponding to the pale cytoplasm. There is a consistent increase in reticulin fibres resulting from humoral factors produced by the hairy cells themselves, which delimit the areas of residual normal haemopoiesis. The erythroid islands often show maturation arrest with a tendency to megaloblastosis. Granulopoiesis is more seriously reduced, perhaps because of abnormal cytokine production. Examination of the spleen shows a particular type of infiltration, which involves exclusively the red pulp with expansion of the cords and engorgement of the sinusoids. The presence of pseudosinusoids is typical; they are lined by hairy cells and contain erythrocytes and leucocytes.

### Immunophenotype

The immunophenotypic profile of HCL is highly characteristic and diagnostically important.[3,60,61] Hairy cells express, almost always strongly, CD20, CD22, CD25 (an activation antigen corresponding to the interleukin 2 receptor), CD11c, CD103 (almost specific for HCL and HCLV) and CD123. They also express, in 80–100% of cases, SmIg at high intensity, FMC7 and B-lineage antigens (CD19, CD20, CD22 and CD24). They are negative for CD5, CD10 and CD23.

### Genetic analysis

Cytogenetic investigation of HCL is made difficult by the low proliferative activity of the pathological cells. Numerical alterations in chromosomes 5 and 7 have been reported. Recently, a recurrent *BRAF2* mutation has been described in almost all cases.

## B-cell non-Hodgkin lymphoma

B-cell non-Hodgkin lymphomas (B-NHL) are solid tumours characterised by a clonal proliferation of B lymphocytes in secondary lymphatic organs. Their clinical course depends on the level of differentiation of the cell of origin and on the topographical site of the clonogenic cell at the moment of the mutation. Generally of lymph node origin, B-NHL can also arise in the spleen or in extranodal tissue and organs such as the gastrointestinal tract, the pharynx, the tonsils, the skin, the central nervous system or the skeleton. Sometimes presentation is with bone marrow infiltration, leucocytosis and the presence of circulating lymphoma cells. Expression as leukaemia is common, particularly in low grade lymphomas, among which are splenic lymphoma with villous lymphocytes, which has some features similar to HCL, MCL and follicular lymphoma, which can be confused with CLL.[62] The majority of other B-NHL can show a leukaemic phase during the course of the disease, with invasion

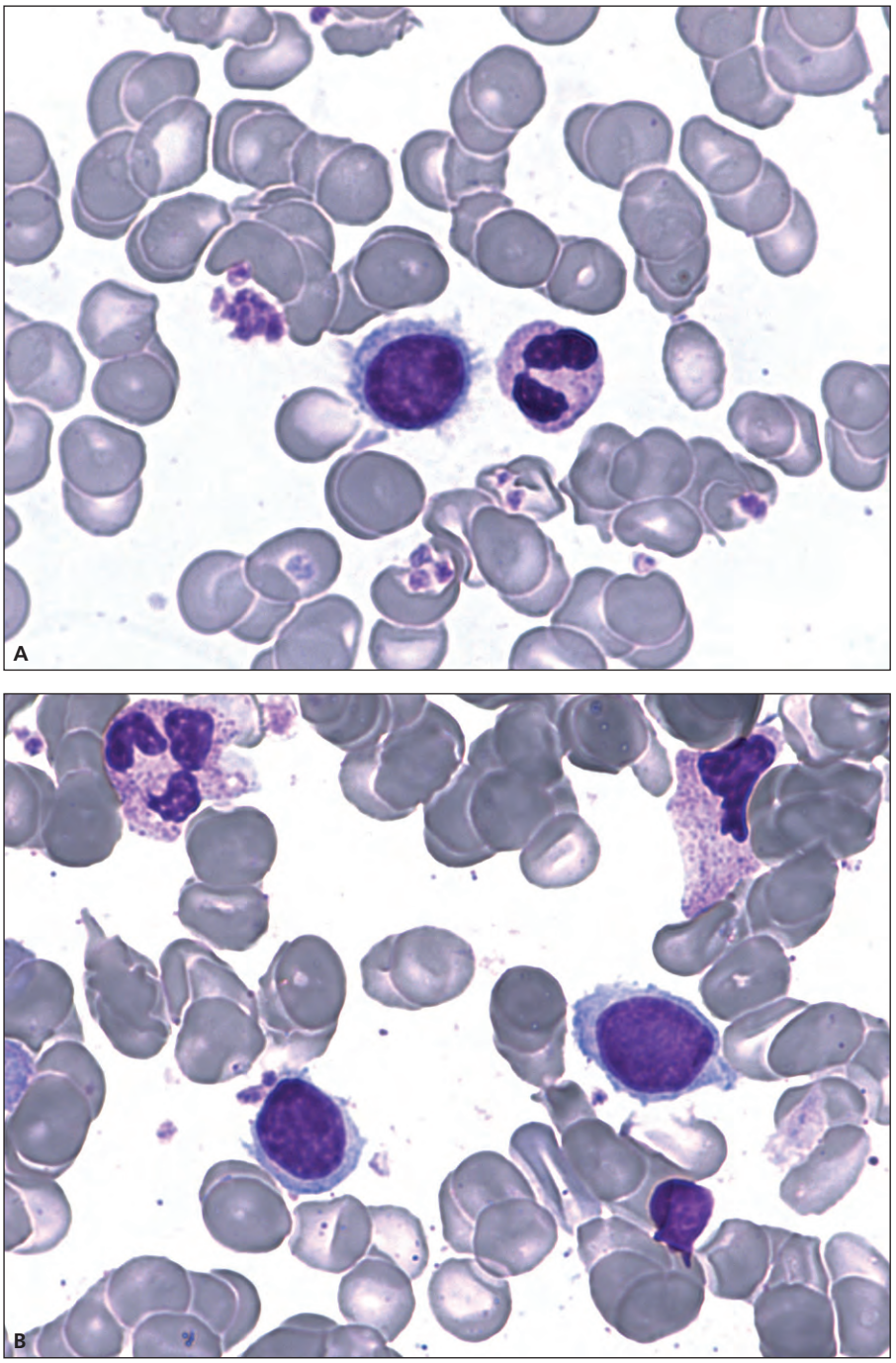



**Fig. 9.27** Morphological features of hairy cells in peripheral blood films. (A) The appearance of this cell is strongly evocative. In addition to the villous projections, the nuclear morphology is very suggestive, oval with compact chromatin. (B) The chromatin is forming small dark areas of condensation in the cell on the left, while it is more open and homogeneous in the larger cell on the right, which has cytoplasm with inconspicuous villi.

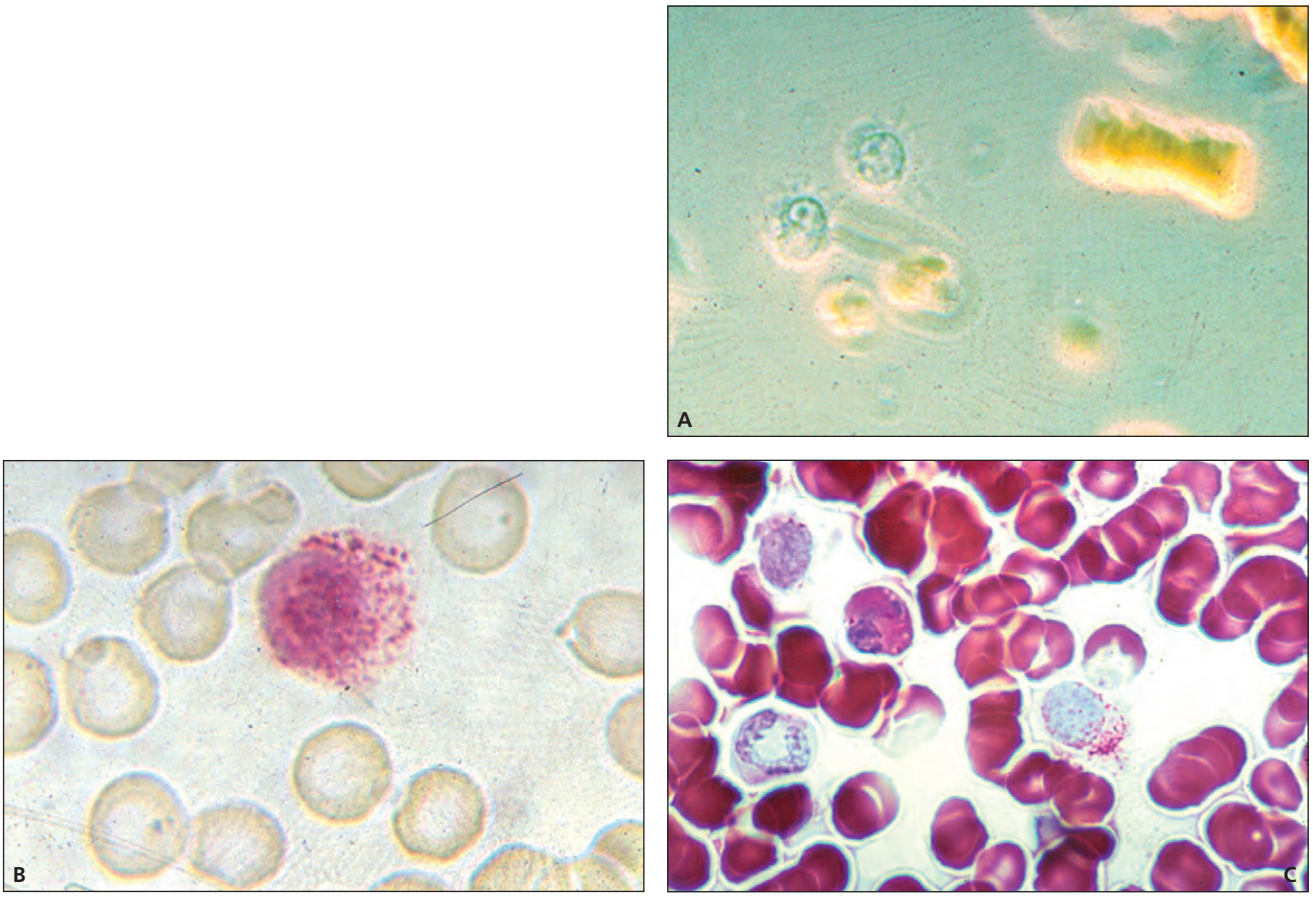



**Fig. 9.28** Characteristics of hairy cells in unfixed, unstained preparations and on cytochemistry. (A) Peripheral blood film, unfixed and unstained, observed by phase contrast microscopy. The villous projections are easily appreciated in their natural state in this type of preparation: they appear longer and more prominent, a result of a refractile halo. The nuclei are also characteristic because of the presence of a clearly outlined membrane and a pale nuclear matrix, in which sometimes small dark nucleoli stand out. (B) Cytochemical stain for acid phosphatase. A hairy cell showing distinct granular positivity. Numerous large reddish granules stand out from the background of the pale unstained cytoplasm. (C) Tartrate-resistant acid phosphatase (TRAP); peripheral blood film. The granular positivity for acid phosphatase, visible in the cytoplasm of the hairy cell on the right, persists when tartaric acid has been added to the substrate.

of the bone marrow and peripheral blood by lymphoma cells. Arbitrarily, the diagnosis of the leukaemic phase of NHL is made when there are more than $5 \times 10^9$/l lymphoma cells in the peripheral blood or more than 25% in the bone marrow.

The 2008 WHO classification includes this group of disorders in the category 'lymphoid neoplasms of mature B cells', which includes also CLL, PLL and HCL.[7]

## Hairy cell leukaemia variant

HCLV has a chronic clinical course, characterised by leucocytosis with the presence of cells that have morphological features intermediate between those of HCL and those of PLL, with an easily aspirable normocellular or hypercellular bone marrow and splenomegaly.[63,64] The differential diagnosis with regard to classical HCL is important, since HCLV shows little response to the therapeutic protocols that are efficacious in classical HCL, but can respond to rituximab and to anti-CD22-immunotoxin.[65]

Even if the cell of origin is similar (an activated B lymphocyte in an advanced maturation stage), the 2008 WHO classification emphasises that HCLV does not show the same biological profile as HCL; it is included as a provisional entity in the category 'splenic B-cell lymphoma/leukaemia, unclassifiable', together with a rare variant of splenic lymphoma designated 'splenic diffuse red pulp small B-cell lymphoma', which shows infiltration of the spleen by monomorphic small B cells and often also involves the peripheral blood and the bone marrow by villous lymphocytes.[66]

### Blood count

The peripheral blood typically shows marked leucocytosis with counts between 40 and $110 \times 10^9$/l, without monocytopenia or neutropenia. Only in 50% of cases is there anaemia or thrombocytopenia, which in general are moderate.

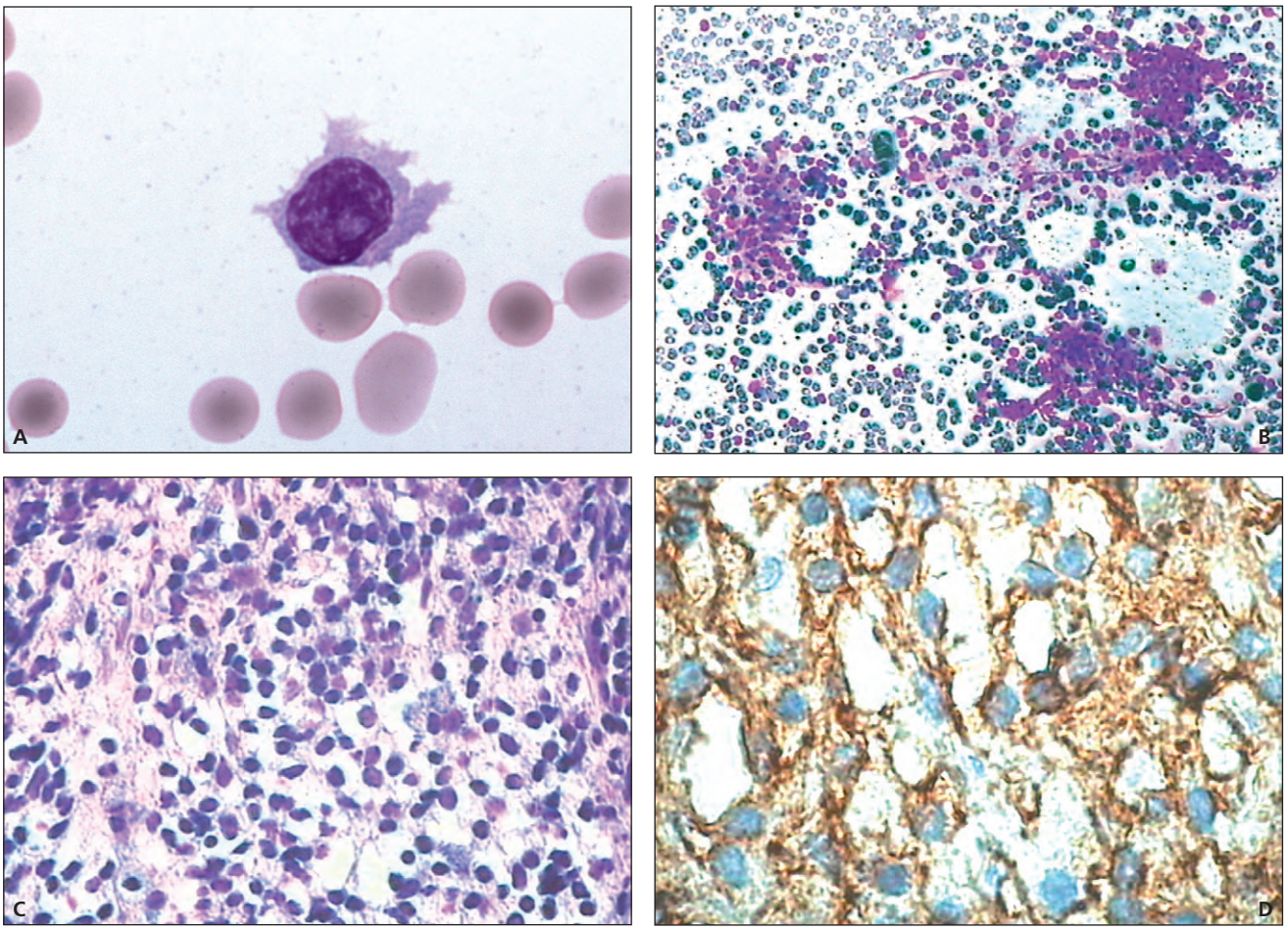



**Fig. 9.29** HCL. (A) Peripheral blood film. Large hairy cell with plentiful stretched out cytoplasm, with good evidence of the fringe of tentacles; the nucleus is oval with pale areas in the compact chromatin. (B) Bone marrow aspirate. Aspirates, even if bone marrow material is obtained, are usually unsatisfactory for evaluation of hairy cells, which form small nodules and are very damaged. (C) Histological section. The infiltrate is much more readily recognised: the hyperchromatic nuclei appear to be separated from each other, without the frequent superimposition that is observed in other leukaemic infiltrates; this is the result of the presence of abundant pale rose cytoplasm. This appearance has been described as resembling fried eggs. (D) Immunohistochemistry of a biopsy section. CD20 staining shows crowding of hairy cells, the cytoplasmic membranes of which are tightly adherent to each other, even though the nuclei are well separated.

### Morphology

The cells of HCLV resemble those of HCL with regard to cytoplasmic characteristics and the cells of PLL with regard to nuclear characteristics (Figs 9.31 through to 9.34). The cytoplasm is generally more abundant and more basophilic than in HCL; from the irregular cytoplasmic margin extend villous projections, which tend to be shorter, thicker, less numerous and further apart. The cell is of similar size or slightly smaller than a hairy cell, but the N:C ratio is higher. The feature most suggestive of the diagnosis is the nuclear morphology, the nucleus being round or oval, rarely notched, with peripheral condensation of the chromatin and the presence of a single, prominent, eccentric nucleolus, very similar to that of a PLL prolymphocyte. Binucleate cells are often seen and also cells with large hyperchromatic nuclei. Sometimes the cells have more dispersed nuclear chromatin and a distorted nuclear outline: this has been described as a blastic or convoluted subtype.[67]

There is TRAP positivity, but not as strong as in HCL; TRAP-negative cases have also been described. On ultrastructural examination, HCLV cells do not show the ribosome-lamellar complex of HCL, but do show numerous fibrillar inclusions.

Since fibrosis is absent, the bone marrow aspirate is always evaluable. The marrow is hypercellular, with predominantly villous lymphocytes identical to those in the blood. Histological sections show an interstitial infiltrate. As for HCL, the spleen shows red pulp infiltration.

### Immunophenotype

The antigenic profile of these cells is analogous to that of PLL, with positivity for CD19, CD20, monoclonal SmIg, CD22 and FMC7. CD25 is always negative, in contrast to classical HCL, while CD11c and CD103 can be positive. There are no specific genetic anomalies.

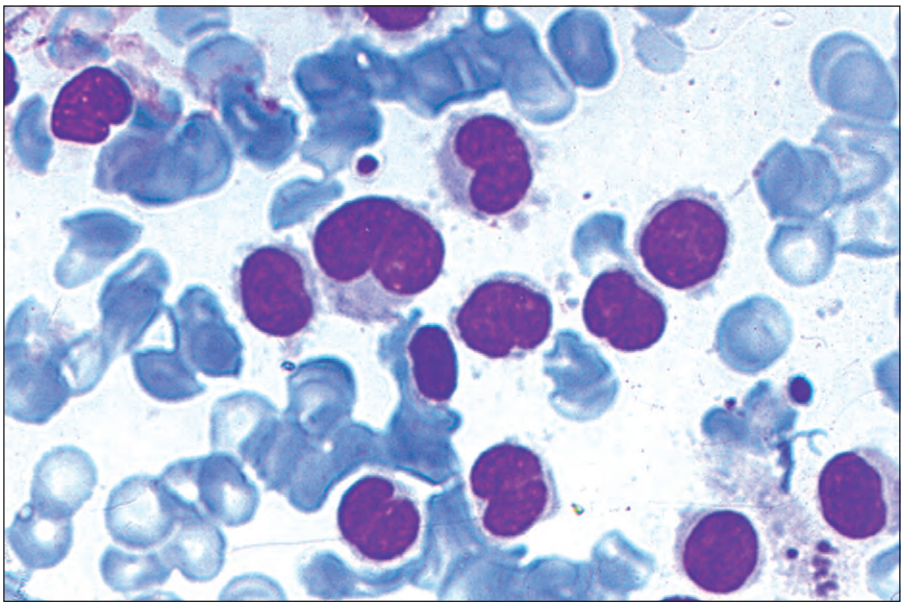


**Fig. 9.30** HCL; bone marrow aspirate. Massive bone marrow infiltration by hairy cells that differ in size and cytoplasmic characteristics: some cells have plentiful cytoplasm while in others it is scanty, with the degree of villosity also varying. The nuclear morphology, however, is very uniform and typical. Almost all the nuclei are bean shaped with a deep indentation in the centre: this continues into a more or less evident groove that appears to cut the nucleus in two.

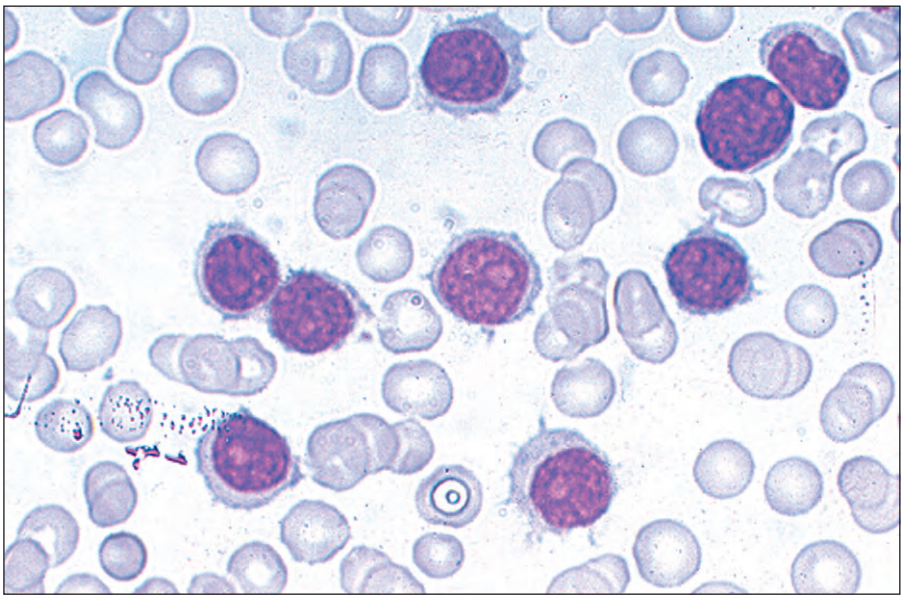


**Fig. 9.31** Hairy cell leukaemia variant (HCLV): peripheral blood film. The morphology of the neoplastic cells of HCLV is usually characteristic so that they are easy to identify, helped by the usual leucocytosis, which means that good quality films can easily be obtained. In this case, the cells are homogeneous and very large, with moderately extended basophilic cytoplasm that almost completely surrounds the nucleus; the chromatin is condensed into small heterogeneous clumps. The nucleolus, characteristic of this lymphoproliferative disorder, is large, pale, prominent and often slightly eccentric. The villous projections are usually thicker and further apart than those of hairy cells.

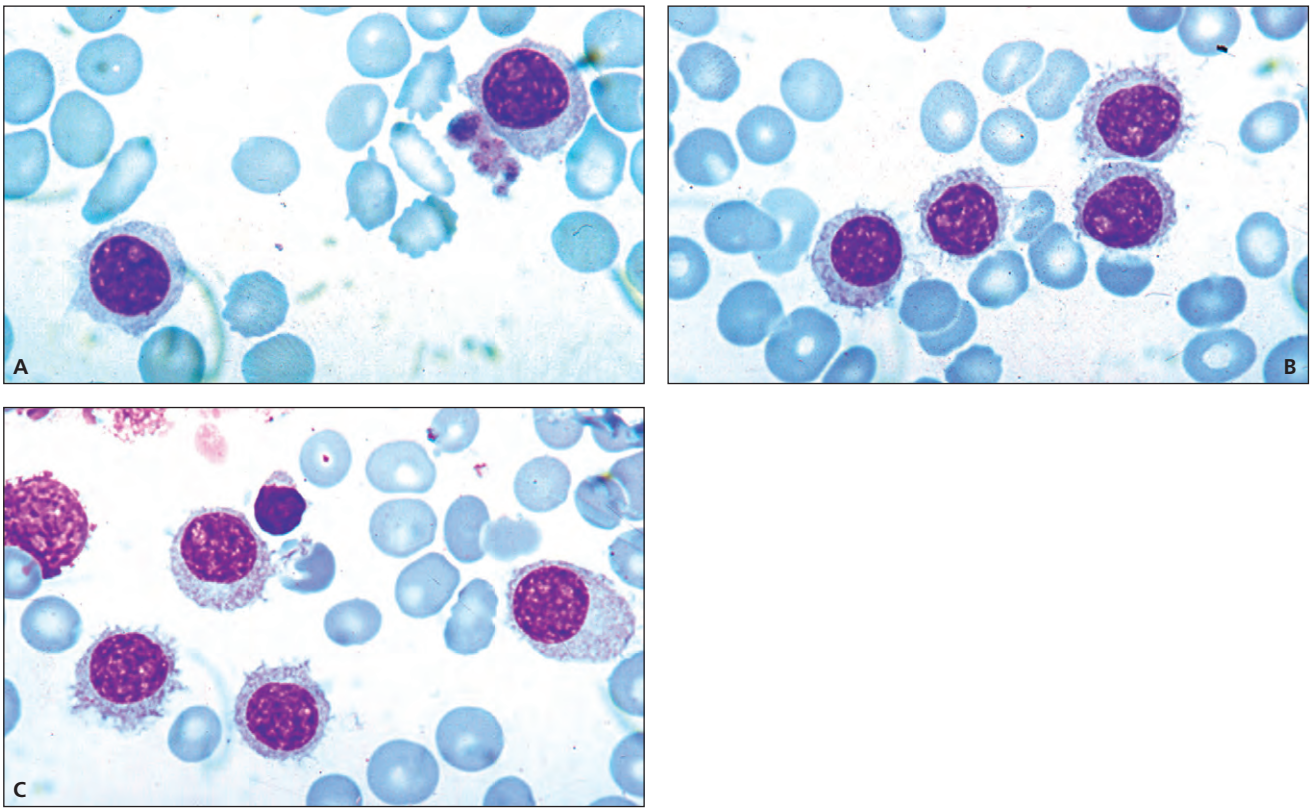



**Fig. 9.32** HCLV: peripheral blood film. (A) Two very characteristic cells, larger than in the case in Fig. 9.31. The cytoplasm is plentiful and greyish, with fine pale circles corresponding to the mitochondria; the villi are short and not very numerous. The nucleus is round and central and has an eccentric nucleolus. (B and C) Making a distinction from PLL requires examination of the area of a well-spread blood film where there is no distortion of cells and where the cytoplasmic villi are therefore well preserved. The consistent roundness of the nucleus, together with the presence of a nucleolus, is an essential criterion in making the distinction from HCL, in which the nucleus can be oval, reniform or, sometimes, shaped like a coffee bean.

## Splenic marginal zone lymphoma (with villous lymphocytes)

This is a rare form of low grade B-cell NHL with a predominantly splenic localisation, which has some analogies with HCL (splenomegaly, moderate or absent lymphadenopathy and villous lymphocytes in the peripheral blood), but differs from it in other aspects (splenic histology showing nodular infiltration in the white pulp, bone marrow invasion is moderate or absent and there is a paraprotein in the serum or a monoclonal free light chain in the urine in more than 75% of patients, with the concentration of the former being less than 2 g/l).[68–71] The median age of diagnosis is above 70 years, with a moderate male predominance. Clinical evolution is very indolent: with splenectomy, one obtains a clinical remission and long survival.

### Blood count

Only about 25% of patients have a mild anaemia and moderate thrombocytopenia at diagnosis; the leucocyte count is moderately elevated, rarely above $40 \times 10^9$/l. In contrast to HCL, there is no monocytopenia. In around 10% of cases, there is haemolytic anaemia or autoimmune thrombocytopenia at presentation.

### Morphology

The lymphocytes that are characteristic of splenic lymphoma are often oval with a size intermediate between those of CLL and of PLL (Figs 9.35 through to 9.42). The nuclei are round or oval, with chromatin condensed into small blocks; sometimes a small nucleolus is visible. Cytoplasm is moderately basophilic and less abundant than in HCL; at the periphery, it extends into short villous projections, concentrated at one pole of the cell or, less often, both. In some cases the cytoplasmic projections are barely evident and the general picture, of small lymphocytes with a compact nucleus in a patient with splenomegaly, is analogous to that of CLL: immunophenotyping is important in those cases with an apparent nucleolus, which otherwise cannot be resolved from B-PLL. A variable proportion of circulating lymphocytes have plasmacytoid features: a greater degree of cytoplasmic basophilia, a pale area corresponding to the Golgi zone and an eccentric nucleus. The TRAP reaction is mainly negative; when it is positive, the reaction is weak and is present in less than 30% of cells. On electron microscopy, ribosome-lamellar complexes, characteristic of HCL, are absent, as are the parallel configurations of endoplasmic reticulum characteristic of plasma cells.

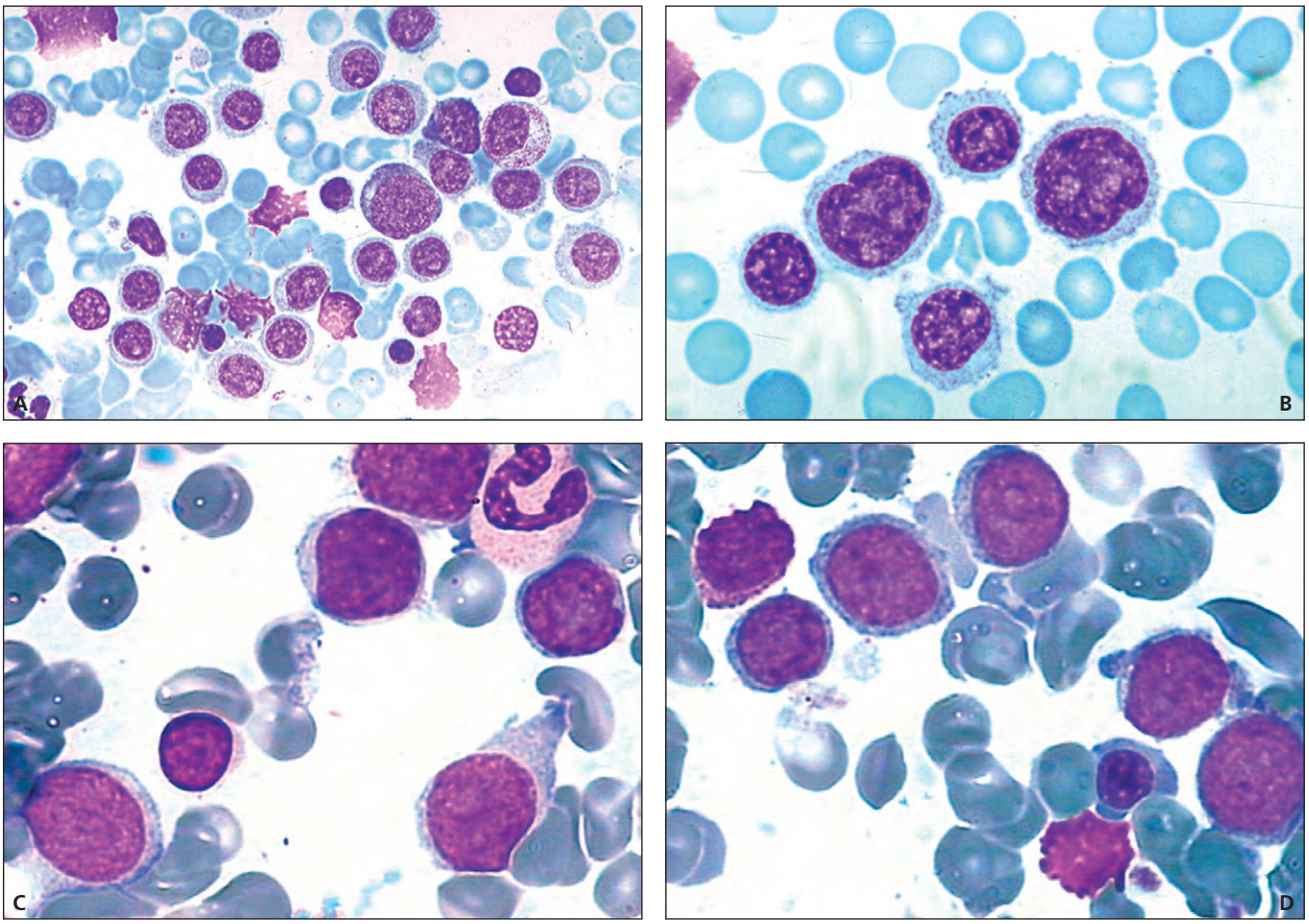



**Fig. 9.33** HCLV; bone marrow aspirate. (A) The marrow of the same patient as shown in Fig. 9.32 is diffusely infiltrated by a homogeneous population of cells with the same features as the circulating cells; in contrast to HCL, their morphology is well preserved, as a result of the absence of fibrosis. Normal haemopoiesis is fairly well preserved: the largest cell, almost at the centre, is a proerythroblast; a little higher and to the right, a basophilic erythroblast and a neutrophil myelocyte, and lower down, some orthochromatic erythroblasts. (B) A small proportion of neoplastic cells (two out of five in this field) are double the size of the others and have a gigantic tetraploid nucleus with an irregular notched outline; the chromatin is irregularly condensed and there are two less distinct nucleoli per nucleus. (C) These cells, in the bone marrow aspirate of another patient, are fairly large. The two cells at the bottom have homogeneous chromatin with nucleoli that are not well defined; the villi, always less easily seen in a bone marrow aspirate than in a blood film, since the cytoplasm is less spread out, are here most evident in the cell high in the centre, which has a more compact dark nucleus, with a high N:C ratio. (B) Another characteristic of this cell population is the relatively intense basophilia of the cytoplasm, which appears less abundant and is only occasionally extending into villous projections of variable length.

The villous projections are of two types: some long and separated from each other, others short and numerous.

While peripheral blood involvement has been observed in 30–75% of cases, bone marrow involvement is almost always present. Histological examination is always indicated and may show focal, nodular or intrasinusoidal infiltration. Plasma cell differentiation is often more obvious in the bone marrow than in the blood. The cytological composition of the bone marrow may be altered by erythroid or megakaryocytic hyperplasia resulting from haemolytic anaemia or autoimmune thrombocytopenia, which are observed in about 15% of patients.

Histological examination of the spleen, often necessary for diagnosis, shows an infiltrate in the white pulp, in which small rounded lymphocytes surround or more often replace the germinal centres, obliterating the mantle zone.[72] The white pulp nodules show the phenomenon of margination, with large cells at the periphery and small cells in the centre. Histological examination of abdominal lymph nodes that are removed during splenectomy show disturbance of the normal architecture such as is also observed in other low grade NHL. The only fairly characteristic feature is the preservation of the lymph node sinuses.

### Immunophenotype

From the immunological point of view, these cells are similar to the prolymphocytes of PLL, expressing B-lineage antigens

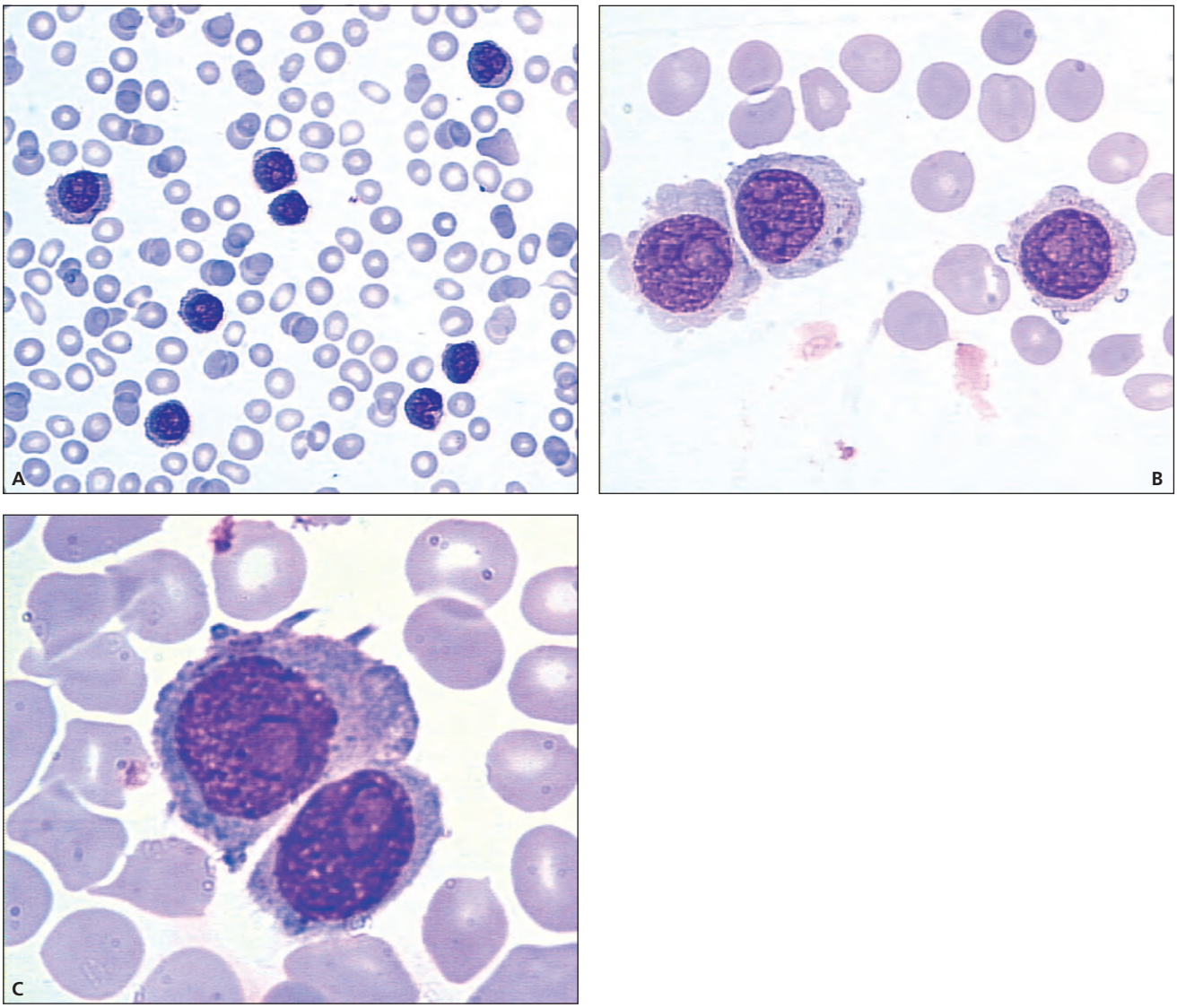



**Fig. 9.34** HCLV: peripheral blood film of another patient. (A) Leucocytosis is usually marked in this condition. Even at intermediate power, some cells show variable fringed cytoplasm and round nuclei with well-delineated nucleoli. (B) The chromatin is granular and the nucleoli are quite prominent. The central cell, which has two nucleoli, also shows some azurophilic cytoplasmic granules; the cytoplasm is very plentiful, pale and with vesicular and villiform projections. (C) Although the cell size is quite variable, the cytological characteristics described above are consistently present and easy to identify. One notes in particular the large size of the nucleoli, the outline of which is emphasised by linear condensation of the chromatin.

(CD19, CD20 and CD24; monoclonal SmIg strongly), CD22 and FMC7; they do not express CD25, which is characteristic of HCL; only a third of cases express CD11c[45]; CD5, typical of CLL, is expressed in a small proportion of cases.[71,73]

### Genetic analysis

Splenic marginal zone lymphoma has a specific, distinct cytogenetic profile. Karyotypic aberrations are common (72% in recent series of cases). The most frequent and specific abnormality is del(7)(q31), but numerous other abnormalities have been described, not rarely multiple.

## Follicular lymphoma

This is the most prevalent NHL in the western world. The neoplastic cells are derived from the follicular centre, being usually a mixture of centrocytes (small cleft cells) and centroblasts (large non-cleft cells). It can be a low grade lymphoma (centrocytic) or, more rarely, intermediate (centrocytic-centroblastic). It is the leukaemic lymphoma most often confused with CLL, from which it can be differentiated by the typical morphology of the circulating small cleft cells and by the immunophenotypic profile (including absence of CD5).[74,75] Incidence is higher in women. The most typical presentation is with asymptomatic

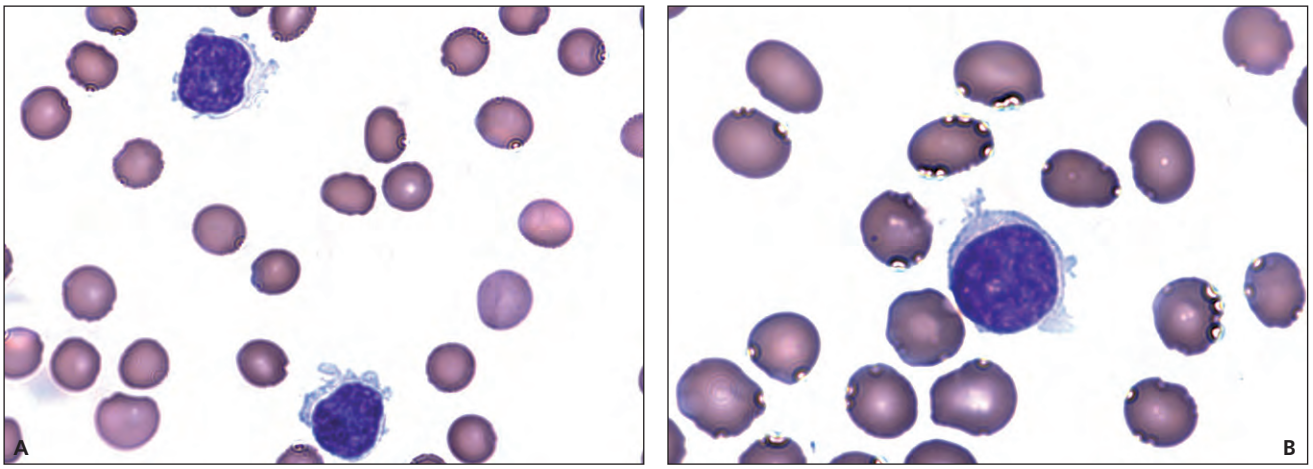



**Fig. 9.35** Splenic lymphoma with villous lymphocytes; peripheral blood film. (A) The leucocyte count is moderately elevated. The neoplastic cells are small lymphocytes with a high N:C ratio. The nucleus is oval with condensed chromatin; the cytoplasm is not very abundant and is of a watery, pale blue colour; it extends from some parts of the cell into morphologically variable projections, some fine, other vesicular. (B) This lymphoma cell also has a mature distinctly outlined nucleus, dense chromatin and a membrane that is extending into a small number of polar villous projections.

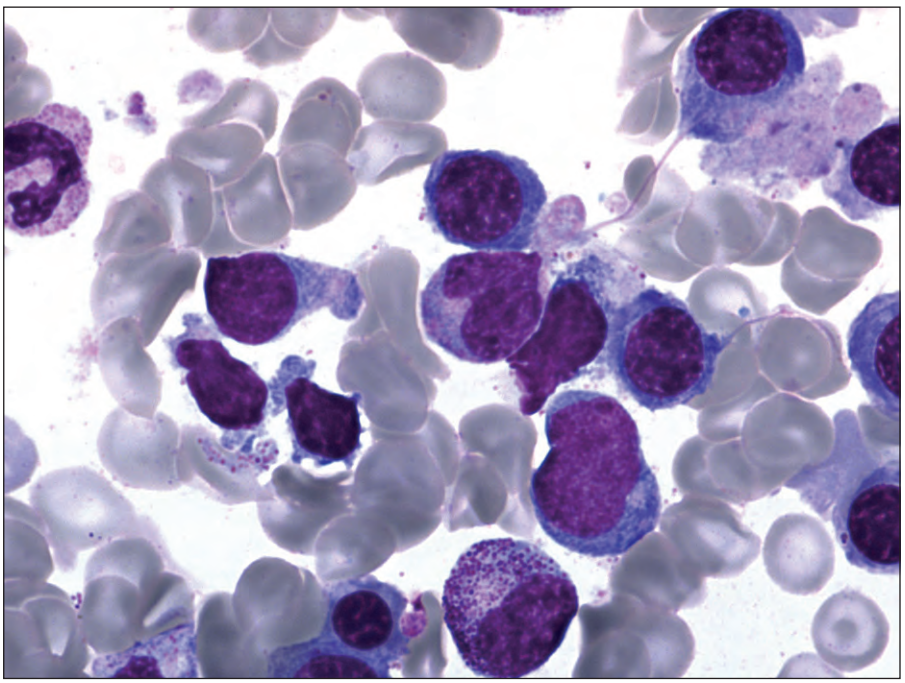


**Fig. 9.36** Splenic lymphoma with villous lymphocytes; bone marrow aspirate; same case as Fig. 9.35. To the left of centre, three oval lymphocytes with compact chromatin and scanty blue cytoplasm with polar projections. A fourth cell is visible to the right, above a larger cell, which has an irregular nucleus and plentiful intensely basophilic cytoplasm. On the right, in the centre and at the top, two pairs or basophilic erythroblasts joined by an internuclear chromatin bridge.

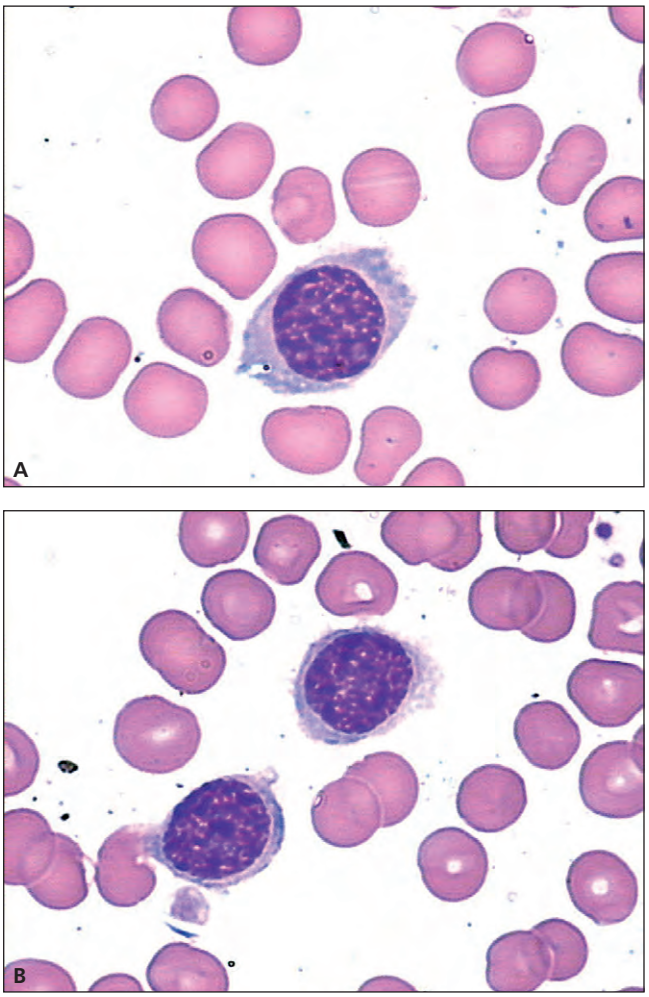



**Fig. 9.37** (A and B) Splenic lymphoma with villous lymphocytes; peripheral blood film. These cells are slightly larger than in Fig. 9.37, with an equally high N:C ratio. The nuclei are round and central with chromatin heterogeneously condensed into small clumps; the cytoplasm is scanty, pale blue and typically ragged at the two poles.

lymphadenopathy. Stage is advanced at presentation, particularly in the form with small cleft cells, which involves the bone marrow in 40–70% of cases at presentation and the peripheral blood in 15–30%.[76,77] The clinical course is indolent with a good response to therapy and survival for many years.

### Blood count

In the leukaemic form the peripheral blood shows leucocytosis, which can be moderate or, rarely, more marked with the leucocyte count sometimes exceeding 200 $\times$ $10^9$/l.

### Morphology

The small cleft cells of follicular lymphoma, the centrocytes, can be present in the circulation even in cases with a normal leucocyte count. The morphology is typical (Figs 9.43, 9.44 and 9.45): they are the smallest nucleated cells with the highest N:C ratio that can be observed in the peripheral blood. They are smaller than the lymphocytes of CLL; the diameter is similar to, or even less than, that of a normal erythrocyte. The cytoplasm is extremely scanty, invisible or reduced to a thin perinuclear rim: the most typical appearance is of small segments separated by a nuclear notch. The nucleus is more or less round, but with the outline often being angulated; the chromatin is homogeneous, dark, uniformly condensed, without visible nucleoli. The typical cleft cells seen on histological sections appear in blood or bone marrow films as cells with a notch that can be barely apparent or so deep as to almost divide the nucleus into two lobes side by side, like a coffee bean. These cells are more resistant to mechanical insults than the lymphocytes of CLL: generally, the blood film does not show smear cells (Gumprecht's shadows).

In the case of follicular lymphoma of mixed centrocytic-centroblastic type, with large and small cleft cells, involvement of the haemopoietic system is less frequent and the appearance of the lymphoid population is more pleomorphic, with some cells being larger with less chromatin condensation (Figs 9.46 through to 9.49).

The bone marrow aspirate shows an infiltrate of centrocytes and centroblasts with morphological features similar to those observed in the peripheral blood. The histological picture is characterised by the presence of focal infiltrates, particularly in the paratrabecular area and tending also to infiltrate interstitially. The presence of numerous large cells with blastic morphology is a sign of aggressive evolution and has a very unfavourable prognostic significance: according to the 2008 WHO classification, cases of this type fall into the category of diffuse large B-cell lymphoma.

### Immunophenotype

The immunophenotype of follicular lymphoma is characterised by expression of CD10 and FMC7, together with B-lineage antigens (CD19, CD20, CD22 and CD79a); it differs from the immunophenotype of CLL in that SmIg is more strongly expressed and CD5 is negative. The lymphoma cells show hyperexpression of BCL2 in the cytoplasm, as a result of the genetic rearrangement that characterises this lymphoma.

### Genetic analysis

There is almost always a characteristic translocation, t(14;18)(q32;q21), which brings the *BCL2* gene into proximity to the *IGH* locus. The genetic anomaly can be recognised also by DNA analysis of the peripheral blood in patients with stage I or II disease in which the blood count and blood film morphology are normal.[78]

## Mantle cell lymphoma

MCL is a low to intermediate grade B-NHL, occurring mainly above the age of 50 years, with the incidence in men being twice that in women.[79,80] It originates in a cell located in the inner mantle zone, which is an immature, virgin B cell, not yet having

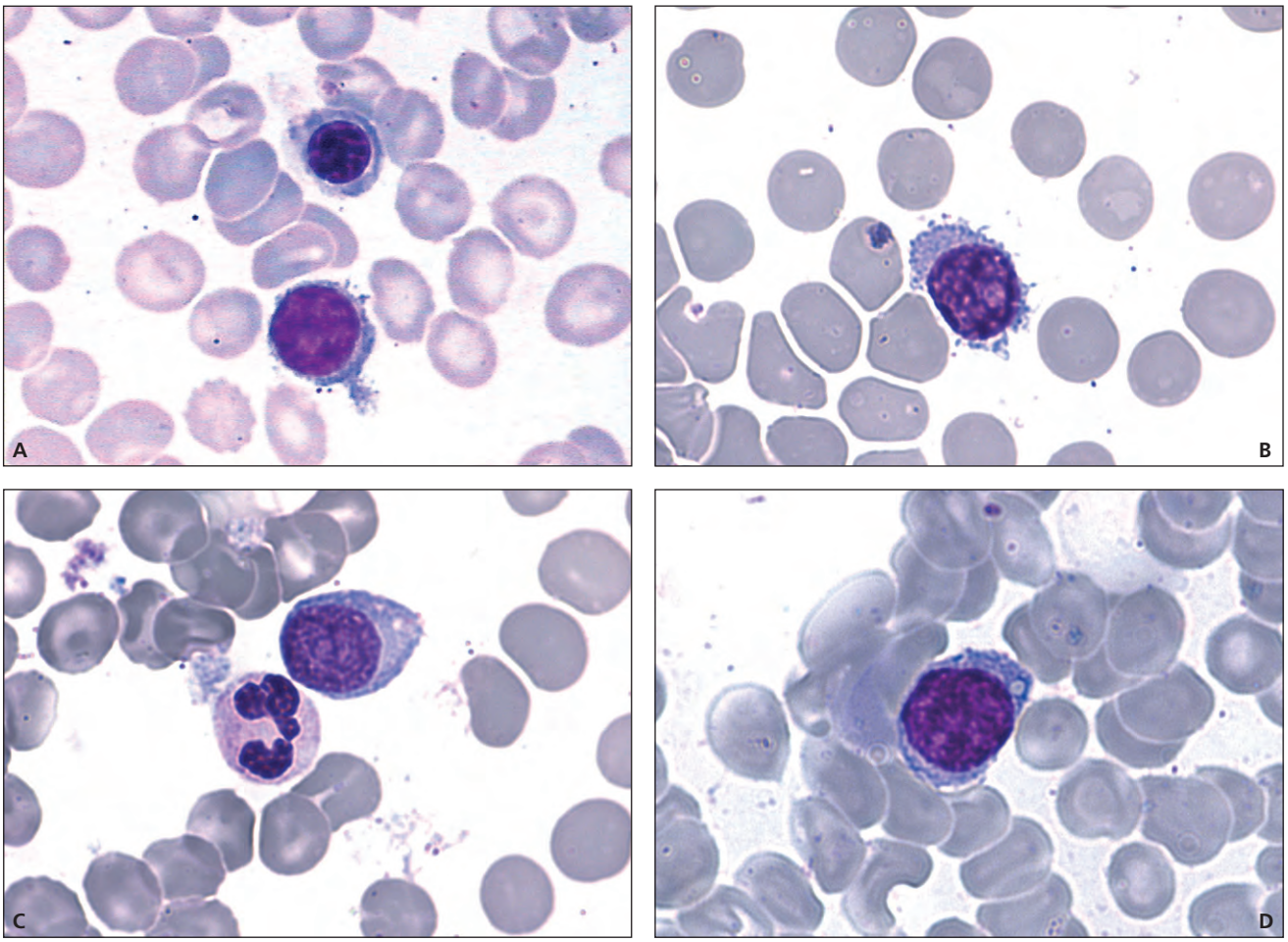



**Fig. 9.38** Morphological heterogeneity of the atypical B lymphocytes of splenic lymphoma with villous lymphocytes; peripheral blood film. (A) High N:C ratio, round compact eccentric nucleus; scanty cytoplasm with a long tuft at one pole and other short villi on the opposite side of the cell. This cell is larger than the polychromatic erythroblast above it. (B) This small lymphoma cell is elongated with an eccentric nucleus with numerous chromatin clumps: the nucleolus is clearly seen, an unusual feature in this lymphoma. The cytoplasm is scanty and extends into short villi, particularly at the two poles of the cell. (C) From the same blood film as (B), adjacent to a mature neutrophil, a larger lymphoid cells with an eccentric nucleus, the outline of a nucleolus and slightly more plentiful cytoplasm, tapering to a blunt point, with a few short filiform projections. (D) From another case with a paraprotein: the morphology of this cell is otherwise typical, but there is a globular immunoglobulin inclusion.

matured in the germinal centre. Presentation is usually with disseminated disease with generalised lymphadenopathy, splenomegaly and systemic symptoms (fever, sweating and weight loss). The clinical course is variable, with a reported median survival between 2.5 and 4 years.

### Morphology

The presence of lymphoma cells in the peripheral blood is common, being seen in up to 70% of cases, but a true leukaemic phase with the WBC above $10 \times 10^9$/l is seen in less than 20% of cases (see Report 9.2) and carries an unfavourable prognosis.[81–83] Bone marrow invasion is present in a third of patients. The characteristic morphology of the lymphomatous population in the peripheral blood and bone marrow of these patients is of heterogeneity and pleomorphism (Figs 9.50 through to 9.54). The predominant cell has a prolymphocytoid appearance, an intermediate size and moderately plentiful cytoplasm. The nucleus has condensed chromatin with one or two small nucleoli; the nuclear outline is irregular because of the presence of indentations, but these are rarely as deep as in follicular lymphoma. Other cytologically diverse cells co-exist: small round lymphocytes, cells with angulated and nucleolated nuclei, small cleft lymphocytes and large cells with a high N:C ratio without nucleoli. The heterogeneity of the picture sometimes recalls the mixed cell type of CLL, even though the proportion of small lymphocytes in MCL is always very low.

In histological sections of the bone marrow, one observes, in contrast to the heterogeneity of the cells in the circulation, a monomorphic infiltration of small and intermediate-sized lymphoid cells with irregular nuclear outlines.

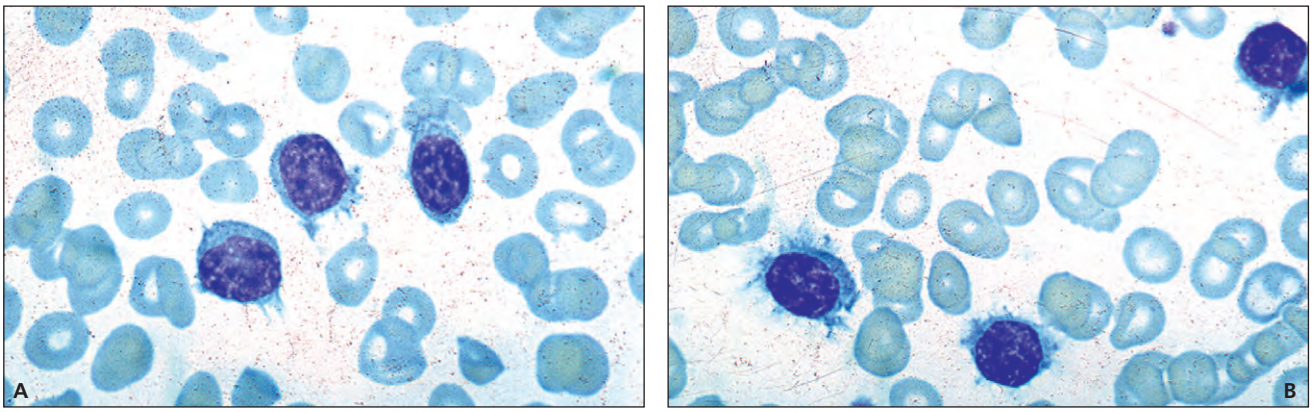



**Fig. 9.39** Splenic lymphoma with villous lymphocytes; peripheral blood film. (A) These lymphoma cells have a similar size, N:C ratio and nucleus to the small lymphocytes of B-CLL; the outline of the cytoplasm, however, resembles that of HCL with obvious villous projections. (B) The villi in this patient are fairly long and extend from only part of the cytoplasmic margin, tending to crowd together at the poles. The cytoplasm is not very abundant and is intensely basophilic. The chromatin is condensed into blocks, almost a mosaic pattern as in B-CLL.

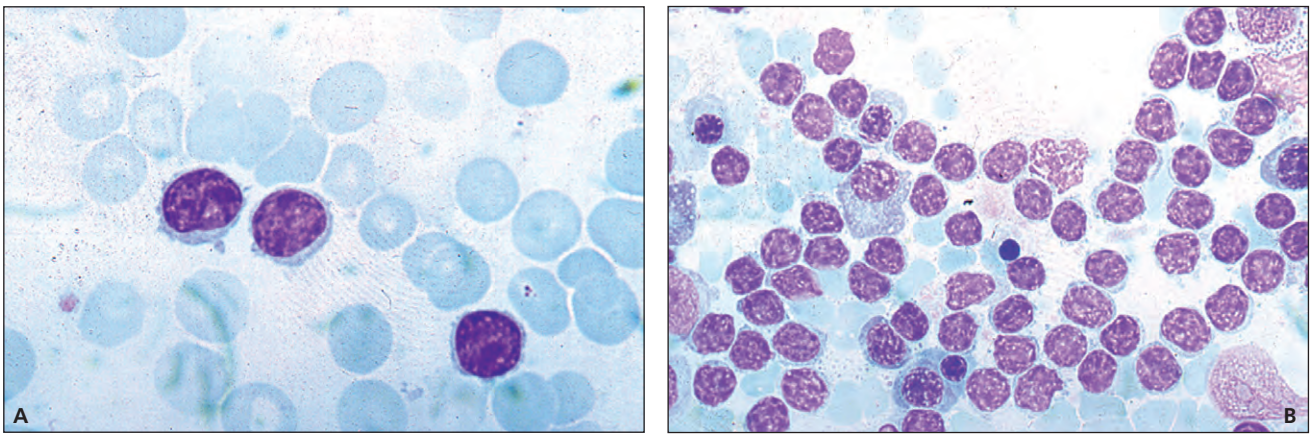



**Fig. 9.40** Splenic lymphoma with villous lymphocytes. (A) Peripheral blood film. The lymphoma cells are small and rounded, with short not very numerous villi: the morphological differential diagnosis with CLL is difficult and requires the support of immunophenotyping (CD5 negative and SmIg strongly positive). (B) Bone marrow aspirate. The marrow is diffusely infiltrated by a monomorphic population of lymphocytes with scanty cytoplasm and nuclear chromatin with a mosaic pattern; the villi are barely evident. There is one plasma cell and several erythroblasts. In the majority of cases of splenic lymphoma, bone marrow invasion is less massive.

Twenty to 30% of cases of MCL represent the blastic variant (blastoid MCL), with leukaemic manifestations being more common and the clinical course more aggressive. The cells resemble lymphoblasts with dispersed chromatin and frequent mitotic figures (Fig. 9.55). The pleomorphic variant, on the other hand, has very heterogeneous cells, often large with pale cytoplasm and oval nuclei or an irregular nuclear outline, and prominent nucleoli[79] (Fig. 9.56).

### Immunophenotype

The immunophenotype resembles that of CLL: strong positivity for B-lineage antigens (CD19, CD20, CD24) and also for CD5; SmIg is more strongly expressed and FMC7 is positive; CD10 is usually negative. Immunohistochemistry shows expression of BCL2 and cyclin D1, the latter explained by the characteristic genetic lesion.

### Genetic analysis

MCL is typified by t(11;14)(q13;q32), which brings the *CCND1* gene, encoding the cell-cycle protein cyclin D1, into proximity to the *IGH* locus. This aberration is the genetic event responsible for the neoplastic transformation.

## Nodal marginal zone lymphoma

This form of lymphoma, also designated monocytoid B-cell lymphoma, is derived from a cell that populates the marginal zone of the follicles of lymph nodes.

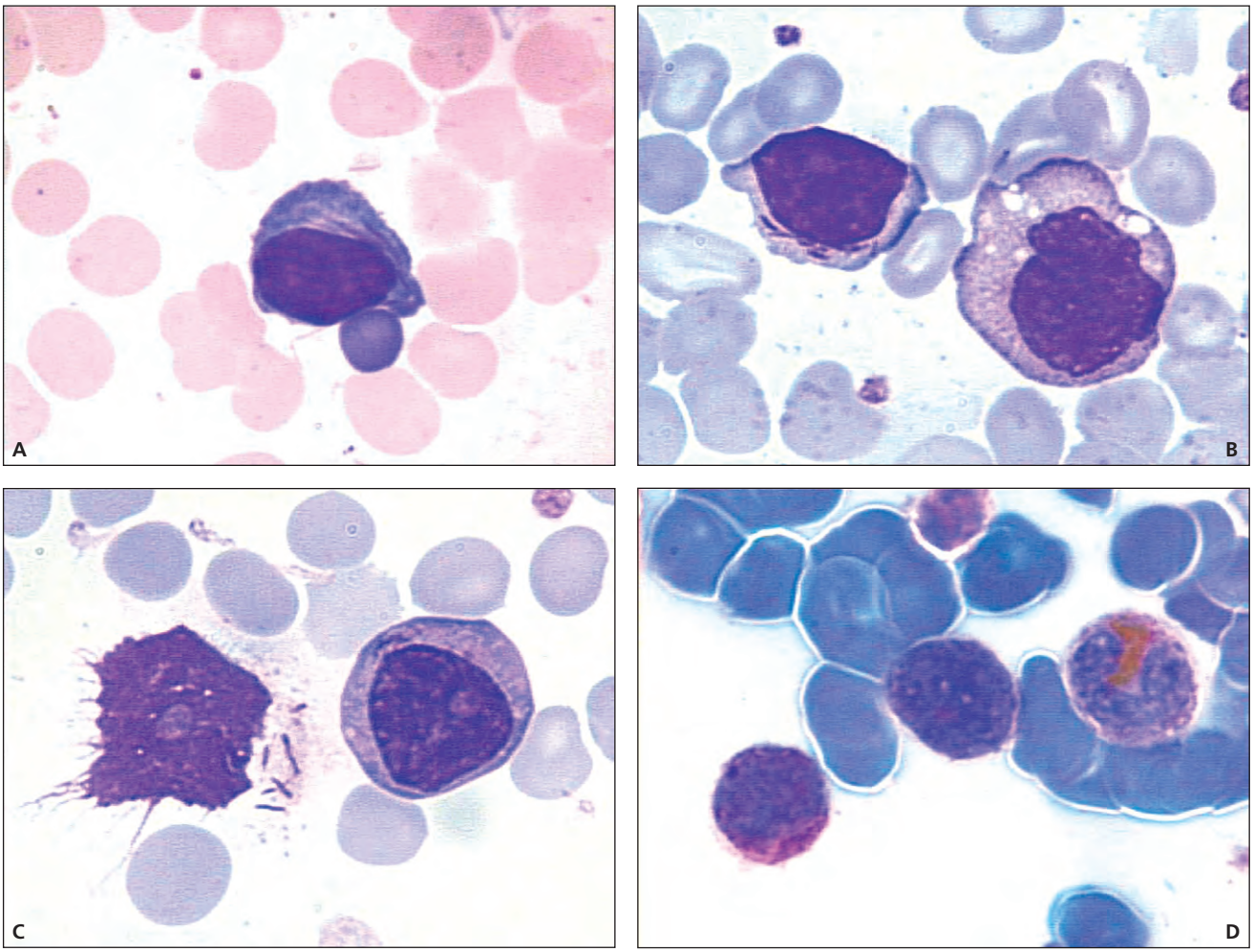



**Fig. 9.41** Splenic marginal zone lymphoma, with needle-like crystalline inclusions resembling Auer rods in the cytoplasm of the neoplastic cells in the peripheral blood film of a patient with an IgM kappa paraprotein (3 g/l). Haematological investigations showed: leucocytes 18.7 × $10^9$/l (atypical lymphocytes 79%, positive for CD19 and CD20 with kappa light chain restriction); Hb 113 g/l; platelets 152 × $10^9$/l. Moderate splenomegaly, maximum diameter 18 cm. (A) The nucleus is compact and oval. The plentiful basophilic cytoplasm contains azurophilic crystals that resemble the Auer rods of promyelocytic leukaemia. A similar rod is visible extracellularly. The lower part of the cytoplasm is forming a globular vesicle representing clasmacytosis. (B) On the left, another cell with an eccentric nucleus, in contact with the cell membrane, with chromatin condensed into indistinct blocks. The free part of the cytoplasm, which is basophilic with peripheral enhancement, contains a number of short thick and pointed reddish rods. On the right, a monocyte with plentiful grey-lilac vacuolated cytoplasm, which has a ground-glass appearance because of the presence of very fine granules. (C) As happens in acute promyelocytic leukaemia, the sharp azurophilic crystals have damaged the cell membrane and appear free, adjacent to the torn nucleus. The cell on the right also shows small azurophilic inclusions. (D) A peroxidase reaction is positive in the metamyelocyte on the right and negative in the cytoplasm of the lymphoma cell; the inclusions were red with a Giemsa stain.

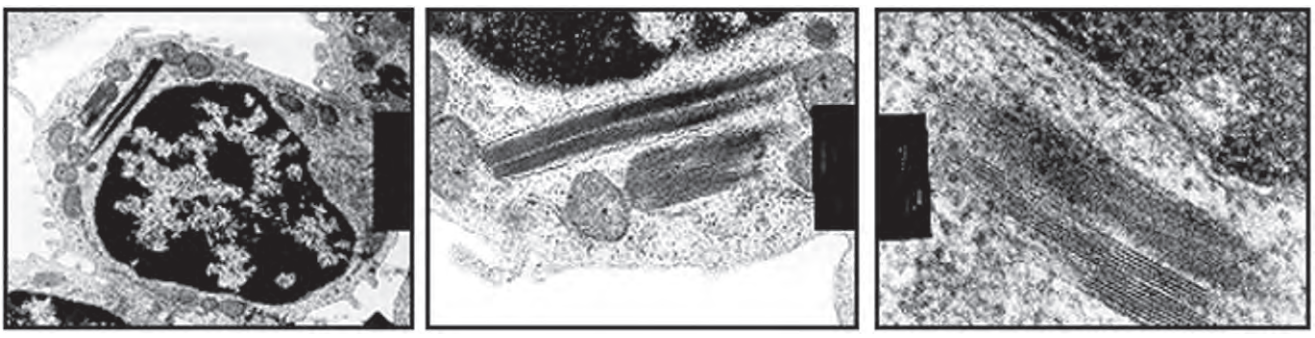



**Fig. 9.42** Splenic marginal zone lymphoma. Characterisation of the rod-shaped cytoplasmic inclusions in the case in Fig. 9.41; peripheral blood. (A–C) Transmission electron microscopy confirms the crystalline structure of the inclusions, formed of thin tubules arranged in parallel bundles, and suggests that their origin is in immunoglobulin molecules. Numerous hypertrophic mitochondria are also visible in the cytoplasm.

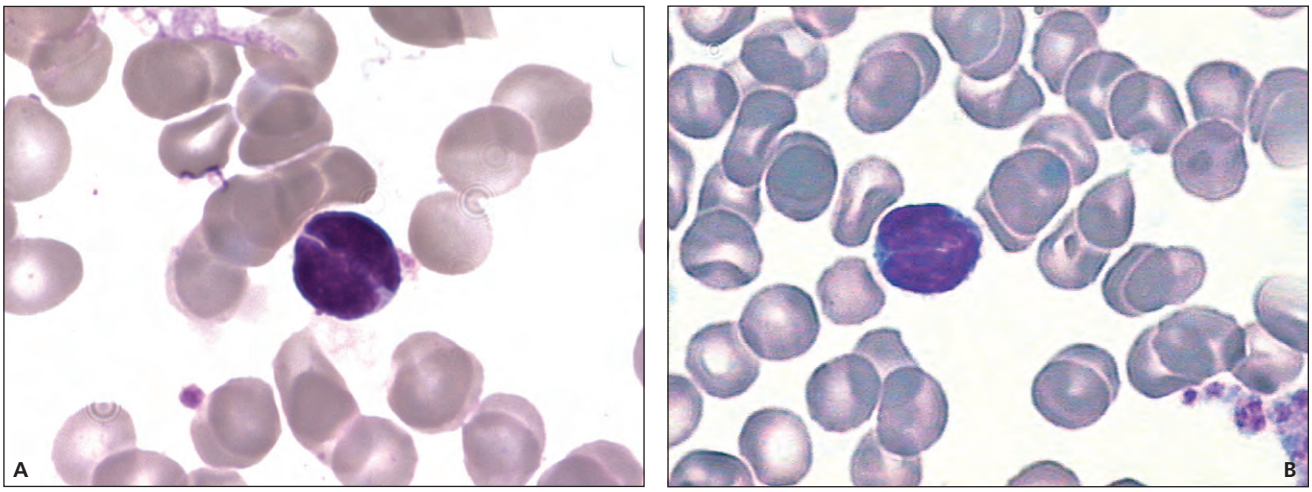
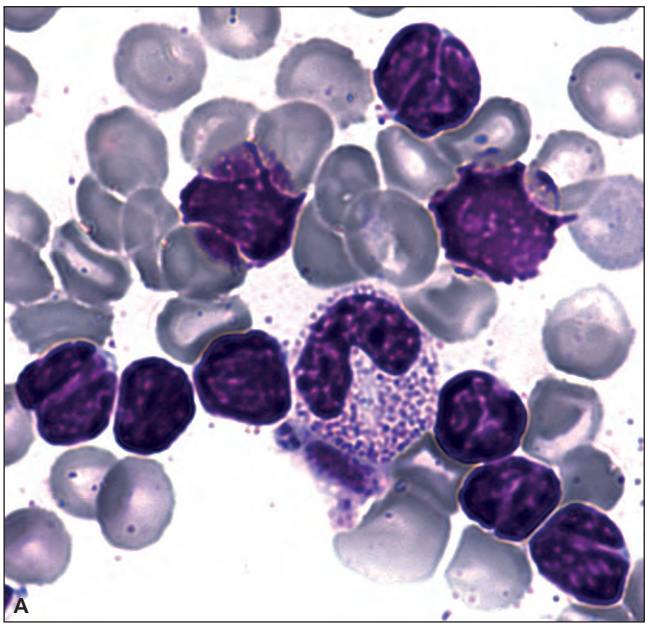
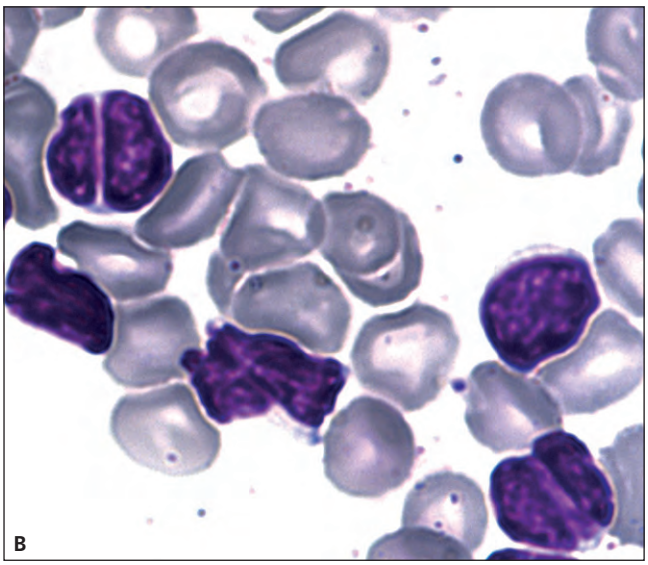
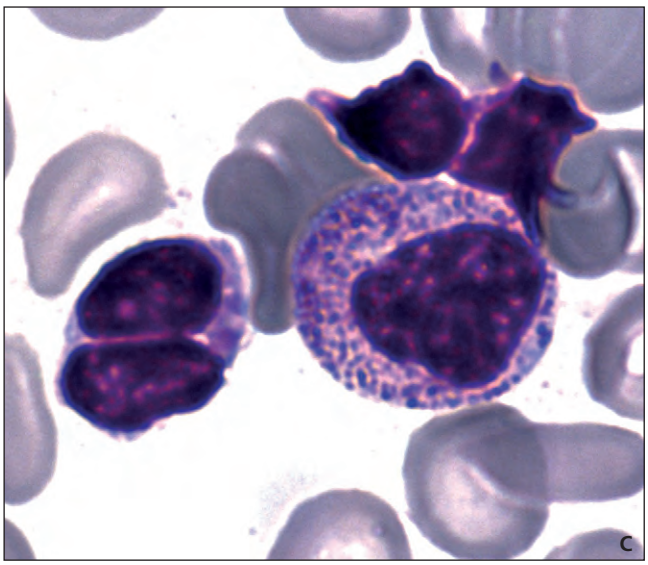



**Fig. 9.43** Cells resembling centrocytes in a follicular lymphoma; peripheral blood film. (A) The cells are slightly larger than the red cells. The coffee bean-shaped nucleus appears to be split into two small semi-spheres by a long straight incision, which traverses it: the two semi-nuclei remain joined in the centre. The cytoplasm is extremely reduced and is visible only in the space left free in the two notches at opposite poles of the cell. (B) The nucleus is split into two lobes, which remain joined on the right. The cytoplasm is scanty and basophilic.

**Fig. 9.44** Follicular lymphoma; bone marrow aspirate. (A) The appearance of these small lymphoid cells is monomorphic and quite characteristic: small (evident by comparison with the band neutrophil), cytoplasm almost invisible and with condensed chromatin and a deep cleft that divides the nucleus. (B) In some lymphocytes (top left), the separation between the two half-nuclei is almost complete; in others (bottom right) there is more the appearance of a valley. (C) On the left, the two half-nuclei adhere to each other by a long linear area, but are joined only by a small bridge that interrupts the fissure. On the right, a normal sized neutrophil myelocyte.



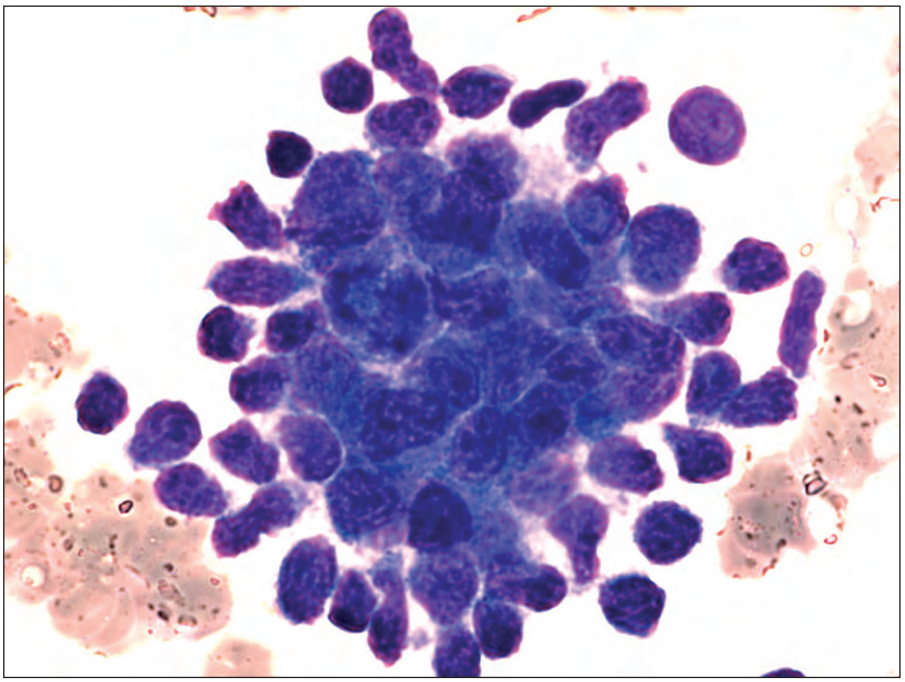


**Fig. 9.45** Follicular lymphoma; bone marrow aspirate. This nodular formation has maintained its cohesion, resisting the negative pressure of the aspiration at the moment when the sample was obtained. The cells that have become detached around the periphery have small hyperchromatic nuclei with almost invisible cytoplasm. In the centre, where the cells are crowded together, there appear to be larger cells with basophilic cytoplasm and nuclei with their chromatin structure preserved.

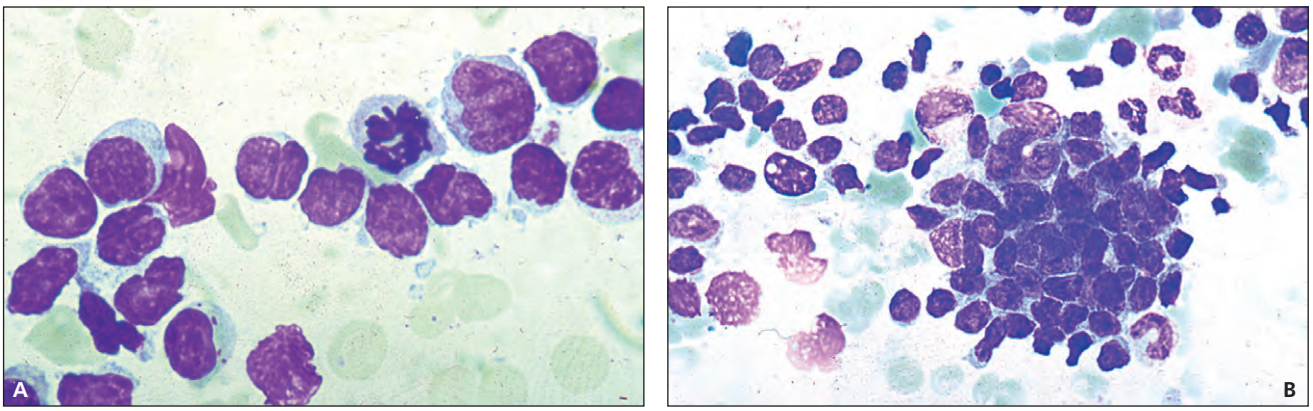



**Fig. 9.46** Follicular lymphoma; bone marrow aspirate. (A)The neoplastic population has heterogeneous morphological characteristics. There is a prevalence of small cleft cells, sometimes with coffee-bean nuclei, together with larger cells with more plentiful basophilic cytoplasm and irregular nuclei. At the bottom, a granular lymphocyte. (B) In the same film as (A), a nodular formation appears to be composed of numerous small cleft cells massed together and superimposed. This appearance can simulate bone marrow metastases from undifferentiated solid tumours. A nodular infiltrate is characteristic of sections of lymph nodes in follicular lymphoma, but is not at all common in the bone marrow.

Bone marrow and peripheral blood involvement is found in 5–10% of cases.[84–86] The neoplastic cells are heterogeneous and pleomorphic with an admixture of small lymphocytes, lymphoplasmacytoid cells and cells with ample ragged cytoplasm and a grooved bean-shaped nucleus. This condition is distinguished from HCL by the absence of bone marrow fibrosis and by the immunophenotype (presence of pan-B antigens, absence of CD5, CD10 and CD25; possible positivity for CD11c and CD43) and by bone marrow histology.

## Lymphoplasmacytic lymphoma and Waldenström macroglobulinaemia

### Lymphoplasmacytic lymphoma

LPL, also designated immunocytoma, is a low grade malignancy. The pathological cell is a small lymphocyte that shows morphological and functional features of frankly lymphoplasmacytic and plasmacytic differentiation, with the frequent presence of a monoclonal band in the serum or urine.[87] Bone marrow involvement is not common and peripheral blood

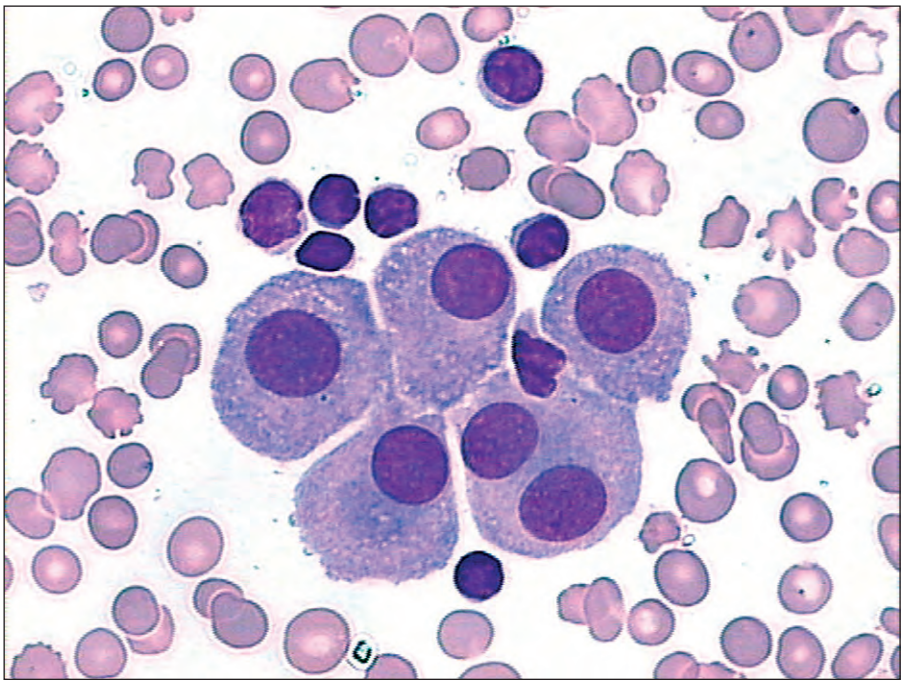


**Fig. 9.47** Follicular lymphoma; pleural aspirate. At the top, small lymphoma cells with compact nuclei, sometimes notched, and scanty cytoplasm. At the bottom, five exfoliated pleural mesothelial cells, one of which is binucleated: their nuclei are round and spongy; the cytoplasm is very plentiful, finely vacuolated and variegated with tinges of rose and lilac.

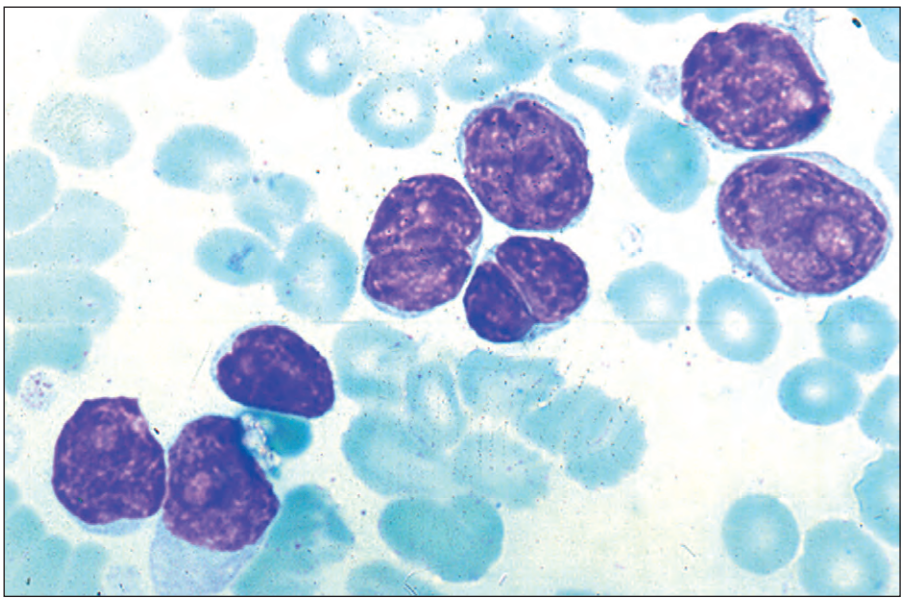


**Fig. 9.48** Follicular lymphoma of mixed cell type (centrocytic-centroblastic); peripheral blood film. The neoplastic population is heterogeneous, with the presence of cleft cells, both small and medium sized, together with cells resembling prolymphocytes and larger cells with homogeneous chromatin and visible nucleoli.

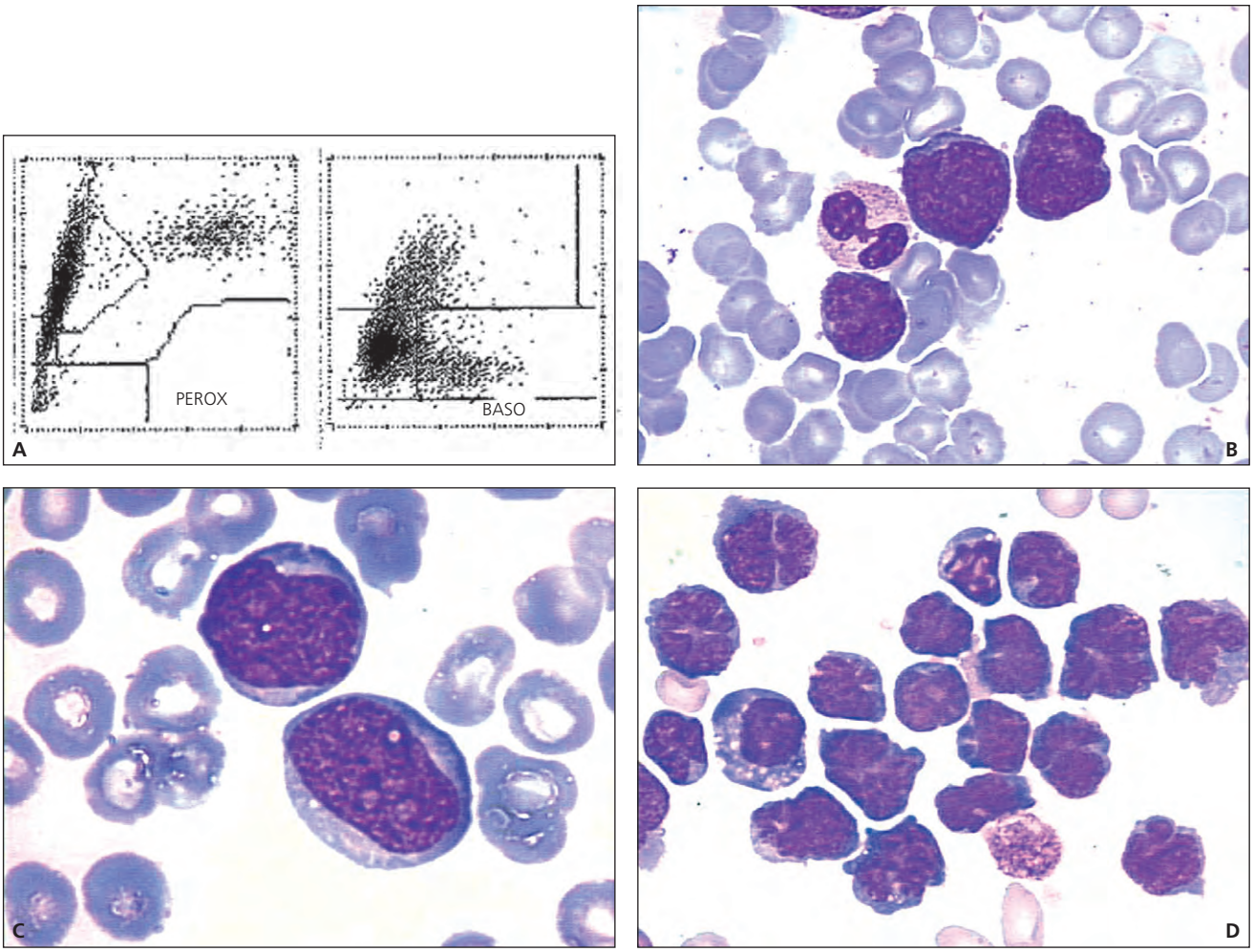



**Fig. 9.49** Follicular lymphoma, centroblastic: presentation with stage IV disease in a patient aged 45 years with fever, hepatosplenomegaly and generalised lymphadenopathy. Blood count: Hb 80 g/l, leucocyte count 18.3 × $10^9$/l (89% 'blasts'), platelets 9 × $10^9$/l. Bone marrow aspirate: very cellular with 31% 'blasts' with irregular nuclei, positive for CD10 and CD19, and a good representation of trilineage haemopoiesis. The diagnosis was made on bone marrow and lymph node sections. FISH was positive for t(14;18). A few days after diagnosis, ptosis appeared together with loss of strength in the lower limbs. (A) The Perox cytogram shows the presence of a considerable population of peroxidase-negative cells in a long thick fusiform shape, inclining to the right in the LUC area and invading the monocyte box; the Baso cytogram shows that the abnormal population is elongating and deforming the MN population, extending into the blast area and also upwards, causing pseudobasophilia. The image recalls that of some acute monoblastic leukaemias. (B) The circulating lymphoma cells are large: the nuclei are irregular; the chromatin is condensed in a very heterogeneous manner without forming large blocks; nucleoli are not apparent; the cytoplasm is scanty and basophilic. (C) Some cells have a more frankly blastic appearance with open chromatin, formed of a thick network, with nucleoli clearly seen. (D) Cytocentrifuge preparation of cerebrospinal fluid (CSF), obtained after the appearance of a neurological disorder, shows the presence of the same lymphoma cells (172/$mm^3$), polymorphic with nuclei that are complex and multilobated, as is often observed in effusions and body fluids other than the blood.

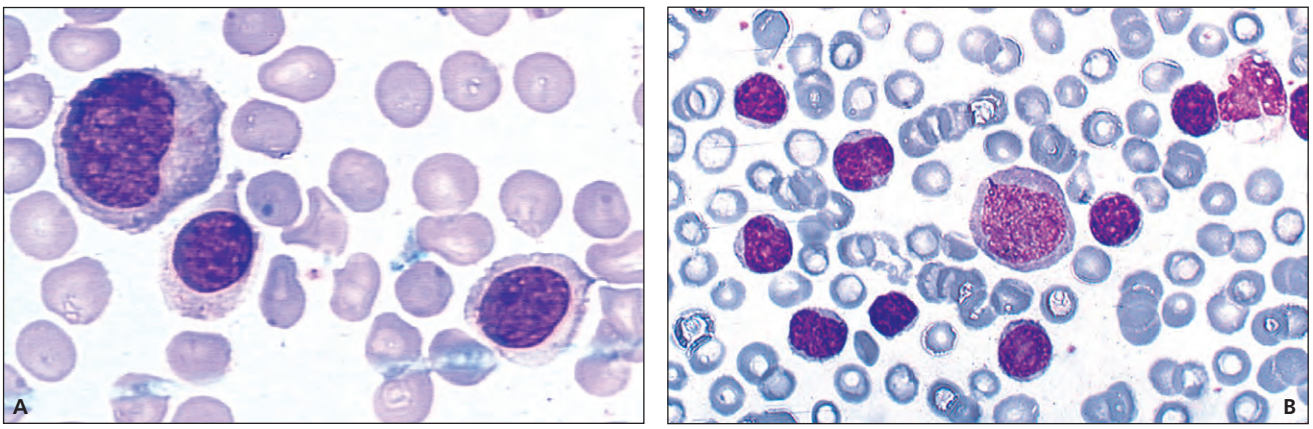



**Fig. 9.50** Mantle cell lymphoma (MCL); peripheral blood films of two patients. (A) The lymphoma cells vary in shape and size. The largest cell, on the left, has plentiful basophilic cytoplasm with a pale area adjacent to the nuclear indentation. The chromatin is condensed into small irregular knots mixed in a disorderly manner with small pale circles; there are at least two nucleoli, but they are not prominent. The two smaller cells, similar to each other, have oval nuclei, clumped chromatin typical of a lymphocyte and pale cytoplasm. (B) A large immunoblast-like cell (granular homogeneous chromatin, two nucleoli and plentiful intensely basophilic cytoplasm) is surrounded by heterogeneous lymphoid cells with condensed chromatin, variably indented nuclei and occasionally a nucleolus. Top right, a vacuolated monocyte.

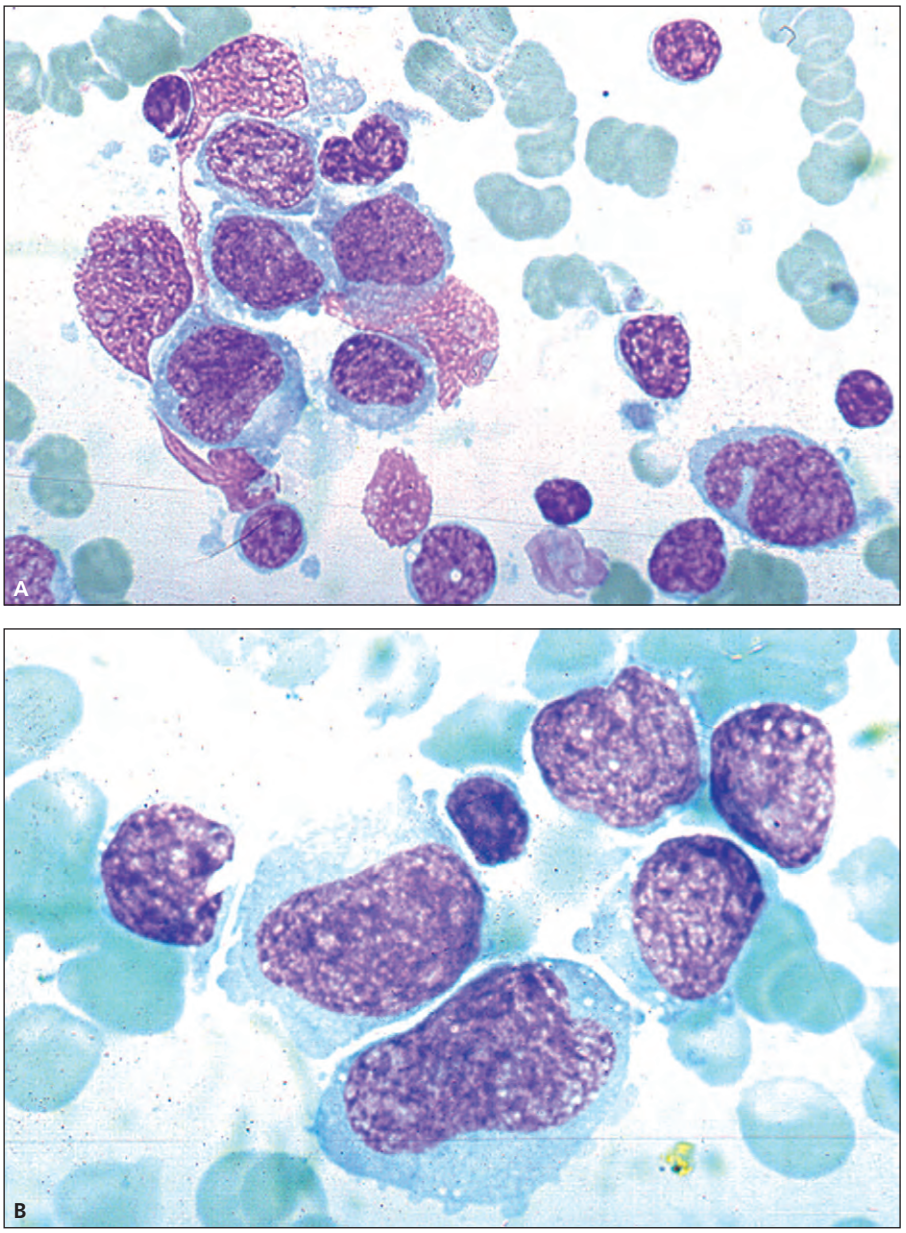



**Fig. 9.51** MCL; bone marrow aspirate. (A) Considerable heterogeneity and polymorphism of the lymphomatous population that is infiltrating the bone marrow. Some cells appear frankly blastic with finely reticular, granular chromatin; others are large with plentiful basophilic cytoplasm and an irregular convoluted or lobulated nucleus; yet others have the appearance of lymphocytes and prolymphocytes. (B) Same preparation as (A). Two large lymphoma cells with plentiful basophilic cytoplasm together with other smaller cells with heterogeneous chromatin and indistinct nucleoli; there is also a small mature lymphocyte.

involvement is unusual. The leucocyte count is rarely above $10 \times 10^9$/l. The neoplastic population is heterogeneous (Figs 9.57, 9.58 and 9.59), covering the full range of maturation from small lymphocytes to mature or atypical plasma cells: the most typical cells are small lymphocytes with plasmacytoid features, such as cytoplasmic basophilia, eccentricity of the nucleus and the presence of an ample pale paranuclear area corresponding to an active Golgi apparatus. Some cells may have amorphous globular or crystalline inclusions. In the bone marrow aspirate, in addition to lymphoplasmacytoid cells in different stages of maturation, there is often an increase in mast cells. LPL has a relationship with splenic lymphoma with villous lymphocytes. Sometimes it is necessary to consider in the differential diagnosis the rare form of CLL in which there is a moderate concentration of a paraprotein.

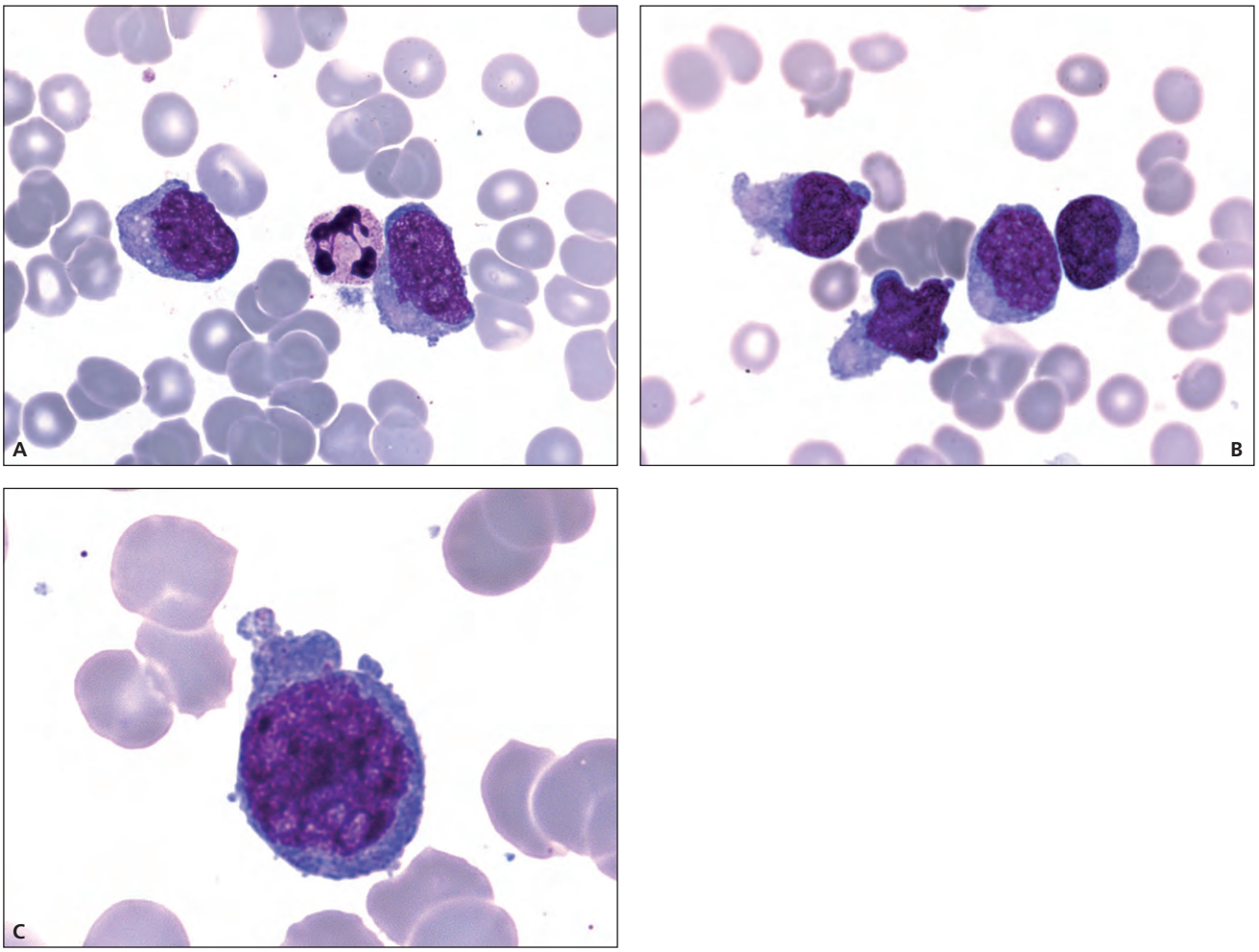



**Fig. 9.52** MCL; peripheral blood film. (A) In comparison with the blast cells of acute myeloid leukaemia, these immature lymphoma cells tend in general to have denser chromatin, with a coarse network or a rough granular appearance deriving from small dark indistinct chromatin clumps. The nuclei, as in these two cells, have an irregular ill-defined outline. The cytoplasm is basophilic and contains small vacuoles. The neutrophil has bizarre nuclear segmentation. (B) One cell has more open chromatin; the other three have compact chromatin and nuclei of quite variable shapes. (C) A large lymphoma cell with ragged basophilic cytoplasm and a nucleus with compact chromatin containing numerous nucleoli.

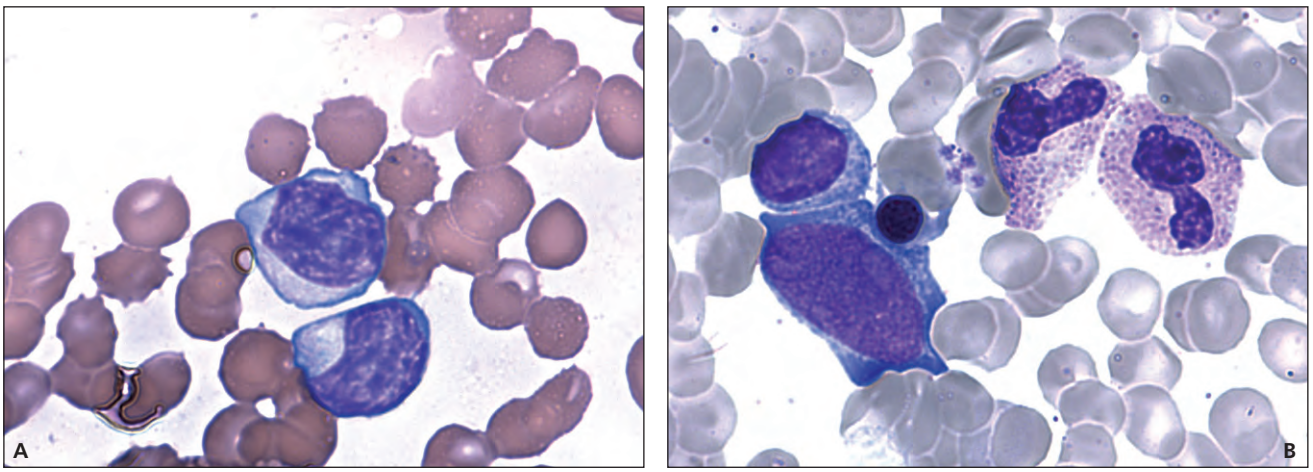



**Fig. 9.53** MCL. (A) Peripheral blood film. Two circulating lymphoma cells with plentiful sky-blue cytoplasm and large nuclei with chromatin condensed into irregular masses between which are pale spaces. (B) Bone marrow aspirate: on the left, a lymphoid cell with a compact nucleus and a large blast with an elongated nucleus, with homogeneous granular chromatin, without clumps or condensation, with three prominent nucleoli; the cytoplasm is intensely basophilic. There is also a small orthochromatic erythroblast, with transparent cytoplasm, and two neutrophil band forms.

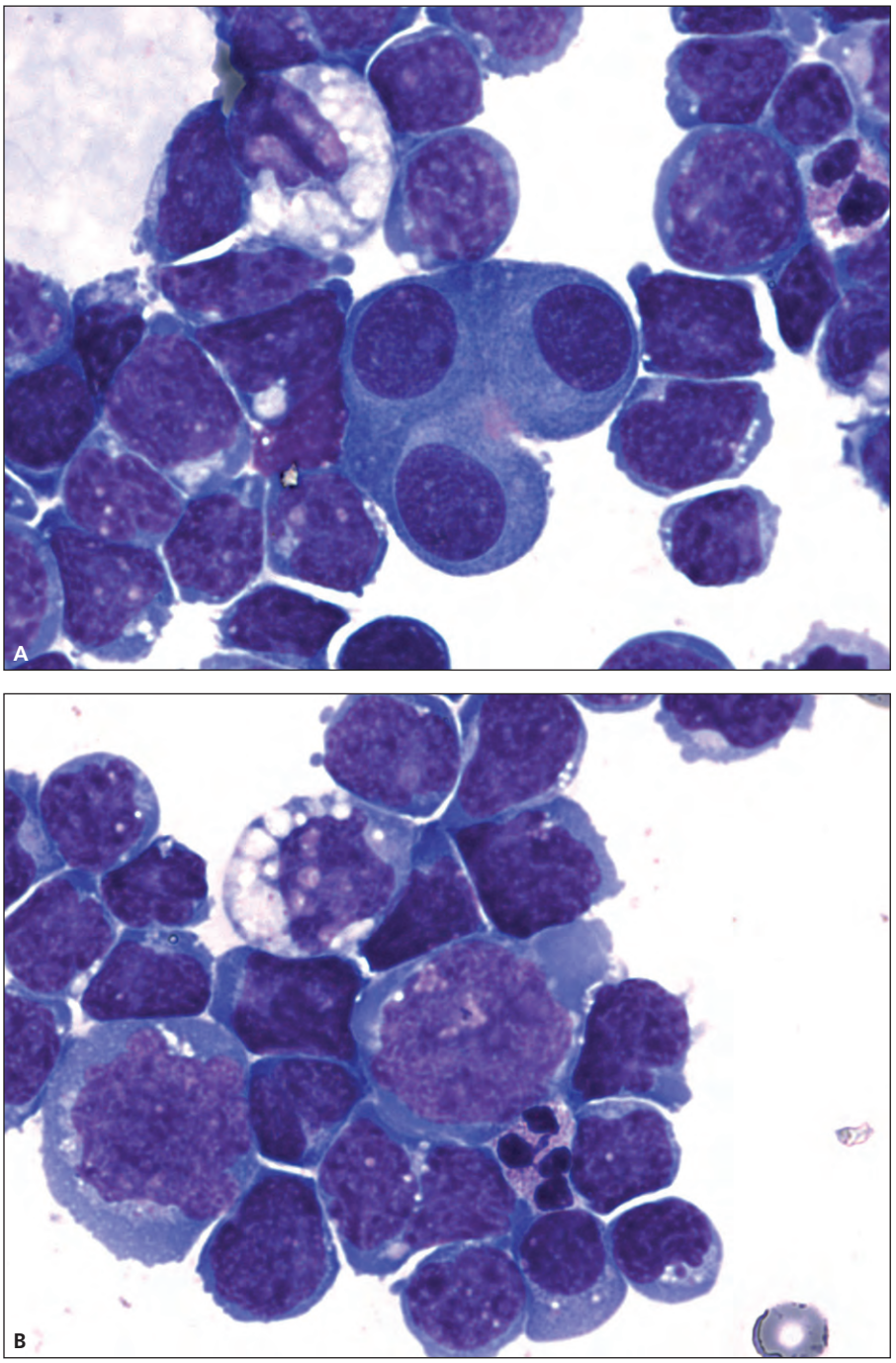



**Fig. 9.54** MCL; same case as Fig. 9.53; pleural effusion. (A) Numerous morphologically heterogeneous lymphoma cells surround a small apparent syncytium of three mesothelial cells. (B) Two of the lymphoma cells appear anaplastic, being large with irregularly shaped hyperdiploid nuclei, with delicate chromatin, multiple nucleoli and intensely basophilic cytoplasm.

The immunophenotype usually shows positivity for pan-B antigens, including CD22, for FMC7 and also for cytoplasmic immunoglobulin and the markers characteristic of plasma cell leukaemia, CD38, CD25 and CD71. CD5, CD10, CD23 and CD5 are not expressed.

### Waldenström macroglobulinaemia

Although continuing to be considered a discrete clinicopathological entity, Waldenström macroglobulinaemia is defined in the 2008 WHO classification, on the basis of the recommendation of a specific expert Consensus Panel,[88] as an LPL with

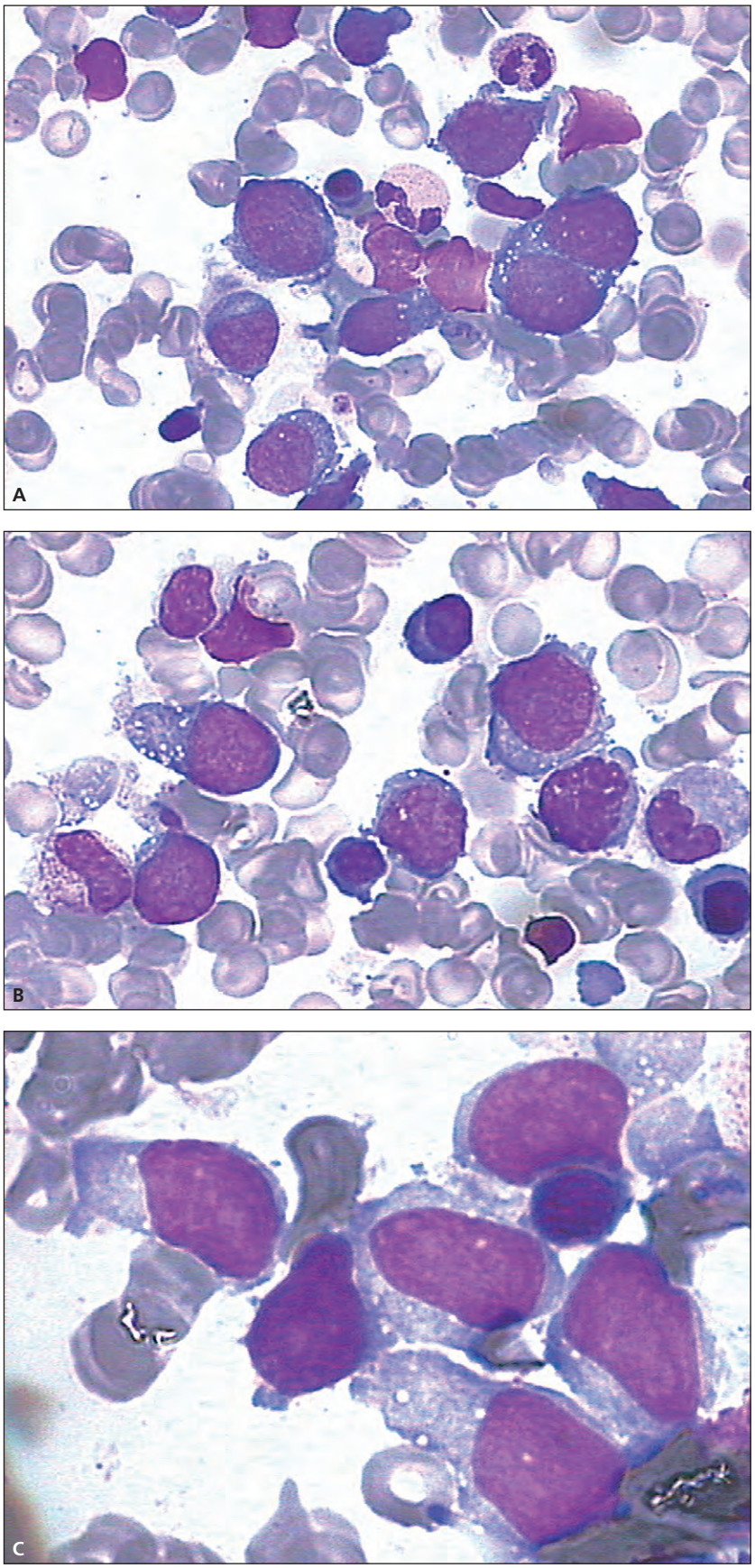



**Fig. 9.55** Patient who presented with generalised lymphadenopathy, anaemia and severe thrombocytopenia; WBC 12.5 × $10^9$/l, with 61% atypical blast-like cells, positive for CD5 and CD20, which also infiltrated the bone marrow (22% of nucleated cells, with some lymphomatous foci). The final diagnosis was blastoid variant of MCL. Bone marrow aspirate. (A) The marrow appears to be invaded by large blast cells, with plentiful intensely basophilic, partly vacuolated cytoplasm and large oval nuclei with dispersed delicate chromatin and nucleoli. On the right, a giant binucleated cell. (B) Various blast cells, the morphology of which resembles that of Burkitt lymphoma cells; two small plasma cells. (C) A small group of blasts with vacuolated blue cytoplasm. The chromatin is very homogeneous and the nucleoli are small and indistinct.

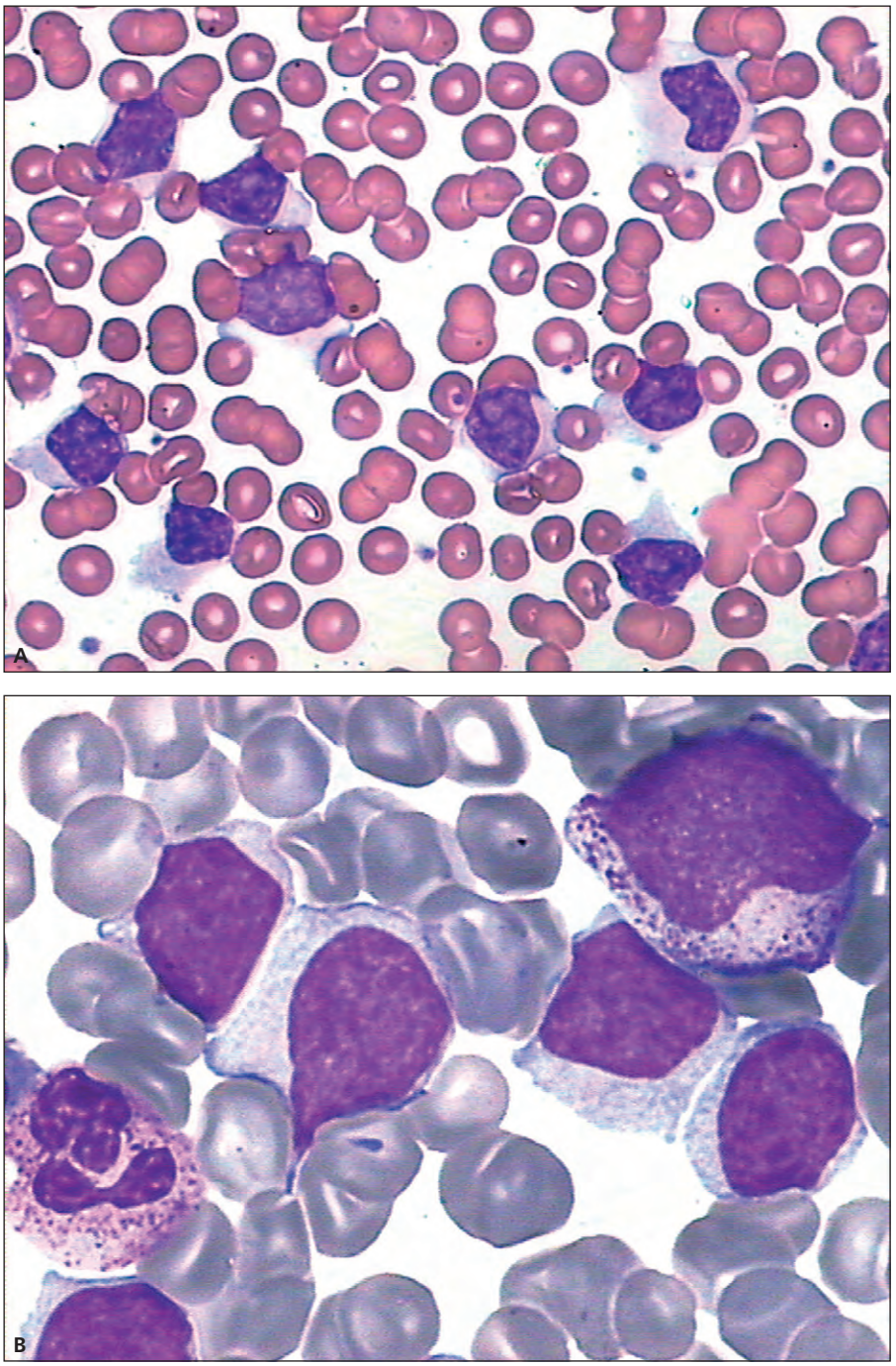



**Fig. 9.56** Another example of the leukaemic form of blastoid MCL, pleomorphic subtype (also known as prolymphocytoid subtype). A routine blood count showed marked leucocytosis, WBC 128 × $10^9$/l. Clinical examination showed minor submandibular and cervical lymphadenopathy. FISH analysis was positive for t(11;14)(q13;q32). (A) Peripheral blood film. The cells, CD5 and CD19 positive, resemble prolymphocytes, but do not have the orderly monomorphism of B-cell PLL. The nuclei vary from medium sized to large, with the nuclear outline sometimes being indented, angulated or folded; the chromatin is irregularly condensed and not homogeneous with the nucleoli often being prominent. There is abundant pale cytoplasm. (B) Bone marrow aspirate. The cells often appear less spread out and the chromatin appears denser; nucleoli are not visible. The pale watery cytoplasm is well represented. On the left, a segmented neutrophil. Top right, a neutrophil myelocyte.

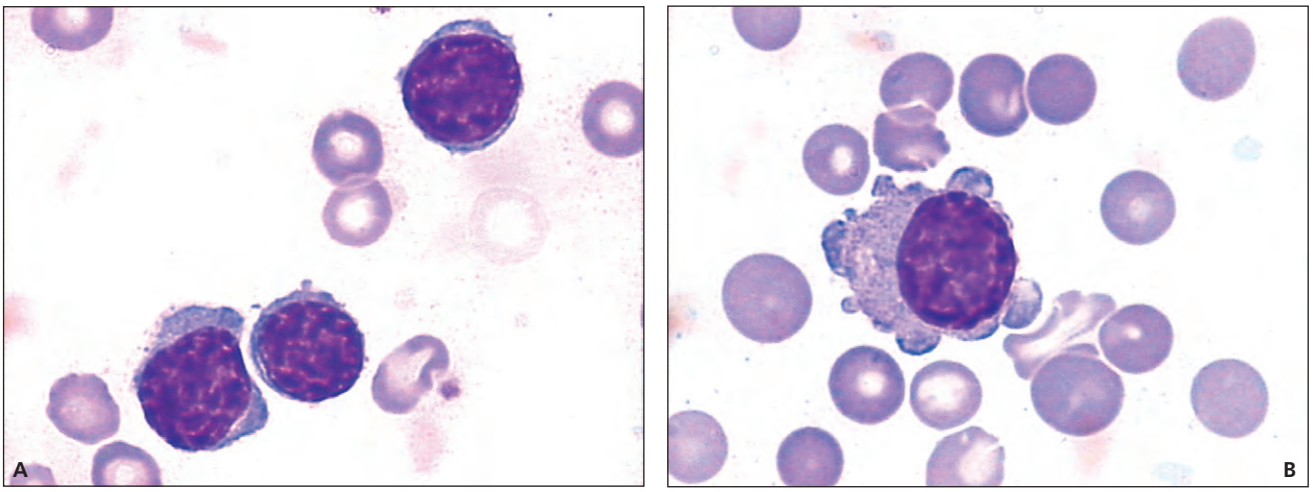



**Fig. 9.57** Lymphoplasmacytic lymphoma (LPL); peripheral blood film. (A) These cells are very similar to the lymphocytes of B-CLL because of the mosaic pattern of the chromatin and the scarcity of the cytoplasm. However, their size, deduced by comparison with the red cells, is greater. (B) Some cells have a lymphoplasmacytoid appearance, with plentiful cytoplasm, which has formed numerous blebs, and an eccentric nucleus with coarsely clumped chromatin.

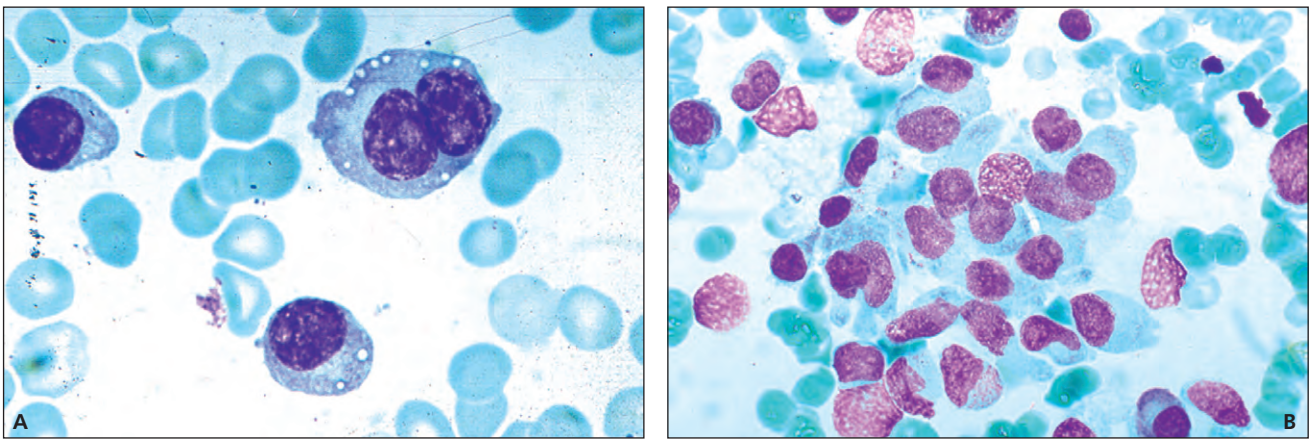



**Fig. 9.58** LPL. (A) Peripheral blood film. Top left, a plasmacytoid lymphocyte, at the bottom a small plasma cell, top right a large binucleated plasma cell. The three cells appear to belong to the same pathological clone since they show striking morphological analogies (eccentric nuclei with chromatin in blocks and intense basophilia) differing from each other mainly in the cytoplasmic characteristics, the cytoplasm in the plasma cell being more plentiful and containing small Russell bodies. (B) Bone marrow aspirate. A cluster of cells with plentiful glassy basophilic cytoplasm, sometimes with prominent arcoplasm; the nucleus is oval and eccentric with delicate granular homogeneous chromatin and frequent nucleoli. The morphology is analogous to that of an immature plasma cell.

involvement of the bone marrow and with an IgM monoclonal gammopathy, regardless of the level of the IgM paraprotein in the serum.[88–90] It is no longer included in the group of plasma cell neoplasms. This condition has cytomorphological and other laboratory peculiarities and therefore will be described separately, as follows.

### *Blood count and peripheral blood film*

LPL that present as Waldenström macroglobulinaemia are generally associated with a normocytic normochromic anaemia that is multifactorial in origin (reduced haemopoiesis, haemolysis and repeated bleeding from the gastrointestinal tract and other mucosal membranes). The WBC is normal or reduced: neutropenia is common. The platelet count can be normal or reduced; around a half of patients have a haemorrhagic diathesis as a result of interference by the monoclonal protein with coagulation factors and with platelet function: defects in adhesion and aggregation, prolonged bleeding time and prolonged thrombin time.

On blood film examination, the red cells show increased rouleaux formation, the rouleaux sometimes forming masses, because of the presence of the macroglobulin, which is also responsible for the greyish-blue background staining (Fig. 9.60). Sometimes there are crystalline precipitates. In rare cases in

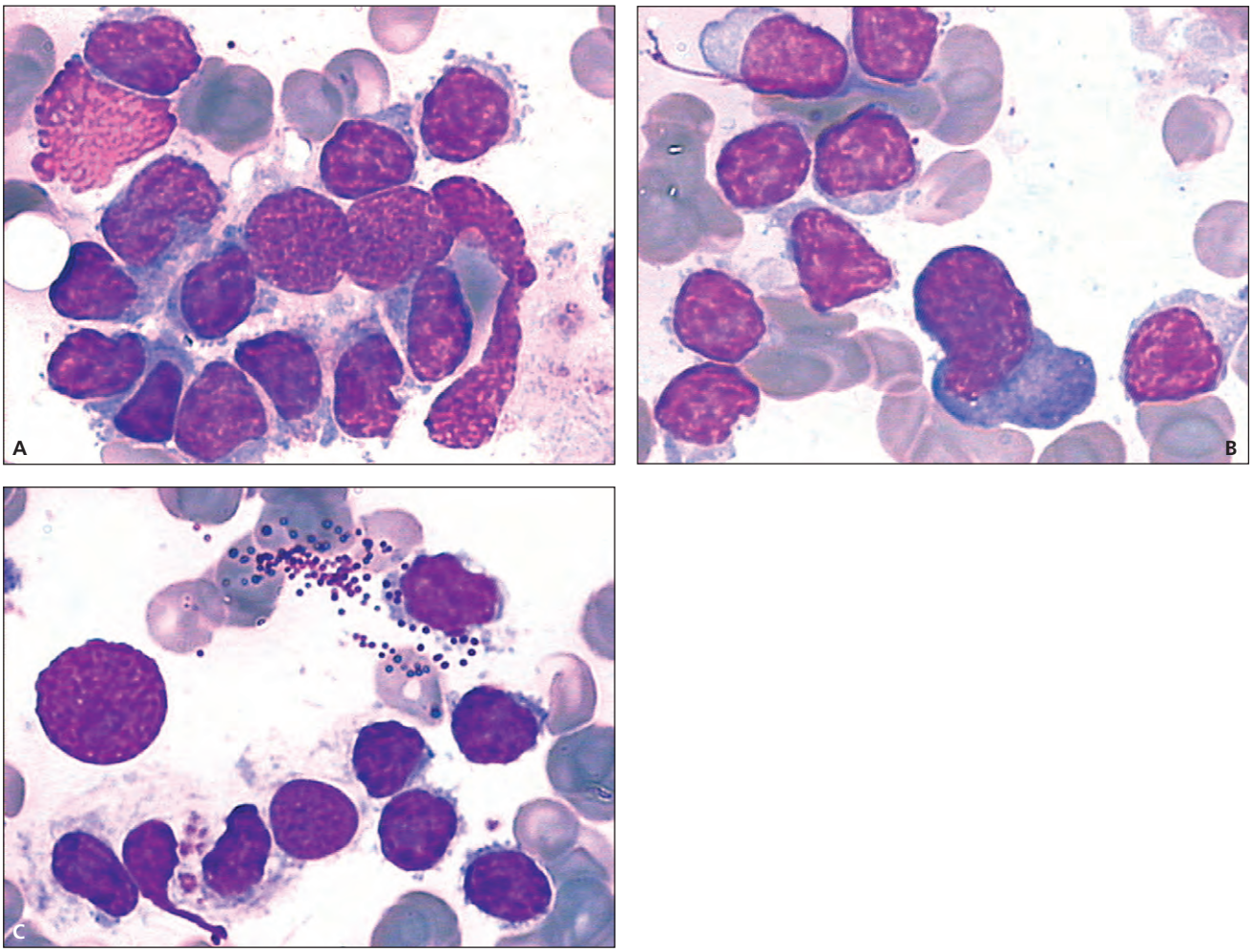



**Fig. 9.59** LPL; bone marrow aspirate. (A) The lymphoma cells have formed a small cohesive aggregate; they are lymphoplasmacytoid by virtue of their basophilic, albeit scanty, cytoplasm. (B) Most of the cells are small lymphocytes with heterogeneously condensed chromatin; one distorted cell is clearly plasmacytoid, with an eccentric nucleus and plentiful blue cytoplasm with arcoplasm. (C) At the top, above numerous mature lymphocytes with scanty basophilic cytoplasm, is the nucleus of a crushed mast cell around which are scattered typical mast cell granules.

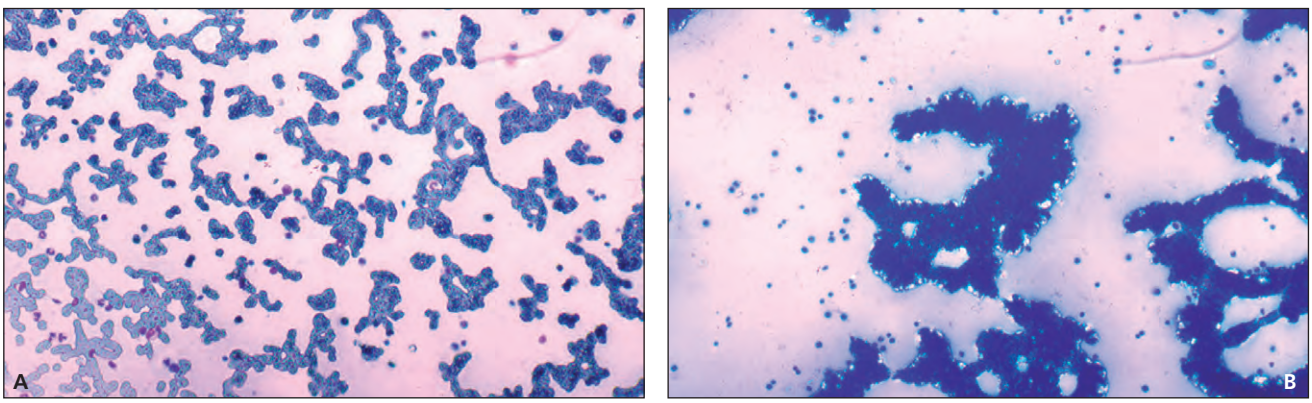



**Fig. 9.60** Waldenström macroglobulinaemia. (A) Peripheral blood film. Marked rouleaux formation. This phenomenon is responsible for the striking increase in the erythrocyte sedimentation rate that characterises this disorder. (B) Bone marrow aspirate. The appearance of the film at low power is often characteristic because of the presence of the blue, greyish or rose background staining attributable to the presence of a veil of amorphous material. The red cells are forming massive rouleaux.

which the monoclonal component is a cryoglobulin, there are protein precipitates which, when numerous, can interfere with cell counts on automated instruments. In these cases, the blood specimen must be brought to a temperature of 37 °C before analysis. The erythrocyte sedimentation rate is always very high. Sometimes there is erythrophagocytosis by circulating neutrophils and monocytes. With disease progression, the neutropenia is accentuated and the peripheral blood may be invaded by lymphoid cells with the same cytological features and immunophenotype as those in the bone marrow.

***Bone marrow morphology***

In some patients there is a dry tap when bone marrow aspiration is attempted, resulting from the marked hyperviscosity of tissue fluids that permeate the matrix of the bone marrow. In other cases there is detection of infiltration by an abnormal lymphoid population (Figs 9.60 through to 9.63), which in more than 50% of patients have cytological features intermediate between those of small lymphocytes and mature plasma cells: lymphoplasmacytoid cells. In around 30% of patients, the neoplastic population shows more plasma cell differentiation, while the remaining cases have lymphocyte morphology similar to that of CLL. There may be intensely PAS-positive cytoplasmic inclusions and Dutcher bodies. In addition to the predominant cell population, the films of patients with macroglobulinaemia always show an increase of morphologically normal small lymphocytes and mature plasma cells; there may also be some immunoblasts. A typical feature is an increase of basophils and, particularly, of mast cells, which in general are closely associated with the neoplastic population.

The erythroid and granulocytic lineages show quantitative alterations; there is often an increase in eosinophils. The megakaryocytes are present in good numbers, often contrasting with the peripheral thrombocytopenia contributing to a haemorrhagic diathesis.

## Heavy chain diseases

This term includes three very rare clinicopathological entities, characterised by the production, by a heterogeneous clonal B-cell population, of one of three types of immunoglobulin heavy chain. In the serum there is a monoclonal component constituted, respectively, by the gamma chain of IgG, the alpha chain of IgA or the mu chain of IgM. The 2008 WHO classification includes these conditions within the group of neoplasms of mature B cells.[91]

Alpha heavy chain disease, also designated immunoproliferative small intestinal disease (IPSID), is a variant of MALT lymphoma and hardly ever involves the bone marrow or the peripheral blood. Gamma heavy chain disease is characterised by a pleomorphic proliferation, mainly in lymph nodes, of mature lymphocytes, lymphoplasmacytoid cells, plasma cells and immunoblasts, together with eosinophils and histiocytes. The infiltration can involve the bone marrow, where sometimes it simulates multiple myeloma because of the prevalence of plasma cells, or the peripheral blood, in which case there is lymphocytosis with atypical plasmacytoid lymphocytes. The pathological cells do not express SmIg but have cytoplasmic gamma heavy chain; they are also positive for CD20 and CD79a (in the lymphoid cells) and for CD138 (in the plasma cells). The very rare mu heavy chain disease is characterised by a neoplastic expansion that is haematologically similar to CLL, with lymphoid cells that are CD5 and CD10 negative, but which express cytoplasmic mu chain and secrete an M component into the serum.

## Diffuse large B-cell lymphoma, not otherwise specified

The 2008 WHO classification lists under the designation diffuse large B-cell lymphoma, not otherwise specified (DLBCL, NOS), numerous very aggressive B-cell neoplasms (see Table 9.1), derived from B-lineage cells at various levels of differentiation.[92] This is the most frequent type of NHL, which sometimes represents transformation of a lower grade lymphoid neoplasm, such as follicular lymphoma or CLL (in the latter instance referred to as Richter syndrome). From the genetic point of view, there are aberrations in *BCL6* in 30% of cases.

DLBCL can involve lymph nodes, but also many other tissues and organs. The bone marrow and peripheral blood can be involved, although rarely as the primary site, by large cells with abundant variably basophilic cytoplasm and large regular or convoluted nuclei with very dispersed loose chromatin and quite prominent easily seen nucleoli (Figs 9.64 and 9.65; see Report 9.3). In these cases, the differential diagnosis with acute leukaemia is not easy and often depends on the demonstration of a mature-B immunophenotype (SmIg, CD19 and CD20). Much more common is a leukaemic phase during disease evolution, occurring at an advanced, often terminal, stage of the disease: in these cases the lymphoma cells are often large, very immature, polyploid or binucleated or very anaplastic[93,94] (Fig. 9.66).

## Burkitt lymphoma

Burkitt lymphoma is an aggressive neoplasm characterised by a monomorphic proliferation of B cells with a mature immunophenotype and, in the majority of cases, with a chromosomal translocation that involves *MYC*.[95] Epidemiology shows that there are three subtypes which have analogous morphological, immunophenotypic and cytogenetic features: the endemic African form, associated with endemic malaria and correlating with EBV infection; the sporadic form, which affects the rest of the world, particularly Europe and North America, much less often associated with EBV infection; the form that occurs in HIV-infected subjects, in which EBV is implicated in around 40% of cases.

The endemic form is leukaemic in about 15% of cases with infiltration of the bone marrow and peripheral blood involvement by blast cells with dispersed chromatin and basophilic vacuolated cytoplasm (Figs 9.67 through to 9.74; see Report 9.4). This picture, indicated in the WHO classification as the

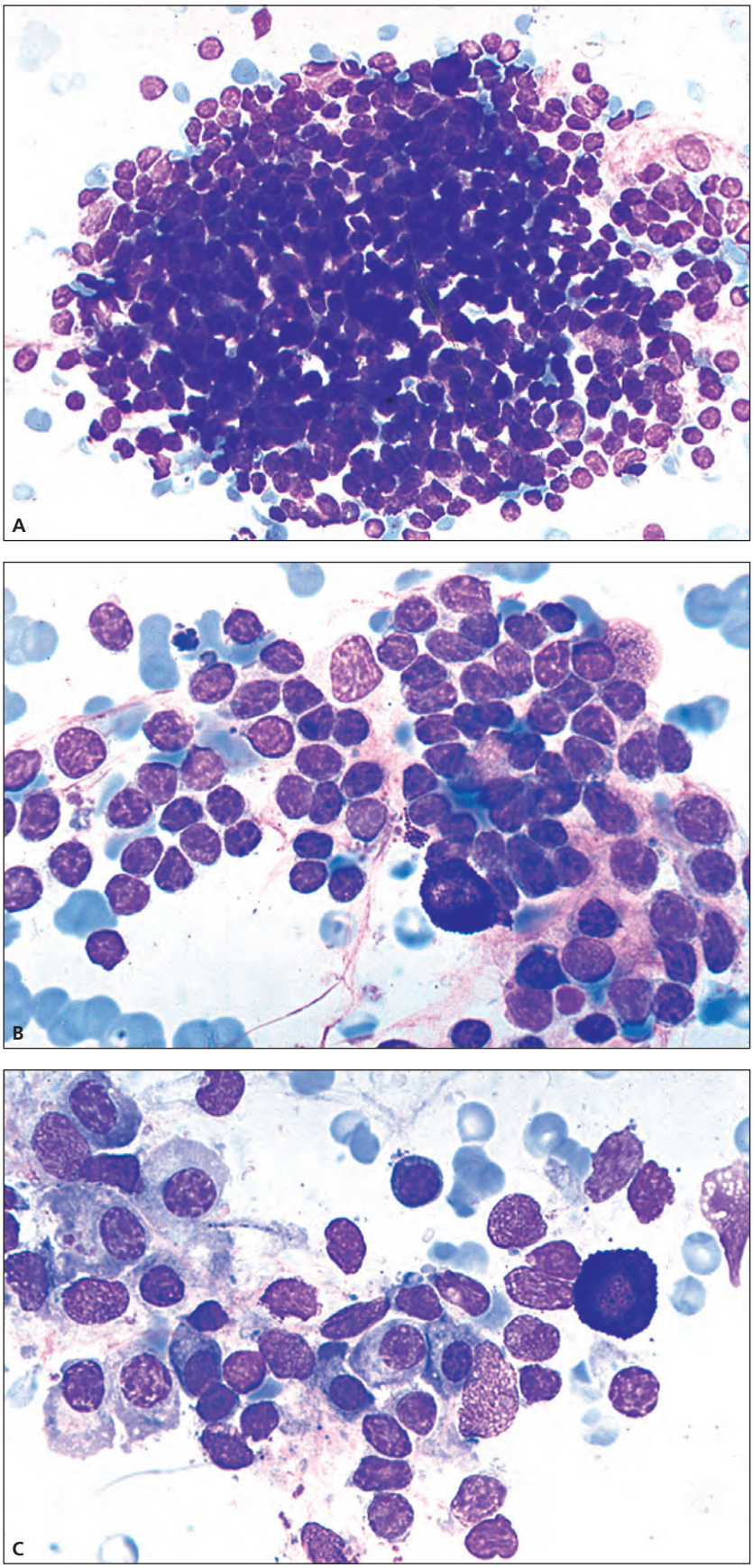



**Fig. 9.61** Waldenström macroglobulinaemia; bone marrow aspirate. (A) Only rarely in cytological preparations can one observe masses of lymphoid cells that are conserving the nodular architecture seen in histological sections. The small cells of this nodule are monomorphic, as is clear in the tail of the film. (B) The pathological population that is diffusely infiltrating the bone marrow appears to be largely composed of small lymphocytes with almost no cytoplasm. One notes the tendency of the chromatin in some cells to condense into polygonal blocks that recall the structure of a plasma cell nucleus. Downwards and towards the right from the centre, a mast cell. (C) In this field the cytological composition is more heterogeneous. One observes, particularly in the centre and on the left, various plasma cells with plentiful weakly basophilic cytoplasm and an eccentric nucleus with chromatin condensed into polygonal blocks; there are other more mature plasma cells with intensely blue cytoplasm; there are also various lymphocytes with scanty cytoplasm, more numerous at the bottom; a mast cell is seen on the right.

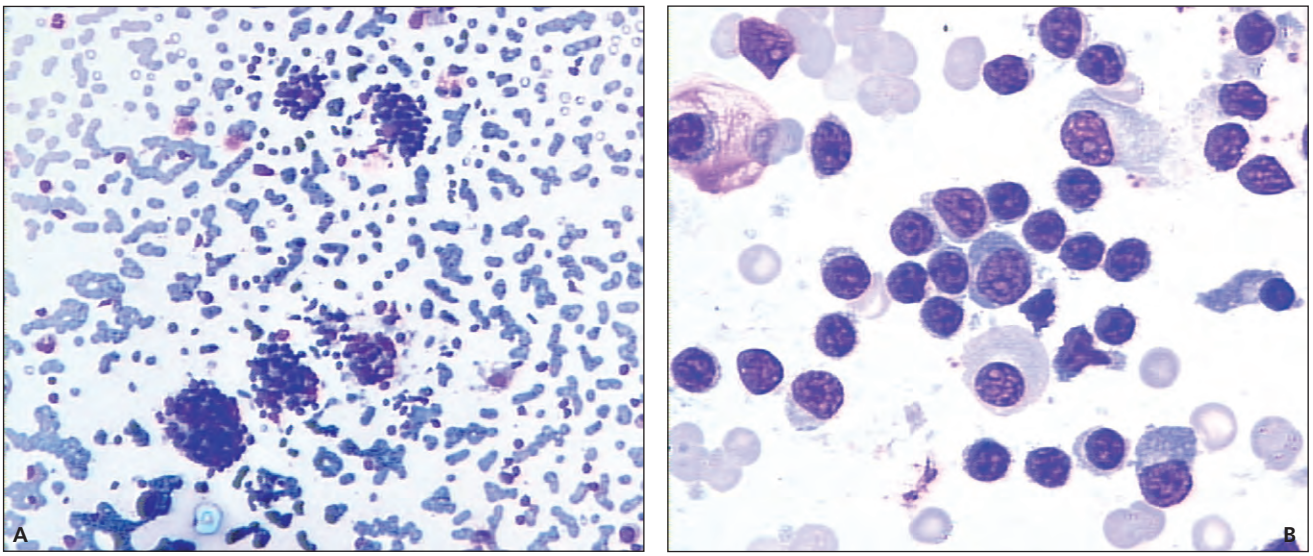



**Fig. 9.62** Waldenström macroglobulinaemia; bone marrow aspirate. (A) A number of nodular formations of variably sized neoplastic lymphocytes. One notes the tendency of the red cells to form rouleaux. This picture could be confused with bone marrow metastases. (B) The lymphoid population infiltrating the bone marrow is mainly composed of small lymphocytes which have nuclei with condensed chromatin; the cytoplasm is generally scanty but sometimes is more extensive and lymphoplasmacytoid. There are three plasma cells, which vary in their morphology and their degree of maturity; in the centre is a nucleolated plasmablast.

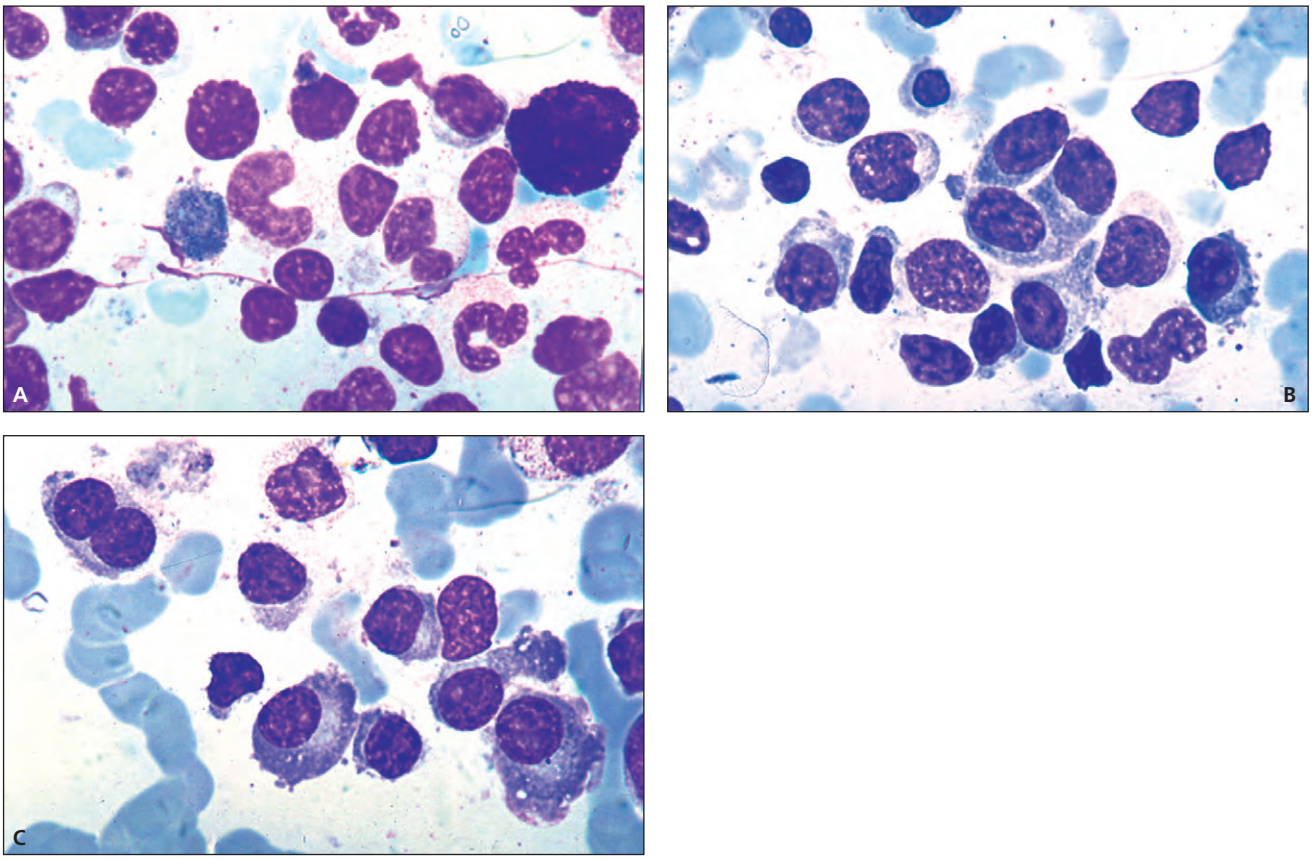



**Fig. 9.63** Waldenström macroglobulinaemia; bone marrow aspirate. (A) Not infrequently, the increased density of the plasma in which the neoplastic cells are suspended favours their rupture during the making of a film. Here there are numerous dense, rounded nuclei without any apparent cytoplasm. Top right, a mast cell with a nucleus that is invisible because of the large number of almost black granules massed in the cytoplasm. (B) The majority of cells have typical lymphoplasmacytoid morphology, with weakly basophilic cytoplasm and an eccentric nucleus with condensed chromatin. (C) In another field of the same film, two plasma cells in the lower area, with plentiful basophilic cytoplasm, a clear Golgi zone and an eccentric nucleus with chromatin condensed into large polygonal blocks. The other cells are mainly plasmacytoid lymphocytes, one of which, top left, is binucleated.

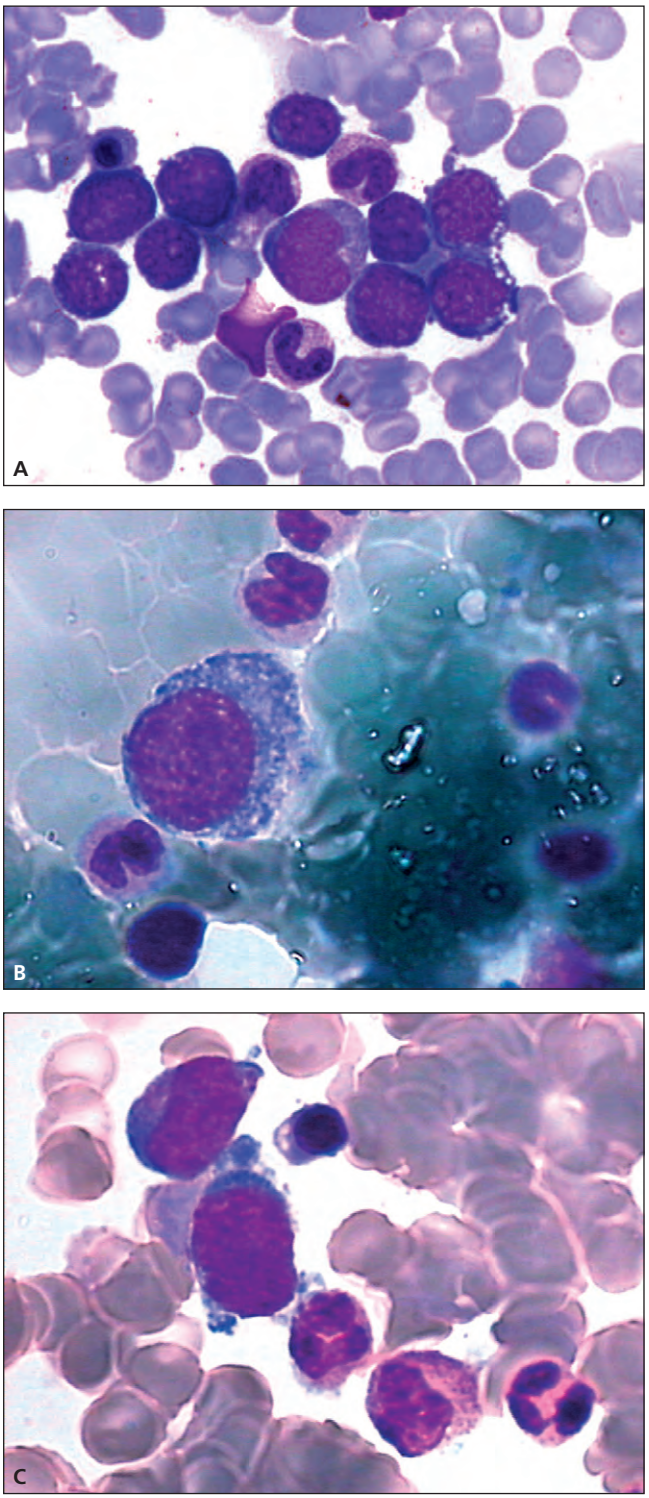



**Fig. 9.64** Diffuse large B-cell lymphoma (different cases); bone marrow aspirates. (A) The lymphoma cells are blast cells of variable size, with intensely basophilic, sometimes vacuolated, cytoplasm, with large oval or indented nuclei, with nucleoli. The N:C ratio is very high. (B) This enormous lymphoma cell has abundant, finely vacuolated, basophilic cytoplasm. The nucleus is a voluminous sphere with heterogeneously condensed compact chromatin. (C) Two neoplastic cells with large oval nuclei and deep blue cytoplasm, which is forming blebs.

leukaemic variant of Burkitt lymphoma,[96] was for a long time classified as L3 ALL in the FAB classification,[97] but should no longer be classified in this manner. The blast cells are large and homogeneous, with a fairly low N:C ratio. The nucleus is round or oval, regular and clearly outlined. The chromatin is fine and homogeneous; one or two prominent, often vesicular nucleoli stand out clearly from the background. The cytoplasm is fairly plentiful, completely surrounding the nucleus and having a very typical appearance with intense basophilia and numerous vacuoles. These morphological characteristics occur in a fairly consistent manner in individual cells. In the bone marrow aspirate, numerous mitoses are always apparent. The cytological features are sufficiently characteristic that the immunophenotypic and cytogenetic features can be predicted.

The histological picture in sections of trephine biopsy specimens or aspirated particles is analogous to that of Burkitt lymphoma in lymph nodes or other tissues. There is a monomorphic infiltrate composed of medium-sized cells with dense chromatin and prominent basophilic nucleoli. The cytoplasm is intensely basophilic with squared off cytoplasm, like the tiles of a mosaic. There are numerous mitotic figures. The typical 'starry-sky' appearance results from the presence of numerous macrophages dispersed through the lymphomatous infiltrate; these are laden with cellular debris, reflecting the high rate of apoptosis and karyorrhexis that characterises this lymphoma.

### Immunophenotype

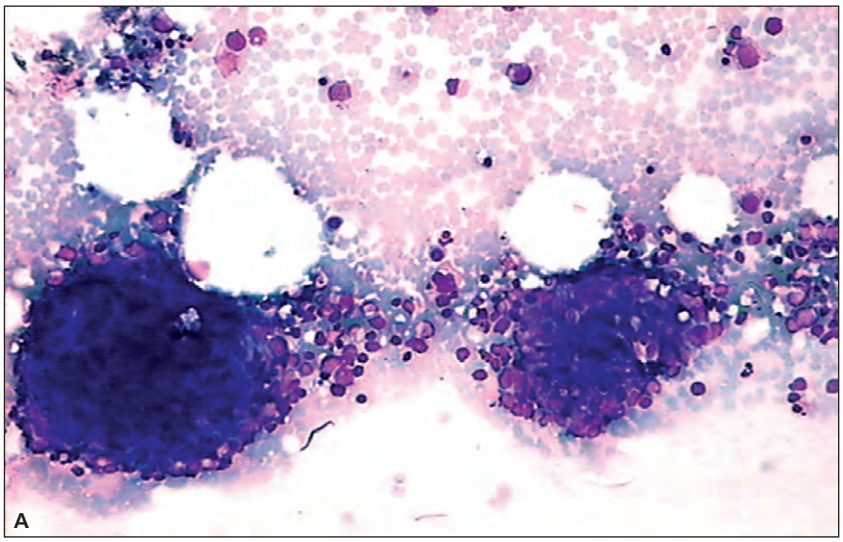
The great majority of these cases express a mature B immunophenotype with absence of CD34 and TdT and the presence of SmIg. In addition, the lymphoma cells are positive for CD20 and CD79a. Dissociation between morphology and immunophenotype has been reported, particularly in adults and in sporadic cases. The presence of EBV-encoded RNA can be demonstrated by FISH in the cases associated with this virus.

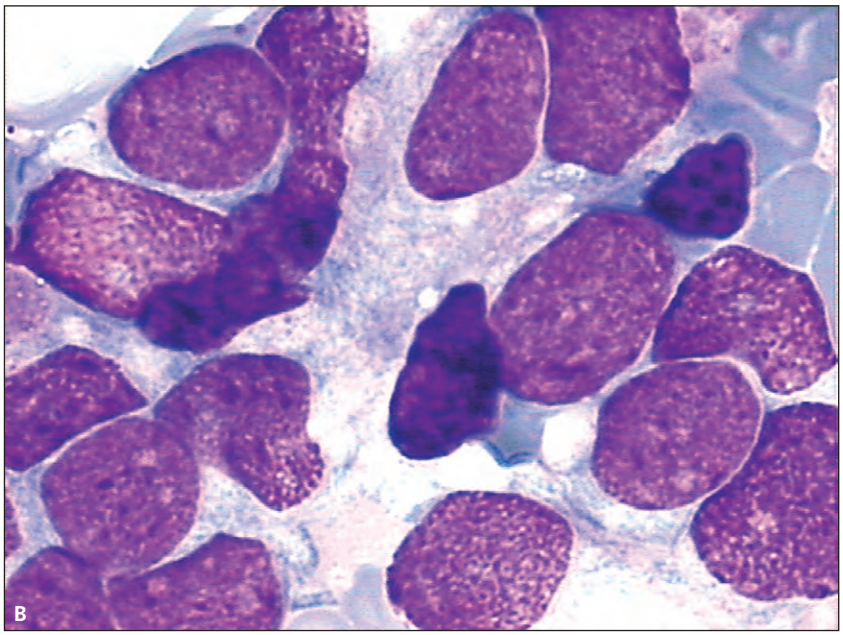
### Genetic analysis

The molecular marker of Burkitt lymphoma is the presence of an aberration that involves chromosome 8 band q24: the *MYC* oncogene at this site is translocated to a region adjacent to the enhancer of an immunoglobulin gene, which favours uncontrolled transcription of *MYC*. The most frequent of these aberrations, seen in 80–90% of patients, is t(8;14)(q24;q32), which brings *MYC* into proximity to the *IGH* locus on chromosome 14; less often the cause is an alternative translocation, either t(2;8) or t(8;22), involving respectively the kappa and the lambda light chain gene.

## Hodgkin lymphoma

Hodgkin lymphoma (HL), which the 2008 WHO classification describes in its diverse forms within a specific category, is characterised by the expansion, initially exclusively in lymph nodes,



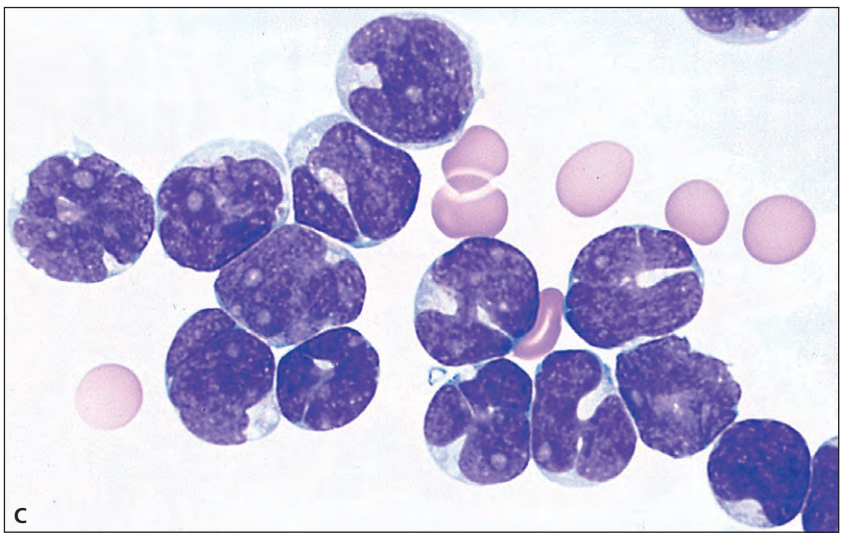




**Fig. 9.65** From a patient with disseminated diffuse large B-cell lymphoma. (A) Bone marrow aspirate. Compact nodular formations composed almost exclusively of neoplastic cells. (B) At higher power, the lymphoma cells have nuclei with dispersed chromatin, which forms a homogeneous tightly woven network; the cytoplasmic margins are poorly defined and give the impression of a syncytium. (C) Cytocentrifuge preparation of CSF. Numerous lymphoma cells have invaded the central nervous system (CNS). In this site, the nuclei assume typical multilobated bizarre forms and the nucleoli are very prominent, but the delicate homogeneous structure of the chromatin is similar to that of the cells that infiltrate the bone marrow.

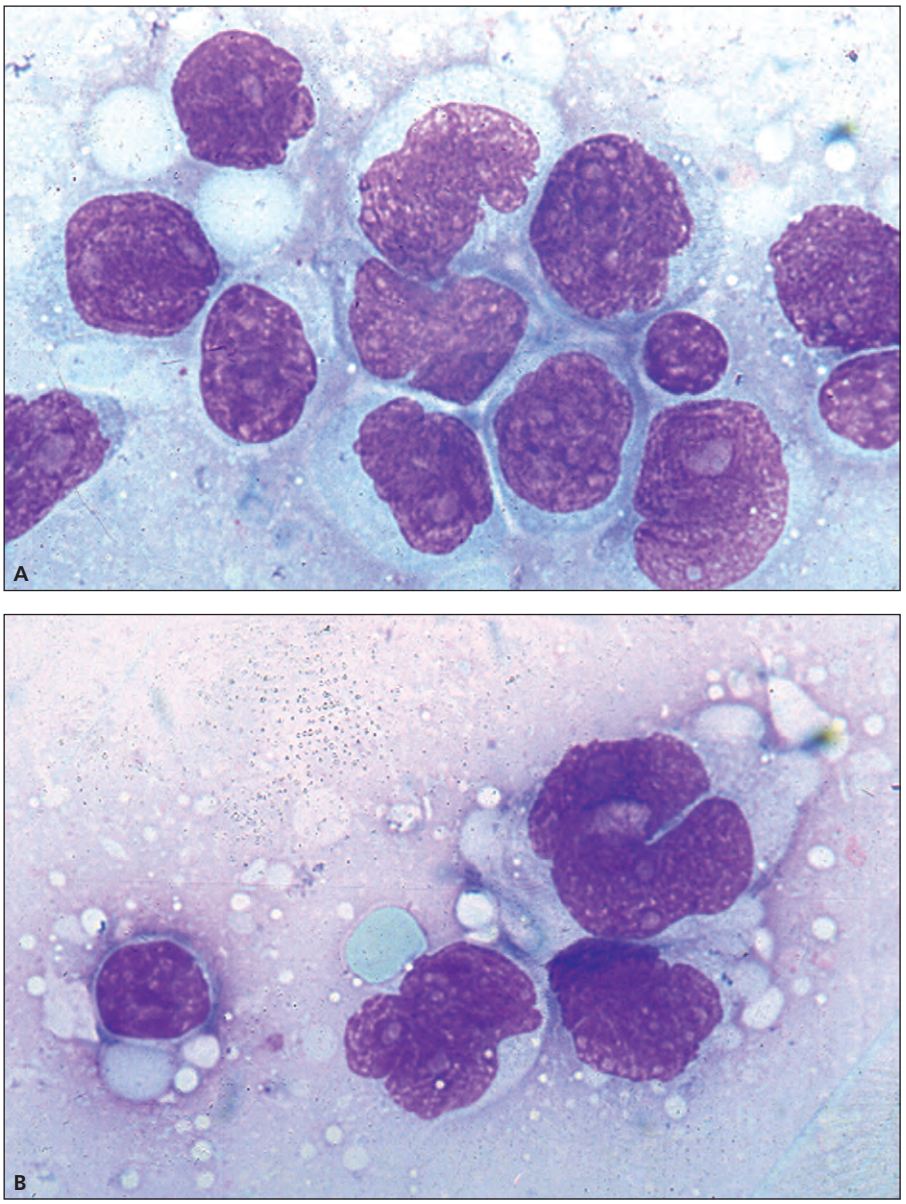



**Fig. 9.66** Diffuse large B-cell lymphoma with a high grade of malignancy; bone marrow aspirate from a patient with peripheral cytopenia in the absence of localisation to superficial lymph nodes. (A) The neoplastic population invading the bone marrow has completely replaced the haemopoietic precursors; it is composed of large anaplastic cells with highly irregular nuclei, hyperdiploid, lobated and convoluted, with large and numerous nucleoli. The chromatin is homogeneous with small areas of condensation. (B) The lymphoma cells appear to be immersed in a dense amorphous matrix, indicating dysproteinaemia. The cytoplasm is pale, weakly basophilic and slightly heterogeneous with one likely example of erythrophagocytosis. The nuclear outline is very irregular.

of a pathological tissue containing a relatively small number of large, multinucleated neoplastic cells, which are dispersed in a heterogeneous matrix of non-neoplastic inflammatory and accessory cells. The normal counterpart of the neoplastic cells is a mature germinal centre B cell. Very rarely this lymphoma arises in a post-thymic T cell. The designation 'Hodgkin lymphoma' encompasses two different conditions: the nodular lymphocyte predominant subcategory and classical HL, the latter in turn divided into four subtypes.[98]

Bone marrow involvement is rare and usually recognised in histological sections: the frequency of involvement varies from 5% in nodular lymphocyte-predominant HL and 3% in nodular sclerosis HL, to 10% in mixed cellularity classical HL (Figs 9.75, 9.76 and 9.77). Bone marrow does not have

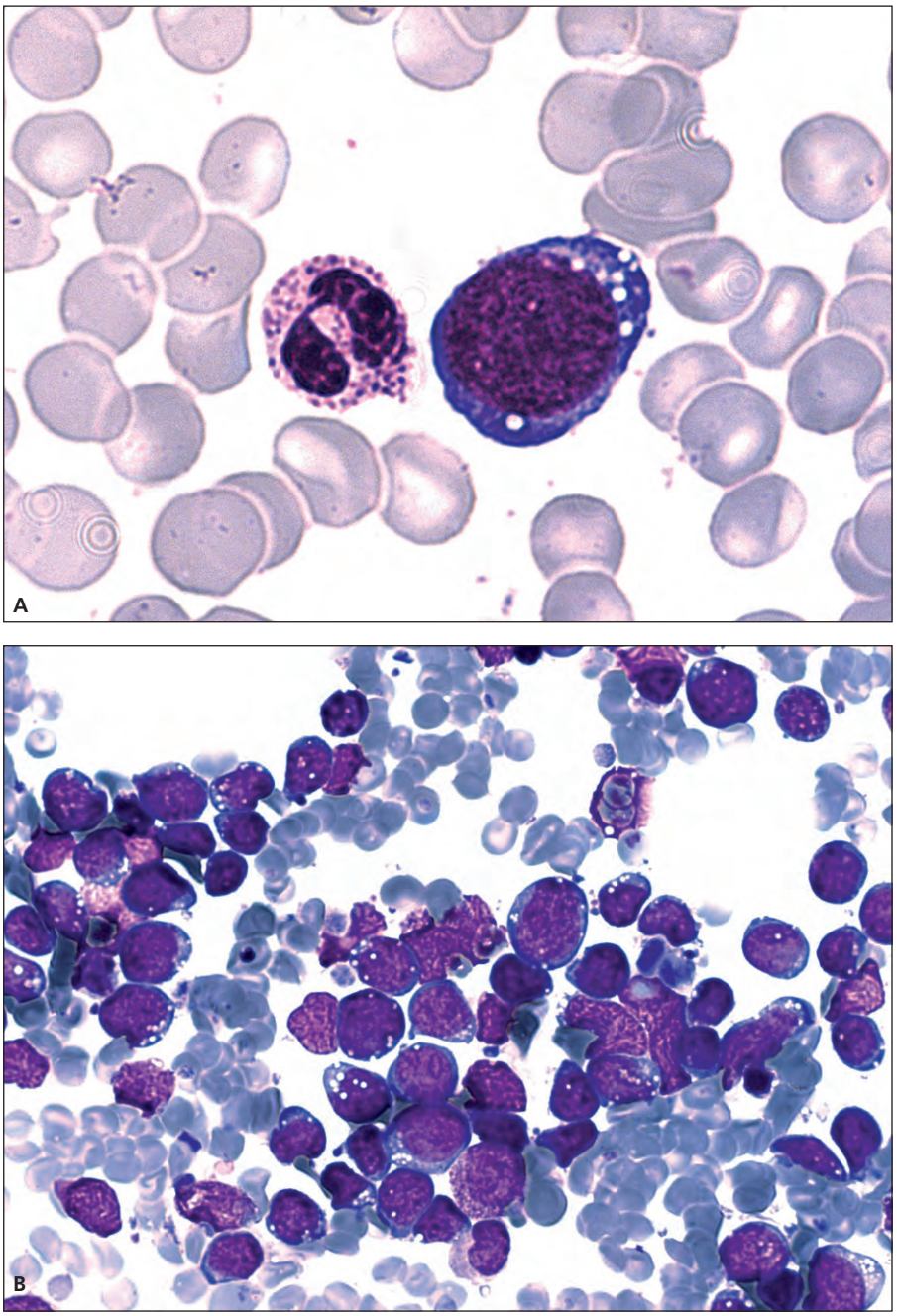



**Fig. 9.67** Burkitt lymphoma, leukaemic variant. From a 26-year-old patient at onset. (A) Peripheral blood film. Adjacent to a segmented neutrophil is a large blastic cell with a round nucleus and chromatin condensed into small dark clumps with delicate indistinct nucleoli. From the intensely basophilic, dark blue cytoplasm stand out seven pale, similarly sized vacuoles. (B) Bone marrow aspirate, hypercellular and composed almost entirely of variably sized blast cells with homogeneous features, similar to those described in (A). The cytoplasm is fairly extensive and is often perforated by optically empty vacuoles.

lymphatic channels and thus the presence of medullary infiltration indicates dissemination through the blood stream and leads to classification as stage IV disease. The Reed-Sternberg cells, observable in variable numbers in the affected tissue, which varies according to the subtype of the disease, are very large and have abundant weakly basophilic cytoplasm. There are two nuclei or a large nucleus composed of two lobes, with an irregular rounded outline. Every nucleus or nuclear lobe contains a giant nucleolus surrounded by a pale halo. The mononuclear variants are called Hodgkin cells. In bone marrow aspirates the Reed-Sternberg and Hodgkin cells are not often seen, but retain the cytological features described above.

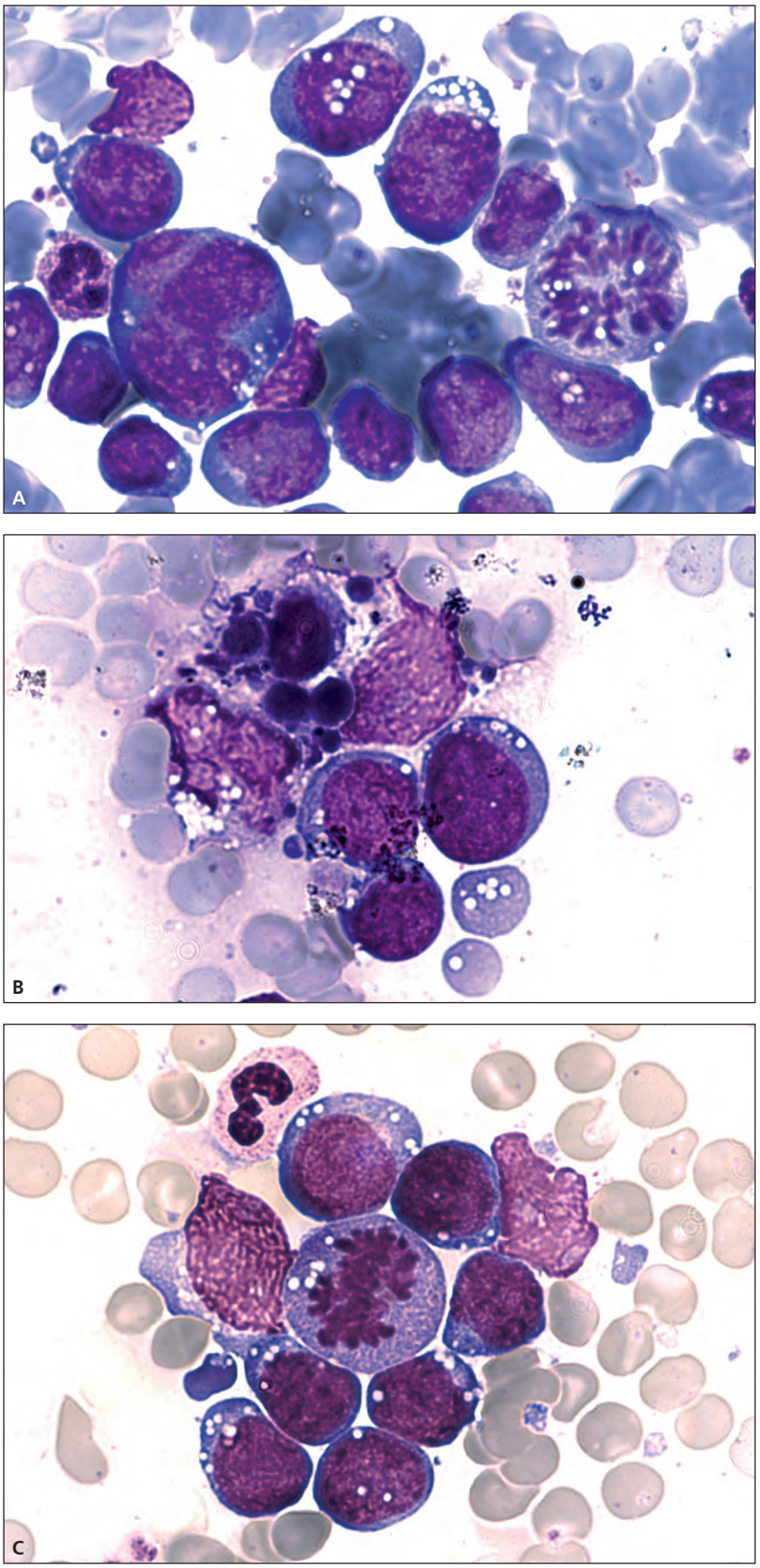



**Fig. 9.68** Burkitt lymphoma, leukaemic variant. From the same patient as Fig. 9.67; bone marrow aspirate. (A) On the left, a gigantic multinucleated blast cell; on the right, a mitotic figure in early metaphase, this also being vacuolated; mitotic figures are often observed in this condition. The other cells are hyperbasophilic blast cells. Vacuolisation is also sometimes observed in nuclei. (B) Top left, a macrophage laden with the residues of lymphoma cells, which have undergone apoptosis or premature death. In the centre, two morphologically typical blast cells. At the bottom, two isolated globules of violaceous vacuolated cytoplasm, detached from blast cells by the process of clasmacytosis: these fragments can lead to an overestimation of the platelet count. (C) A small group of blast cells, cohesive but easily distinguished from each other, with a cell in the centre in metaphase. To the right and to the left, other small free fragments of cytoplasm.

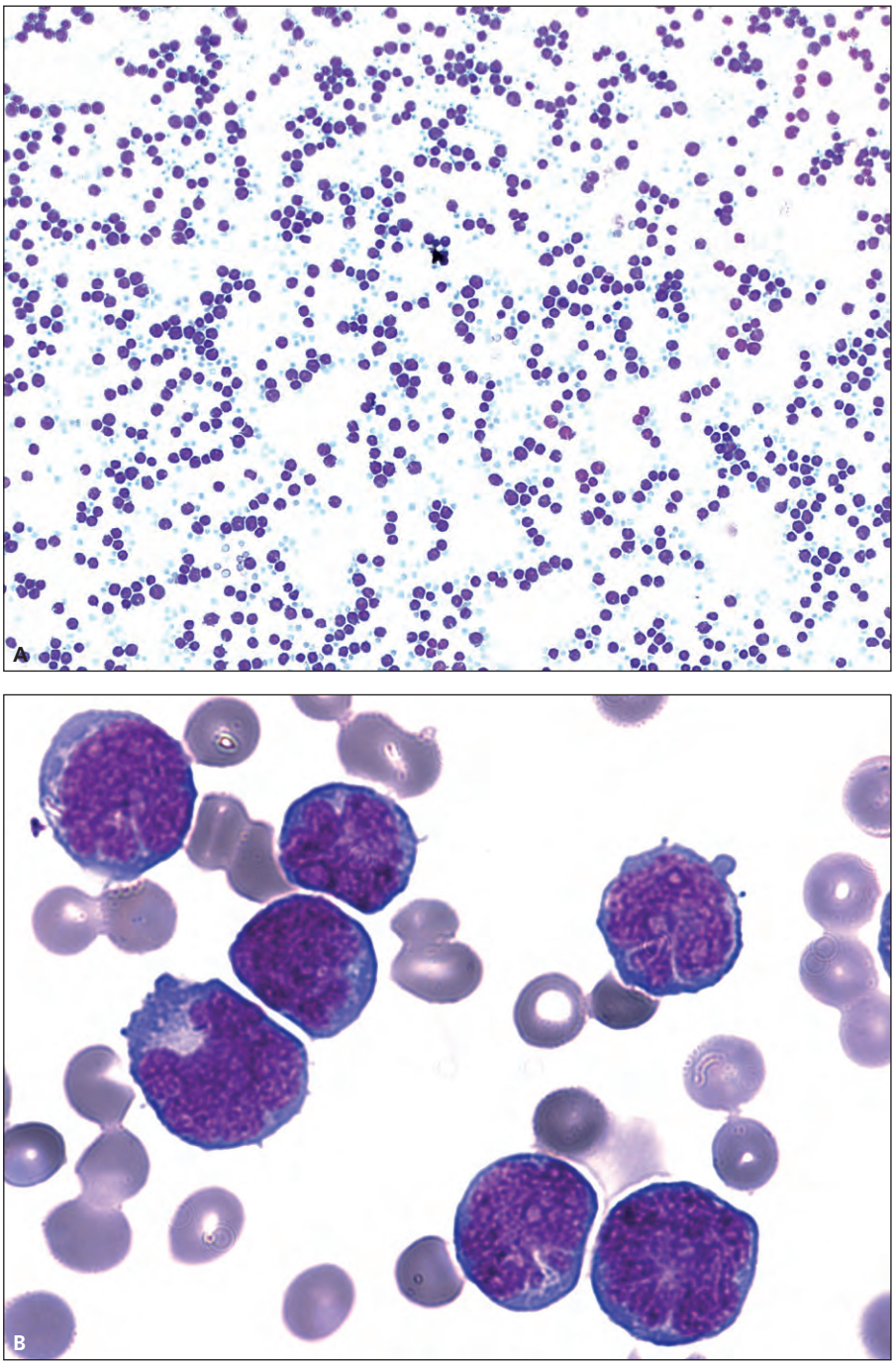



**Fig. 9.69** Burkitt lymphoma, leukaemic variant, CNS localisation in the same patient as Fig. 6.98; cytocentrifuge preparation of CSF. (A) At low power, the CSF contains red cells and a carpet of variably sized blast cells. (B) The lymphoma cells have intensely basophilic cytoplasm, similar to that seen in the bone marrow cells, but they lack vacuoles. The nuclear morphology is altered, as usually occurs when blood cells are found in other tissue fluids.

Bone marrow localisation of HL is not rare in HIV-infected individuals, who are usually young adults with systemic symptoms. Primary bone marrow involvement is rare, but in this context can occur.[99,100] In these patients, histological sections usually show a cellular marrow with reduced haemopoiesis and the presence of typical large cells with bilobed or gigantic nuclei, surrounded by lymphocytes, plasma cells, macrophages and eosinophils.

In classical HL, the neoplastic cells are typically positive for CD30 and CD15, and negative for CD45. B-lineage markers are expressed in a weak and inconsistent manner.

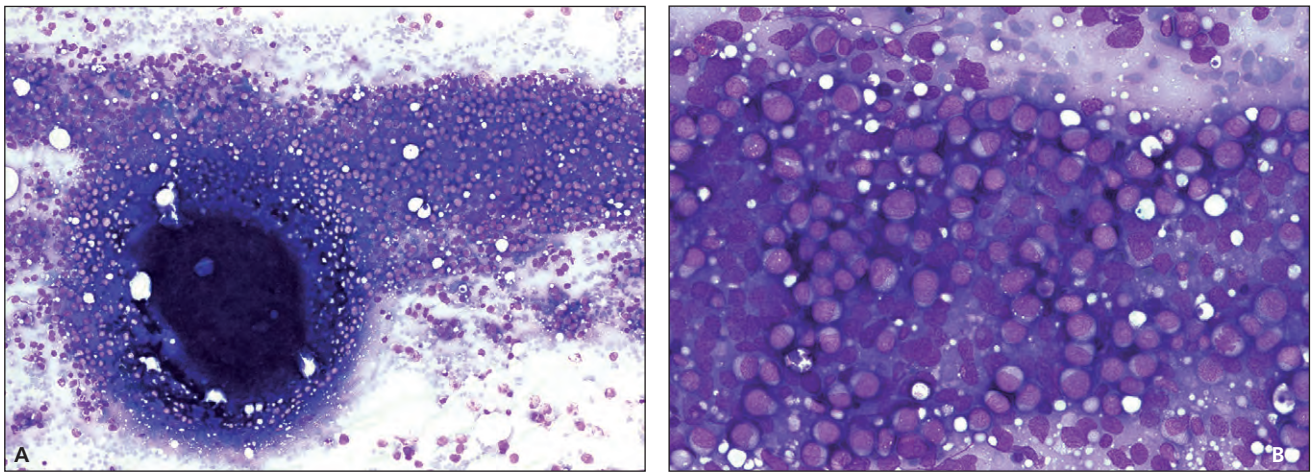



**Fig. 9.70** Massive bone marrow infiltration in a case of Burkitt lymphoma; bone marrow aspirate. (A) At low power, the fragment is composed of a compact mass of lymphoma cells with intensely blue cytoplasm. (B) The morphological homogeneity of the neoplastic population is evident despite the crowding of cells that characterises the preparation. On the right, a mitotic figure.

## Neoplasms of mature T and NK cells

Lymphomas of mature or peripheral T cells include numerous and very different disorders, categorised in the 2008 WHO classification as shown in Table 9.1. The main forms with leukaemic expression in this group of neoplasms are T-PLL, T-cell large granular lymphocytic leukaemia (T-LGLL), chronic lymphoproliferative disorder of NK cells, aggressive NK cell leukaemia, adult T-cell leukaemia/lymphoma (ATLL) and Sézary syndrome (SS) (Table 9.3). One is dealing with disorders that result from a genetic lesion (identified with certainty only in T-PLL) occurring in a peripheral, post-thymic T cell. The immunophenotype is of a mature T cell, these conditions being distinguished from T-ALL by the absence of markers of T lymphoblasts, such as CD34, TdT and CD1, and by the strong expression of CD45, membrane CD3 and, variably, CD4, CD8 or both. In these patients, clinical presentation may be non-specific, resulting from cytopenia (asthenia, infections or haemorrhage), or with cutaneous manifestations (in SS and, less often, in ATLL and T-PLL). In other cases, an incidental observation of lymphocytosis leads to the diagnosis. In the absence of recurrent genetic aberrations (except for T-PLL), the diagnosis must be based on integration of data derived from the blood count, cytology (often clearer in the blood film than in bone marrow aspirates or sections) and immunophenotypic analysis. The differentiation from reactive lymphocytosis is sometimes difficult, particularly in large granular lymphocytic leukaemia, and can require molecular confirmation by demonstration of clonality; PCR or Southern blot analysis of the beta or gamma TCR genes characteristically shows rearrangement in all T-lineage cases.

### T-cell prolymphocytic leukaemia

This is a rare lymphoproliferative disorder: it represents around 20% of PLL and around 30–40% of leukaemias of T lineage.[101–103] The normal counterpart of the T prolymphocyte is hypothesised to be a lymphocyte at an intermediate stage of differentiation between the cortical thymocyte and the mature T lymphocyte. T-PLL occurs in adults above the age of 30 years with a median age of 65 years and a slight male predominance. At onset there is splenomegaly which, in contrast to B-PLL, is associated in 40–50% of cases with hepatomegaly and lymphadenopathy. Papular cutaneous lesions are present in 20% of patients. The leucocyte count is almost always above $100 \times 10^9$/l, and reaches $200 \times 10^9$/l in almost half of patients; in reported cases the range has been from 35 to $1000 \times 10^9$/l.[47] Anaemia and thrombocytopenia are always significant. The clinical course is more aggressive than that of B-PLL, with little response to treatment and a median survival of less than a year. A minority of cases, however, have an indolent course.

#### Morphology

From the cytological point of view, the prolymphocytes that invade the peripheral blood and massively infiltrate the bone marrow can be indistinguishable from B prolymphocytes (Figs 9.78 and 9.79): there is plentiful cytoplasm and the nucleus has a central nucleolus. However, their T nature can often be suspected, particularly in peripheral blood films, from their smaller size and the more intense basophilia of the less plentiful cytoplasm. The N:C ratio is higher. Two morphological variants have been described[103]: the first, present in two thirds of patients, is characterised by medium-sized prolymphocytes, nuclei with condensed chromatin and an irregular outline, prominent nucleoli and intensely basophilic cytoplasm with large cytoplasmic projections, referred to as 'blebs'; in the other remaining cases, the prolymphocytes are smaller and can have a convoluted or cerebriform nucleus, similar to that of a Sézary cell, with barely visible nucleoli (Fig. 9.80). The morphological heterogeneity is not associated with any difference in clinical

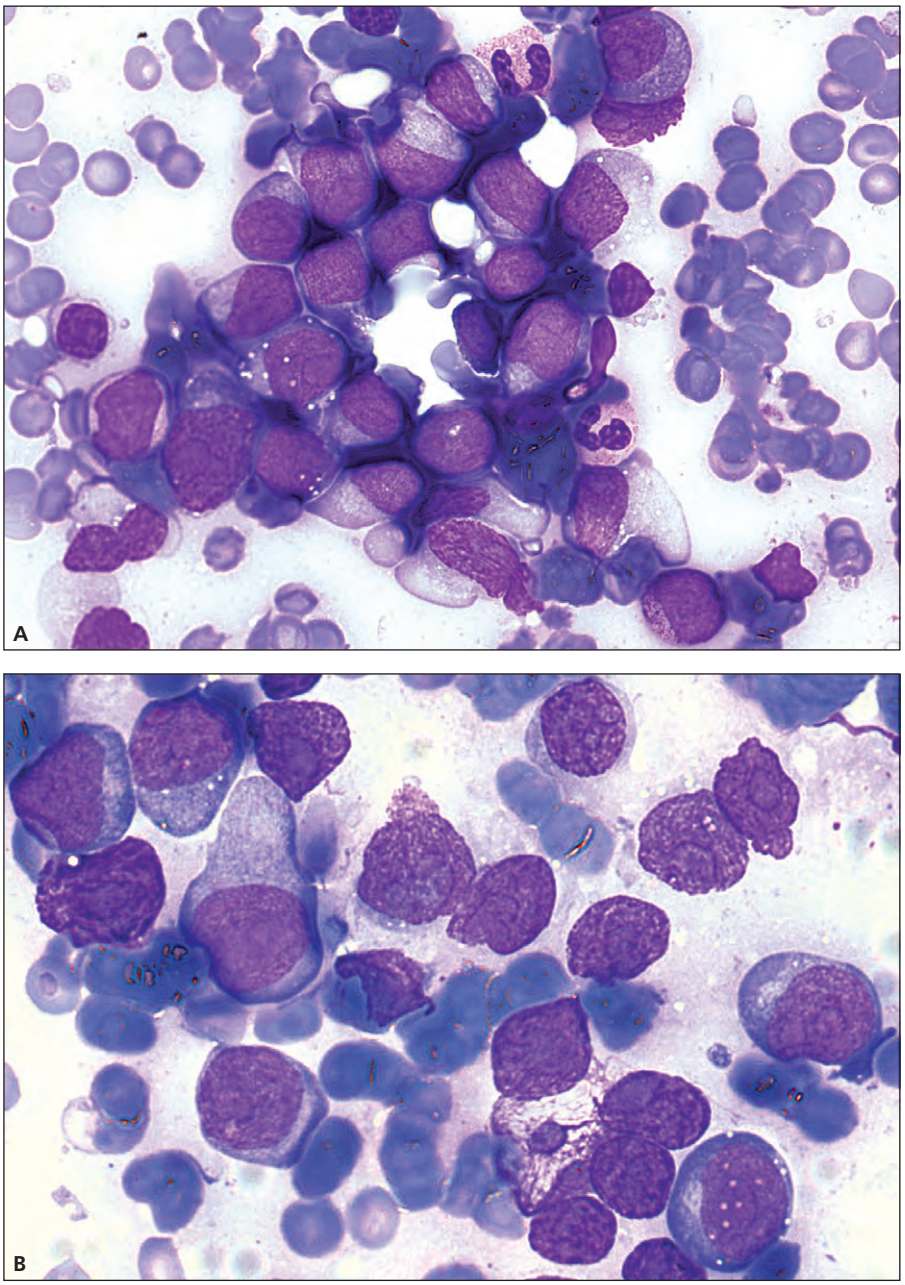


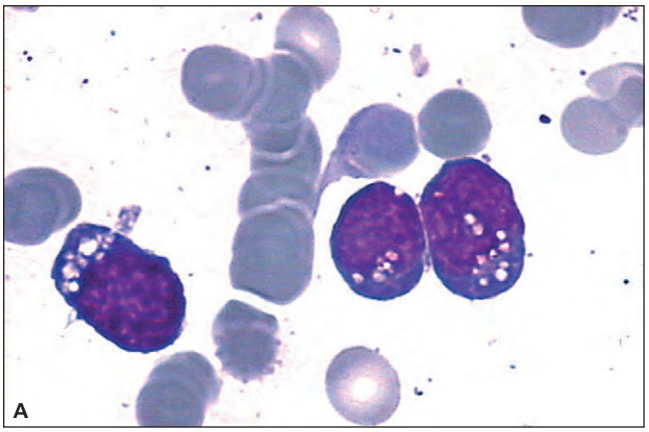

**Fig. 9.71** Burkitt lymphoma, leukaemic variant, from the same patient as Fig. 9.70; bone marrow aspirate. (A and B) In the better spread part of the film the blast cells have plentiful but less intensely stained cytoplasm. The nuclei have a variable outline, often angulated, with very fine, delicate, loose, homogeneous chromatin. The nucleoli are not at all prominent and often are indistinct. Only a few cells are vacuolated. In the cells that are more intensely stained, a pale paranuclear Golgi zone is apparent.

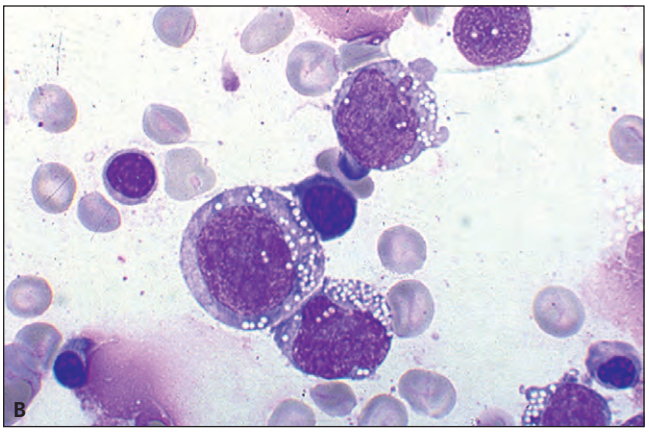
behaviour or immunological profile.[104] Cytochemistry often shows positivity for ANAE and ANBE.[105] Bone marrow aspiration and trephine biopsy are not always equally informative: the bone marrow shows interstitial or diffuse infiltration by prolymphocytes, so massive that the cytological details are less evident. Lymph node infiltration is diffuse and usually paracortical, while in the spleen infiltration is of the red pulp, which can obliterate the white pulp.

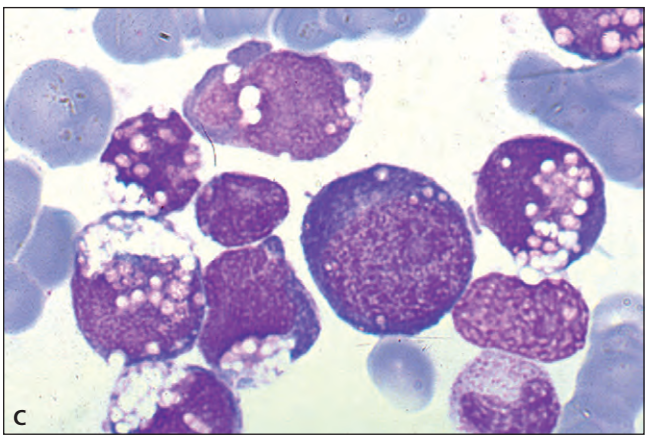
### Immunophenotype and genetic analysis

T prolymphocytes express the antigens of mature post-thymic T lymphocytes: CD2, CD3, CD5 and CD7. The majority of cases have a CD4-positive, CD8-negative T-helper phenotype; 25% of patients, however, have cells that are CD8 positive and CD4 negative, while in 15% there is co-expression of these two markers. Positivity for CD52 indicates that treatment with alemtuzumab (anti-CD52) may be useful. Immunohistochemistry

shows strong expression of the *TCL1* (T-cell leukaemia/lymphoma 1) oncogene, which is a specific and sensitive marker of T-PLL. This gene is involved in the chromosomal aberrations involving chromosome 14 band q32, the most common of which is inv(14)(q11q32), present in 80% of cases, which brings *TCL1* into proximity to the *TCRA* gene, causing activation of *TCL1*. Other genetic alterations have been identified with molecular techniques or FISH.[47]




## Large granular lymphocytic leukaemia

Large granular lymphocytes (LGL) are lymphocytes of medium size, with a low N:C ratio (Figs 9.81, 9.82 and 9.83); the nucleus is eccentric, rounded or angulated, with mature chromatin, without visible nucleoli. There is ample very pale cytoplasm with a greyish tint or weak basophilia; fine or coarse red-violet azurophilic granules are clearly visible, these corresponding to hypertrophic lysosomes. Sometimes there are morphologically similar cells, but without visible granules; probably these do have granules but below the level of resolution of the light microscope. LGL show strong cytochemical positivity for acid phosphatase and for beta-glucuronidase, indicating a high content of acid hydrolases. Peroxidase is consistently negative. The cytoplasmic granules contain numerous other proteins with a cytolytic action, such as perforin and granzyme B. By electron microscopy, the granules are electron dense and have a diameter of 0.1–0.7 μm; in the cytoplasm there are parallel tubular structures formed by massing together of microtubules. LGL represent 5–15% of circulating lymphocytes in normal blood, with there sometimes being a higher proportion in the bone marrow. Their percentage increases in viral infections and in any condition associated with immunological activation. From the immunophenotypic point of view, lymphocytes with LGL morphology can be divided into two subpopulations:

- Cytotoxic suppressor T cells, which express the T-cell antigens CD2, CD3, CD7 and CD8, and also CD11b, CD11c,

**Fig. 9.72** Other cases of the leukaemic form of Burkitt lymphoma. (A) Peripheral blood film. Typical circulating Burkitt cell, cytologically very similar to that shown in Fig. 9.67A, although from a different patient. (B) Bone marrow aspirate. The three blast cells from another patient have a delicate, finely granular chromatin network, with a slight lack of homogeneity as a result of alternating zones of greater or lesser chromatin condensation. Every nucleus contains two to four not very distinct nucleoli. The cytoplasm is moderately plentiful and in the large cell completely surrounds the nucleus; the basophilia is moderate with no apparent Golgi zone but with numerous small vacuoles resembling a bee hive; the blast at the top shows cytoplasmic protrusions. The larger cell has morphological analogies with a promegaloblast of pernicious anaemia – the regular oval nucleus with granular cytoplasm and the cytoplasmic characteristics: the distinction is based on the morphological features of other cells present in the film (one notes the irregular nuclear outline of another two blasts, not compatible with their being erythroid cells) and on immunophenotyping. The vacuoles of dysplastic erythroblasts are less numerous and in general are more heterogeneous in size than those of Burkitt cells, which are very similar to each other. (C) This case is characterised by the presence of very numerous vacuoles, which completely perforate the cytoplasm. Also here the chromatin is homogeneous, finely granular or stippled. Nucleoli are not evident. The larger cell, in the centre, is a normoblastic proerythroblast: the chromatin forms a coarser network, with a large central nucleolus with perinucleolar enhancement; the nucleus is round and regular; the cytoplasm is the characteristic ultramarine of the erythroid series, more intense than that of Burkitt cells, and shows a small paranuclear area of arcoplasm and some fine vacuoles.

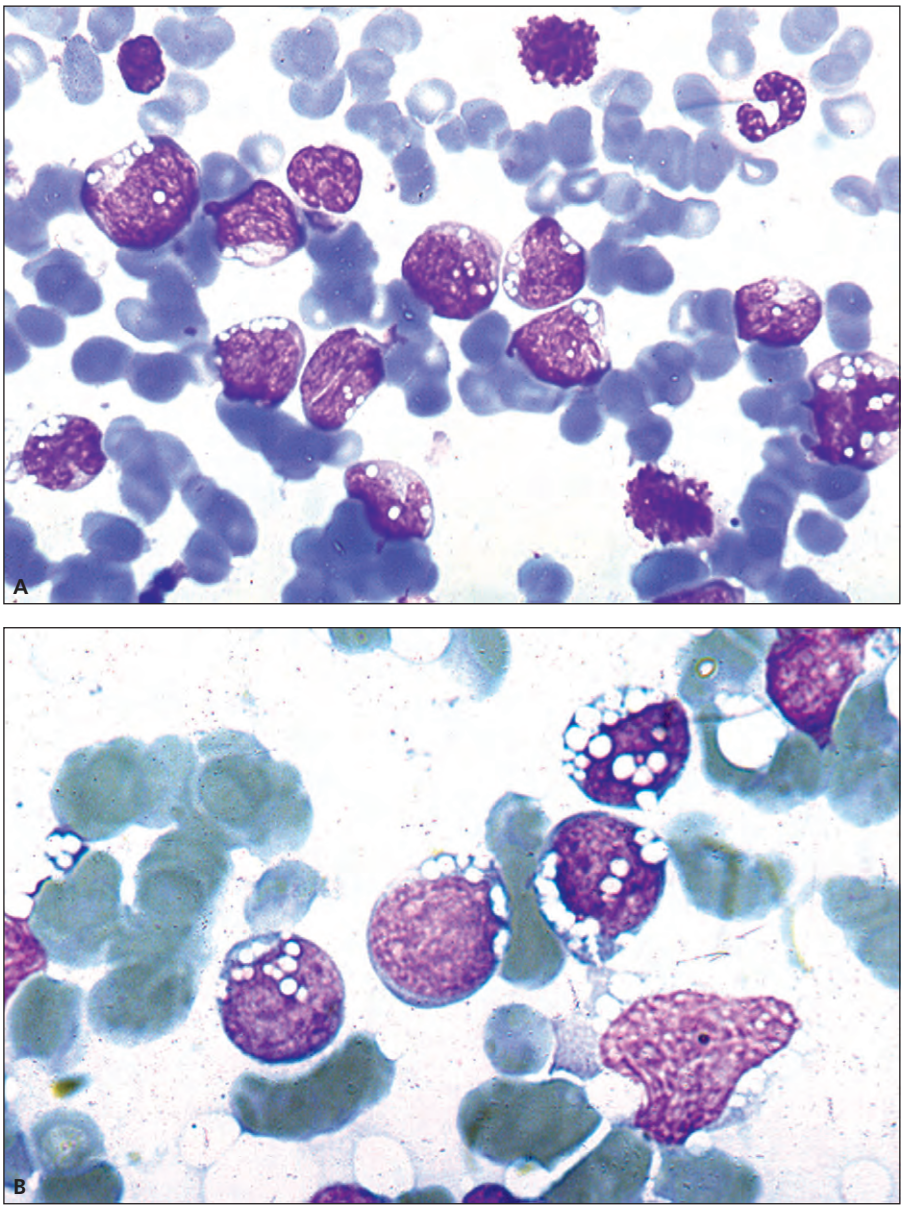



**Fig. 9.73** Other cases of Burkitt lymphoma in leukaemic phase. (A) Bone marrow aspirate in a patient with overt AIDS. In the peripheral blood there is marked leucopenia (WBC 1.5 × $10^9$/l), with rare vacuolated blasts. The bone marrow infiltrate is above 90%. The nuclear outline of the bone marrow blast cells is less regular than usual, with notches and angulation; chromatin condensation is more markedly heterogeneous than usual. (B) Heavily vacuolated lymphoma cells in the bone marrow aspirate of another patient with AIDS.

CD16, CD56 and CD57; they are TCR alpha/beta positive and on molecular analysis show rearrangement of the TCR genes;

- NK cells, which are distinguished from the preceding category by the lack of membrane CD3 expression and the expression of CD4 rather than CD8; TCR genes are not rearranged.

Following the first description of the clinical entity characterised by proliferation of LGL with associated neutropenia,[106] numerous examples of lymphoproliferative disorders of LGL, with various designations, were published; the FAB classification of lymphoproliferative disorders inappropriately designated these cases as T-CLL.[1] The forms now recognised in the 2008 WHO classification are T-cell large granular lymphocytic leukaemia (T-LGLL), representing a clonal expansion of cytotoxic T lymphocytes, and the rarer disorder of NK cells (described below).

In the presence of an absolute increase in the number of LGL in the peripheral blood, the first alternatives to consider are a

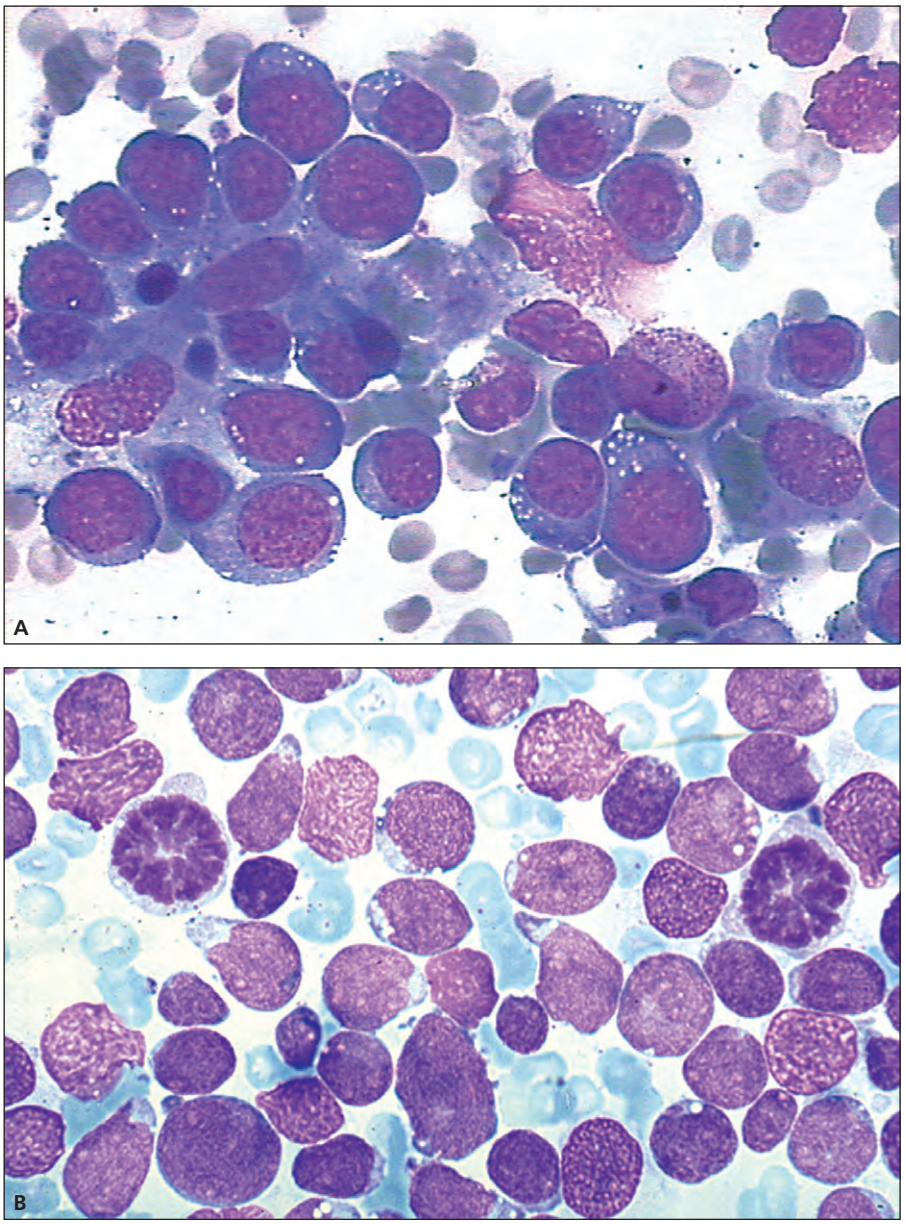



**Fig. 9.74** Another two cases of Burkitt lymphoma; bone marrow aspirate. (A) Hypercellularity with massive bone marrow infiltration by intensely basophilic, moderately vacuolated blast cells. On the left, a group of blast cells are arranged in a rosette, around the spongy bean-shaped nucleus of a macrophage, in the cytoplasm of which there are two barely visible hyperchromatic degenerated nuclei. (B) Also in this case, the marrow is totally replaced by blastic appearing lymphoma cells, with fine homogeneous chromatin, admixed with some lymphocytes. There are two mitotic figures.

reactive proliferation and a clonal lymphoproliferative disorder. The persistence of increased numbers, usually, but not always, more than $2 \times 10^9$/l, for more than six months with no apparent cause raises the diagnostic suspicion of T-LGLL. This is a heterogeneous disorder, which affects individuals between the ages of 45 and 75 years, men and women equally; the clinical course is often indolent. In addition to an increase of mature LGL, which rarely number more than $20 \times 10^9$/l, there is often an autoimmune neutropenia, which can be severe, often but not consistently associated with anaemia and thrombocytopenia.[107,108] The frequent association with autoimmune phenomena, such as rheumatoid arthritis and thyroiditis, and the presence of autoantibodies and circulating immune complexes leads to the hypothesis that prolonged immune stimulation is important in pathogenesis. Splenomegaly results from the accumulation of LGL in the cords and sinuses of the red pulp with hyperplasia of the white pulp follicles. Disease evolution is generally very slow, with a stable clinical picture for many years and

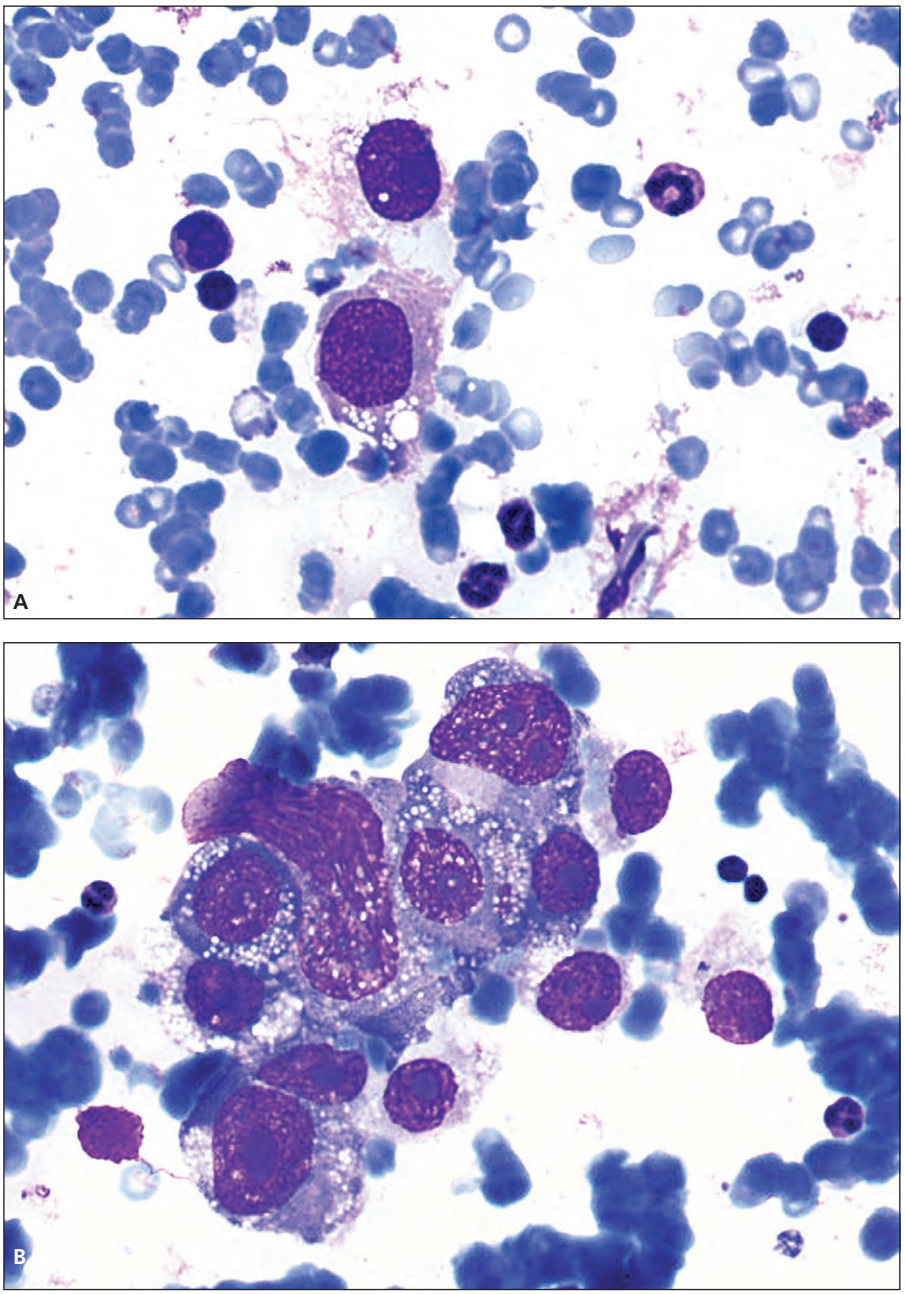



**Fig. 9.75** Hodgkin lymphoma; bone marrow aspirate from a patient with advanced disease. (A) In a hypocellular aspirate there are two giant cells, which are the mononuclear variant of the Reed-Sternberg cell, known as Hodgkin cells. The cytoplasm is pale lilac and appears frayed and very vacuolated. The nuclei are oval with sieve-like chromatin, composed of numerous pale areas, each containing a single voluminous dark violet nucleolus with a homogeneous structure. (B) Apparently syncytial conglomerate of colossal Hodgkin cells (in comparison with the size of the two neutrophils at the right and left margins, which appear miniscule). The cytoplasm has indistinct limits, a variable degree of basophilia and an appearance resembling a beehive because of the presence of very numerous fine vacuoles. The nucleoli are very large, blue-violet and sometimes multiple. This picture could be confused with that of metastases of non-haemopoietic neoplasms.

the possibility of spontaneous remission. Acute evolution is rare.

The bone marrow aspirate can be hypocellular, normocellular or hypercellular and often shows a left shift in granulocyte maturation and a moderate infiltration, rarely above 50%, of LGL. The immunophenotype is generally typical, as described above, with expression of CD16 and CD57. Recurrent genetic abnormalities have not been described. Confirmation of clonality by TCR gamma analysis can be decisive in distinguishing LGLL from either oligoclonal or polyclonal reactive conditions, such

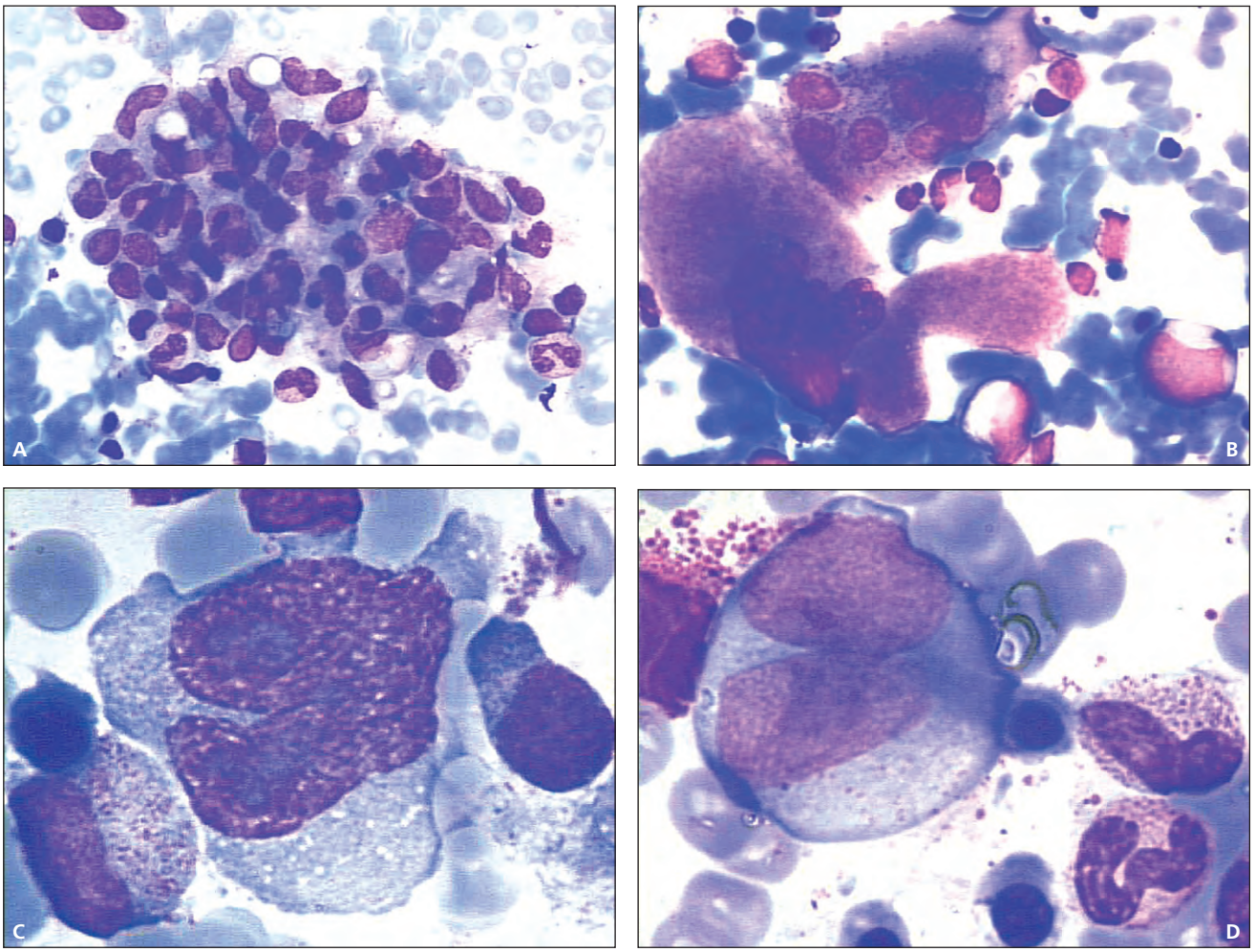



**Fig. 9.76** Hodgkin lymphoma; bone marrow aspirate from a patient with multiply relapsed and disseminated disease. (A) The marrow is hypocellular with an abundance of stromal cells, which here appear to be forming a granulomatous nodule; this does not, however, appear to contain any neoplastic cells. (B) On the left, a large mature megakaryocyte. At the top, an osteoclast with multiple slightly oval nuclei and variegated cytoplasm containing many dark granules. On the right, a large cell with a blastic appearance, not further identifiable. (C) Gigantic Reed-Sternberg cell with a large bilobed nucleus. Each lobe appears to contain a large azure nucleolus. (D) Large Reed-Sternberg cell with a double nucleus and fine homogeneous chromatin. The nucleoli are indistinct. The cytoplasm is very plentiful, basophilic, bloated.

as those observed after haemopoietic stem cell transplantation. Clonal LGL expansion without disease evolution, considered to be reactive, is observed following transplantation and in association with indolent B-cell leukaemias such as CLL and HCL.[107]

### Chronic lymphoproliferative disorder of NK cells

These rare and heterogeneous conditions are usually seen in adults with a median age of 60 years, without EBV involvement and with no ethnic predisposition, thus differing from acute NK cell leukaemia.[108] They are characterised by an increase in the peripheral blood, sustained over time and with no apparent cause, of lymphocytes with the morphology of typical LGL and with an NK immunophenotype (membrane CD3 negative, cytoplasmic CD3 epsilon positive; positivity for CD2, CD16 and CD56; negativity for CD4 and CD57, and variable expression of CD8; TCR genes are not rearranged). The clinical course is similar to that of T-LGLL, but with less severe neutropenia. Other types of autoimmune or neoplastic disease can co-exist.

### Aggressive NK cell leukaemia

This is a rare and serious leukaemia, correlating with EBV infection and more frequent in Asia. It affects young adults (median age 39 years), more often males, with an acute or fulminant clinical course, characterised by severe and progressive symptoms, related in part to cytokine production: fever, even in the absence of infection, hepatosplenomegaly, jaundice, ascites, superficial and deep lymphadenopathy, disseminated

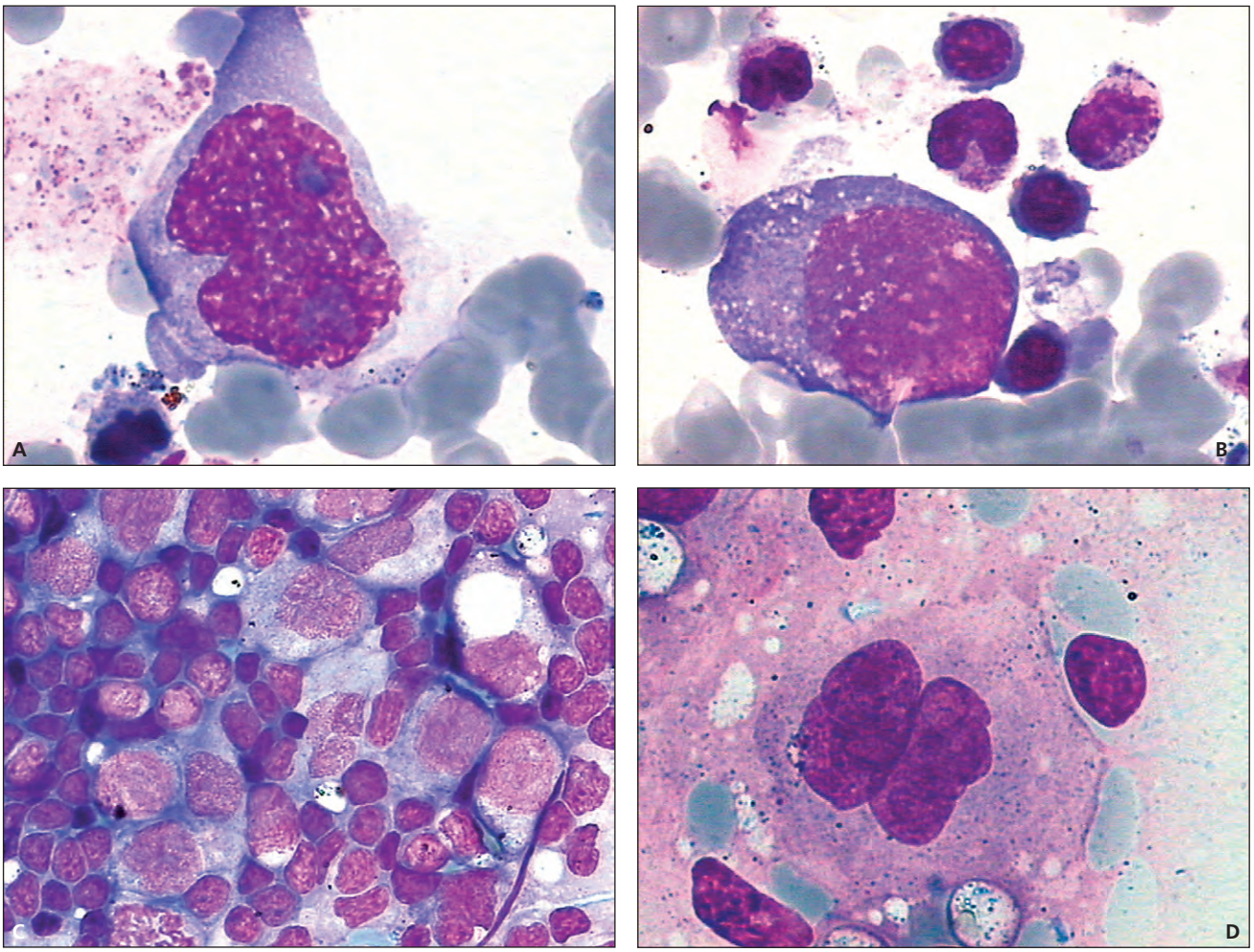



**Fig. 9.77** Hodgkin lymphoma, with primary bone marrow localisation in a patient with HIV infection. (A and B) Hodgkin cells recognisable in a bone marrow aspirate. (C) Lymph node imprint. There are numerous giant mononuclear Hodgkin cells with large pale nuclei and weakly basophilic cytoplasm, admixed with small lymphocytes. (D) Reed-Sternberg cell with characteristic cytological features, from the same lymph node imprint as (C).

**Table 9.3** Lymphoproliferative disorders of mature T/NK cells: differential diagnosis of the forms that have frequent and relevant involvement of the bone marrow

| | Bone marrow | Leucocyte count ($\times 10^9$/l) | Morphology | Immunophenotype | Genetics |
|---|---|---|---|---|---|
| T-cell prolymphocytic leukaemia | 100% | Very high, often >100 | Irregular basophilic prolymphocytes, cerebriform variant | CD4+, CD8− (60%) CD4−, CD8+ (25%), CD4+, CD8+ (15%) | inv(14)(q11q32) |
| Large granular lymphocyte leukaemia | 100% with partial infiltration | Variable | LGL with mature nucleus | CD4-, CD8+, CD16+, CD56+ | None known |
| Chronic mature lymphoproliferative disorder of NK cells | 100% | Variable | LGL with mature nucleus | CD3−, CD4+, CD8−, CD16 and CD56 variable | None known |
| Aggressive NK cell leukaemia | Very common | Variable, can be very high | Variable, from LGL to blasts with convoluted and azurophilic granules | CD16+, CD56+ | None known |
| Adult T-cell leukaemia/ lymphoma | Frequent | Atypical cells constant, not correlating with the bone marrow | Quite variable with flower cells | CD4+, CD5+, CD25+, HTLV-1+ | |
| Sézary syndrome | Variable, often moderate infiltration | Variable, can be very high | Sézary cells, large or small with cerebriform nuclei | CD4+, CD8−, CD7+, CD158k+ | |

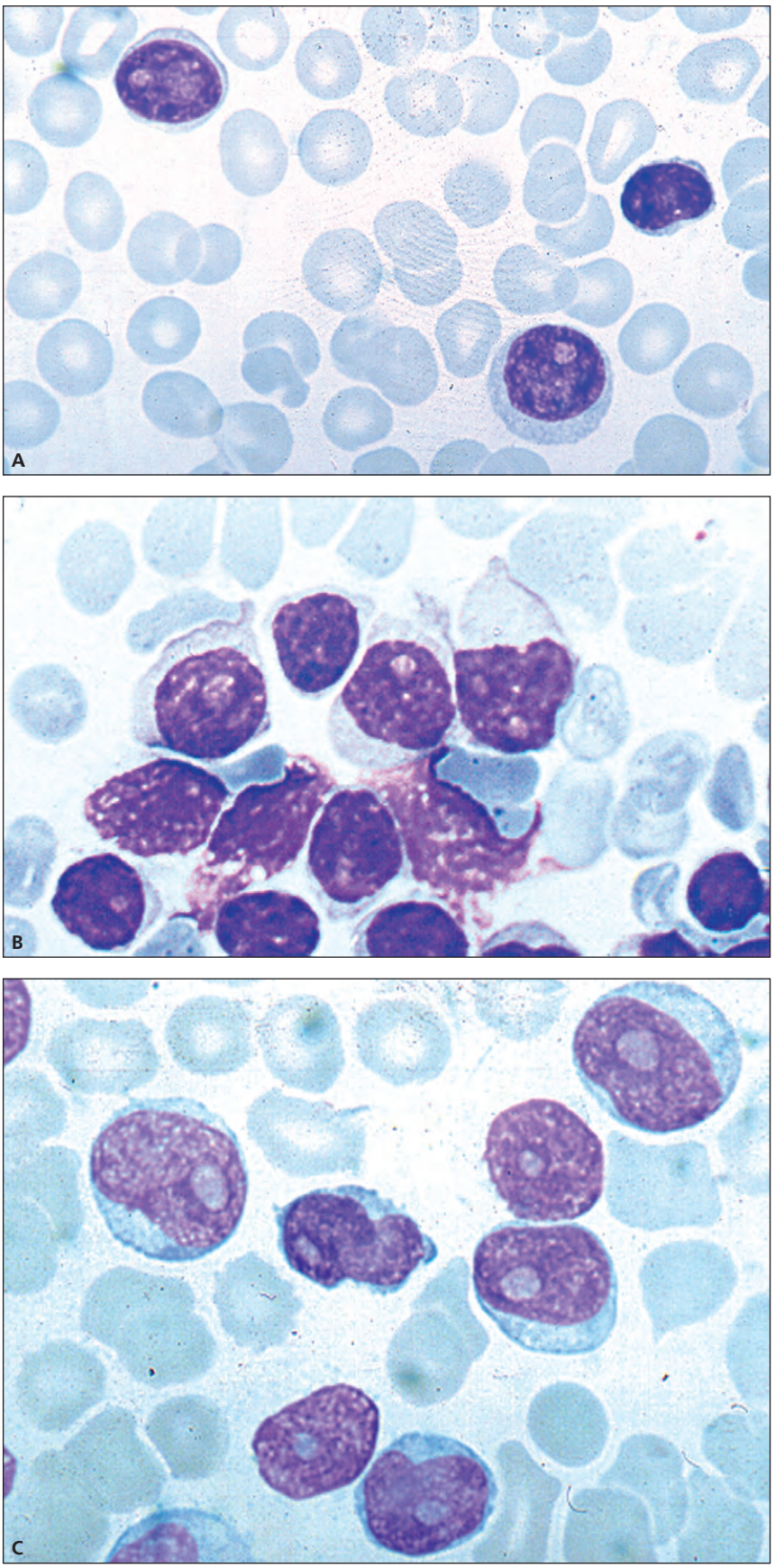



**Fig. 9.78** T-cell prolymphocytic leukaemia (T-PLL); peripheral blood film. (A) Two small prolymphocytes with a fairly high N:C ratio. The cytoplasm is less plentiful than in B prolymphocytes and is uniformly basophilic. The nuclei are round with irregularly condensed chromatin, with pale stamped-out nucleoli, eccentric and not large. On the right, a small mature lymphocyte. (B) The cells are small and have fairly scanty cytoplasm with some short protrusions. The nuclear outlines in some cells are angulated or irregular with notches and indentations; the chromatin is compact and the nucleoli are more visible in the cells that are more spread out. (C) In this patient the leucocyte count was very high ($313 \times 10^9$/l) and the prolymphocytes co-expressed CD2, CD3, CD4 and CD8. Characteristics that can help to distinguish T prolymphocytes from B prolymphocytes are the shape of the nucleus, which is not regularly rounded but rather is often notched, the basophilia and the less plentiful cytoplasm. The nucleoli are very pale, with a clearly incised or stamped-out outline, in a granular chromatin matrix.

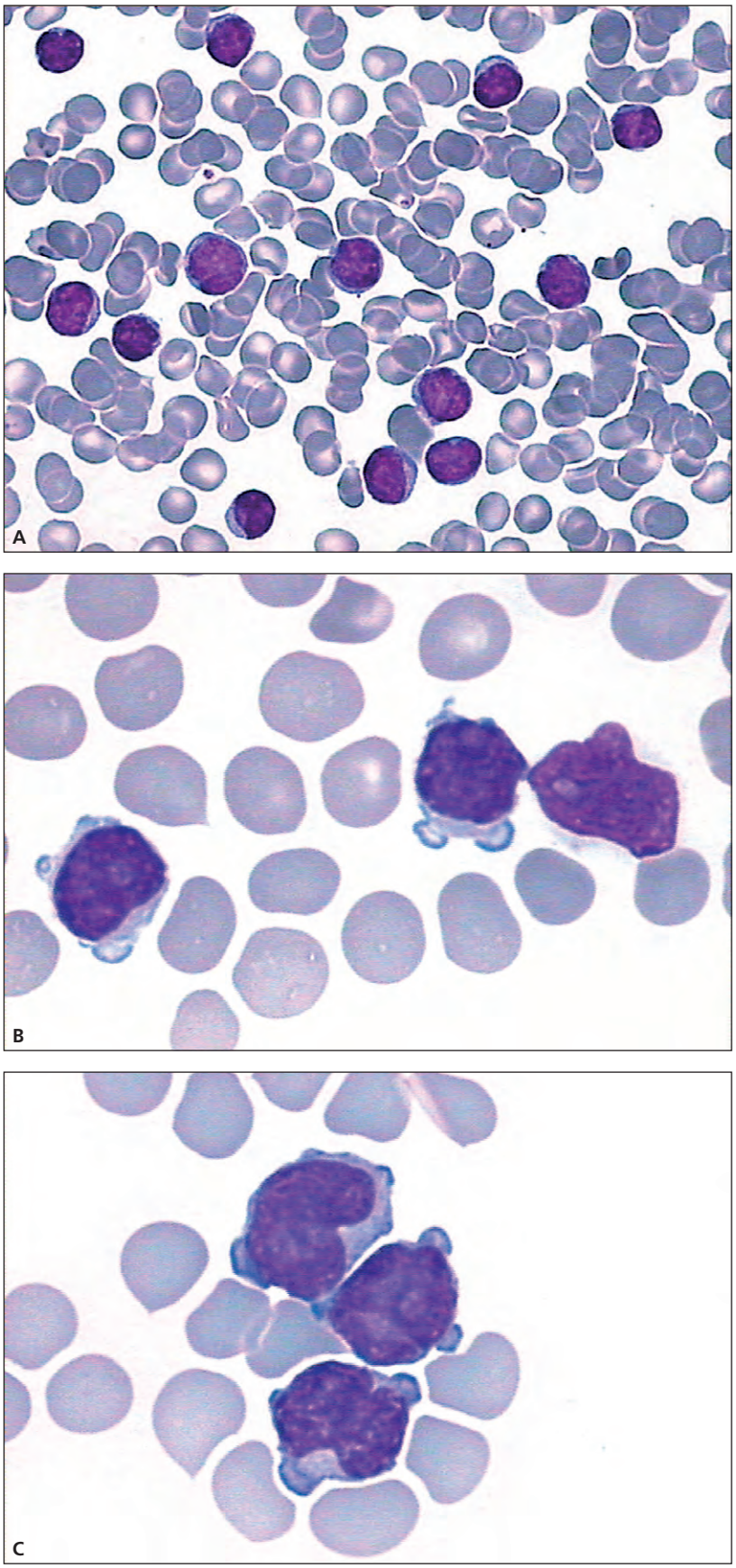



**Fig. 9.79** T-PLL; peripheral blood film. (A) The leucocyte count is increased and the prolymphocytes are very numerous, heterogeneous in size, but mainly small with a high N:C ratio. (B and C) Small T prolymphocytes, recognisable from the nucleolus, which is not prominent but is visible as a circular area of pallor in the otherwise solidly compact chromatin. As is typical in T-PLL, the nuclear profile is irregular and the cytoplasm is scanty and basophilic, with a tendency to extend into apparently vesicular blebs.

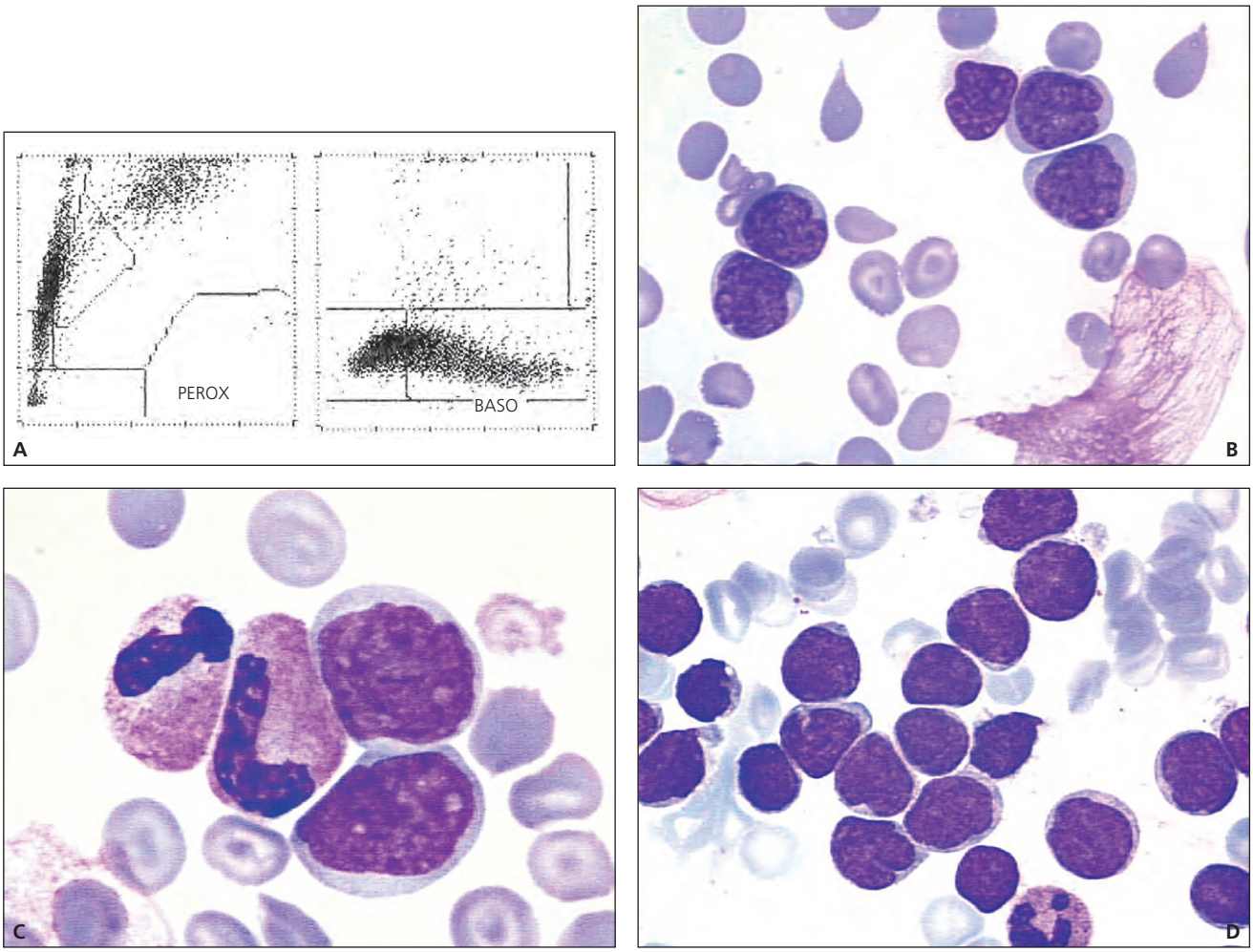



**Fig. 9.80** T-PLL variant with cerebriform nuclei, in the past designated 'Sézary cell leukaemia'; peripheral blood film. Patient aged 86 years, presenting to the Emergency Medicine Department with dyspnoea, asthenia and marked pallor. (A) Blood count: Hb 49 g/l; platelets 94 × $10^9$/l; WBC 22.6 × $10^9$/l. 90% of white cells in the blood appear in the Perox cytogram as large peroxidase-negative cells and in the Baso cytogram as mononuclear cells with fairly dense chromatin, forming a horizontal oval that invades the blast area only slightly. (B and C) Peripheral blood film. The cells were negative for CD4, CD19 and CD56, and positive, among others, for CD5, CD7 and CD8. These are large cells with plentiful basophilic cytoplasm and a nucleus that is convoluted or cerebriform, with multiple folds. There are some dacrocytes and target cells. (D) The bone marrow aspirate appears completely replaced by medium-sized to large cells with distorted folded nuclei, with cytological features similar to those described in the peripheral blood film.

intravascular coagulation, haemophagocytic syndromes and multiorgan failure.[107,108] Median survival is two months.

Anaemia and thrombocytopenia are common and there is usually leucocytosis, which can be moderate or very marked, with a variable percentage of LGL, which are variable in appearance, often less mature than normal LGL and sometimes frankly blastic, with large nuclei with an irregular outline, not very compact chromatin and visible nucleoli. There is plentiful pale or weakly basophilic cytoplasm containing fine or coarse azurophilic granules (Fig. 9.84). The same cells are present in variable numbers in the bone marrow, associated with increased haemophagocytic macrophages and features of myelitis and necrosis.

Immunophenotyping shows expression of CD2, CD16, CD56 and cytoplasmic CD3 epsilon, being similar to the immunophenotype of chronic lymphoproliferative disorder of NK cells. Expression of CD4 and CD8 is variable. The TCR genes are in germline configuration.

### Adult T-cell leukaemia/lymphoma

ATLL was first recognised in Japan. It has a characteristic geographical distribution with endemic foci in the south-west of Japan, in the Caribbean, on the south-east coast of the United States and in some areas of Brazil, West Africa and Iran. Non-endemic cases have also been reported. All cases are infected by the human T-cell lymphotropic virus 1 (HTLV-1).

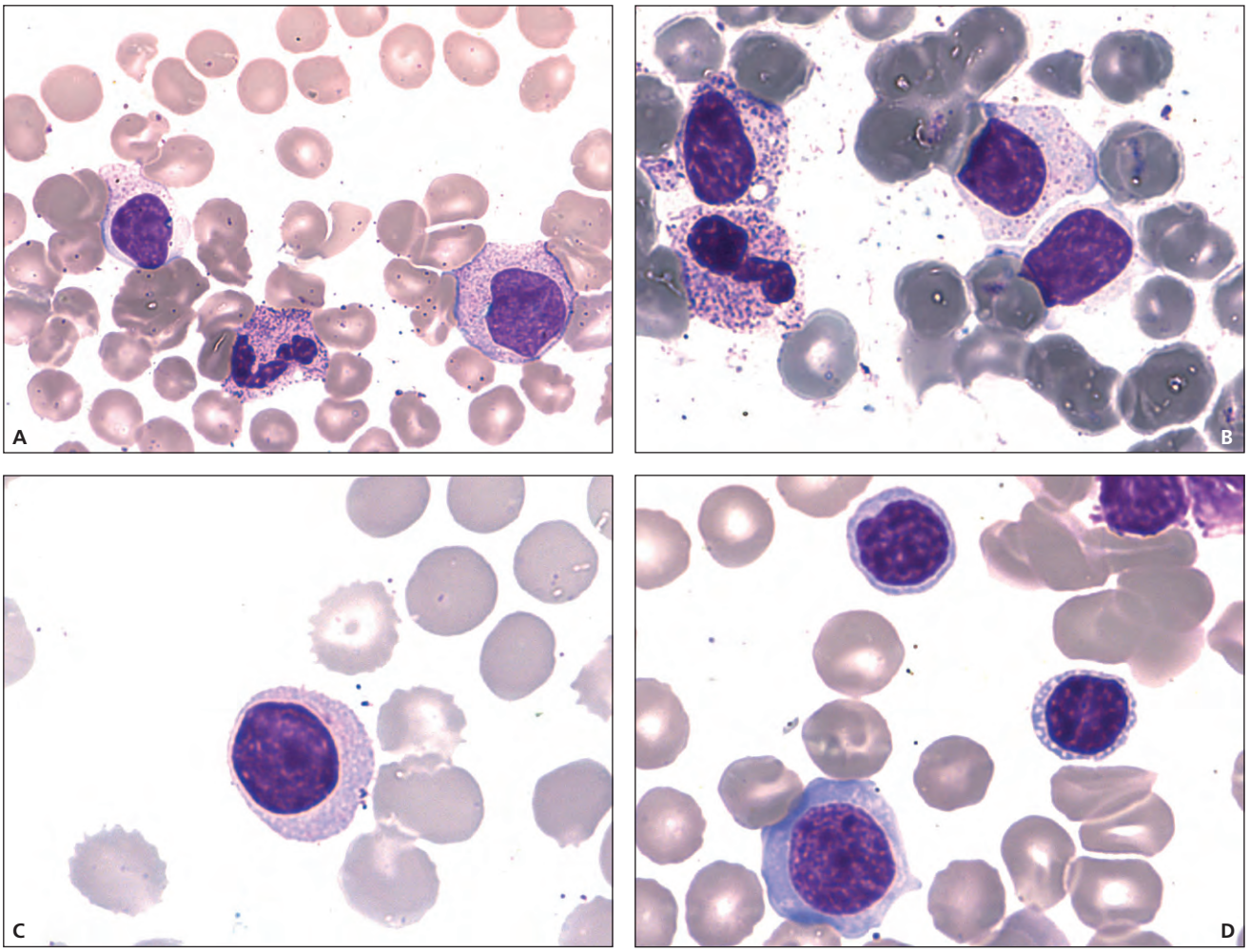



**Fig. 9.81** Large granular lymphocytic leukaemia (LGLL); peripheral blood film. (A) On the left, a lymphoid cell with plentiful pale cytoplasm containing numerous fine granules; the nucleus is compact, with chromatin condensed into blocks. To the right, a large granular lymphocyte (LGL), which appears less mature, with more plentiful, pink cytoplasm; the nucleus is large, notched and angulated with a minor degree of chromatin condensation. (B) To the left, a metamyelocyte and a band form neutrophil. To the right, two LGL with different amounts of cytoplasm. (C) Also this cell, in which granules are not identifiable, is an LGL, as indicated by the plentiful pale lilac cytoplasm. (D) This leukaemic population shows a certain degree of cytological heterogeneity: at the bottom, a large cell with plentiful basophilic cytoplasm, the cell sufficiently delicate that it has been deformed by the surrounding red cells. The other two cells do not have visible granules: that on the right has a corona of small vacuoles. The structure of the chromatin is, however, similar to that of the other LGL shown in (A–D).

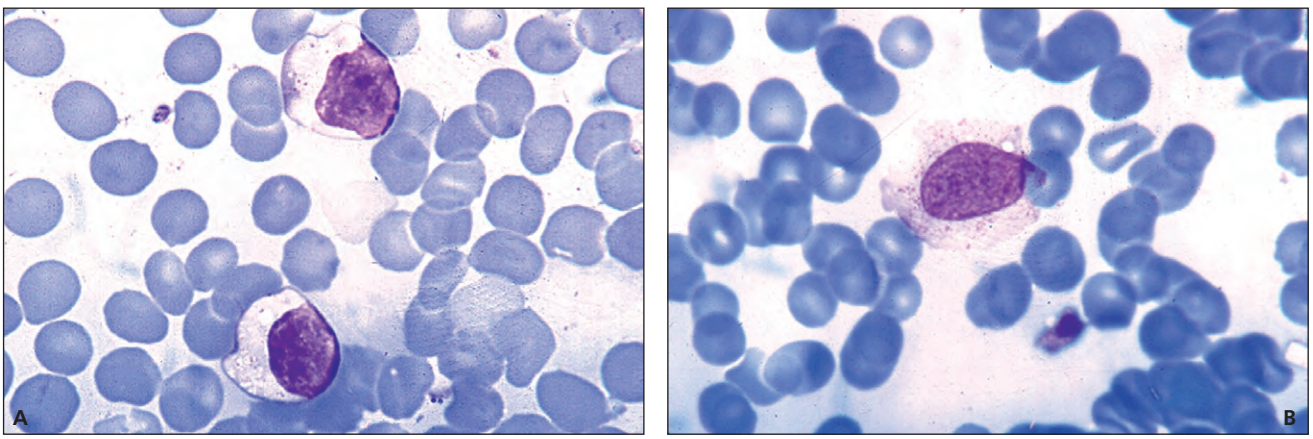



**Fig. 9.82** LGLL; peripheral blood film. In this patient, the leukaemic cells were CD3 negative, CD4 positive and CD8 negative. (A) The nuclei are rounded with a chromatin structure similar to that of normal lymphocytes. There is ample cytoplasm lacking homogeneity, resembling blown glass: the mild cytoplasmic basophilia is more intense at the periphery and appears interrupted by indistinct vacuoles and isolated fine azurophilic granules. Some granules are within vacuoles. (B) Other cells, such as those shown here, have the same characteristics except that the nucleus is more immature, with heterogeneous chromatin with only small areas of condensation and ill-defined nucleoli. The azurophilic granules are particularly numerous.

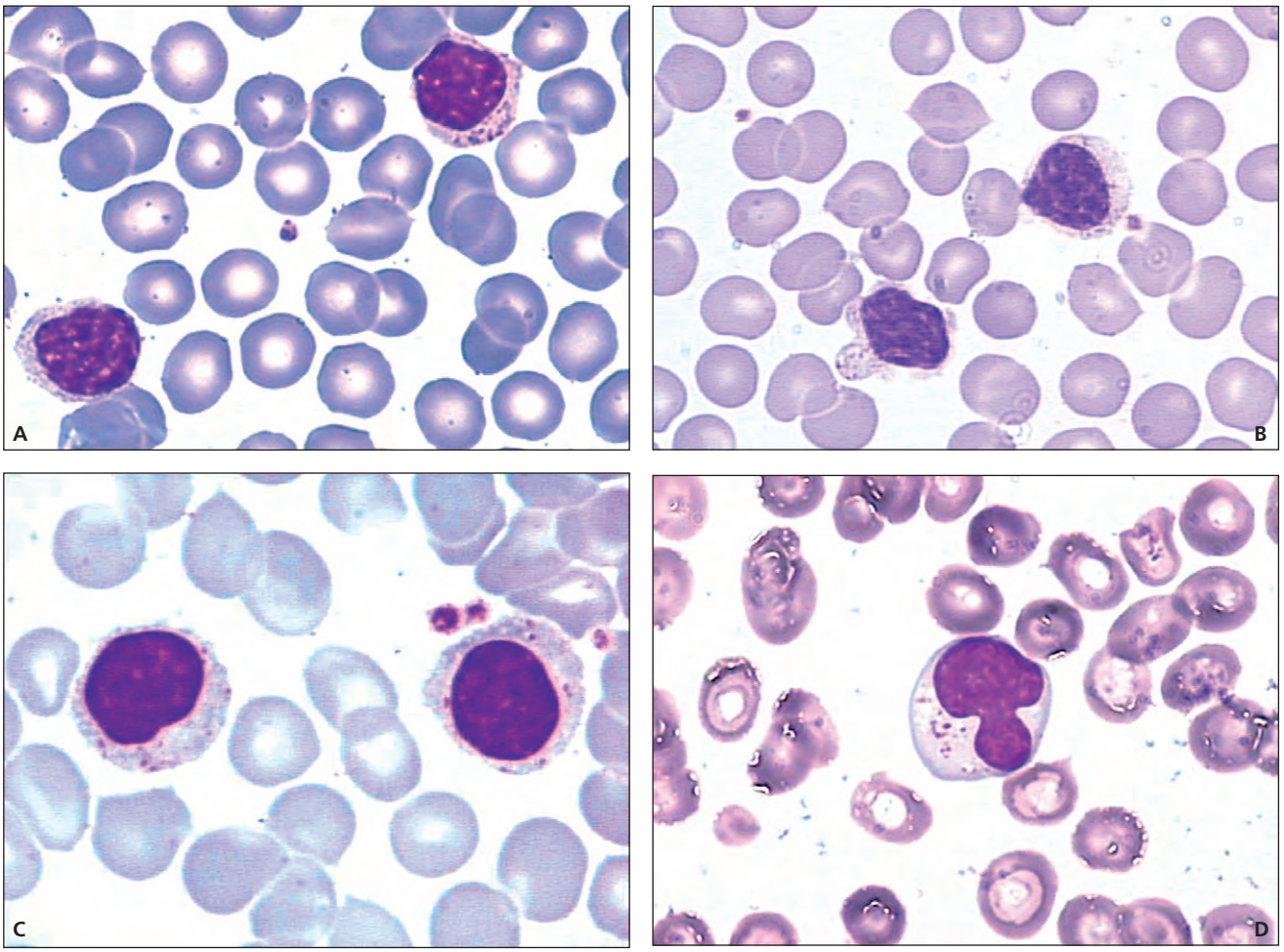



**Fig. 9.83** LGLL; images of LGL in a peripheral blood film from another patient. (A) Small cells. There are visible granules, particularly in the cell top right. (B) Two medium-sized LGL with mature nuclei and ample pale cytoplasm. (C) The chromatin of this cell is dark and compact. The nucleus of the cell on the left is eccentric and bean shaped, with a clear notch: an appearance very typical for LGL. (D) LGL with a lobated nucleus and a few large dark granules within the pale cytoplasm.

Symptomology is heterogeneous, with extremely variable haematological and clinical features: the latter include hypercalcaemia, lymphadenopathy, hepatosplenomegaly, cutaneous and bone lesions, fever, eosinophilia and increased serum LDH.[109–111] Hypercalcaemia has been attributed to overexpression of genes that promote osteoclast differentiation, such as *RANKL*, *WNT5A* and *PTHLH*. Various forms of the disease have been described: a subclinical asymptomatic form, characterised exclusively by the presence of mild lymphocytosis with a low percentage of atypical cells; chronic lymphocytosis with an indolent clinical course and survival of more than two years; a lymphomatous form with mainly extramedullary localisation; an acute, clinically aggressive form, with a frankly leukaemic haematological picture associated with hypercalcaemia, extramedullary involvement and survival of no more than six months. In asymptomatic seropositive patients, sometimes DNA analysis with probes for the HTLV-1 virus or the demonstration of rearrangement of TCR genes permits T-cell clonality to be demonstrated.

### Morphology

The peripheral blood often shows a moderate leucocytosis with an increase of atypical lymphocytes, sometimes associated with neutrophilia and eosinophilia. Anaemia and thrombocytopenia are variable. The morphology of circulating lymphoma cells is very variable, with small, medium-sized or large cells, but often sufficiently characteristic to suggest the diagnosis (Figs 9.85, 9.86 and 9.87). The most typical cells have been called flower cells or cloverleaf cells because of the convoluted or lobulated appearance of the nucleus: they can be identified on light microscopy with as many as five to six lobes, between which it is almost always possible to see the cytoplasm. The chromatin is generally dense, hyperchromatic and without visible nucleoli. Some cells, however, have immature dispersed chromatin,

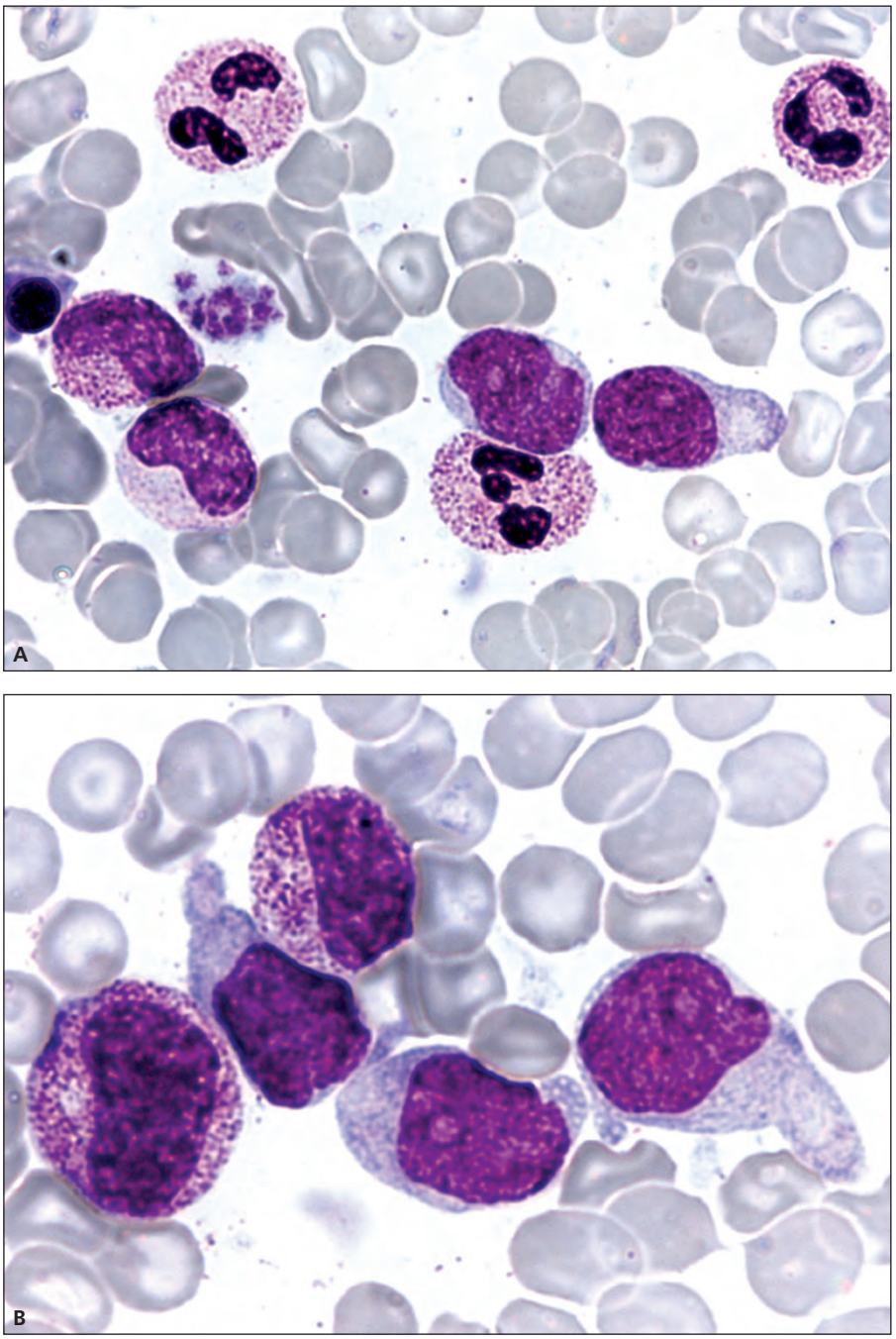



**Fig. 9.84** Aggressive NK leukaemia; bone marrow aspirate. (A) In the centre and on the right, two of the leukaemic cells that partially infiltrated this marrow. The cytological features are those of small to medium-sized blast cells, with basophilic cytoplasm containing no visible granules and a nucleus with an indented outline, worm-like chromatin and a pale, well-delineated nucleolus. (B) Another three blast cells with similar characteristics; in some cells the cytoplasm has an elongated polar projection giving a racquet or hand-mirror shape; the other two cells are neutrophil myelocytes.

giving a more frankly blastic appearance. The cytoplasm is well represented, weakly basophilic and without granules. In some patients, the cytological picture is pleomorphic, with cells of all sizes and the presence of anaplastic, giant and bizarre cells with convoluted nuclei.

On ultrastructural examination, the majority of cells show nuclear irregularity with a small nucleus and the presence of areas of chromatin condensation. The cytoplasm shows a small, well-developed Golgi zone and lysosomal granules often grouped together; sometimes there are masses of microfibrils in

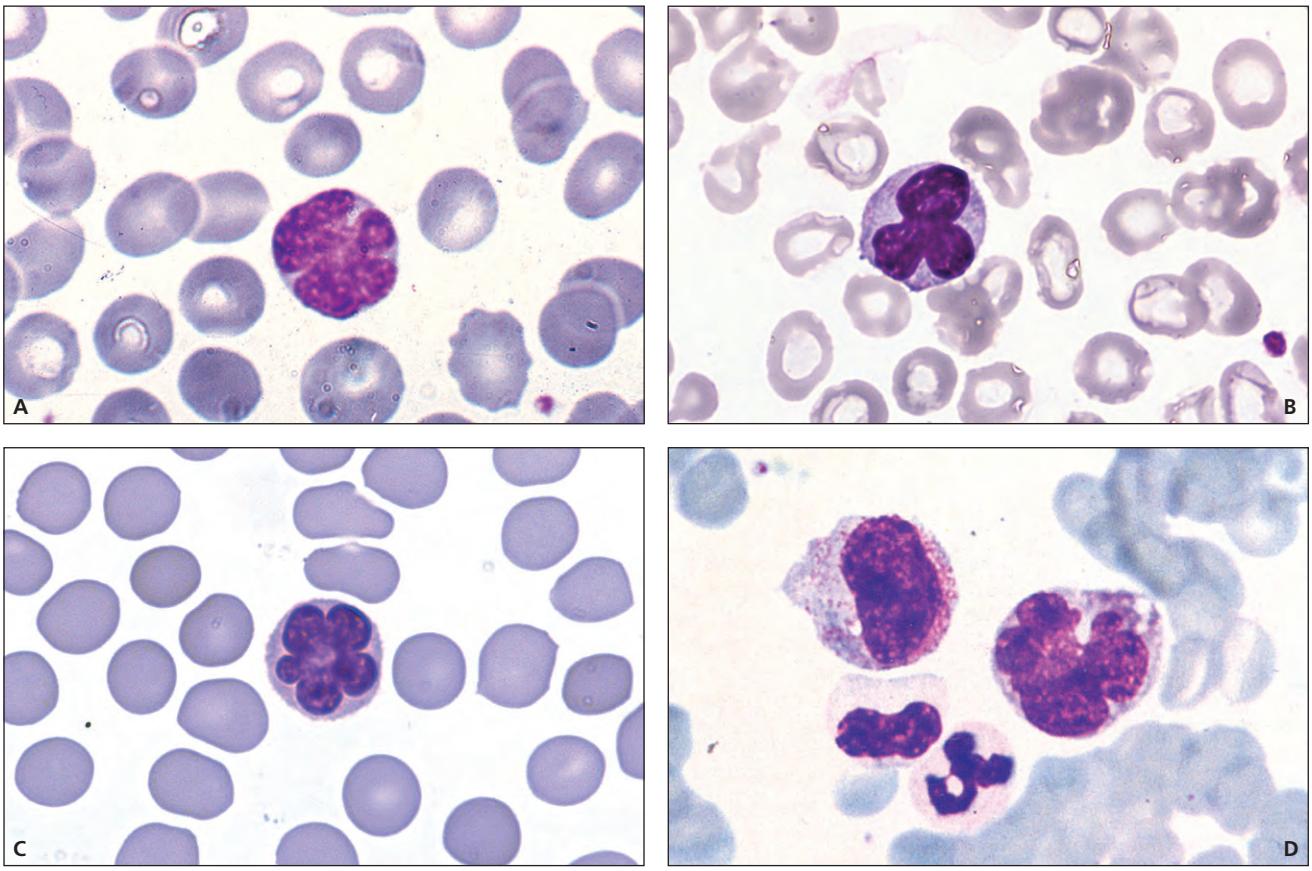



**Fig. 9.85** Adult T-cell leukaemia/lymphoma (ATLL). (A) Peripheral blood film. Typical 'flower cell' with numerous petals separated by deep inlets in which the basophilic cytoplasm is visible. The chromatin is condensed into small clumps and nucleoli are not seen. (B and C) Peripheral blood of two other patients: lymphoid cells with a lobated nucleus, trilobed or flower shaped, with compact chromatin. (D) Bone marrow aspirate. Patient with no lymphoma cells in the peripheral blood and limited bone marrow infiltration (15%). The atypical cells, such as that on the right, are large with irregular multilobated nuclei.

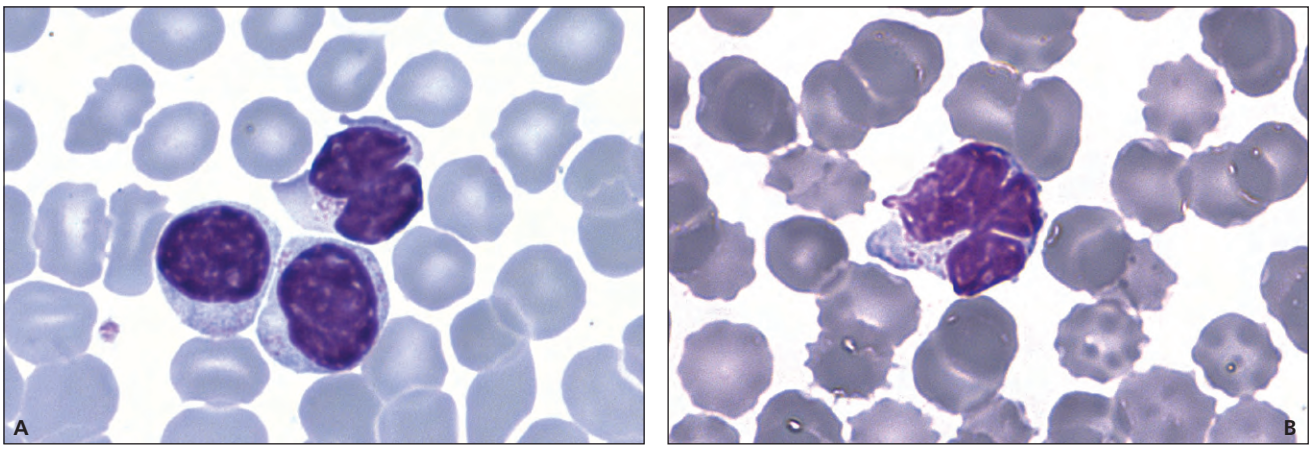



**Fig. 9.86** ATLL; peripheral blood film. (A) The leukaemic cells have the appearance of small lymphocytes with chromatin condensed into large clumps and basophilic cytoplasm, often containing fine granules. Some nuclei are reniform or indented. (B) Other cells, these also with fine cytoplasmic granules, have the characteristic lobulated nucleus of the flower cell. The red cells in this part of the film show an artefactual echinocytic deformation, more marked than in (A).

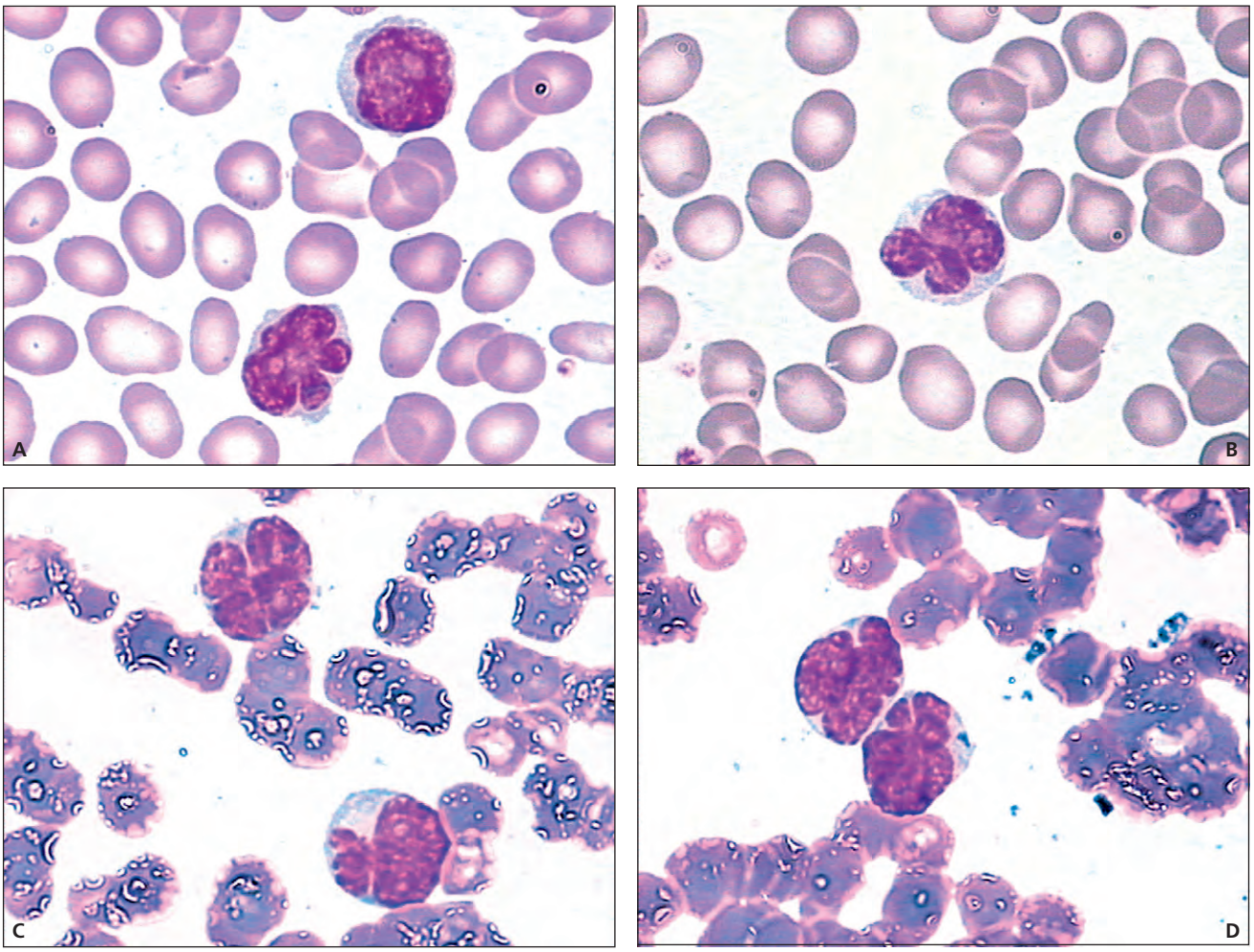



**Fig. 9.87** ATLL; peripheral blood films of different patients. (A and B) There are typical flower cells with irregularly multilobated nuclei. (C and D) The lobulated nuclear appearance of the leukaemic T cells is retained in this film, which has been dried too slowly so that the erythrocyte morphology is lost.

the perinuclear zone. Cells show focal paranuclear acid phosphatase activity and positivity for beta-glucuronidase and ANAE. Bone marrow infiltration correlates with the degree of leucocytosis. Lymph nodes are diffusely infiltrated. The skin shows perivascular dermal infiltration.

### Immunophenotype and genetic analysis

The cells of ATLL are positive for the post-thymic T-cell markers CD2, CD3, CD4 and CD5; in addition, they are strongly positive for CD25. Rare cases expressing CD8 have been described, either isolated CD8 expression or associated with CD4. Many cases express CD52, making alemtuzumab therapy possible. The TCR genes are rearranged. The neoplastic cells have HTLV-1 integrated into their genome.

## Primary cutaneous T-cell lymphoma

The clinical forms of T-cell lymphoma that show tropism for the skin are mycosis fungoides and Sézary syndrome. The latter, rather than being a leukaemic manifestation of mycosis fungoides, is now considered to be a distinct clinicopathological entity. The normal cellular counterpart, however, is the same: a mature CD4-positive T cell characterised by specific tropism for the epidermis. These are rare disorders that appear after the sixth decade of life with the incidence in men being twice that in women. In mycosis fungoides the clinical picture is exclusively of cutaneous lesions, plaques and nodules, with a tendency to ulcerate, resulting from infiltration by medium-sized to small T lymphocytes with hyperchromatic cerebriform nuclei. Characteristic, but not pathognomonic, are the accumulations of lymphoma cells, known as Pautrier microabscesses, recognisable in the epidermis. Evolution is slow with eventual formation of tumours.

The dermatological picture of Sézary syndrome is that of generalised erythroderma, often hyperpigmented and exfoliative. The clinical course is aggressive, with a five-year survival of less than 5%. The 2008 WHO classification defines a diagnostic triad of:

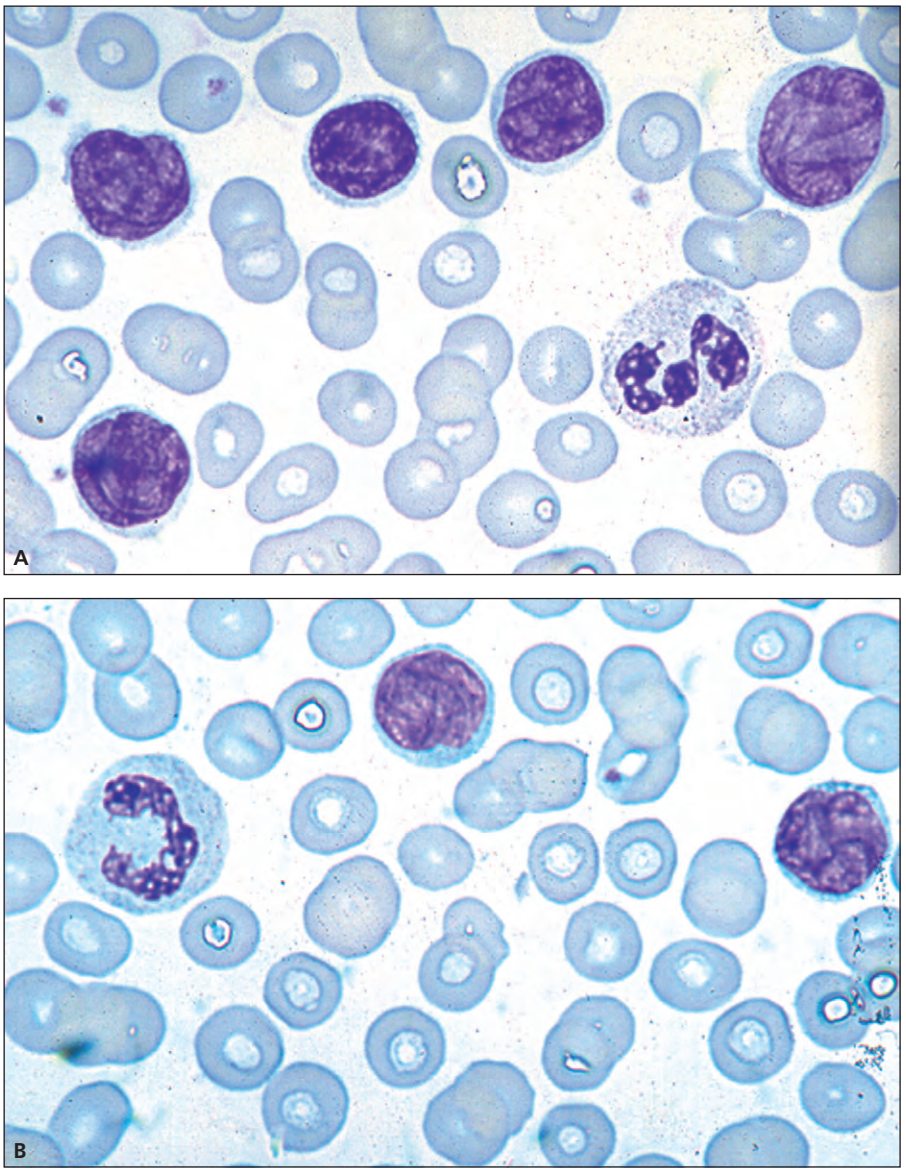



**Fig. 9.88** Sézary syndrome; peripheral blood film. Female patient with marked generalised erythroderma and an elevated leucocyte count (64 × $10^9$/l). (A) One lymphocyte (the second cell from the left in the top row) shows normal chromatin condensation into irregular clumps. The other four lymphoid cells are typical Sézary cells. The convoluted cerebriform nuclear appearance can be seen clearly in the larger cell in the top right corner; this is not so much evident in the nuclear outline, which shows only small notches, but rather in the grooves, folds and valleys that traverse the surface of the nucleus. (B) The clarity of the nuclear morphology of these small cells makes it possible to imagine the convoluted nucleus in three dimensions. The lobes of these nuclei are folded tightly together, without clear open fissures, in which it would be possible to see the cytoplasm.

- Erythroderma;
- Generalised lymphadenopathy;
- The presence of neoplastic clonal T cells, Sézary cells, with a cerebriform nucleus in the peripheral blood, the skin and the lymph nodes, which satisfies at least one of the following criteria:
  - Absolute count in the peripheral blood above 1.0 × $10^9$/l;
  - Presence in the circulation of an increased percentage of CD4-positive T cells with a CD4/CD8 ratio above 10 and/or associated with a loss of one or more T-lineage antigens.[112]

## Morphology

Sézary cells are present in variable numbers in the blood film, ranging from a few cells to a frankly leukaemic picture with marked leucocytosis (Figs 9.88 through to 9.91), often accompanied by moderate eosinophilia. A very low percentage of similar cells can also be observed in the blood of healthy control subjects, in patients with non-neoplastic skin conditions and in other cutaneous lymphomas.[113,114] The count, with the support of flow cytometric immunophenotypic analysis and clonality studies of TCR genes using PCR or Southern blot, is

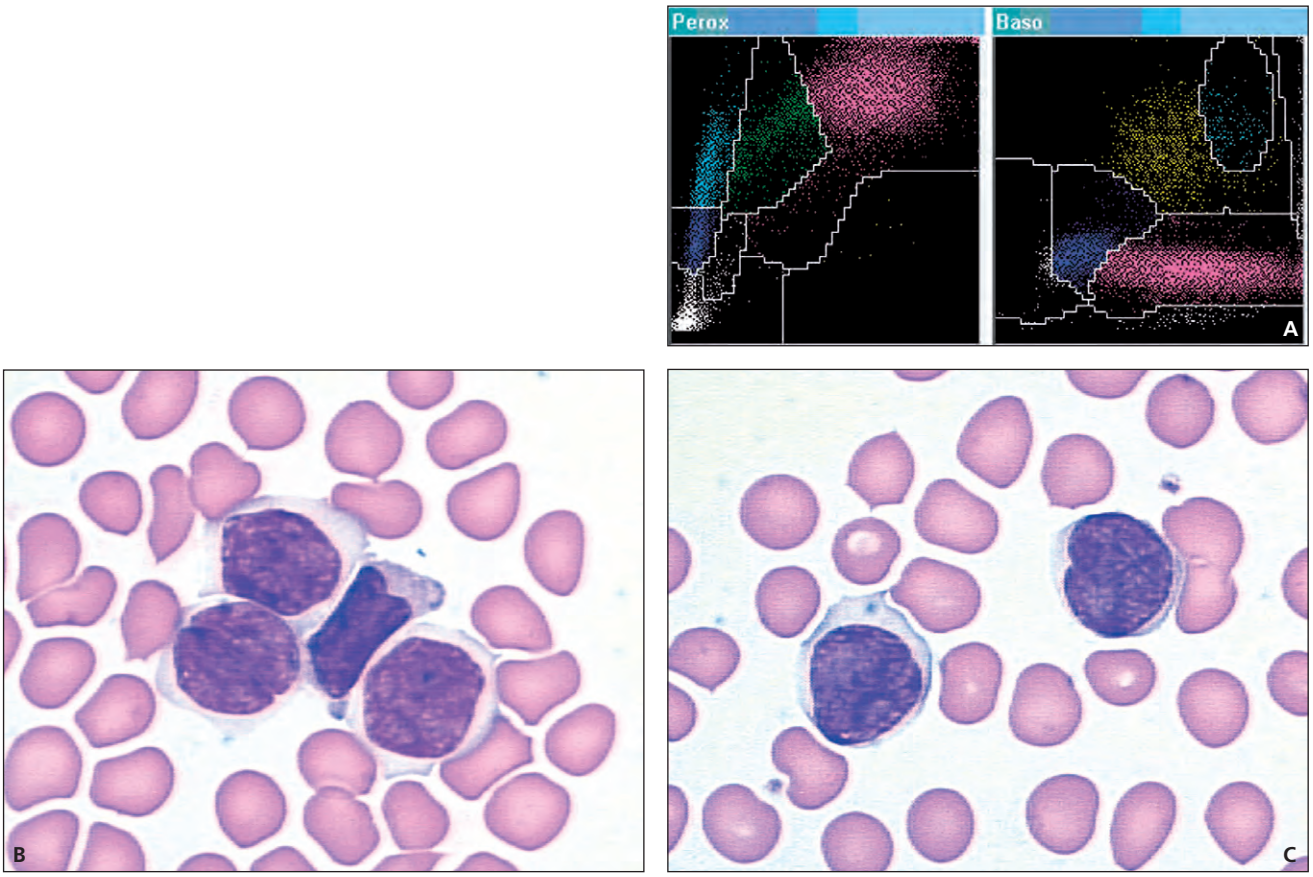



**Fig. 9.89** Sézary syndrome in a woman aged 64 years. WBC 15.7 × $10^9$/l, with neutrophilia (71%); Hb 127 g/l; platelets 364 × $10^9$/l. (A) The Perox cytogram shows increased LUC (9.6%), which correspond numerically to the cells that in the Baso cytogram are detached from the MN and PMN clusters and are invading the basophil area as a circumscribed cluster, causing pseudobasophilia (7.8%). (B and C) In the peripheral blood film there are Sézary cells (three or four in image [C]), which are medium sized to large with a convoluted cerebriform nucleus; the cytoplasm is fairly extensive and almost colourless.

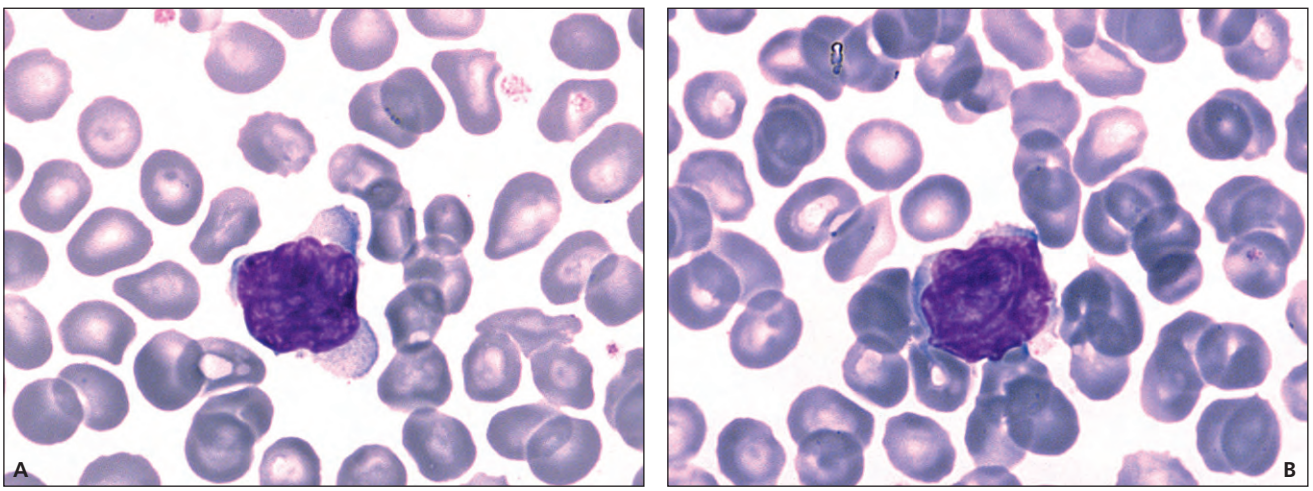



**Fig. 9.90** Sézary syndrome; peripheral blood films. (A and B) Large Sézary cells with scanty basophilic cytoplasm and voluminous nuclei with folds and convolutions.

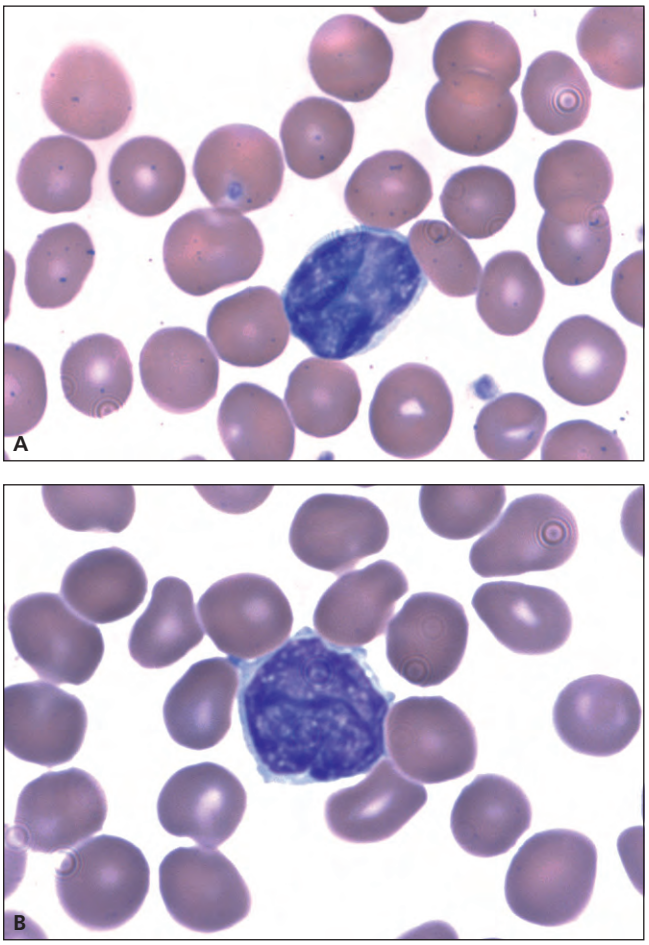



**Fig. 9.91** Sézary syndrome; peripheral blood films. (A and B) Small to medium-sized cells: the nuclear morphology, with folds, furrows and valleys, is also very characteristic.

used to establish for each patient a 'blood' stage,[115,116] indicated as:

- B0: absence of significant involvement of the peripheral blood; less than 5% of circulating lymphocytes are Sézary cells (subdivided into B0a if clonality analysis is negative and B0b if it is positive);
- B1: low tumour burden in the peripheral blood; Sézary cells are more than 5% (B1a and B1b based on the absence or presence of evidence of clonality);
- B3: high tumour burden; the absolute count of Sézary cells (or of cells with an appropriate immunophenotype) is higher than $1.0 \times 10^9$/l and investigation for clonality is positive.

Classically, two types of Sézary cell have been described, large and small. Some cases show mainly small cells, while in others there is a mixture of large and small. The characteristic cytological feature of both is the morphology of the nucleus: this is round or oval and very irregular as the result of deep very narrow clefts with lobes superimposed and folded, this giving a convoluted appearance to the nucleus, classically described as cerebriform. Nucleoli are absent or barely visible; the chromatin is condensed and hyperchromatic. The depth of the indentations and the narrow space between the lobes means that the underlying cytoplasm is not visible: this feature is useful in the differential diagnosis with ATLL. The small cells, also known as Lutzner cells, have a diameter of 8–10 μm, equivalent to that of a small lymphocyte. They are more difficult to recognise on light microscopy because the nuclear structure is very compact and the evidence of irregularity may only be apparent in three dimensions, focusing with the microscope and paying careful attention to the nuclear surface where grooves and slits are apparent. The N:C ratio is very high; the cytoplasm is scanty, weakly basophilic, sometimes with small PAS-positive vacuoles. Large Sézary cells are more easily recognised; they have a diameter similar to that of a monocyte (15–20 μm), scanty basophilic agranular cytoplasm and a high N : C ratio. The nucleus is very large, occupying almost the entire cell; its convoluted appearance is very evocative. Ultrastructural examination shows the cerebriform nucleus very clearly.

In bone marrow aspirates, Sézary cells can be absent or very infrequent or, in cases with leucocytosis, they can be very numerous. In histological sections of trephine biopsy specimens, the infiltrate, when present, can be dispersed and interstitial, to the extent that it can be identified in many cases only with the help of immunohistochemistry. The morphology of the cells that infiltrate the skin, lymph nodes and other tissues is analogous to that of the circulating cells.

### Immunophenotype and genetic analysis

Sézary cells have the phenotype of mature helper T cells, expressing CD3, CD4 and CD8; CD7 is typically absent or weak. Very rarely, CD8 is expressed instead of CD4. A new sensitive and specific marker is CD158k, which is also useful for monitoring for minimal residual disease. All cases express TCR alpha/beta. The results of flow cytometry must be interpreted with caution, since up to 20–30% of T cells with this characteristic immunophenotype have been reported in normal subjects and in patients with various benign skin conditions.

Recurrent cytogenetic alterations have not been identified. Using hybridisation techniques, a high frequency of unbalanced translocations and deletions involving various chromosomes have been identified. TCR genes show clonal rearrangement.

## Other T-cell leukaemia/lymphomas that can involve the blood and marrow

Involvement of the bone marrow and, less often, the peripheral blood with a true leukaemic picture can occur in angioimmunoblastic T-cell lymphoma, anaplastic large T-cell lymphoma, particularly ALK (anaplastic lymphoma kinase)-positive cases, hepatosplenic T-cell lymphoma, enteropathy-associated T-cell lymphoma and T-cell lymphoma, not otherwise specified.[117] Invasion of the bone marrow and blood occurs particularly

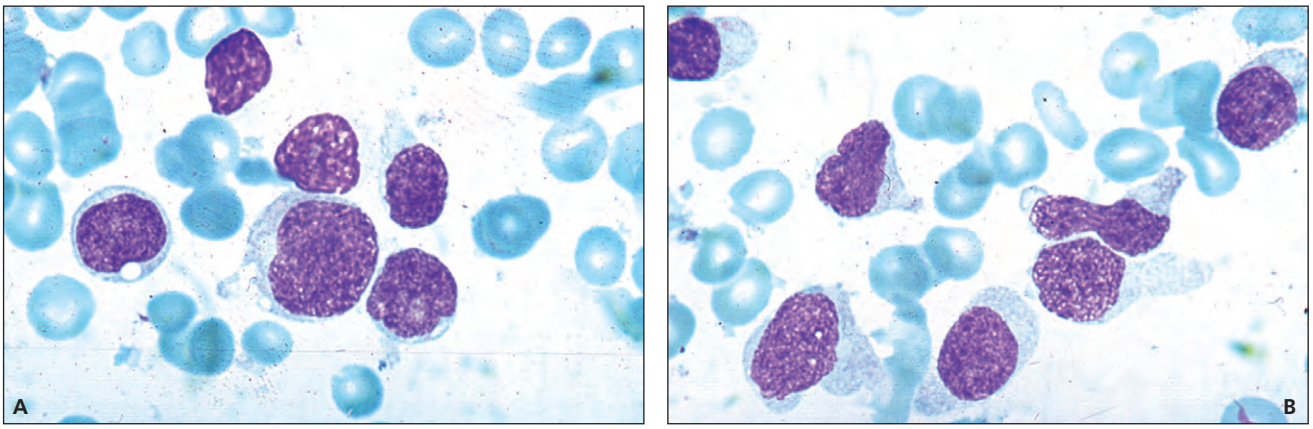



**Fig. 9.92** T-cell leukaemia/lymphoma; bone marrow aspirate. (A) The lymphoma cells have the appearance of blast cells of various sizes with granular chromatin, occasional indistinct nucleoli and a variable amount of weakly basophilic cytoplasm. (B) Same film as (A). The cells have more plentiful, grey-blue cytoplasm; the granular appearance of the chromatin is particularly evident, whereas nucleoli are not seen.

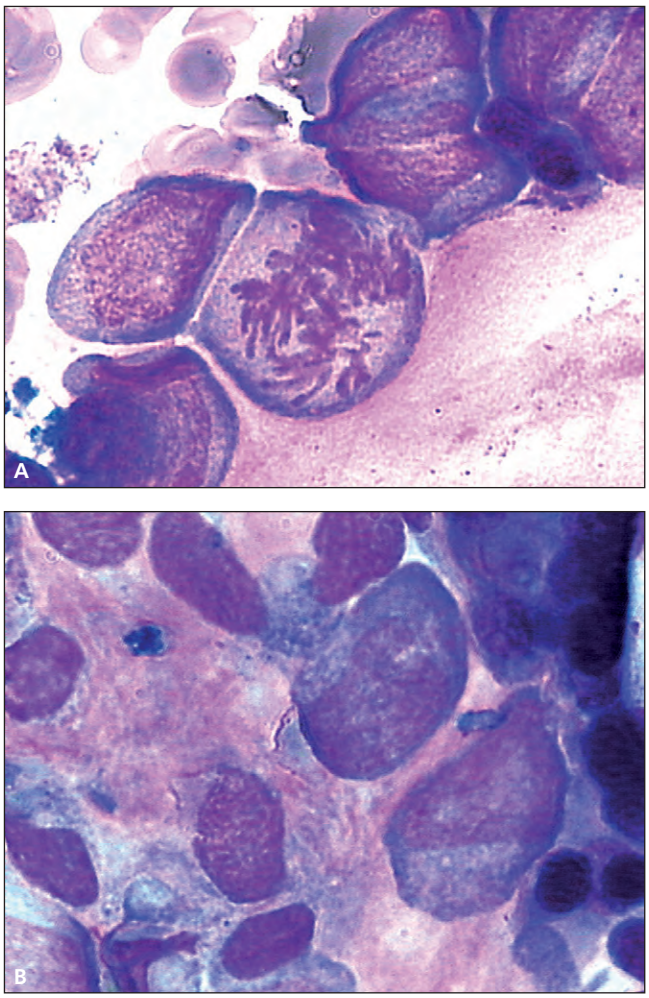



**Fig. 9.93** T-cell leukaemia/lymphoma; bone marrow aspirate. (A) The lymphoma cells that have infiltrated the bone marrow, but not the peripheral blood, are large blast cells with immature chromatin: in this small field there is a hyperdiploid mitotic figure. (B) The cytoplasm of the two lymphoma cells is basophilic and the nucleus is typically blastic. There is a rose-coloured, acidophilic, background staining, which when superimposed on part of the cytoplasm gives a false impression of granularity.

during disease evolution, with lymphoma cells showing diverse characteristics, depending on the histological subtype (Figs 9.92 through to 9.98): small and medium-sized lymphoid cells with moderate morphological heterogeneity in angioimmunoblastic lymphadenopathy and pleomorphic small cell lymphomas; large blastic cells with polymorphic nuclei, dispersed chromatin, visible nucleoli and basophilic cytoplasm in hepatosplenic T-cell lymphoma and anaplastic large cell lymphoma.

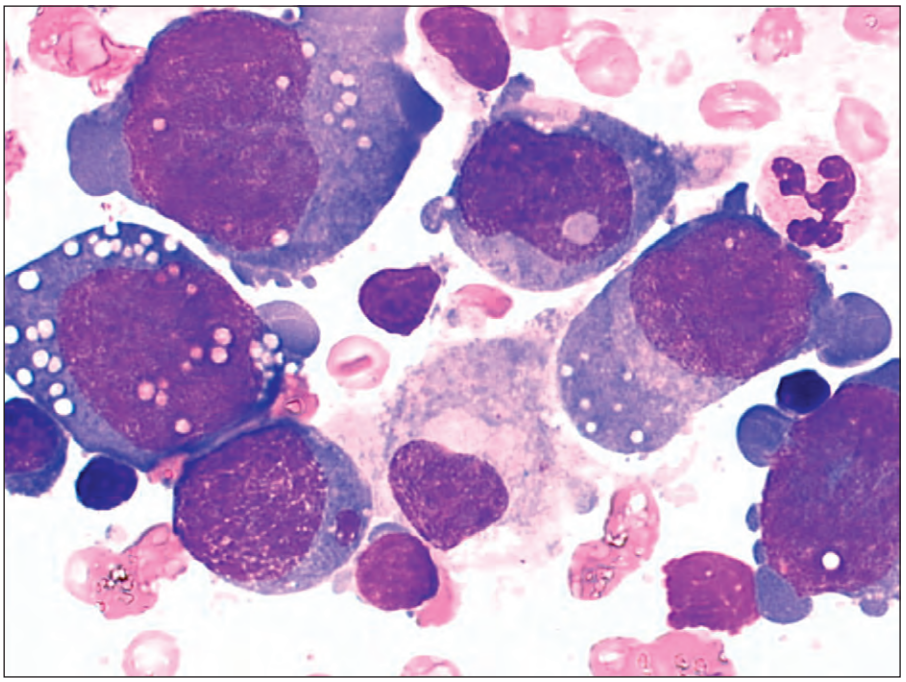


**Fig. 9.94** Anaplastic large T-cell lymphoma, ALK-positive; bone marrow aspirate. The majority of cells infiltrating the bone marrow in this patient are gigantic with abundant basophilic, heavily vacuolated cytoplasm. The chromatin of the enormous nuclei is homogeneous, dispersed in very fine granules; the nucleoli are numerous, but not very distinct. These cells were positive for CD2, CD5, CD4 and, typically, CD30; with immunohistochemistry, furthermore, they stained specifically with anti-ALK antibodies. The chromosomal rearrangement, t(2;5), present in the great majority of these cases, leads to the formation of an *NPM1-ALK* fusion gene.

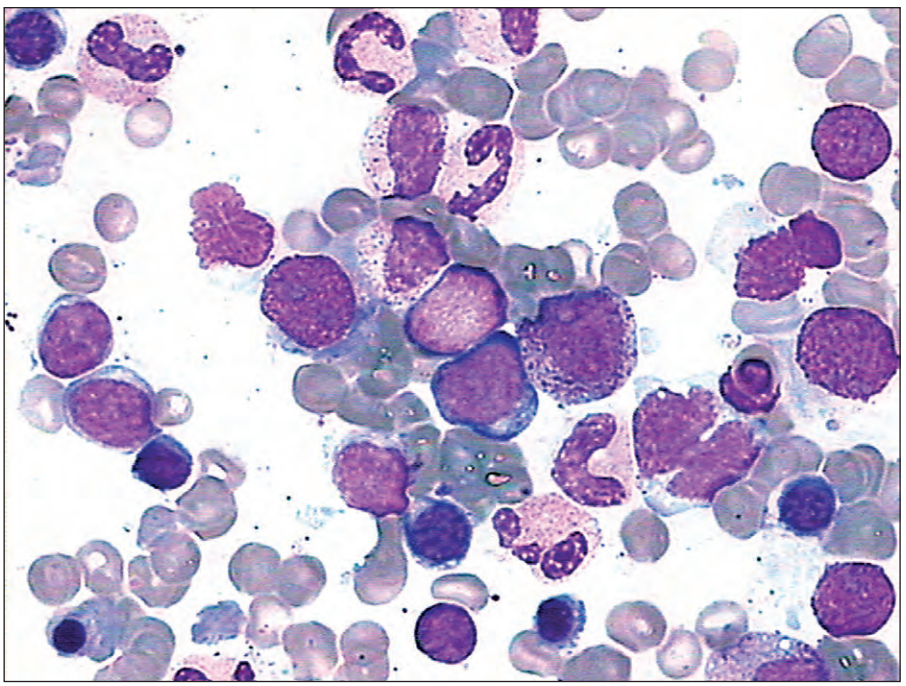


**Fig. 9.95** Hepatosplenic T-cell lymphoma with bone marrow involvement. Bone marrow aspirate from a 29-year-old student with thrombocytopenia (platelet count 60 × $10^9$/l); there were no atypical cells in the peripheral blood; there was moderate hepatomegaly and marked splenomegaly. 30% of cells were immature peroxidase-negative cells, which were negative for CD19 and CD5 and positive for CD2, CD3, CD7, CD16 and TCR gamma/delta.

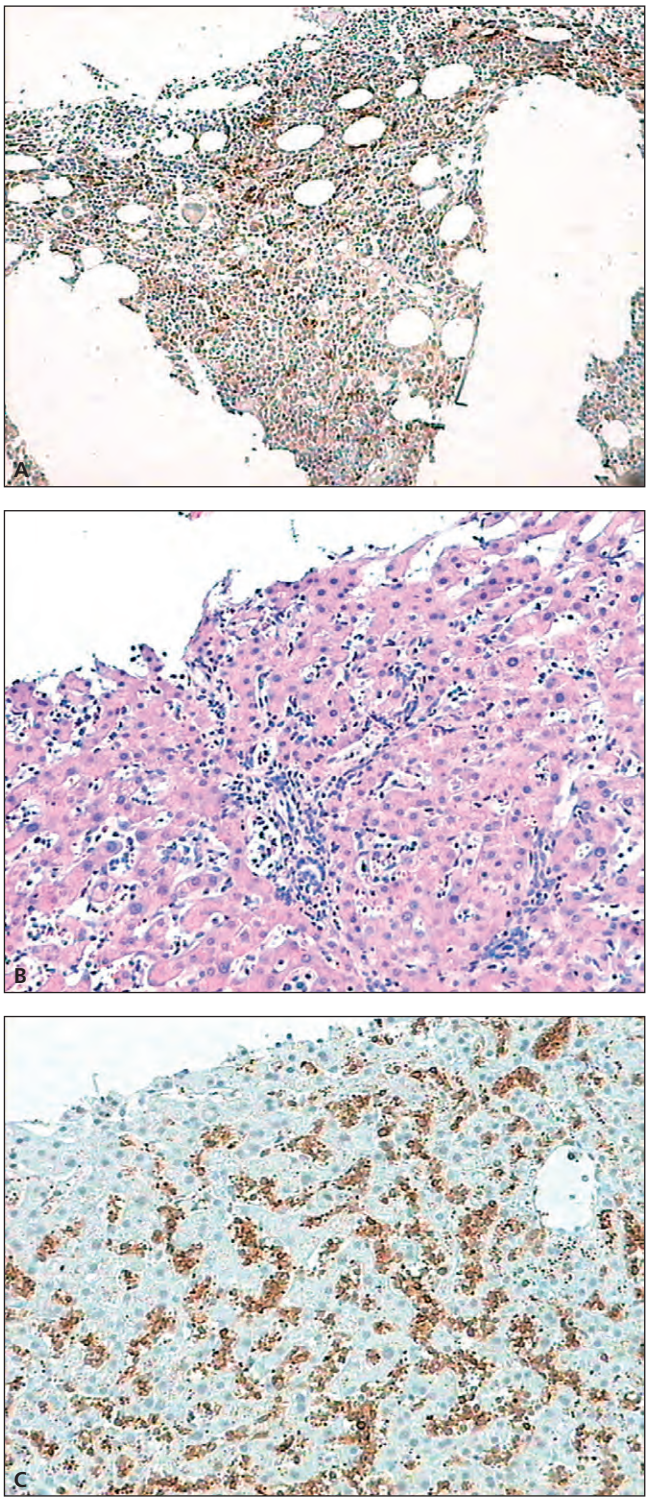
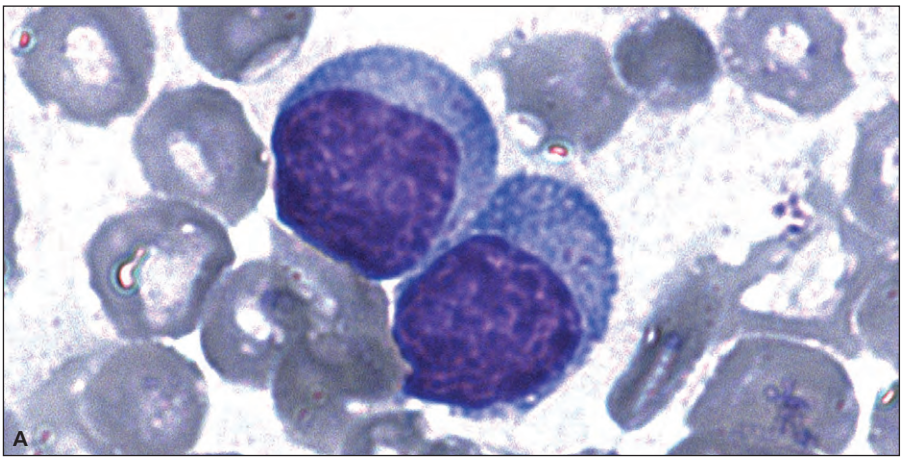
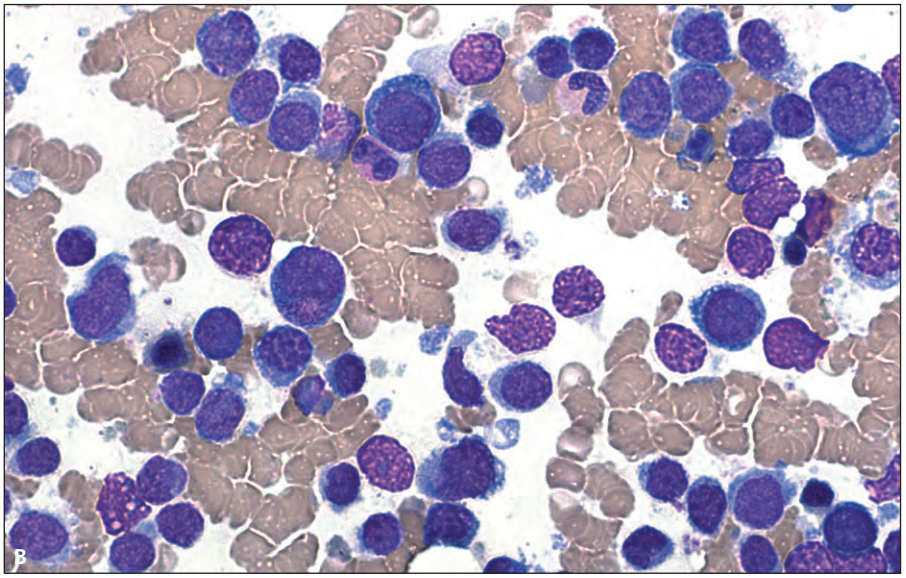



**Fig. 9.96** Hepatosplenic T-cell lymphoma; same case as Fig. 9.95. (A) Bone marrow trephine biopsy section, immunoperoxidase for CD3: normal cellularity, infiltration by irregularly shaped atypical cells, which have plentiful cytoplasm, are CD3 positive and show a mainly intrasinusoidal distribution. (B) Histological section of a fragment of a liver biopsy specimen. Between the rows of polygonal pink hepatocytes, there is a diffuse, mainly intrasinusoidal, infiltrate of lymphoid cells. (C) Immunohistochemistry for CD3 shows very clearly the intrasinusoidal distribution of the hepatic infiltrate, a characteristic pattern of infiltration in this lymphoma. An autologous haemopoietic stem cell transplant in this patient induced a complete remission followed by fatal relapse five years later.



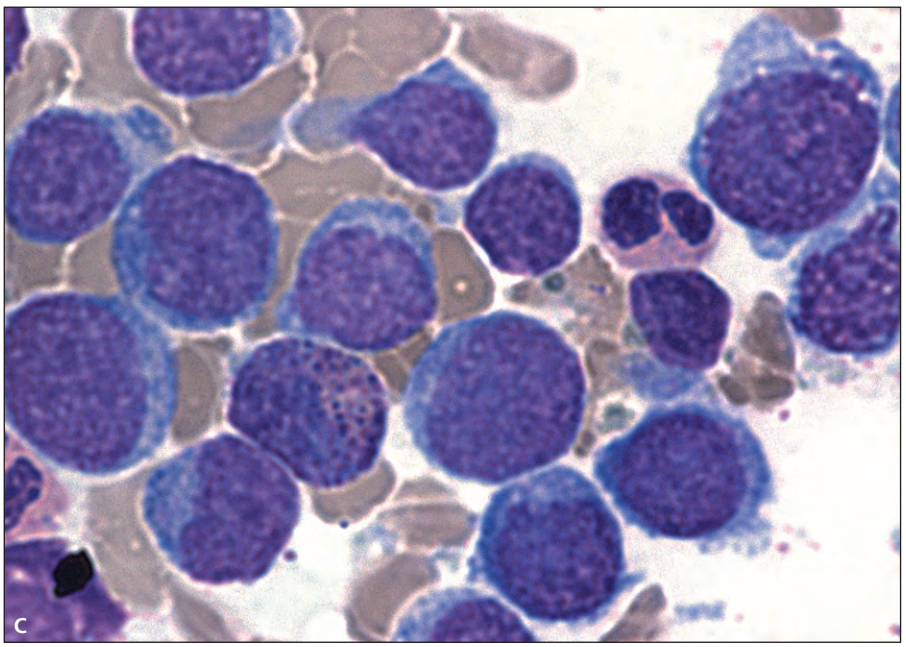




**Fig. 9.97** Enteropathy-associated T-cell lymphoma; from a 60-year-old man with disseminated disease after multiple relapses. This rare neoplasm originating in an intraepithelial T cell only rarely involves the haemopoietic system. (A) Peripheral blood film. Large lymphoma cells with basophilic cytoplasm and a markedly eccentric nucleus with a fairly regular outline and chromatin condensed into blocks. The blood count showed severe pancytopenia (WBC $1.8 \times 10^9$/l; Hb 87 g/l; platelets $77 \times 10^9$/l); immunophenotyping showed expression of CD3, CD7, CD8 and CD103, but not CD5. (B) Bone marrow aspirate. The bone marrow is hypercellular and massively infiltrated by a population of variably sized lymphoma cells, with variable amounts of intensely basophilic cytoplasm. (C) Mainly cells with scanty cytoplasm and large nuclei with chromatin condensed homogeneously into small clumps; nucleoli are visible in some of these, as in the larger cell top right. To the left of the centre, an eosinophil myelocyte with large dark granules.

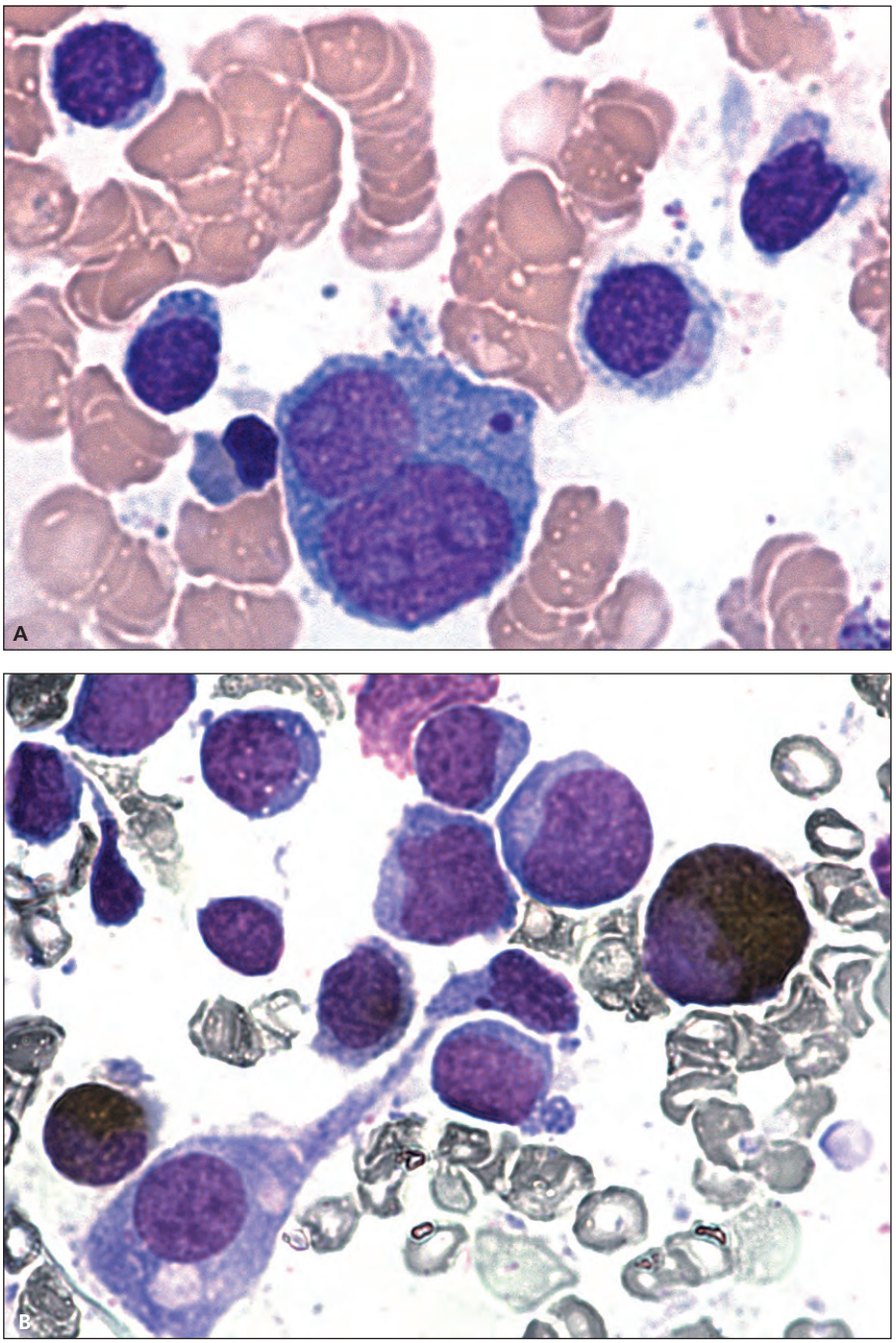
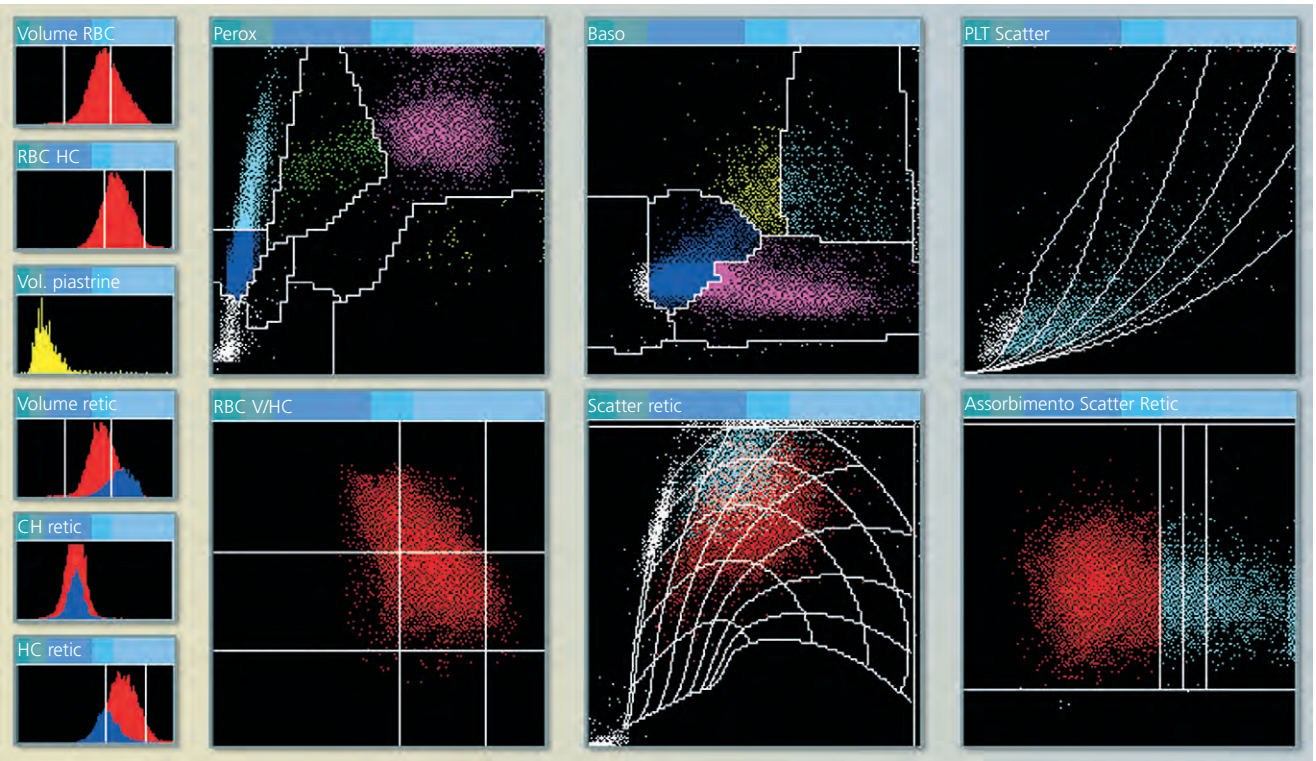



**Fig. 9.98** Enteropathy-associated T-cell lymphoma; bone marrow aspirate from the same case as Fig. 9.97. (A) A giant binucleated cell with basophilic cytoplasm and prominent nucleoli, distinguished from a Reed-Sternberg cell of Hodgkin lymphoma by the asymmetry of the two nuclei and the presence of two clearly distinguished nucleoli in the lower nucleus (which appears tetraploid). (B) Peroxidase stain. Peroxidase is strongly positive in two granulocyte precursors to the right and to the left, and is negative in the lymphoma cells. Peripheral to the main theme, but of exceptional interest, is the cell seen bottom left, which has all the morphological characteristics of an enterocyte, including straight edges and what appears to be a globule of mucous.*

*The translator wonders if it might be a macrophage with unusual morphology.

## REPORT 9.1 B-cell chronic lymphocytic leukaemia with associated autoimmune haemolytic anaemia



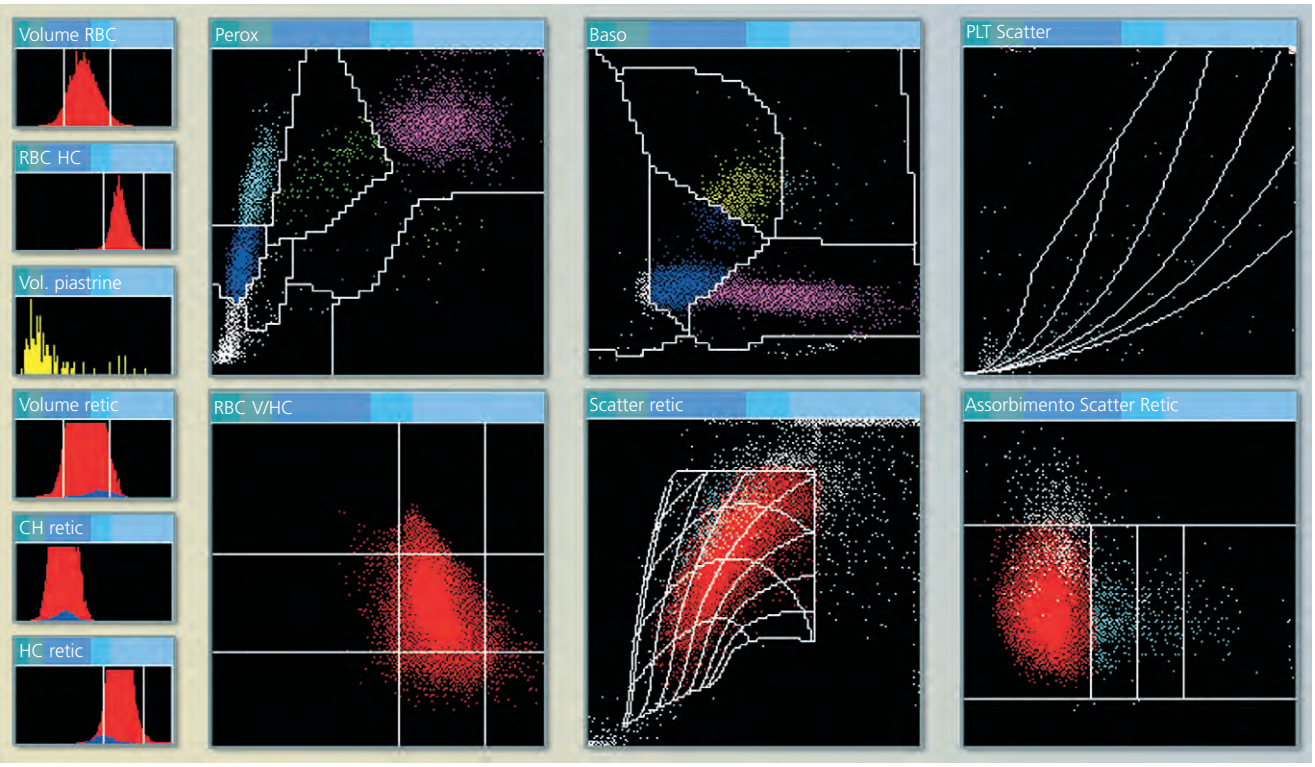

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 46 | g/l | |
| RBC | 124 | $\times 10^{12}$/l | * |
| HCT | 0.145 | l/l | * |
| MCV | 116.8 | fl | * |
| MCH | 36.9 | pg | * |
| MCHC | 316 | g/l | * |
| CHCM | 321 | g/l | * |
| RDW | 17.7 | % | * Anisocytosis + |
| HDW | 4.34 | g/dl | * Anisochromasia |
| HYPO | 17.4 | % | Hypochromia +++ |
| HYPER | 1.2 | % | |
| MICRO | 0.2 | % | |
| MACRO | 37.7 | % | Macrocytosis +++ |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 19.2 | % | * RTCADA |
| RET | 238.1 | $\times 10^{9}$/l | * RTC-FS |
| CHr | 38.0 | pg | |
| IRF (M+H) | 34.6 | % | |
| PLT | 173 | $\times 10^{9}$/l | * PLTORN |
| MPV | 11.6 | fl | * |
| PDW | 51.4 | % | |
| Large platelets | 8 | $\times 10^{9}$/l | |
| MPC | 20.1 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 76.12 | | |
| WBC Perox | 74.15 | | |
| NEUT | 7.11 | 9.3 | |
| LYMPH | 61.16 | 80.3 | |
| MONO | 0.46 | 0.6 | |
| EOS | 0.09 | 0.1 | |
| BASO | 0.86 | 1.1 | |
| LUC | 6.45 | 8.5 | Atyp ++ |
| NRBC | 0.00 | 0.0 | |
| LI | 1.83 | | |
| MPXI | 4.8 | | |
| MN | | 83.2 | |
| PMN | | 14.7 | |
| BLASTS | | 0.4 | |
| Perox HPX | | 0.4 | |
| Perox SAT | | 0.1 | |

This patient had suffered from B-CLL for some years. The report shows marked leucocytosis with an Atyp flag but no Blast flag. In the Perox cytogram, a peroxidase-negative population forms a flame-shaped cluster slightly inclined to the right and extending upwards from the lymphocyte box; 90% of the leukaemic cells are classified as lymphocytes and 10% as LUC; there are also numerous neutrophils forming a homogeneous cluster in a normal position. In the Baso cytogram, the MN head forms a dense and compact oval, which extends upwards causing a slight pseudobasophilia. There is a very severe anaemia, with macrocytosis and a marked increase in reticulocytes; the MCH is increased, correlating with the MCV, but the MCHC is normal. In the Scatter retic cytogram the reticulocytes, coloured blue, are localised in the upper left part of the erythrocyte population, since they are larger and have a lower haemoglobin concentration than mature red cells; further still to the left, one notes a small cigar-shaped cluster coloured white, produced by small leukaemic lymphocytes.

**Morphology.** Differential count: neutrophils 8%, lymphocytes 91.5% (CD19+, CD5+ and CD23+), monocytes 0.5%. The red cells show anisopoikilocytosis with macrocytosis and the presence of numerous microspherocytes. The direct Coombs test was positive.

## REPORT 9.2 Leukaemic form of mantle cell lymphoma



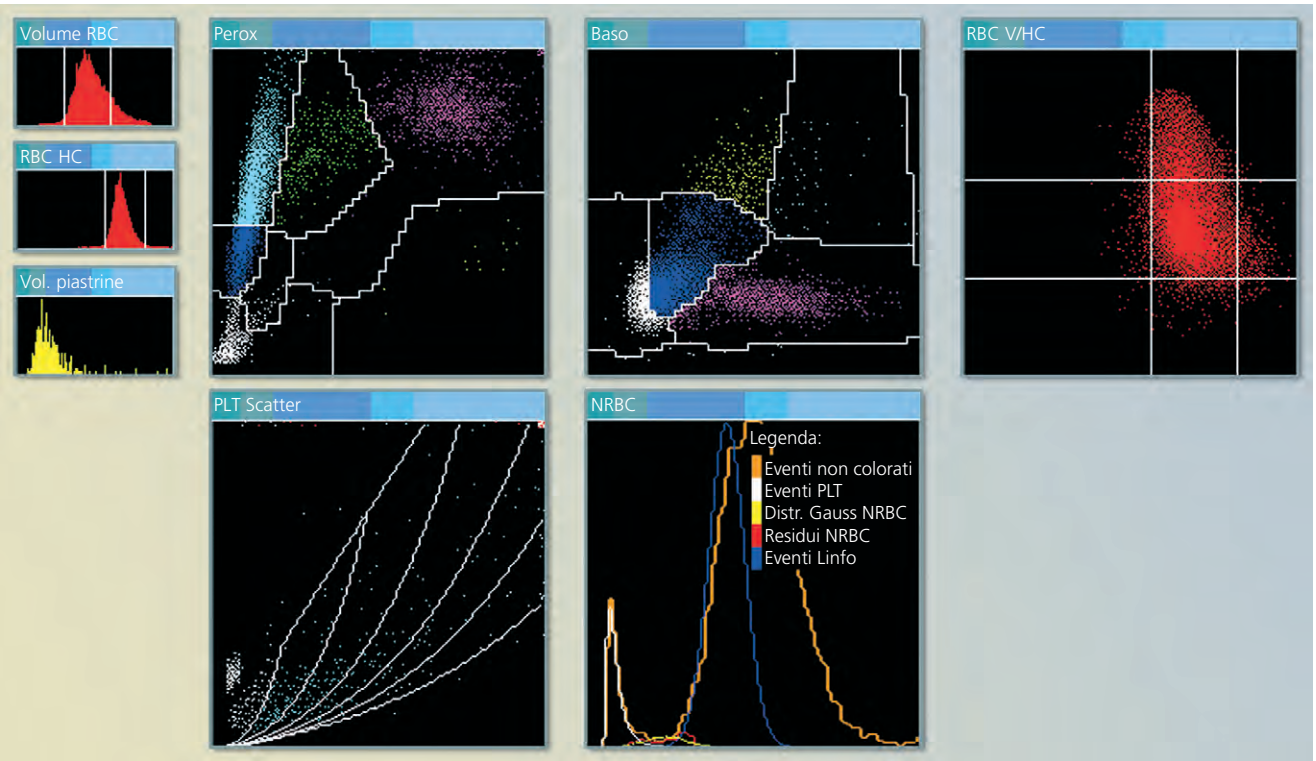

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 105 | g/l | |
| RBC | 3.56 | $\times 10^{12}$/l | |
| HCT | 0.310 | l/l | |
| MCV | 87.2 | fl | |
| MCH | 29.5 | pg | |
| MCHC | 338 | g/l | |
| CHCM | 336 | g/l | |
| RDW | 20.4 | % | |
| HDW | 2.73 | g/dl | Anisocytosis ++ |
| HYPO | 1.6 | % | |
| HYPER | 0.8 | % | |
| MICRO | 5.3 | % | Microcytosis + |
| MACRO | 3.1 | % | Macrocytosis + |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 1.69 | % | |
| RET | 60.2 | $\times 10^{9}$/l | |
| CHr | 30.9 | pg | |
| IRF (M+H) | 17.4 | % | |
| PLT | 16 | $\times 10^{9}$/l | * PLT-NO |
| MPV | 12.3 | fl | * |
| PDW | 45.1 | % | |
| Large platelets | 13 | $\times 10^{9}$/l | LPLT ++ |
| MPC | 18.5 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 6.36 | | |
| WBC Perox | 6.13 | | |
| NEUT | 2.27 | 35.6 | |
| LYMPH | 3.00 | 47.2 | * |
| MONO | 0.17 | 2.7 | |
| EOS | 0.04 | 0.6 | |
| BASO | 0.53 | 8.3 | * B-SUSP |
| LUC | 0.89 | 13.9 | * Atyp+++ |
| NRBC | 0.00 | 0.0 | |
| LI | 1.95 | | |
| MPXI | 4.1 | | |
| MN | | 53.9 | |
| PMN | | 36.5 | |
| BLASTS | | 1.9 | Blasts + |
| Perox HPX | | 0.5 | |
| Perox SAT | | 0.2 | |

In the Perox cytogram there is an elongated LUC cluster, which is separated, from the lymphocyte cluster by a slight narrowing; the neutrophil cluster is normal with regard to number and position. In the Baso cytogram there is a spherical cluster of signals clearly separated from the nuclei of MN and PMN: this corresponds to the lymphoma cells, the cytoplasm of which has partly resisted the lysing agent. This is recognised as an anomaly by the system and leads to a B-SUSP flag. A small proportion of MN events are found to the left of channel 8 and, in the presence of LUC >4%, leads to a Blast + flag. The platelet count may be inaccurate, as indicated by the flag PLT-NO, which indicates the risk of interference by red cell fragments or, as is more probable in this patient, by leucocytes; it has lead to a LPLT (large platelets) flag.

**Morphology.** White cell differential count by microscopy: neutrophils 26%, lymphocytes 61%, monocytes 2%, atypical and immature lymphoid cells 11%. The larger and less mature cells correspond numerically to the cells classified as LUC in the Perox channel and as basophils (pseudobasophilia) in the Baso channel.

## REPORT 9.3 Leukaemic phase of diffuse large B-cell lymphoma



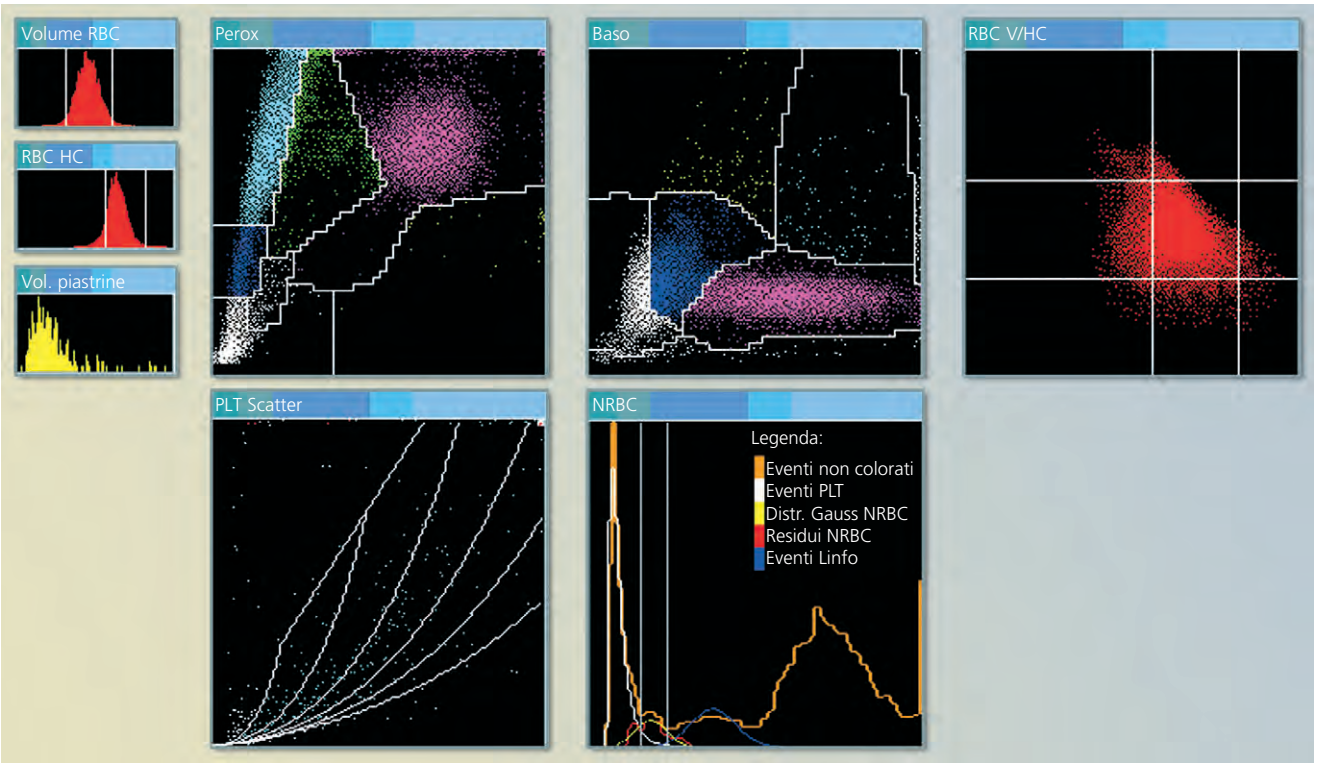

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 77 | g/l | |
| RBC | 2.28 | $\times 10^{12}$/l | |
| HCT | 0.226 | l/l | |
| MCV | 99.1 | fl | |
| MCH | 33.6 | pg | |
| MCHC | 339 | g/l | |
| CHCM | 337 | g/l | |
| RDW | 22.2 | % | Anisocytosis +++ |
| HDW | 2.86 | g/dl | |
| HYPO | 1.2 | % | |
| HYPER | 1.3 | % | |
| MICRO | 1.1 | % | |
| MACRO | 15.5 | % | Macrocytosis +++ |
| RBC fragments | 0.00 | $\times 10^{12}$/l | |
| RBC ghosts | 0.01 | $\times 10^{12}$/l | |
| RET | 0.78 | % | |
| RET | 17.8 | $\times 10^{9}$/l | |
| CHr | 29.3 | pg | |
| IRF (M+H) | 12.4 | % | |
| PLT | 33 | $\times 10^{9}$/l | |
| MPV | 13.2 | fl | |
| PDW | 57.5 | % | |
| Large platelets | 3 | $\times 10^{9}$/l | |
| MPC | 21.1 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 12.09 | | |
| WBC Perox | 11.47 | | |
| NEUT | 1.44 | 11.9 | |
| LYMPH | 3.06 | 25.3 | |
| MONO | 0.40 | 3.3 | |
| EOS | 0.02 | 0.2 | |
| BASO | 0.29 | 2.4 | |
| LUC | 6.88 | 56.9 | Atyp+++ |
| NRBC | 0.00 | 0.0 | |
| LI | 1.77 | | |
| MPXI | 9.3 | | |
| MN | | 84.2 | |
| PMN | | 12.8 | |
| BLASTS | | 16.6 | Blasts+++ |
| Perox HPX | | 0.9 | |
| Perox SAT | | 0.0 | |

Anaemia and thrombocytopenia. The leucocyte count is slightly increased, but the appearance of the Perox cytogram is very abnormal because of the presence of a large population of large peroxidase-negative cells, which are found mainly in the LUC box and, to a lesser extent, in the lymphocyte box. In the Baso cytogram the head of the MN population is deformed towards the left because of the presence of signals derived from low density nuclei; there is a slight pseudobasophilia. The PANDA morphology is P0b, D1. The erythrocyte population is macrocytic.

**Morphology.** The cells recognised by the instrument as Blasts/Atyp appear in the blood film as peroxidase-negative blast cells with large polymorphic nuclei and plentiful agranular cytoplasm. The immunophenotype is CD19+, CD5− and CD10−.

## REPORT 9.4 Leukaemic variant of Burkitt lymphoma




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 110 | g/l | |
| RBC | 3.72 | $\times 10^{12}$/l | |
| HCT | 0.334 | l/l | |
| MCV | 89.6 | fl | |
| MCH | 29.6 | pg | |
| MCHC | 330 | g/l | |
| CHCM | 320 | g/l | |
| RDW | 16.3 | % | Anisocytosis ++ |
| HDW | 3.23 | g/dl | |
| HYPO | 8.3 | % | Hypochromia ++ |
| HYPER | 1.2 | % | |
| MICRO | 2.4 | % | |
| MACRO | 1.4 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.02 | $\times 10^{12}$/l | |
| RET | 0.47 | % | |
| RET | 17.5 | $\times 10^{9}$/l | |
| CHr | 31.5 | pg | |
| IRF (M+H) | 4.2 | % | |
| PLT | 35 | $\times 10^{9}$/l | |
| MPV | 14.0 | fl | |
| PDW | 41.6 | % | |
| Large platelets | 9 | $\times 10^{9}$/l | |
| MPC | 18.5 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 18.4 | * | |
| WBC Perox | 17.62 | * | |
| NEUT | 9.72 | * | |
| LYMPH | 0.76 | * | |
| MONO | 1.46 | * | |
| EOS | 0.04 | * | |
| BASO | 0.13 | * | |
| LUC | 6.02 | * | Atyp +++ |
| NRBC | 0.31 | 1.7 | NRBC + |
| LI | 1.82 | | |
| MPXI | −2.5 | | |
| MN | | 46.8 | |
| PMN | | 51.4 | |
| BLASTS | | 36.3 | Blasts +++ |
| Perox HPX | | | |
| Perox SAT | | | |

Moderate anaemia with severe thrombocytopenia. In the Perox cytogram, in addition to a cluster of neutrophils, present in the normal position but increased in number, there is an abnormal population of very large peroxidase-negative cells, extending from the lymphocyte box into the LUC area and invading to a minimum extent also the monocyte box. This appearance, which can be designated P1 according to the PANDA classification, is a false positive, since the cells are totally peroxidase negative; crossing the threshold is actually due to the large size of their nuclei and to the presence of vacuoles, which lead to minimal but significant light absorption. In the Baso channel the neutrophils are clustered in a normal appearing tail, while the MN head is deformed downwards and to the left, being classified as PANDA D1, causing a high instrumental blast count.

**Morphology.** Differential white cell count by microscopy: neutrophils 49%, lymphocytes 6%, monocytes 1%, blasts 44%. The leukaemic cells are large and uniform, with plentiful basophilic vacuolated cytoplasm, and a characteristic mature B immunophenotype. The neutrophils are cytologically normal. There are some detached cytoplasmic fragments.

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CHAPTER 10

# Plasma Cell Proliferative Disorders

Proliferative disorders of plasma cells represent a group of neoplasms that generally originate in the bone marrow and are characterised by:

- Proliferation of bone marrow plasma cells, originating as a clonal expansion of a single cell; this results from mutation of a B lymphocyte at the final stage of maturation, following heavy chain switch and the somatic hypermutation as a result of the antigenic selection that occurs in the germinal centre;
- Abnormal secretion by the plasma cells of a homogeneous immunoglobulin, with a specific idiotype, known as a paraprotein or M component (monoclonal component).

These conditions, also known as monoclonal gammopathies, have a very heterogeneous clinical expression, from 'monoclonal gammopathy of undetermined significance', which is phenotypically benign, to clinically silent myeloma and symptomatic myeloma causing bone destruction, suppression of haemopoiesis and renal damage (Table 10.1). Also included in this group of diseases are primary or light-chain-associated amyloidosis, in which the fundamental pathogenetic event is the deposition of altered immunoglobulin in tissues, and the heavy and light chain deposition diseases.

Waldenström macroglobulinaemia, previously included in this group of disorders, is now classified together with other lymphoplasmacytic lymphomas and heavy chain deposition diseases in the category of non-Hodgkin lymphoma with secretion of immunoglobulin.[1]

## Monoclonal gammopathy of undetermined significance

This condition is characterised by the presence of an M component in the serum at a concentration below 30 g/l, with only a low level of bone marrow plasmacytosis and neither signs of organ damage (hypercalcaemia, anaemia, osteolytic lesions, amyloidosis) nor other neoplasm of B lymphocytes (Table 10.1).[2,3] Monoclonal gammopathy of undetermined significance (MGUS) is recognised in 3% of the population over the age of 50 years and in 5% of those over 70 years. The male : female ratio is 1.5 : 1.0.

The production of the M component in MGUS is the result of the subclinical proliferation of a clone of plasma cells, which is neoplastic but clinically benign, and can be a forerunner of multiple myeloma or an overt lymphoma. Progression occurs in 1% of cases per year. Epidemiological studies indicate that in a variable proportion, between 20% and 25%, of individuals with MGUS there is progression to multiple myeloma in an interval varying from 10 to 20 years.[4] The risk is higher in cases with a higher concentration of the M component and when the paraprotein is IgM or IgA. IgG and IgA MGUS progress to multiple myeloma. IgM MGUS progresses to lymphoplasmacytic lymphoma or Waldenström macroglobulinaemia.

In these patients, haematological tests are normal or show only abnormalities related to concomitant disorders. The percentage of plasma cells in the bone marrow aspirate is increased, on average about 3%, but by definition less than 10%; otherwise the bone marrow is quantitatively and qualitatively normal; only rarely, in histological sections, is it possible to observe small focal accumulations of plasma cells. In general, the plasma cells are mature with condensed chromatin and basophilic cytoplasm; occasionally there is moderate atypia (binuclearity, presence of nucleoli, increased amount or cytoplasm or cytoplasmic inclusions).

By fluorescence *in situ* hybridisation (FISH), it may be possible to detect either a translocation involving the immunoglobulin heavy chain (*IGH*) locus at 14q32 or deletion of 13q.

## Multiple myeloma

Multiple myeloma, in the World Health Organization (WHO) classification known as plasma cell myeloma, is a B-lineage neoplasm in which there is a clonal proliferation of plasma cells. The story of myeloma starts in 1848 when two Englishmen, Watson and McIntyre, separately sent to the chemist Henry Bence-Jones the urine of their patient, receiving in reply a report of an unusual substance in the urine, which subsequently took the name 'Bence-Jones protein'. In 1850, McIntyre, after the death of the patient and a subsequent autopsy, described the clinicopathological features and the related chemical abnormalities under the name *Mollities et fragilitas ossium*. In 1873, von Rustitzki had coined the name 'multiple myeloma', basing it on histological observations of the pathological tissues. In 1889, Kahler described in minute detail the clinical course of

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**Table 10.1** Disorders that can be associated with the production of a monoclonal paraprotein

**Plasma cell neoplasms**
- Multiple myeloma (plasma cell myeloma)
- Plasmacytoma
- Plasma cell leukaemia
- Waldenström macroglobulinaemia
- Heavy chain diseases
- Light chain deposition diseases
- Primary amyloidosis
- POEMS syndrome
- Monoclonal gammopathy of undetermined significance (MGUS)

**B-lineage lymphoproliferative disorders**
- Splenic lymphoma with villous lymphocytes
- Lymphoplasmacytic lymphomas
- Chronic lymphocytic leukaemia
- Prolymphocytic leukaemia
- Hairy cell leukaemia
- Other non-Hodgkin lymphomas

**Other disorders**
- Cold haemagglutinin disease
- Cryoglobulinaemia
- Autoimmune disorders
- Chronic infections and inflammatory disorders
- HIV infection
- Parasitic diseases
- Carcinoma
- Carcinoid tumour
- Organ transplantation
- Peripheral neuropathy
- Cardiovascular disorders
- Polycythaemia vera
- Gaucher disease

the illness (from which is derived the eponym that is no longer used, Kahler disease). Only in 1902 was it observed, by Wright, when describing the myelomatous tissue, that the disorder resulted from an expansion of plasma cells – the same cell or one very similar to that described by Waldeyer in 1875 in normal bone marrow, which had been given the name plasma cell because of its plentiful cytoplasm.

## Epidemiological and clinical features

Globally, multiple myeloma represents 10–15% of new diagnoses of a haematological neoplasm, equal to 4 cases per 100 000 population per year. The frequency is higher in men (7.1 cases per 100 000 in men in the United States and 4.6 per 100 000 in women), and in African-Americans is twice the rate in Caucasians. The incidence is lower in Chinese and in other Asiatic populations. It is rare under the age of 40 years (although exceedingly rare paediatric cases have been reported) with the median age at diagnosis being 61 years in men and 62 years in women.[5]

The aetiology is unknown, but epidemiological studies have demonstrated a relationship to ionising radiation and benzene, and to occupational exposure to chemicals in petrochemical industries, agriculture, rubber and plastics. A genetic predisposition is demonstrated by the increased risk of developing the disorder in first-degree relatives of patients with myeloma. Protracted antigenic stimulation could favour the development of benign plasma cell clones, with malignant transformation occurring in one of these cells: this hypothesis is supported by the increased risk of myeloma described in patients with HIV infection, other chronic infections and autoimmune and allergic conditions, but this remains controversial.[4]

In the development of the clinicopathological features of myeloma, a relevant role has been attributed to an interaction between the neoplastic plasma cells and the medullary microenvironment, mediated by adhesion molecules and cell surface receptors, able to promote the growth, migration and drug-resistance of the neoplastic cells.[5] The osteolytic lesions, in particular, depend on activation of osteoclasts by a delicate interaction of cytokines, with reabsorption dominating over osteogenesis. The neoplastic plasma cells produce interleukin (IL)-6, which is a potent growth factor for these tumour cells, favouring the increase of the myelomatous mass (autocrine stimulation), and for vascular endothelium, stimulating angiogenesis.[6]

After a clinically silent period of variable length, during which the only manifestation may be an increased erythrocyte sedimentation rate (ESR) and the presence of an M component in the serum, possibly with proteinuria, the disease becomes clinically manifest with symptoms resulting from the plasma cell infiltration and the abnormal production of a monoclonal protein, which damages particularly haemopoietic tissue, the kidneys and the skeleton. The cardinal clinical features that typify multiple myeloma, which can be indicated by the acronym CRAB (calcium, renal, anaemia, bone), are:
- Anaemia due to impaired haemopoiesis and possibly also thrombocytopenia and neutropenia;
- Hypercalcaemia with multifocal osteolytic lesions, best demonstrated by magnetic resonance imaging (MRI), which also reveals the bone marrow involvement; the bone lesions often lead to pain and pathological fractures;
- Chronic renal insufficiency, resulting from damage to tubules by free light chains in the urine;
- Possibly a hyperviscosity syndrome with neurological manifestations;
- Immunodeficiency, with increased susceptibility to infection, particularly bacterial, as a result of the reduced production of normal immunoglobulins and possibly also leucopenia.

These manifestations occur in various combinations, producing diverse clinical syndromes ranging from forms with few or no symptoms and slow evolution (indolent or smouldering myeloma), in which immediate treatment is not essential, to symptomatic forms with active and progressive disease and finally forms with very aggressive, rapidly progressive disease (anaplastic myeloma, plasma cell leukaemia).

The clinical staging of multiple myeloma needs to reflect particularly the extent and the tendency to evolution of the neoplastic plasma cell mass. Staging can follow the scheme proposed

**Table 10.2** WHO diagnostic criteria for plasma cell neoplasms[1,9,11]

**Monoclonal gammopathy of undetermined significance (MGUS)**
- Criterion 1: serum M component <30 g/l
- Criterion 2: bone marrow plasmacytosis <10%
- Criterion 3: absence of organ damage or insufficiency (hypercalcaemia, renal insufficiency, osteolytic lesions) and of other features associated with myeloma (hyperviscosity, amyloidosis, recurrent infections)

**Asymptomatic myeloma (smouldering myeloma)**
- Criterion 1: serum M component ≥30 g/l and/or clonal bone marrow plasmacytosis of 10% or higher
- Criterion 2: absence of organ damage or insufficiency (hypercalcaemia, renal insufficiency, osteolytic lesions) and of other features associated with myeloma (hyperviscosity, amyloidosis, recurrent infections)

**Symptomatic myeloma**
- Criterion 1: serum or urine M component (a minimum level is not required but the concentrations found are generally >30 g/l for IgG myeloma, >25 g/l for IgA myeloma, or >1 g/24 hours for urinary light chain)
- Criterion 2: clonal bone marrow plasmacytosis ≥10% of nucleated cells
- Criterion 3: organ damage or insufficiency (hypercalcaemia, renal insufficiency, osteolytic lesions) or other features associated with myeloma (hyperviscosity, amyloidosis, recurrent infections)

NB. All the criteria must be met simultaneously (with the exception of non-secretory myeloma, which lacks the M component).

**Table 10.3** Methods for the diagnosis of plasma cell neoplasms

**Detection and characterisation of a monoclonal protein**
- Serum protein electrophoresis
- Immunofixation for IgG, IgA and IgM
- Quantitation of serum immunoglobulins by nephelometry
- Total 24-hour urinary protein with electrophoresis and investigation for Bence-Jones protein
- Immunofixation on 24-hour urine collection
- Investigation of free serum light chain ratios
- Examination of a peripheral blood film

**Investigation of the pathological cell population**
- Bone marrow aspirate
- Bone marrow trephine biopsy (with immunohistochemistry for CD138 and cytoplasmic kappa and lambda chains)
- Flow cytometric immunophenotyping for detection of clonality (not applicable for measuring the percentage of plasma cells in the marrow)

**General evaluation of organ function**
- Full blood count
- Serum chemistry, particularly creatinine and calcium
- Imaging of the skeleton (including MRI and PET-CT)

**Evaluation of prognosis**
- Serum albumin
- Serum beta-2 microglobulin
- Lactate dehydrogenase
- C-reactive protein (only to assess the likelihood of infection)
- Bone marrow cytogenetic analysis (karyotype, FISH)

**Further evaluation of selected patients (not required routinely)**
- Investigation for amyloid by needle biopsy of abdominal fat or bone marrow, or biopsy of organs suspected of being involved
- Targeted biopsy of isolated skeletal lesions
- Serum viscosity (if the M component is IgM or is >70 g/l or if there are suspicious clinical features)
- Immunofixation for IgD and IgE
- MRI with the STIR technique for oligosecretory and non-secretory myeloma

by Durie and Salmon in 1975[7] based on major criteria (bone marrow plasma cells above 30% and a significant amount of a serum paraprotein) and minor criteria (a lesser degree of plasmacytosis and a lower concentration of serum paraprotein, associated hypogammaglobulinaemia). Since 2005, patients can be clinically staged using the new International Staging System, based on serum albumin concentration and beta-2 microglobulin, which has been shown to give more reliable prognostic information.[8]

## Diagnostic criteria

The triad of bone marrow plasmacytosis, an M component and signs of organ damage (anaemia, renal insufficiency and skeletal lesions) permit a diagnosis of multiple myeloma: however, these three elements are not always present at disease onset and conversely they can occur in conditions other than myeloma.

The diagnostic criteria for myeloma required in the WHO classification,[1] based on the work of several consensus groups,[9–11] are shown in Table 10.2.

The diagnostic evaluations indicated in patients with suspected myeloma are shown in Table 10.3. In addition to the WHO criteria, which mainly reflect the plasma cell mass, consideration is now given to other factors that are prognostically very important, which relate to the biology of the tumour, particularly an increase in beta-2 microglobulin, lactate dehydrogenase (LDH), C-reactive protein (CRP) and soluble IL-6 receptor and a reduced concentration of serum albumin.

## The M component

The presence in the serum of an M component causes an alteration in the electrophoretic profile of proteins in the serum and/or urine. However, a monoclonal peak can also be observed in various other plasma cell dyscrasias (Table 10.3). When myeloma is suspected, the paraprotein is assessed by immunofixation and quantification. Paraproteins have a single light chain type (kappa or lambda) and a single heavy chain type, which can belong to any class (IgG in 70%, IgA in 20%, IgM in <1% and IgD or IgE quite rarely). In 5–10% of patients, the M component is a free light chain. It is very common for normal immunoglobulins to be reduced; in light chain myeloma, the suppression involves all three major classes of immunoglobulin. It is quite exceptional for there to be free heavy chain or a biclonal or, indeed, triclonal M component. Non-secretory myeloma, in which the M component is lacking, represents only a small proportion of cases, around 1–3%.

Quantification of free light chain in the serum, made possible by the recognition of epitopes that are hidden in the complete molecule, associated with the demonstration of imbalance in the kappa : lambda ratio, has recently become a useful complement for measuring the tumour mass, particularly when the M component is present in a low concentration or is absent, and

also in patients with renal insufficiency, in whom quantification of 24-hour urinary Bence-Jones protein is not reliable.

The quantity and type of the paraprotein correlates with the clinical picture and with the course of the illness. For example, in the rare IgM myeloma, the concentration of the M component is elevated and there is an increased risk of infection related to the reduction of normal immunoglobulins, but the clinical course is usually slow. In patients with IgA myeloma, on average, survival is shorter and the clinical picture is characterised by hypercalcaemia and a significant occurrence of hyperviscosity attributable to the tendency of IgA to polymerise. In IgD myeloma, extraosseus lesions, such as hepatosplenomegaly, lymphadenopathy and amyloidosis, predominate.

## Blood count and peripheral blood film

At diagnosis, the majority of patients have a normocytic normochromic anaemia with haemoglobin concentration (Hb) between 70 and 100 g/l, with a low reticulocyte count.[12] Uncommon cases of macrocytic anaemia[13] and rare cases of polycythaemia[14,15] have been reported. The severity of the anaemia and reticulocytopenia correlate with the extent of bone marrow infiltration and are accentuated by dilution caused by the increased plasma volume. In addition, the presence of chronic renal insufficiency leads to a hyporegenerative anaemia because of reduced erythropoietin production. The tendency to form rouleaux causes a notable increase in the ESR; however, in the presence of an M component that is a cryoglobulin, this can be zero.

The platelet count is often normal or slightly reduced at presentation; platelet life span can be shortened as a result of intravascular activation by the abnormal protein. Platelet function can also be compromised by increased plasma viscosity, which interferes with the activation of factors V, VII and VIII and with the formation of complexes with prothrombin and fibrinogen.

The leucocyte count is usually normal, although sometimes there is a mild leucopenia: in half of cases, the differential count shows neutropenia with relative lymphocytosis. In rare cases there is marked neutrophilia, sometimes resulting from the production of granulocyte colony-stimulating factor (G-CSF) and IL-6 by the neoplastic plasma cells, and sometimes because of an association, which has often been described but remains controversial, between myeloma and chronic neutrophilic leukaemia.[16,17]

It is characteristic for the whole film to show erythrocytes stacked up into rouleaux. This is the result of the monoclonal protein which, when it is present in a very high concentration, also leads to increased background staining of the blood film because of a thin film of amorphous material that is diffusely weakly basophilic (Figs 10.1, 10.2 and 10.3). This phenomenon can compromise an appreciation of subtle morphological abnormalities. To prepare films without this artefact, it is possible to dilute the sample four fold in isotonic saline. The same procedure can be followed in determining the blood group, in crossmatching for blood transfusion and also for making a distinction between rouleaux formation and

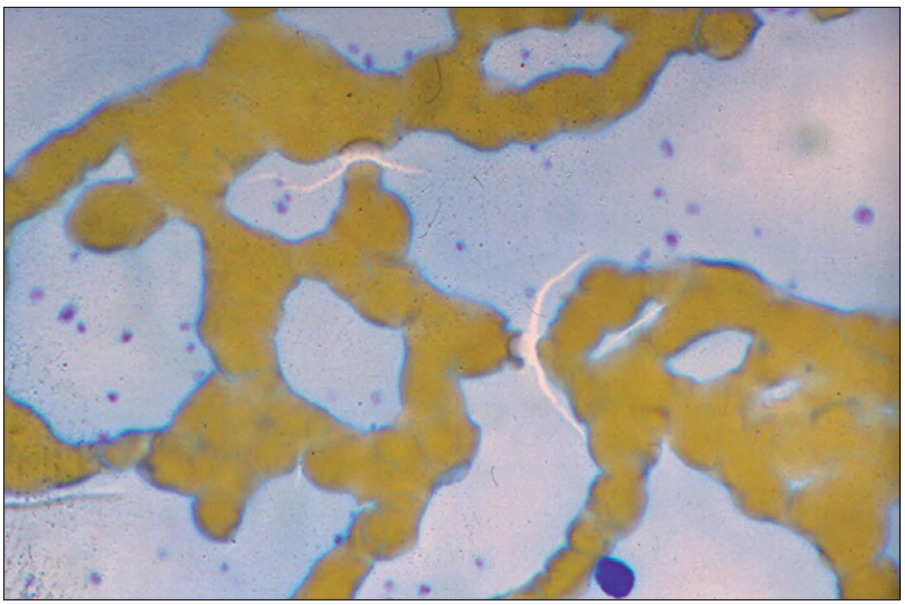


**Fig. 10.1** IgG multiple myeloma; peripheral blood film. The red cells are massively involved in rouleaux formation, forming long stacks that cross over each other and appear to be immersed in a homogeneous grey and blue material, which also incorporates the platelets. The density of the proteinaceous background can be better appreciated by the contrast that is apparent when there are thin slits in this background through which the original clear background of the microscopic field can be seen.

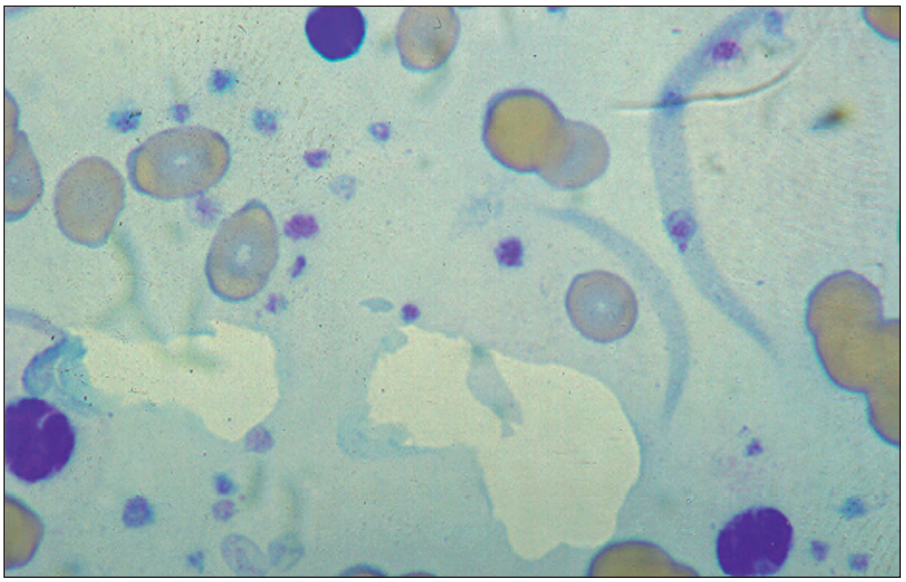


**Fig. 10.2** IgG multiple myeloma; peripheral blood film. The amorphous proteinaceous material is detaching in strands at certain points, so that the contrast with the clean pale background permits it to be fully appreciated. There are various comma- and sickle-shaped precipitates that are superimposed on the homogeneous deposit that provides the background of the film.

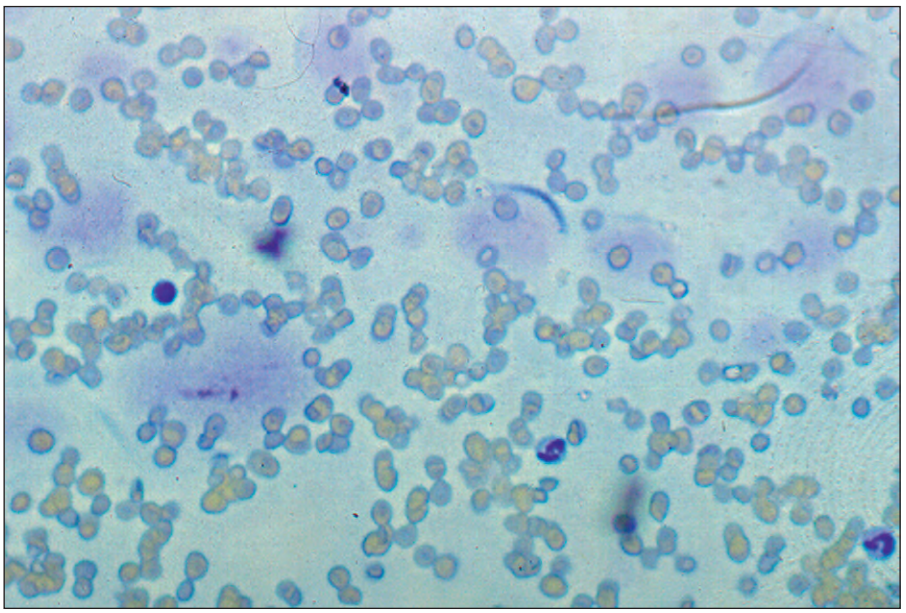


**Fig. 10.3** IgA multiple myeloma; peripheral blood film. The paraprotein concentration in this patient is not very high following chemotherapy that has induced a partial remission; however, the paraprotein has the characteristics of a cryoprotein and has precipitated in the film in the form of amorphous masses with indefinite, nebulous outlines. A little above the centre, a comma-shaped deposit. These appearances are not pathognomonic for multiple myeloma, but indicate a dysproteinaemia, often related to hypergammaglobulinaemia.

agglutination resulting from a cold agglutinin, which persists after dilution of the sample. The presence of a paraprotein can interfere with cell counts on impedance instruments, particularly causing a factitious thrombocytosis.[18,19] Sometimes one can detect the presence of a cryoprecipitate within phagocytic vacuoles in the cytoplasm of neutrophils and monocytes.

It is important to examine the film for circulating plasma cells which, if present, can be analysed by flow cytometry to establish clonality. The recognition of circulating plasma cells is rare, if one excepts cases of plasma cell leukaemia.

There may be atypical lymphocytes, such as plasmacytoid lymphocytes (Downey cells type I). These cells have a round or

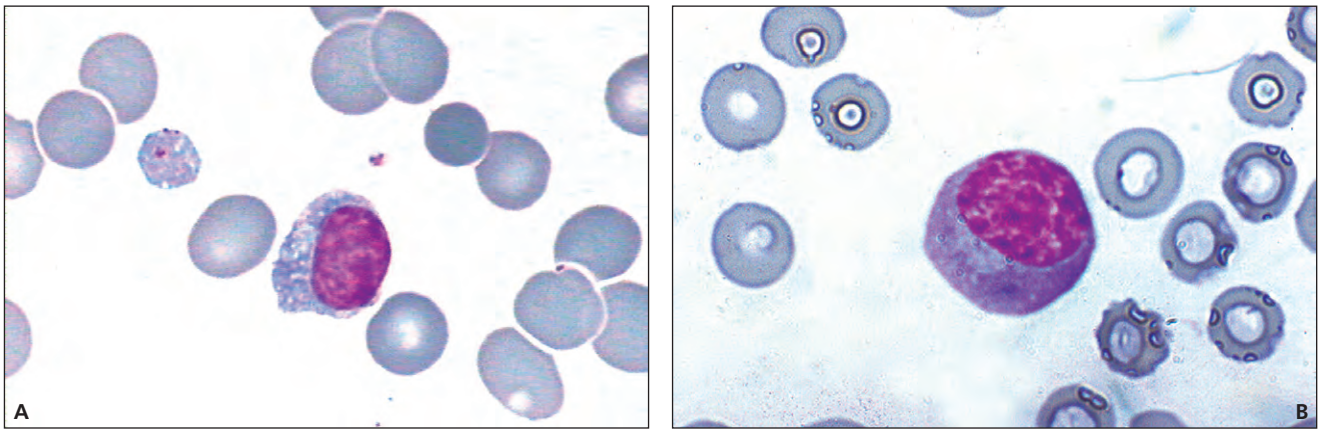



**Fig. 10.4** Lymphoplasmacytoid cells in the peripheral blood of two patients with multiple myeloma. (A) This lymphocyte has a higher nucleocytoplasmic (N:C) ratio than a plasma cell. The basophilic cytoplasm is not homogeneous, there being a large pale paranuclear area and also numerous small non-staining areas, perhaps corresponding to hypertrophic mitochondria. The chromatin structure is heterogeneous, with large areas of condensation. The main axis of the eccentric nucleus is parallel to that of the cell. Top right, a spherocyte. (B) The greater abundance of the cytoplasm and the condensation of the chromatin into clumps make the morphology of this plasmacytoid lymphocyte closer to that of a plasma cell. There are no morphological criteria for assigning cells with these characteristics to a myeloma population. Lymphoplasmacytoid morphology is, in fact, often seen in conditions of immune stimulation. It must be remembered that multiple myeloma is a bone marrow disease and its diagnosis always requires examination of a bone marrow aspirate film.

oval nucleus in an eccentric position (Fig. 10.4), with chromatin having intermediate characteristics between those of a small lymphocyte and those of a mature plasma cell; nucleoli are generally absent. The cytoplasm is plentiful and moderately basophilic, sometimes with small vacuoles, but the nucleocytoplasmic (N:C) ratio is higher than that of a mature plasma cell. The presence in the peripheral blood of B lymphocytes already oriented to plasma cell differentiation, which have left the germinal centres of lymphoid tissues to colonise the bone marrow, cannot be detected by any morphological feature: they can, however, be demonstrated by immunophenotyping, which shows intermediate positivity for CD38. The presence of variably sized azurophilic granules in the cytoplasm of some lymphoid cells reflects their T or NK nature.[20]

The presence in the circulation of B lymphocytes and plasma cells (Fig. 10.5) with the same clonal origin as the myeloma cells in the bone marrow has been demonstrated in more than 20% of patients. By using flow cytometric analysis, one can also demonstrate both aneuploidy and B-cell clonality.[21] The number of circulating plasma cells, measured by this technique, has been recognised as a predictive factor for survival in newly diagnosed myeloma.[22]

In patients with non-secretory myeloma one does not observe the abnormalities that result from the presence of a serum paraprotein (rouleaux, background basophilia and increased ESR), and in these cases peripheral blood examination can be completely normal.

Routine haematological analysis is also useful in myeloma patients during follow-up. The values for Hb and leucocyte, neutrophil and platelet counts, for example, are useful for following the response to treatment with high dose thalidomide.[23] The counts of haemopoietic progenitors and immature reticulocytes produced by automated analysers are a useful complement to the count of CD34-positive cells for establishing the optimal time for collection of autologous stem cells from the peripheral blood.[24–26]

## Bone marrow

Multiple myeloma usually behaves as a selectively bone marrow neoplasm. The recognition and quantification of bone marrow plasma cells, together with the characterisation of the M component, is the second cardinal diagnostic and prognostic criterion. Bone marrow films are usually very cellular, with a good representation of normal haemopoietic precursors, which are admixed with a plasma cell population, which is very heterogeneous with regard to numbers, morphology and topography (Figs 10.6 through to 10.9).

According to the WHO criteria,[1] it is sufficient to demonstrate a clonal plasmacytosis in the bone marrow without any specified minimal number being required: a count of above 10% of nucleated cells has been suggested, but in fact around 5% of patients with symptomatic myeloma (diagnosed on the basis of osteolytic lesions or an M component $>30$ g/l) have a lower count. In a large case series, 96% of patients with a diagnosis of multiple myeloma had a plasma cell percentage equal to or above 10%, with a median value of 50%.[12]

Numerous researchers have related the prognosis to the percentage infiltration at diagnosis, this directly reflecting the neoplastic mass: the patients who at presentation have more than 50% plasma cells have a significantly shorter survival than other patients.[27–29] Recently, the percentage of bone marrow plasma cells has again been confirmed as an important prognostic

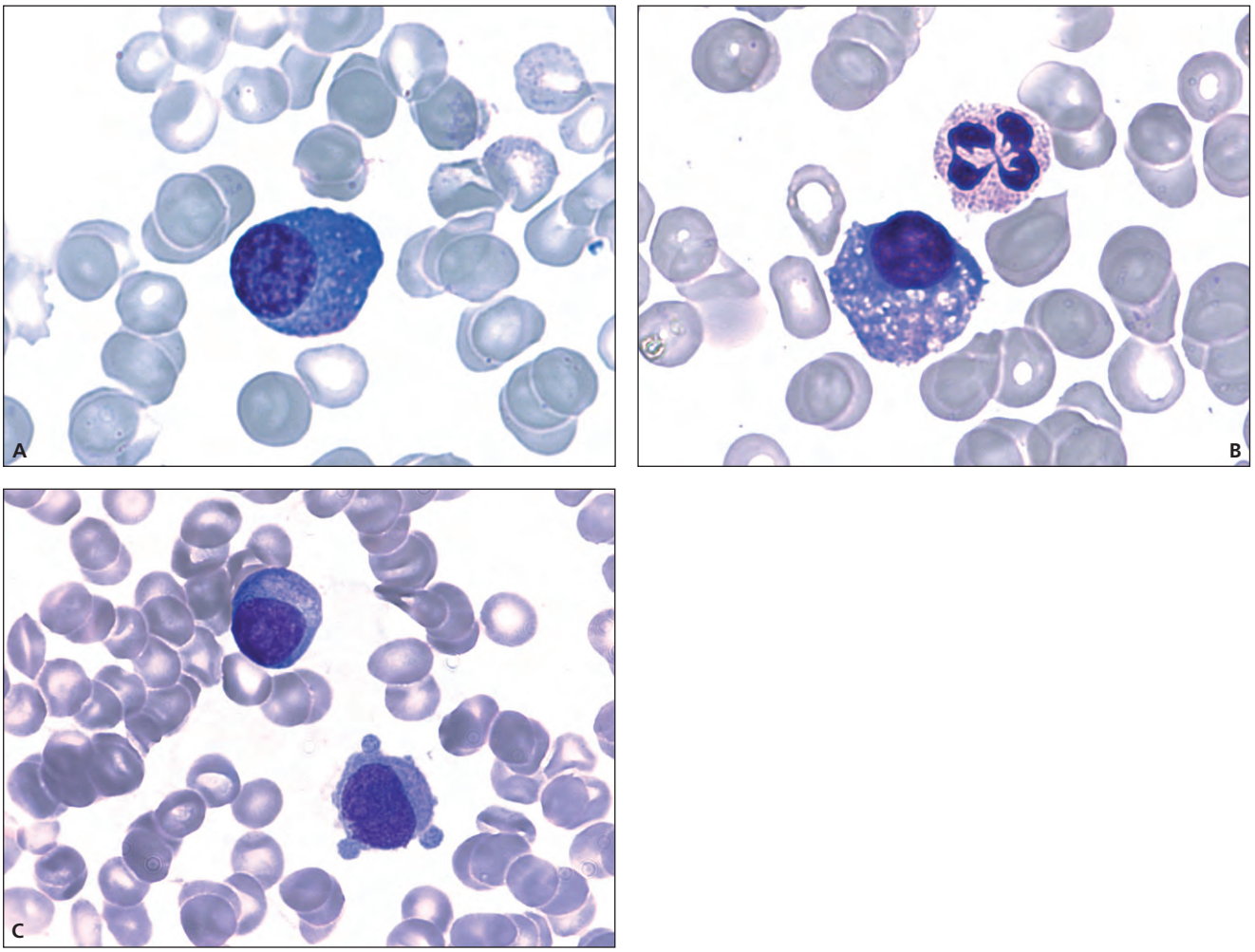



**Fig. 10.5** Circulating plasma cells in the peripheral blood films of three different patients with multiple myeloma. (A) Oval-shaped mature plasma cell, with plentiful intensely basophilic, ultramarine cytoplasm, with pale paranuclear arcoplasm, which appears as if riddled by holes. The nucleus is oval and eccentric with the major axis perpendicular to that of the cell. The chromatin is condensed into dark masses that are more numerous at the periphery. (B) In this plasma cell, the nucleus is small, round and very eccentric with chromatin markedly condensed into a small number of large peripheral masses. The cytoplasm is perforated by pseudovacuoles, mainly non-staining, but sometimes showing a pink tinge reflecting the presence of immunoglobulin. (C) At the top, a mature plasma cell with plentiful arcoplasm and a relatively high N:C ratio. At the bottom, an immature plasma cell with an eccentric nucleus and dense homogeneous chromatin, with two visible nucleoli, and lighter blue cytoplasm that is extending into rounded blebs.

factor, as an indicator of tumour mass, along with the serum concentration of beta-2 microglobulin, the presence of hypercalcaemia, the severity of anaemia and the presence of more than three lytic lesions.[30]

Myelomatous infiltration is not associated, at least in the early stages, with severe anomalies in haemopoietic lineages. The granulocytic series does not show any qualitative abnormalities at presentation, but is always reduced in inverse proportion to the tumour mass. There is often an increase in eosinophils, related to anomalous cytokine production. The erythroid series almost always shows reduced maturation: in some patients, even at the time of diagnosis, there is a drastic reduction in erythropoiesis, which some authors have related to alterations in cytokines such as IL-1 and IL-6.[31] Megakaryocytes are rarely reduced and sometimes are actually increased, this being related to increased IL-6: even in the presence of significant myelomatous infiltration, the number of megakaryocytes remains relatively well preserved in comparison with other lineages; thrombocytopenia, as discussed above, is generally the result of increased consumption.[32]

The proportion of lymphocytes is not increased, even if it is fairly common to find lymphoid aggregates. The consistent presence of osteoclasts and osteoblasts provides the morphological evidence of active bone turnover[33] (Fig. 10.7C).

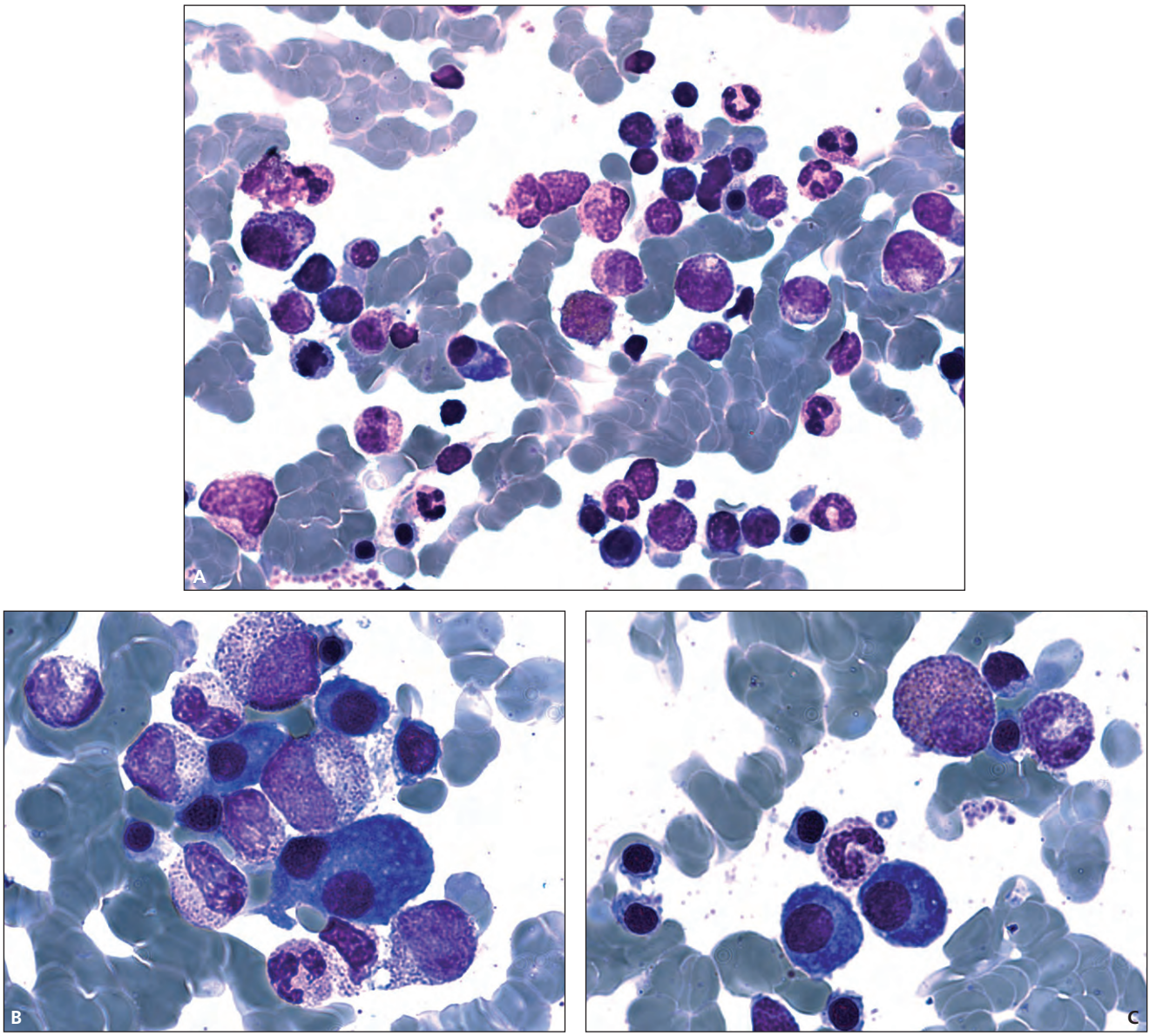



**Fig. 10.6** Bone marrow aspirate of a patient with IgG multiple myeloma, with low level bone marrow infiltration. (A) Good cellularity with granulocytic and erythroid precursors showing normal maturation. There is one mature plasma cell in the centre, immediately to the left of an eosinophil granulocyte; it is easily recognised from its pear shape, polar nucleus, cytoplasmic basophilia and the presence of a well-developed paranuclear semilunar Golgi zone. Other cells of the plasma cell series are rounded with less cytoplasmic development: one is present, for example, close to the lower edge of the image, a little to the right of centre. (B) In the centre of this small group, a mature promyelocyte immediately adjacent to a large binucleated plasma cell and slightly deforming its upper margin. The cytoplasm of this plasma cell is hypertrophic, very blue, with a faint Golgi zone between the two nuclei, which are round, not central and with clumped chromatin. Above the promyelocyte, another two smaller plasma cells. The other cells are four erythroblasts and nine well-formed cells of granulocyte lineage. (C) This pair of plasma cells have cytological features indistinguishable from those of normal plasma cells, with the exception of there being less chromatin condensation. At the top, an eosinophil myelocyte. There are also four orthochromatic erythroblasts.

## Morphology of normal and pathological plasma cells

Morphological features of maturity or immaturity do not represent an absolute criterion for the differential diagnosis of patients with moderate infiltration, since many cases of multiple myeloma at diagnosis have an infiltrate of plasma cells that are morphologically indistinguishable from their normal equivalents (Figs 10.10, 10.11 and 10.12). These are ovoid cells with a length greater than 15–25 μm and a diameter of 7–15 μm; the nucleus is oval and always eccentric, almost polar: the major axis of the nucleus is generally oriented perpendicularly to the major axis of the cell as a whole. The nucleolus is not visible

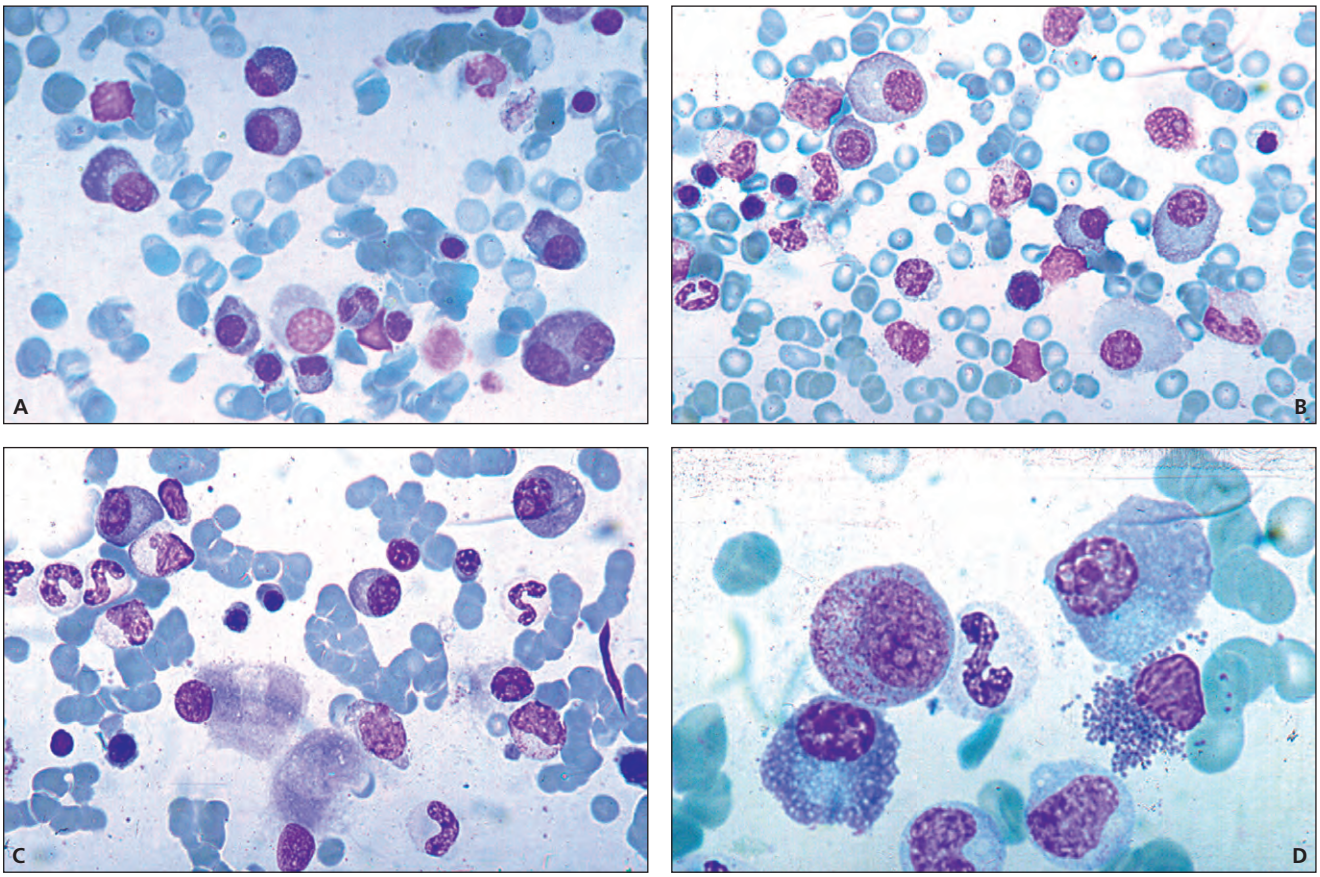



**Fig. 10.7** Partial infiltration of the bone marrow by plasma cells with heterogeneous morphology and maturity from different cases of myeloma and monoclonal gammopathy of undetermined significance (MGUS). (A) Typical plasma cell morphology, with eccentric nuclei with chromatin in blocks, a large Golgi zone and intensely basophilic cytoplasm. Only a proportion of cells show a larger nucleus with less condensed chromatin but fully mature cytoplasm (asynchronous maturation). The nuclei of the binucleated plasma cell at the bottom right also show less compact chromatin: the nuclei are very eccentric with their major axes being at an angle of about 90°. (B) Not very marked bone marrow plasmacytosis (25%) in a case of IgG myeloma. The plasma cell morphology is almost normal, with moderate hypertrophy of the cytoplasm, which in some cells appears less basophilic; some cells have nucleoli. There are various polychromatic and orthochromatic erythroblasts, some neutrophil metamyelocytes and some band neutrophils. In cases such as this, the morphological picture is not sufficient for a firm diagnosis of myeloma and confirmation from clinical and histological features is required. (C) IgG myeloma; bone marrow aspirate. Towards the top, three immature plasma cells, the two at the sides with plentiful basophilic cytoplasm and the one at the centre with a more elevated N:C ratio. At the bottom in the centre, two osteoblasts in which the eccentricity of the nucleus and the cytoplasmic basophilia recall the morphology of plasma cells: cytological characteristics that permit a distinction are the presence in the osteoblasts of a large Golgi zone that is clearly separated from the nucleus, the spongy appearance of the nucleus and the indistinct outline of the cytoplasm. There are various granulocyte precursors, non-segmented neutrophils and erythroblasts. (D) Two plasma cells in the bone marrow aspirate of a patient with MGUS and 4% plasma cells. The bottom left cell is mature and appears normal: the nucleus is eccentric and has eight blocks of peripherally sited chromatin; its major axis is perpendicular to that of the cell; the cytoplasm contains a large Golgi zone, which occupies the centre of the cell; there are numerous small vacuoles. The larger cell, top right, shows mild hypertrophy without atypia: the nucleus has the suggestion of two nucleoli and the cytoplasm is pale; at the bottom, there is a large globular eccentric Golgi zone. To the left, a neutrophil promyelocyte with a visible nucleolus, basophilic cytoplasm and an orderly distribution of azurophilic granules; in the centre, a neutrophil band form and to the right, a non-segmented eosinophil with damaged cytoplasm.

with panoptic stains, although it is always demonstrable by electron microscopy. The chromatin is condensed into large polygonal clumps (on average between seven and nine), characteristically arranged close to the nuclear membrane (cartwheel formation). It is not rare to see plasma cells with two or more nuclei (Fig. 10.13).

Plasma cell karyokinesis can occur: often it represents non-mitotic nuclear division which occurs by progressive central narrowing of the nucleus or by twinning and detachment of the twinned portion (nuclei of different sizes), not followed by cell division. The eccentricity of the plasma cell nucleus can be related to the presence of a very large, unstained or weakly acidophilic Golgi zone almost always situated in proximity to the nucleus (the pale paranuclear area also being referred to as the arcoplasm); this is an expression of a very well-developed Golgi apparatus in cells of this lineage. The remaining

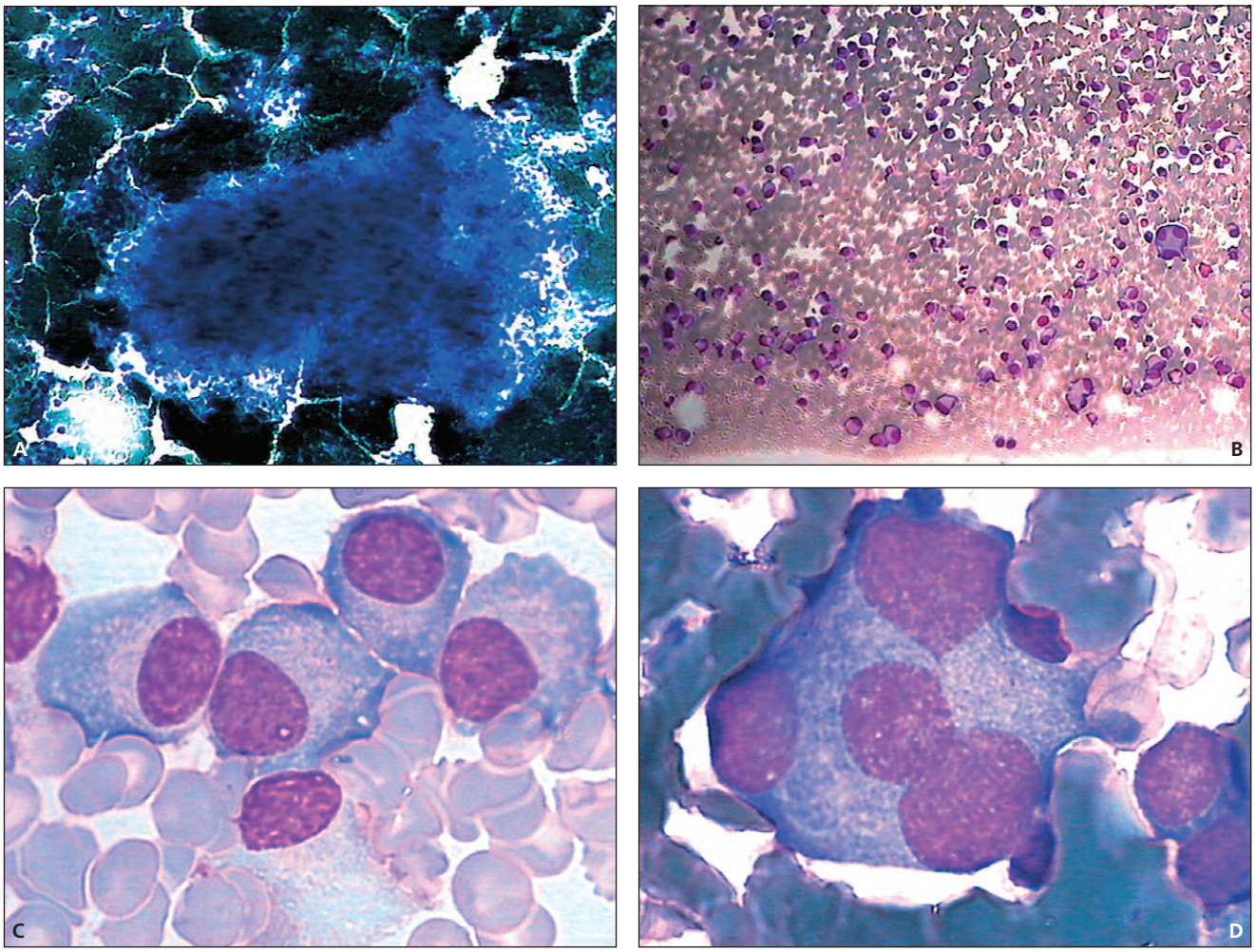



**Fig. 10.8** Bone marrow aspirate of a patient with IgA myeloma with marked plasma cell invasion of the marrow (60% of nucleated cells). (A) At low power, this bone marrow fragment appears to be composed exclusively of a nodule of basophilic cells without any stromal elements. (B) At higher power, the plentiful cells appear to be mainly plasma cells, which are medium sized to large with plentiful weakly basophilic cytoplasm, pale pink or violet, with the nucleus flattened at one pole. A cell on the right is gigantic and has three nuclei. (C) Four plasma cells showing an intermediate degree of maturation, in which the cytoplasmic basophilia, reinforced at the periphery, contrasts with the large area of pink paranuclear arcoplasm. In the cell on the left, which has an elliptical nucleus, the perpendicular relationship of the axes of the nucleus and the cell is particularly clear. (D) Giant plasma cell with four nuclei. The upper nucleus and that in the centre appear to be still joined by a thin filament, which appears to pinch off a part of the margin of the upper nucleus. The chromatin is homogeneous and granular, without clumps.

cytoplasm is intensely basophilic because of the presence of numerous granules of ribonucleoprotein attached to the external surface of the sacs of the endoplasmic reticulum, forming the rough endoplasmic reticulum, which is also highly developed in these cells. It is also possible to detect pale round or oval structures in the cytoplasm, which are the negative images of mitochondria and secretory granules.

The principal cytochemical characteristic of the plasma cells is the intense pyroninophilia attributable to the high cytoplasmic content of RNA; cytochemical reactions for protein (Danelli or Weiss-Tsou-Seligman reaction) are also intensely positive; uncommonly polysaccharide is demonstrable with a periodic acid-Schiff (PAS) stain. The peroxidase reaction is negative, while there is moderate activity for acid and alkaline phosphatases.

It is not uncommon for there to be inclusions of various types in the cytoplasm or the nucleus.[34] Hyaline vacuoles that vary in number and size and more or less homogeneous round masses can be present, being designated Russell bodies after the author who first described them in 1890, interpreting them as parasites present in neoplastic conditions. Russell bodies are spherules, which on a May-Grünwald-Giemsa stain can appear blue, violet or pink, but more often their contents are dissolved and they appear as unstained vacuoles (Fig. 10.14). They represent an

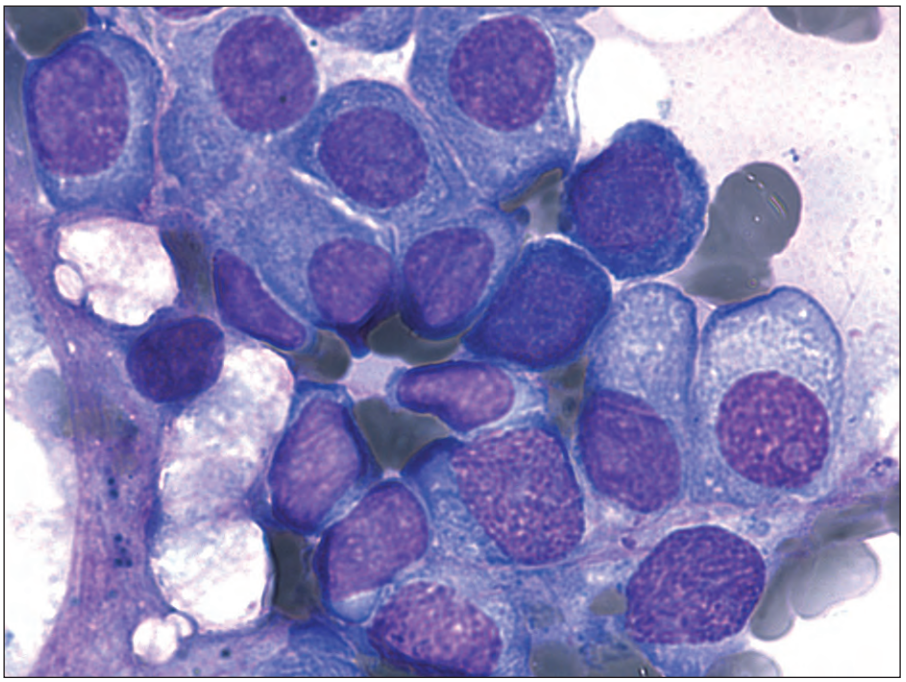


**Fig. 10.9** IgG multiple myeloma; bone marrow aspirate. A mass of plasma cells in a bone marrow fragment, heterogeneous with regard to morphology and maturity. The apparent variability in chromatin condensation is attributable in part to the variable degree of spreading of different plasma cells. The four at the top, to the left of centre, are fairly compact, but their nuclei nevertheless show the presence of a nucleolus. In the two most spread out, bottom right, the chromatin appears granular, without clumps, and the nucleoli, which number one or two per cell, are prominent; the cytoplasm in these cells appears pale because of the extensive non-homogeneous arcoplasm. At the bottom in the centre, three lymphocytes.

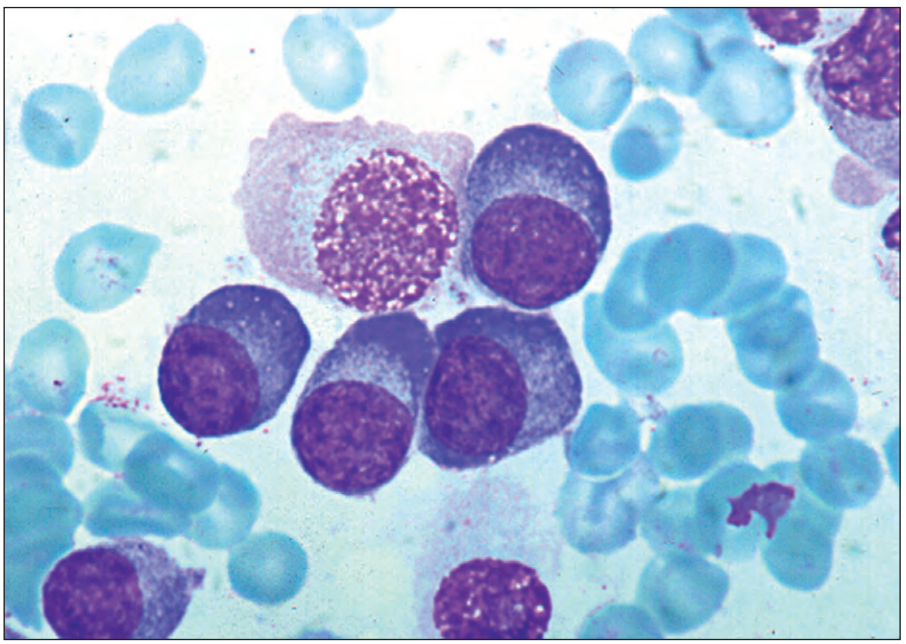


**Fig. 10.10** Multiple myeloma; bone marrow aspirate. A small nest of plasma cells with mature, intensely basophilic cytoplasm with a minimal pinkish tinge attributable to the presence of abundant glycoprotein admixed with the ribosomes; the paranuclear Golgi zone is pale and large; there are some small pale vacuoles corresponding to hypertrophied mitochondria. The nucleus is slightly less mature than normal; only the cell more to the left in the central group shows clear condensation of chromatin into polygonal blocks, the others showing an intermediate degree of condensation. The largest and palest cell, top left, is a plasma cell that has suffered mechanical trauma during spreading of the film: the nucleus is similar to that of a cell in early prophase.

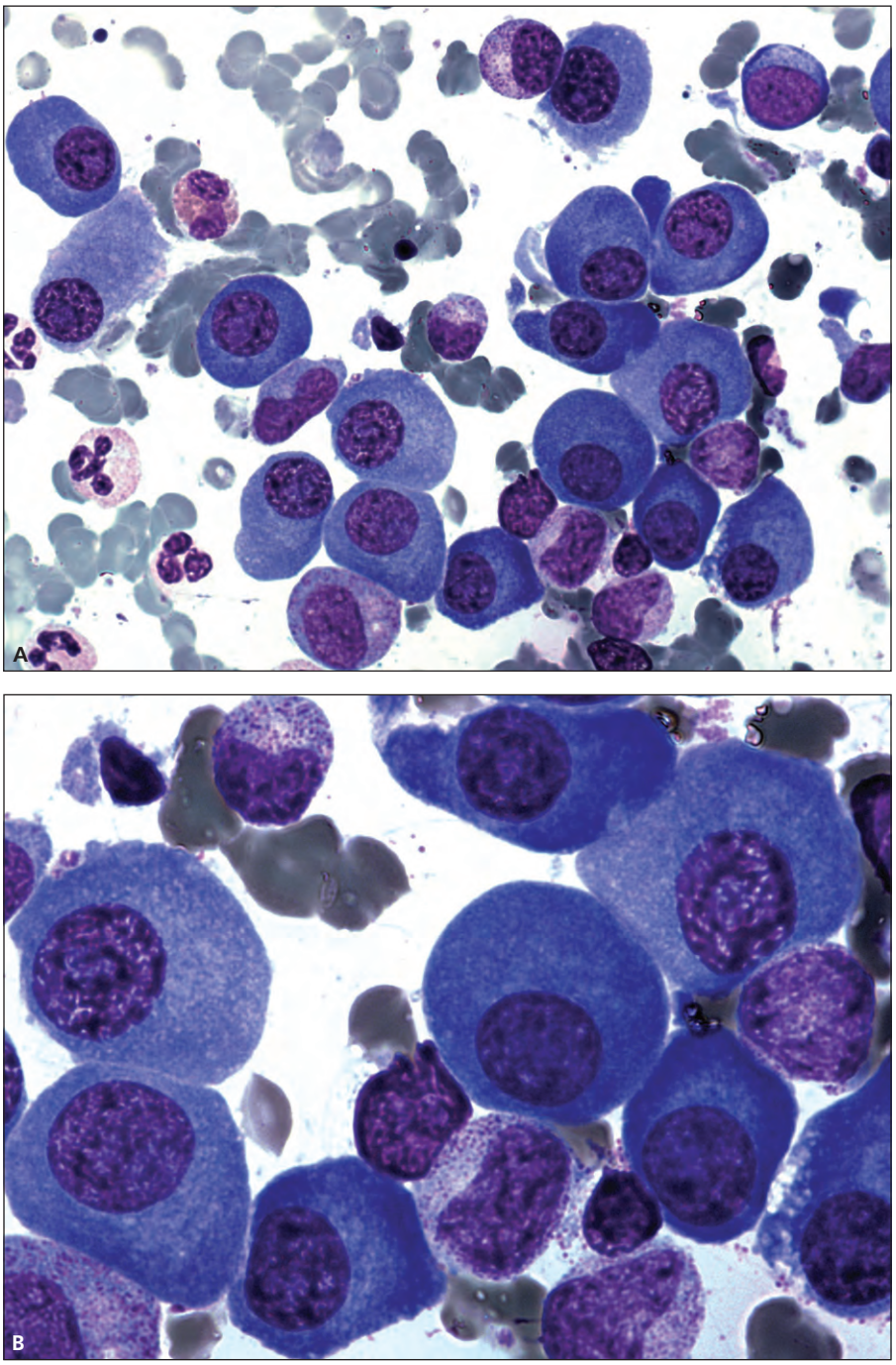



**Fig. 10.11** IgG multiple myeloma; bone marrow aspirate. Massive homogeneous infiltration by mature plasma cells and plasma cells showing an intermediate level of maturation with plentiful hypertrophic blue cytoplasm. (A) The plasma cells are very large (in comparison with the neutrophils and the myelocyte, top centre, and the promyelocyte that is touching the lower border of the image), but are fairly heterogeneous. The morphology appears repetitive, with plentiful basophilic cytoplasm, an eccentric nucleus and a clearly defined cellular outline, but showing a certain variability in shape: the smaller cells appear more mature, with a small, round, clumped, very eccentric nucleus and intensely basophilic cytoplasm; the larger cells have chromatin condensed into small heterogeneous clumps and less homogeneous cytoplasm with major development of the Golgi zone. The cell to the left, with rectangular cytoplasm, could be an osteoblast without a Golgi zone or a damaged plasma cell, but from the nuclear morphology it is more likely to be the latter. (B) A small group of gigantic plasma cells, mainly having an intermediate degree of maturation. The condensation of nuclear chromatin varies from one cell to another.

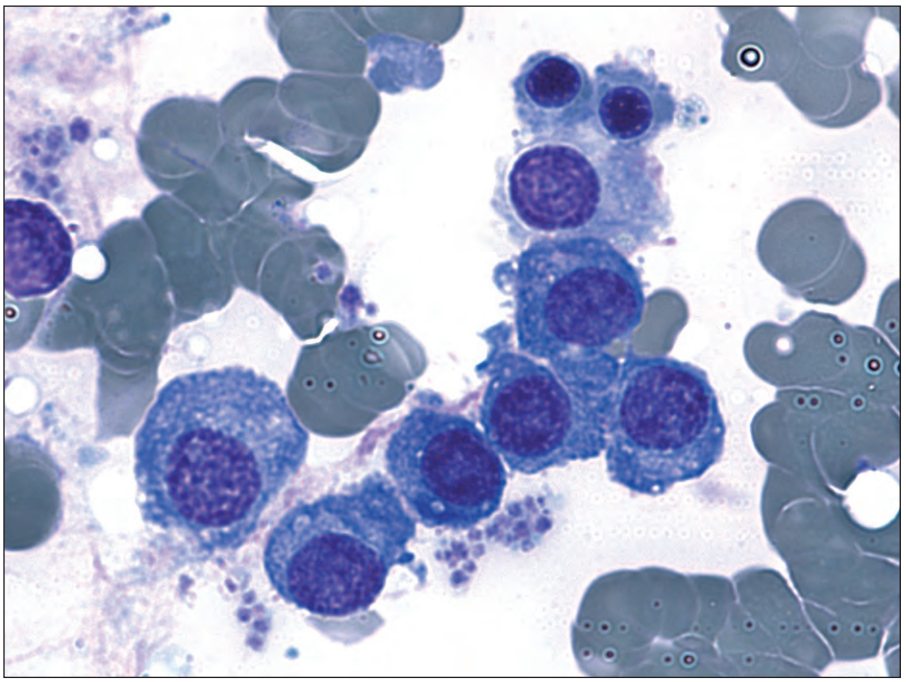


**Fig. 10.12** Mature plasma cells in the bone marrow aspirate of a patient with IgG myeloma. As in Fig. 10.11, there is a certain variability with regard to size and degree of maturation, but in the context of generally homogeneous morphology. The cytoplasmic outline, however, is often irregular and sometimes ragged and forms projections. There are a number of small masses of platelets and, at the top and a little to the right, two polychromatic erythroblasts.

accumulation of mucopolysaccharide and immunoglobulin within the lumen of dilated saccules of the endoplasmic reticulum. Mott cells (Figs 10.14 through to 10.17) are plasma cells with a morular or 'bunch of grapes' appearance as the result of cytoplasm that is packed with vacuoles corresponding to Russell bodies, optically empty or containing amorphous weakly basophilic or acidophilic material, which sometimes surround the nucleus and totally hide it. These globules are of variable size and appear clearly delimited from the surrounding cytoplasm, with a glass-like transparency or being weakly bluish or pink. The nucleus is always eccentric and the chromatin can be finely reticular or coarsely clumped.

Dutcher bodies are inclusions similar to vacuoles, single or multiple, which appear to be intranuclear and sometimes resemble large nucleoli (Figs 10.18 and 10.19; see also Figs 10.22F and 10.47). Differentiating them morphologically from nucleoli is not always easy (see Fig. 10.45B). By electron microscopy, it can be seen that the formation of Dutcher bodies is the result of the dilation of a cytoplasmic cistern, which invaginates into the interior of the nucleus and superimposes itself on it. Such inclusions therefore have the same significance as Russell bodies.[34,35] They are intensely PAS positive.

It is generally considered that cytoplasmic inclusions are also present in reactive plasmacytosis, while nuclear inclusions are more characteristic of neoplastic plasma cells. Many of these inclusions are composed of immunoglobulin molecules; some authors have reported that immunoglobulin can be absent, while others sustain that the molecules of immunoglobulin are so dense that the specific antibodies cannot penetrate and the histochemical evidence of their nature is thus not available. Other types of inclusion can be present in the cytoplasm of plasma cells in patients with diverse conditions, not necessarily neoplastic: amyloid, haemosiderin or PAS-positive material. Snapper-Schneid granules (Figs 10.13A and E and 10.20) are more frequent after chemotherapy and are the result of a non-specific reaction with hypertrophic lysosomes. One can also find, in the cytoplasm, genuine immunoglobulin crystals (Fig. 10.21): they are generally large, rarely needle-like, staining similarly to Auer rods on panoptic stains, but easily distinguishable from them by a negative peroxidase reaction.

In some plasma cells, there is very plentiful, spread out cytoplasm of a grey-violet colour verging on red: for this reason they are called flame cells or flaming plasma cells (Figs 10.13F, 10.22 and 10.23). This appearance results from the accumulation in the cytoplasm of more immunoglobulin than is present in other cells: the violaceous colour is the result of superimposition in variable proportions of the blue of ribosomes and the red of glycoprotein. Often the immunoglobulin molecules are concentrated beneath the cytoplasmic membrane, so that with panoptic stains the reddish staining is concentrated at the periphery.

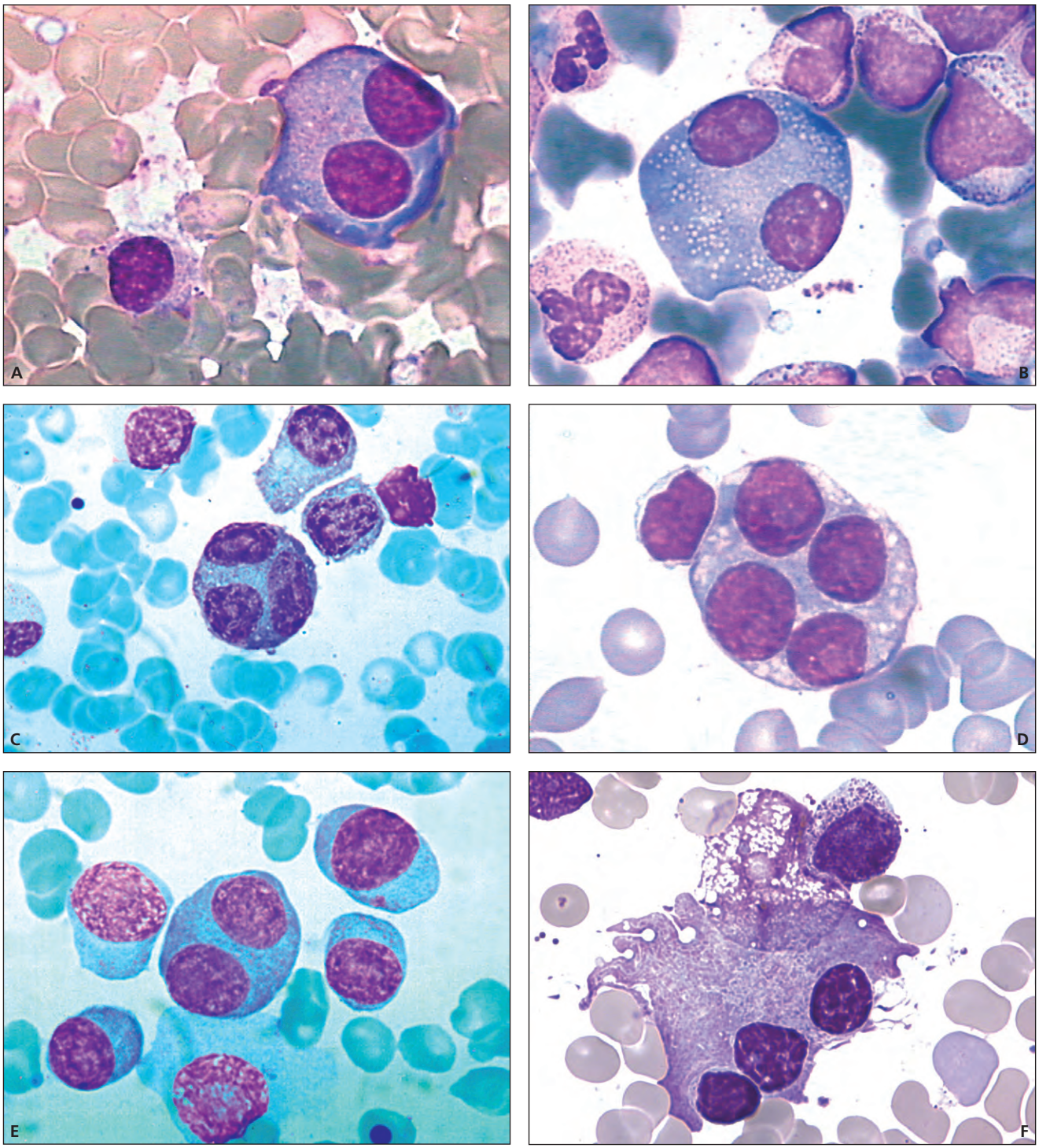
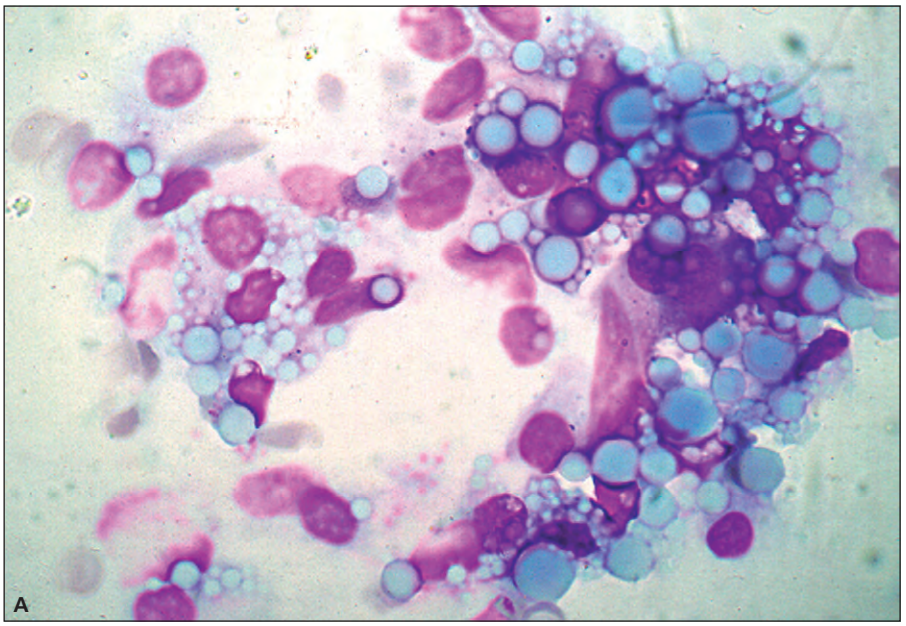
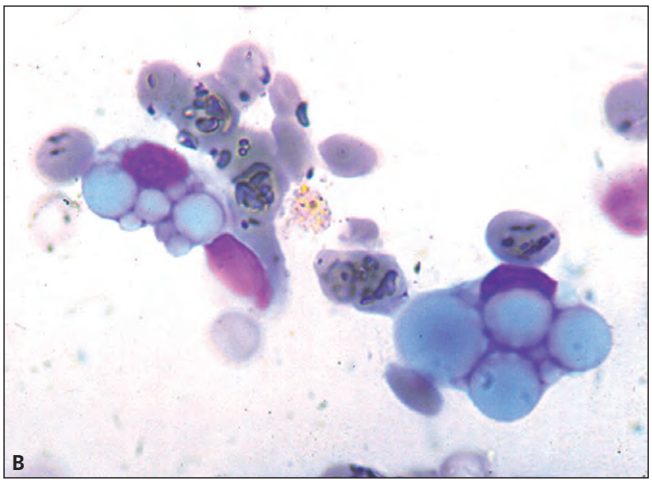



**Fig. 10.13** Multinucleated plasma cells in the bone marrow aspirates of different patients with multiple myeloma. (A) A large binucleated plasma cell. The outline of the nuclei is imperfectly ovoid and the chromatin shows the usual condensation into clumps, partially abutting the nuclear membrane. The cytoplasm is basophilic and not homogeneous; it contains fine dust-like reddish granules. (B) Although from a different patient, this large binucleated cell is very similar to that in (A). The nuclei are more elliptical and adhere to a good part of the cell membrane at opposite poles of the cell. The cytoplasm is extensively perforated by vacuoles of various sizes, the colour of which sometimes verges on red, revealing the accumulation of glycoprotein. (C) Trinucleate plasma cell with minor hypertrophy of the cytoplasm. The nuclei are quite close to the inner surface of the cell membrane. (D) In this plasma cell there are four irregularly rounded nuclei, arranged at the poles of the greyish, heavily vacuolated cytoplasm. On the left, a lymphocyte. (E) Various plasma cells of intermediate maturity, with slightly clumped chromatin, one of which is binucleated. In the smallest cell, on the right, there are fine purple granules. (F) Large binucleated plasma cell with clumped chromatin and plentiful cytoplasm that is ragged, almost forming tentacles. The violaceous deposits of immunoglobulin seem like small drops of various shapes scattered on a weakly basophilic background. The nucleus at the bottom, which seems to be inside the plasma cell, in reality is probably a small lymphocyte enveloped by the cytoplasm of the plasma cell. At the top, a dysplastic myelocyte, with disorderly granules and cytoplasm that is still basophilic.



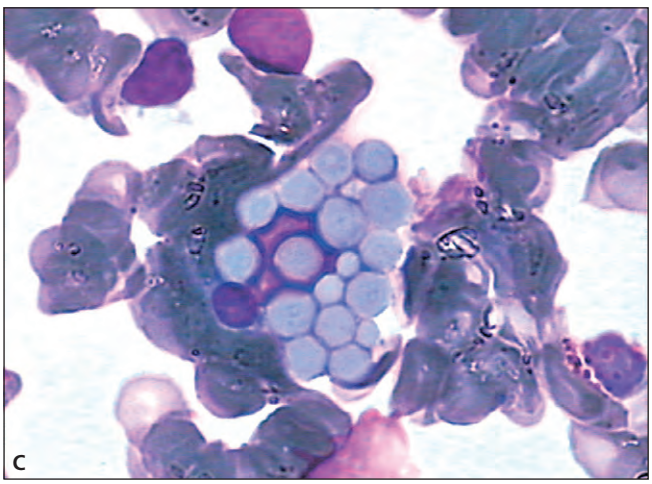




**Fig. 10.14** IgG multiple myeloma; bone marrow aspirate. The Russell bodies that pack the cytoplasm of these plasma cells appear as spherules that are heterogeneous with regard to size but homogeneous in structure, glass like and pale blue. (A) In a small nest of plasma cells it is possible to see the particular abundance of Russell bodies of various sizes, resembling bunches of grapes. The nuclei are mainly mature and there is no anaplasia, but the replacement of the haemopoietic parenchyma is almost total. Cases such as this were previously sometimes referred to as Mott cell myeloma. (B) The Russell bodies flatten and partly cover the nuclei. The appearance of this Mott cell is very atypical. Neoplastic transformation is associated, in cases such as this, with anomalies in the secretion of immunoglobulin, which accumulates in dilated saccules of the endoplasmic reticulum. (C) From a different patient, a Mott cell with typical morphology very similar to that described in (A) and (B).

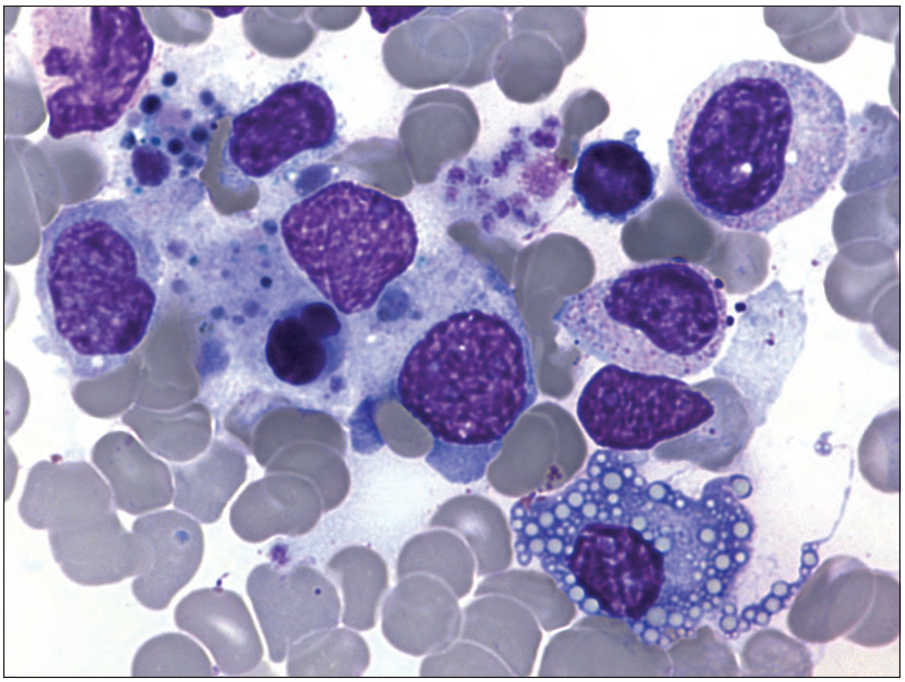


**Fig. 10.15** Multiple myeloma during treatment; bone marrow aspirate. Bottom right, a Mott cell with nuclear chromatin that is strongly condensed into hyperchromatic clumps, between which a nucleolus can just be discerned. The Russell bodies extend into a long thin cytoplasmic tail. In the centre, a large binucleated macrophage that contains cellular detritus in various stages of decomposition. To its left, a monocyte. Top right, a neutrophil myelocyte.

These cells are present in significant numbers, but not exclusively, in IgA multiple myeloma: the intracytoplasmic material is composed of immunoglobulin molecules that are highly polymerised so that they cannot be secreted normally. Other morphological features of accumulation of glycoprotein in the cytoplasm of plasma cells include the presence of signet ring cells in which optically empty vacuoles fuse to fill the entire cytoplasm; in other cases, the cytoplasm appears to have a network of small vacuoles, recalling the appearance of liposarcoma cells (Fig. 10.24).

Mott cells and flaming plasma cells are common in the bone marrow of patients with multiple myeloma, but they are not specific, being seen also in reactive conditions when there is generalised and prolonged stimulation of the immune system. They are generally interpreted as thesaurocytes or storage cells, but not in the strict sense of this term, since they do not have digestive enzymes that would be necessary to break down the excessive load of immunoglobulin molecules and are inevitably destined to die because of this excessive accumulation. True storage cells of macrophage origin are often found in the bone marrow aspirates of patients with myeloma, sometimes having the appearance of multinucleated pseudo-Gaucher cells or of sea-blue histiocytes similar to those that are observed in chronic granulocytic leukaemia. The presence of some less mature plasma cells also does not necessarily indicate a diagnosis of multiple myeloma.

## Monomorphism and heterogeneity, atypia and immaturity in myeloma cells

The morphological features described above do not provide unequivocal evidence of myeloma, even if they are frequently observed in this condition. A more specific feature, in the sense of indicating neoplasia, is the recognition of plasma cells with atypical features of immaturity, polymorphism and anaplasia, particularly in association with the presence of more than 10–20% bone marrow plasma cells. A morphological criterion that is useful for distinguishing a normal or reactive plasma cell from a neoplastic cell is the presence in the latter of N:C asynchrony: the nucleus has immature chromatin and one or more nucleoli, while the cytoplasm is basophilic and shows the normal features of a mature cell, with considerable development of the rough endoplasmic reticulum.

The cytological picture of multiple myeloma is characterised by considerable morphological variability between patients and sometimes in a single patient: together with plasma cells that are morphologically indistinguishable from normal cells (see Figs 10.6, 10.10 and 10.12), which are called 'Marshalko cells' by histopathologists, there are cells showing various degrees of immaturity, with gross nuclear or cytoplasmic atypia, possibly including frankly anaplastic forms (Figs 10.23 and 10.25 through to 10.30).

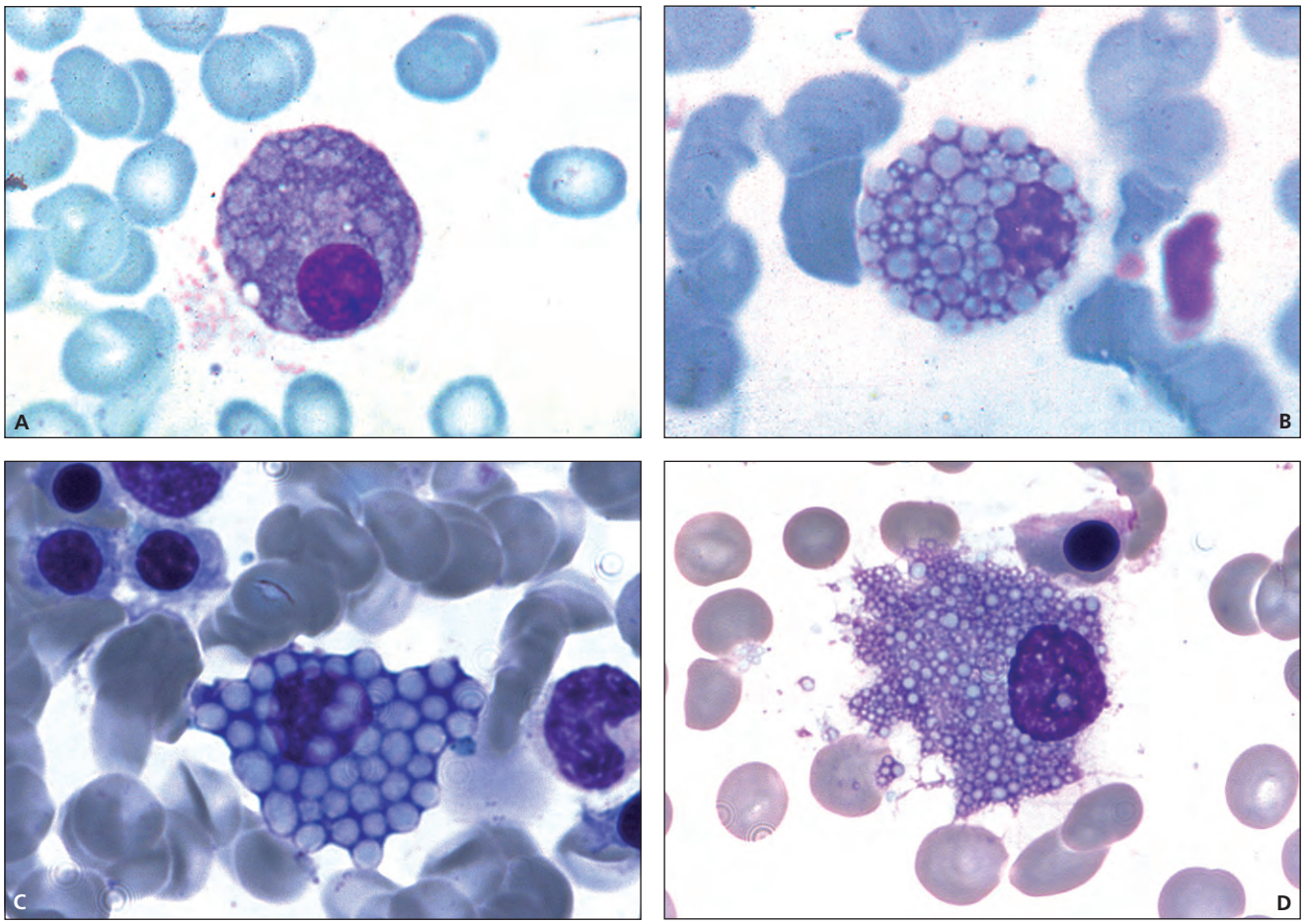



**Fig. 10.16** Mott cells in the bone marrow aspirate of various patients without multiple myeloma. (A) A plasma cell with some features of a thesaurocyte with an accumulation of glycoprotein in the spaces of the endoplasmic reticulum. The reticulated appearance of the cytoplasm, which has a reddish tinge, is characteristic of an intermediate phase in the development of a Mott cell. From a patient with MGUS. (B) Fully formed Mott cell in the bone marrow of a young woman with immune thrombocytopenic purpura. The Russell bodies are homogeneous with regard to structure, but heterogeneous with regard to size, weakly basophilic with a very clear outline; they are so numerous that they almost completely fill the cytoplasm and partially cover the nucleus. The recognition of occasional Mott cells with this regular, orderly appearance is not rare in subjects with immune activation and does not signify myeloma. (C) The large Russell bodies in this cell completely fill the cytoplasm and are superimposed on the nucleus. From a patient with monoclonal gammopathy and rheumatoid arthritis. (D) Typical Mott cell, with an eccentric nucleus and ragged cytoplasm, in a patient with HIV infection. A single Russell body appears to be superimposed on the nucleus.

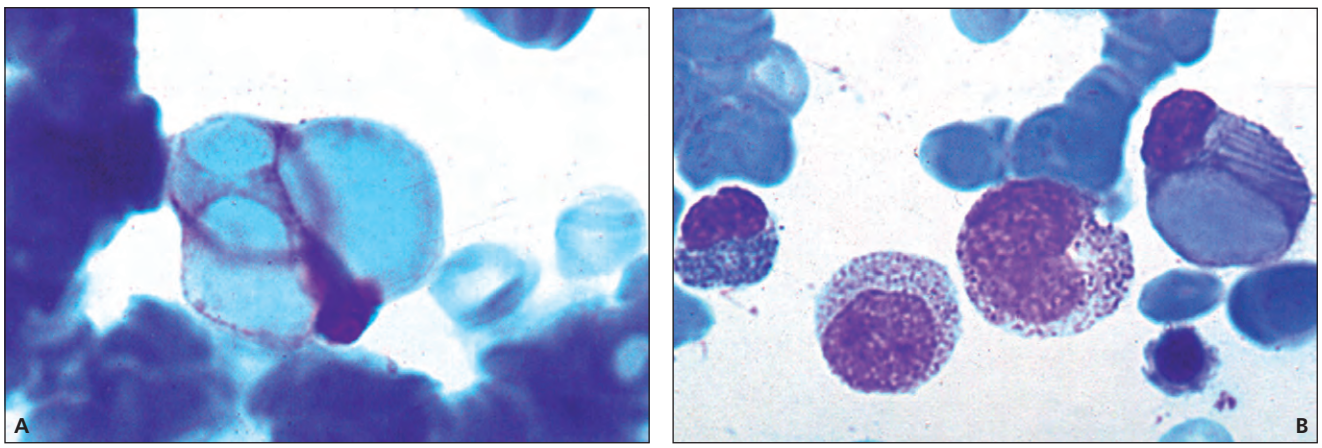



**Fig. 10.17** Multiple myeloma: bone marrow aspirate. The cytoplasm of the plasma cells in this patient is completely occupied by enormous, well-circumscribed inclusions. (A) The dilation of the ergastoplasm in this case is extreme. The nucleus is flattened and pyknotic. (B) The plasma cell in the right part of this image has a mature nucleus compressed at the periphery of the cell. Two thirds of the cytoplasm is occupied by an enormous, structurally amorphous, homogeneous Russell body, while the other third is occupied by parallel needle-like crystals: this image also shows the result of transformation of the structure of the endoplasmic reticulum in which the glycoprotein molecules of immunoglobulin accumulate and precipitate.

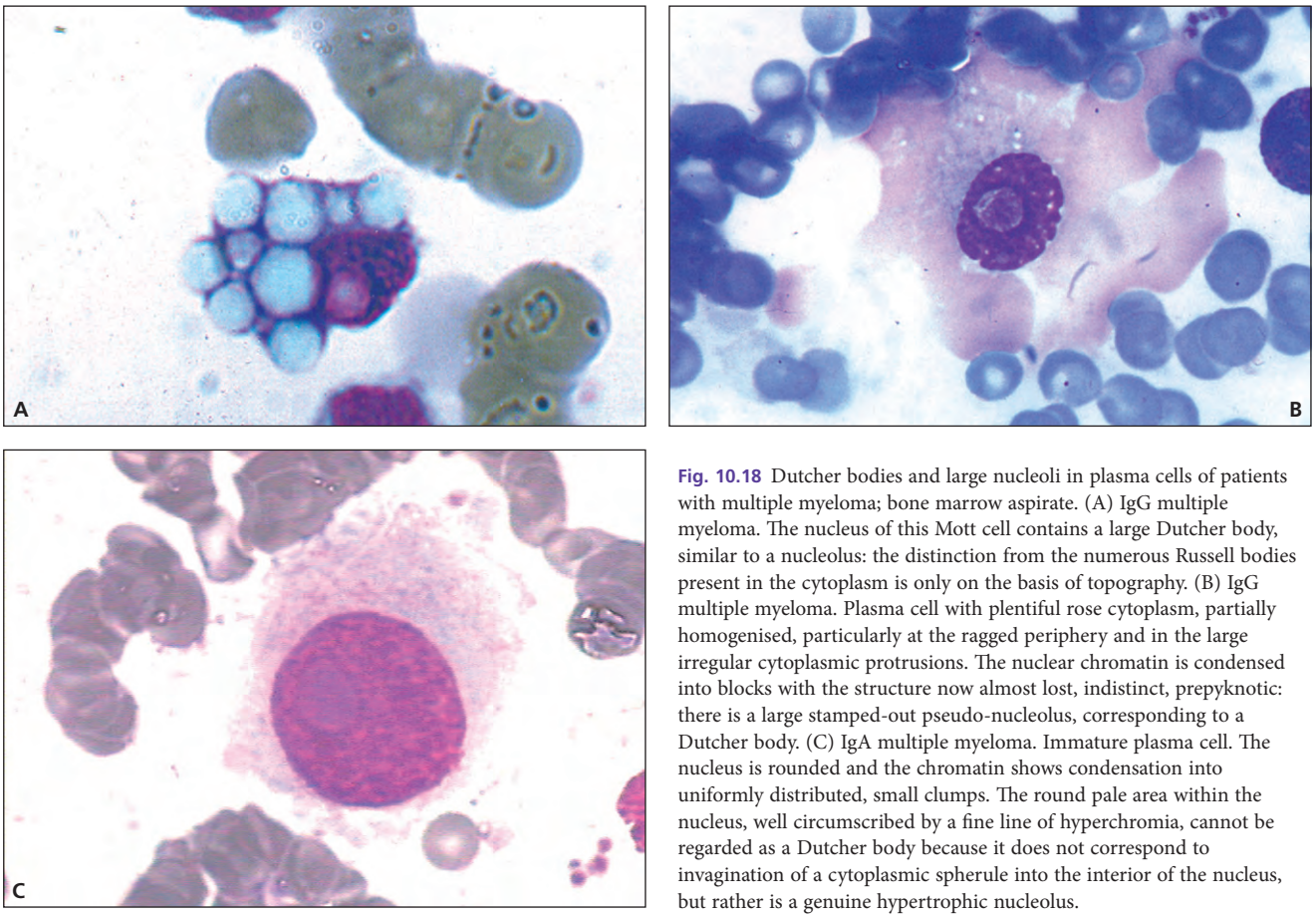



**Fig. 10.18** Dutcher bodies and large nucleoli in plasma cells of patients with multiple myeloma; bone marrow aspirate. (A) IgG multiple myeloma. The nucleus of this Mott cell contains a large Dutcher body, similar to a nucleolus: the distinction from the numerous Russell bodies present in the cytoplasm is only on the basis of topography. (B) IgG multiple myeloma. Plasma cell with plentiful rose cytoplasm, partially homogenised, particularly at the ragged periphery and in the large irregular cytoplasmic protrusions. The nuclear chromatin is condensed into blocks with the structure now almost lost, indistinct, prepyknotic: there is a large stamped-out pseudo-nucleolus, corresponding to a Dutcher body. (C) IgA multiple myeloma. Immature plasma cell. The nucleus is rounded and the chromatin shows condensation into uniformly distributed, small clumps. The round pale area within the nucleus, well circumscribed by a fine line of hyperchromia, cannot be regarded as a Dutcher body because it does not correspond to invagination of a cytoplasmic spherule into the interior of the nucleus, but rather is a genuine hypertrophic nucleolus.

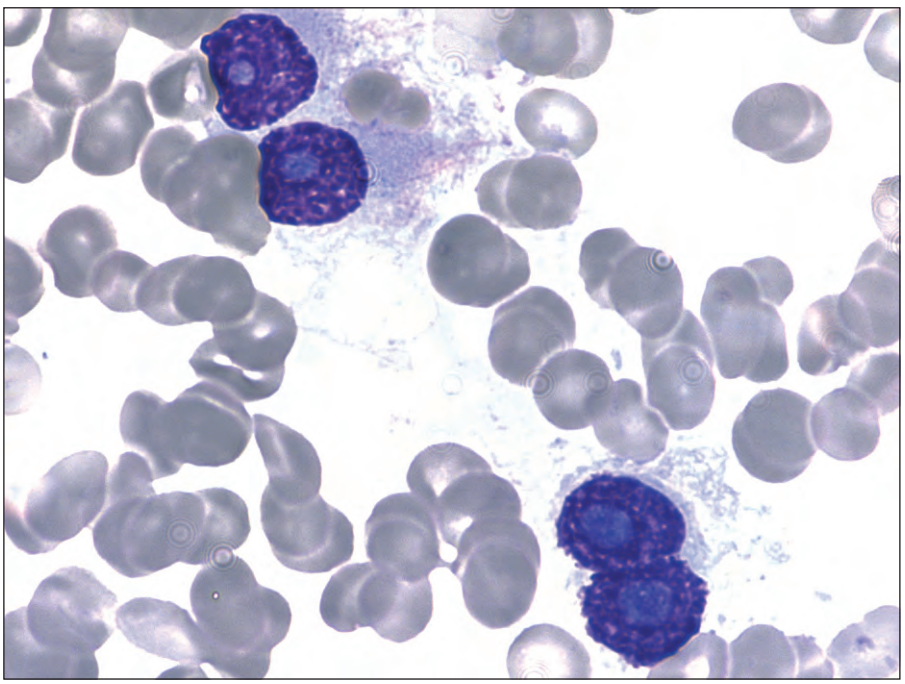


**Fig. 10.19** Multiple myeloma; bone marrow aspirate. Two binucleated plasma cells with atypical morphology: the cytoplasm is scanty, weakly basophilic and frayed; the nuclei have chromatin that is condensed into numerous clumps, more numerous and dark at the periphery, and each contains a large Dutcher body.* One of the cells has engulfed two red cells, which appear shrunken and hyperchromic.
*The translator comments that this image shows the difficulties in being sure that an intranuclear inclusion is a Dutcher body. Each of the four nuclei in this image contains a similar inclusion. Could they be giant nucleoli?

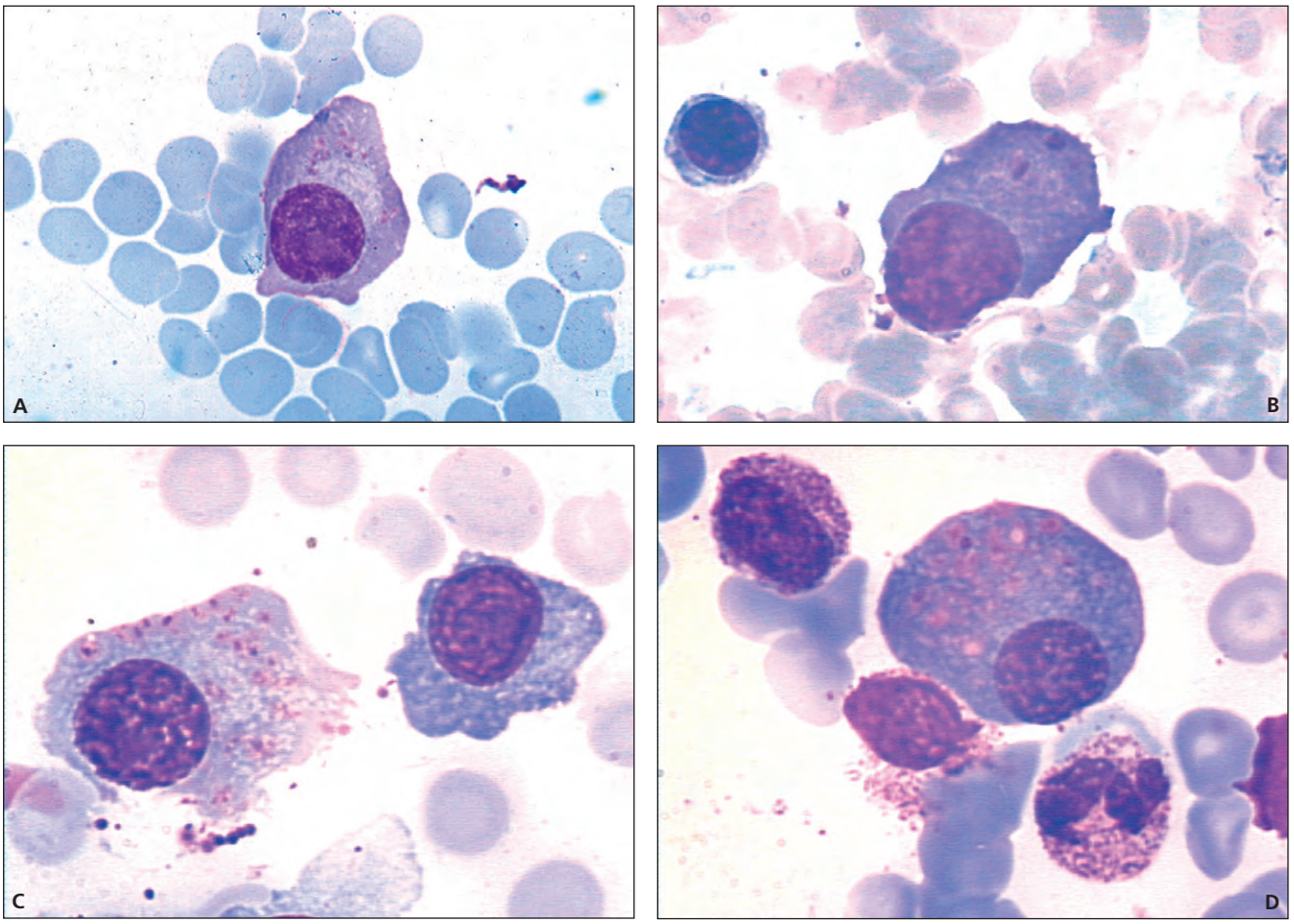



**Fig. 10.20** Plasma cells with cytoplasmic granules; bone marrow aspirates in different patients with multiple myeloma. (A) Medium-sized immature plasma cell with a large round nucleus with chromatin condensation commencing at the periphery. The cytoplasm is plentiful and violaceous and contains a large Golgi zone in the form of arcoplasm plus a number of distinct azurophilic granules, definable as Snapper-Schneid granules and corresponding to peroxidase-negative hypertrophied lysosomes. (B) Mature plasma cell containing large granules, one of which is similar to the short thick Auer rods that can be seen in some cases of promyelocytic leukaemia. A negative peroxidase reaction, however, easily distinguishes the two types of inclusion. (C) The largest plasma cell, on the left, has ample flaming cytoplasm, rich in dark red granules, large and well isolated from each other. (D) IgA multiple myeloma. Mature appearing plasma cell with hypertrophic cytoplasm containing formations that resemble vacuoles full of acidophilic material and also granules with identical tinctorial characteristics, resulting from the lack of removal of the abundant immunoglobulin produced in the rough endoplasmic reticulum, responsible for the intense background basophilia.

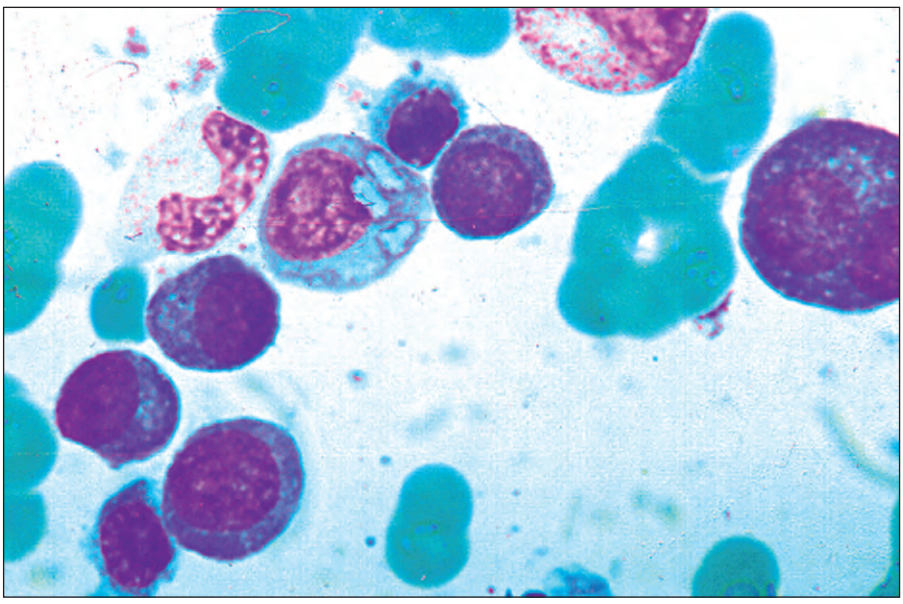


**Fig. 10.21** Multiple myeloma; bone marrow aspirate. There are various plasma cells with intensely basophilic cytoplasm and a nucleus, sometimes two nuclei, with variable chromatin condensation. The most immature cell shows a visible nucleolus surrounded by a circle of condensed chromatin and has less basophilic cytoplasm containing three crystalline inclusions, the significance of which is analogous to that of Russell bodies.

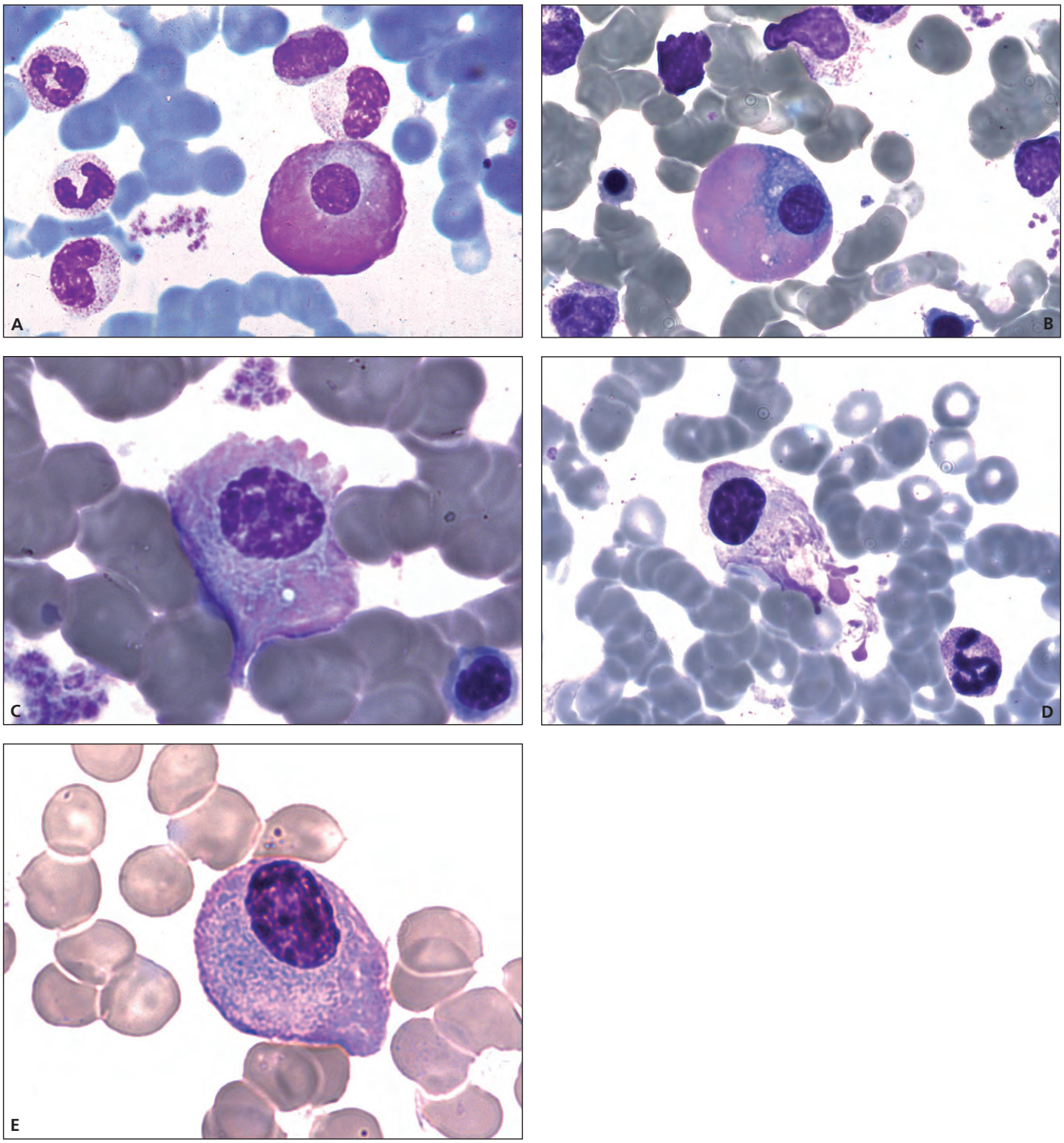



**Fig. 10.22** Plasma cells with flaming cytoplasm in different cases of multiple myeloma; bone marrow aspirates. (A) From a patient with IgA myeloma in complete remission after chemotherapy. The glycoprotein responsible for the very dense and homogeneous reddish colour has accumulated particularly on the opposite side of the cell to that occupied by the paranuclear Golgi zone, which remains pale and weakly basophilic. The nucleus is round and mature, slightly off centre, with chromatin condensed into blocks. (B) Large flaming plasma cell from a different patient with IgA myeloma. The characteristic morphology is identical to that in (A), with the exception of a greater degree of eccentricity of the nucleus. The acidophilic material has accumulated peripherally opposite the nucleus. (C) The accumulation of pink-staining glycoprotein appears in this cell to be fragmented and certainly corresponds to the saccules of dilated endoplasmic reticulum. At the top of the cell, it seems to evoke an unsuccessful attempt to secrete immunoglobulin. The chromatin is very condensed into large blocks. (D) Also in this cell (from the same preparation as [B]), the non-secreted immunoglobulin has accumulated in intracytoplasmic saccules and large submembranous violaceous vesicles, in part apparently detached, like flames, from the body of the cell. (E) From the same preparation as (B), mature plasma cell with cytoplasm that is heterogeneous with regard to structure and staining characteristics. The cytoplasmic margin is flattened by compression by surrounding red cells.

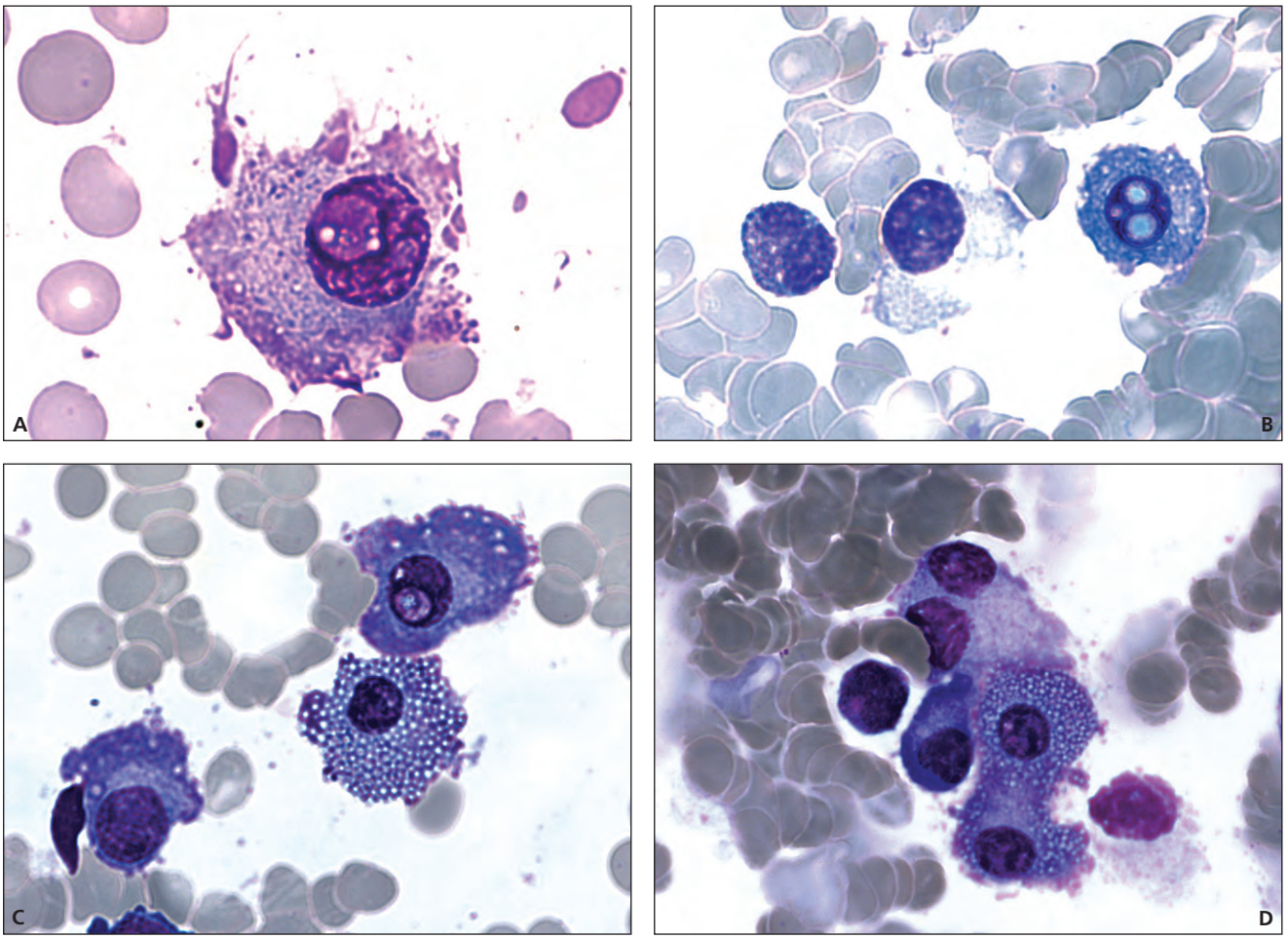



**Fig. 10.23** Morphological heterogeneity of plasma cells in a patient with IgG multiple myeloma; bone marrow aspirate. (A) In addition to the flaming cytoplasm, this plasma cell is characterised by the presence of an intranuclear Dutcher body containing small pale vacuoles.* (B) The plasma cell on the right is rounded and basophilic, with a central nucleus and two Dutcher bodies. (C) Top right, a large plasma cell characterised by plentiful basophilic cytoplasm with a ragged margin which appears to be fragmenting into reddish drops, very obvious arcoplasm and an intranuclear Dutcher body. Immediately below, a Mott cell with cytoplasm packed with small vacuoles. Bottom left, a plasma cell of intermediate maturity, with a nucleus having less condensed chromatin and ample paranuclear arcoplasm. (D) At the top, a flaming binucleated plasma cell. Immediately beneath it, a mature basophilic plasma cell and a binucleated Mott cell, the lower nucleus of which is markedly eccentric and almost surrounded by a pale hypertrophic Golgi zone.
*The translator wonders if this could be a giant nucleolus rather than a Dutcher body. Image (B), however, shows two undoubted Dutcher bodies. Again, the difficulty of making this distinction is shown.

In some cases the picture is monomorphic and the bone marrow appears to be diffusely infiltrated by plasma cells arrested at the same level of maturation: the neoplastic population in these cases can be composed mainly of mature cells, mainly of cells at an intermediate level of maturation (basophilic cytoplasm, more immature nucleus with chromatin less condensed and nucleoli present), mainly of blastic cells (plasmablasts) or mainly of less differentiated lymphoplasmacytoid cells (Table 10.4). A typical lymphocyte-like or small plasmacytoid lymphocyte morphology has been described in a considerable number of patients with t(11;14)(q13;q32); these are also associated with an atypical immunophenotype.[36]

In other cases, referred to as asynchronous myeloma or polymorphic myeloma, there is a marked lack of morphological homogeneity, with the co-existence of variable proportions of cells at the stages of maturation described above, ranging from morphologically normal plasma cells to plasmablasts.

It is not rare to see severe cytological anomalies, including cells that are bizarre, monstrous, anaplastic, giant and multinucleated, polyploid or with asymmetric nuclei produced by twinning; nuclei with small excrescences; multilobated nuclei; obvious and multiple nucleoli; immature cells with scanty weakly basophilic cytoplasm, which are dedifferentiated having lost the capacity to synthesise immunoglobulin; mitotic figures.

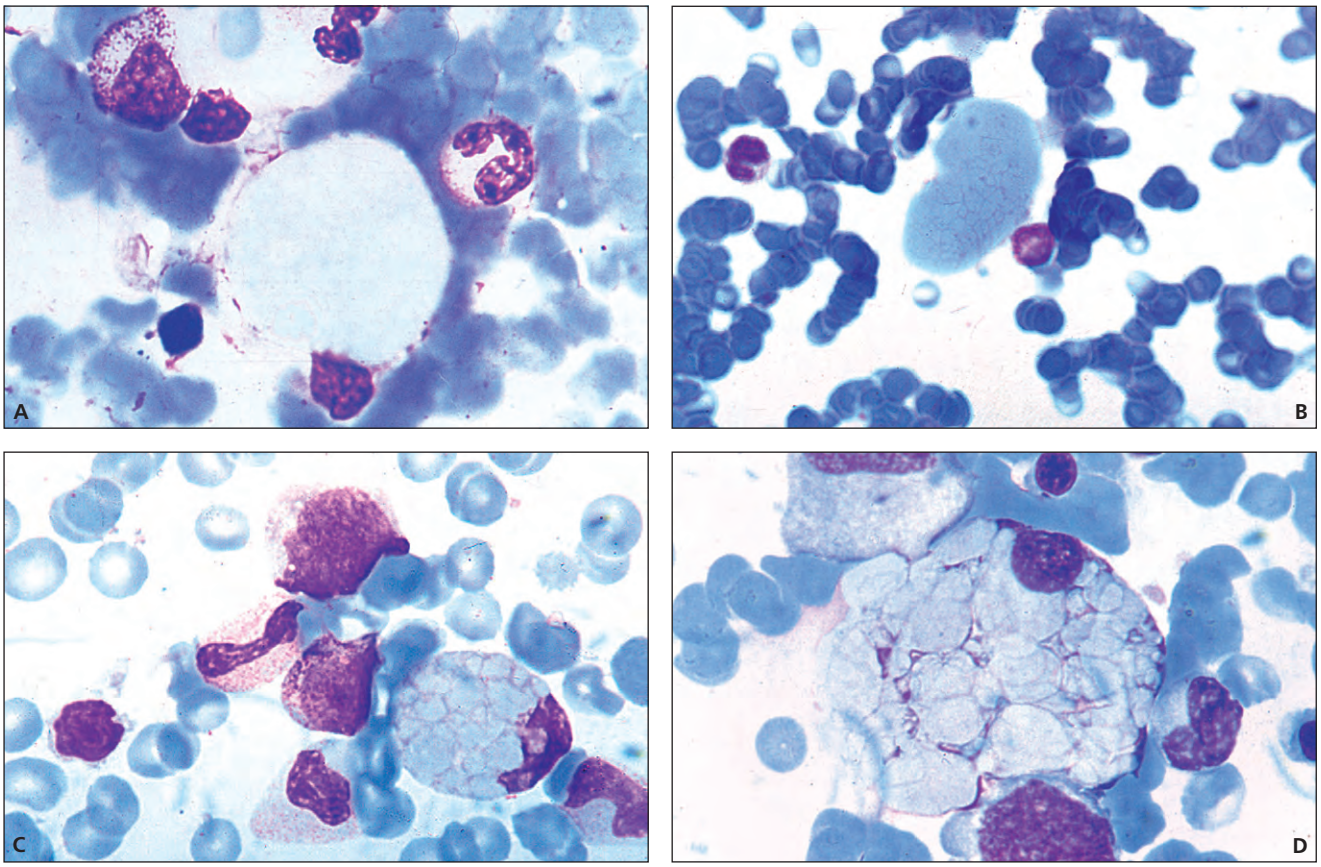



**Fig. 10.24** Plasma cell with storage features: bone marrow aspirate. (A) Plasma cell with pale hypertrophic cytoplasm, resembling a signet ring (from the same patient as Fig. 10.17). The structure of the cytoplasm is invisible after staining with May-Grünwald-Giemsa and the appearance of the cell is very similar to that of a large histiocyte with an eccentric nucleus and very dense chromatin. (B) In this cell with storage features (present in the same film as [A]), the cytoplasm appears detached from the nucleus, but maintains intact the homogeneous basophilic contents, which have a reticular appearance as a result of the visibility of the sacs of the endoplasmic reticulum. (C) A plasma cell that resembles a histiocyte or a liposarcoma cell in the bone marrow aspirate of a patient with relapsed acute lymphoblastic leukaemia. The cytoplasm is hypertrophic and loaded with globules resembling irregular and heterogeneous Russell bodies, which compress and deform the nucleus. The cell in the centre at the top is a lymphoblast. (D) Another pseudoliposarcomatous plasma cell in the marrow of a patient with MGUS. The dilated saccules that pack the cytoplasm are irregular, transparent and superimposed on each other; their outline is visible because of an unusual background acidophilia.

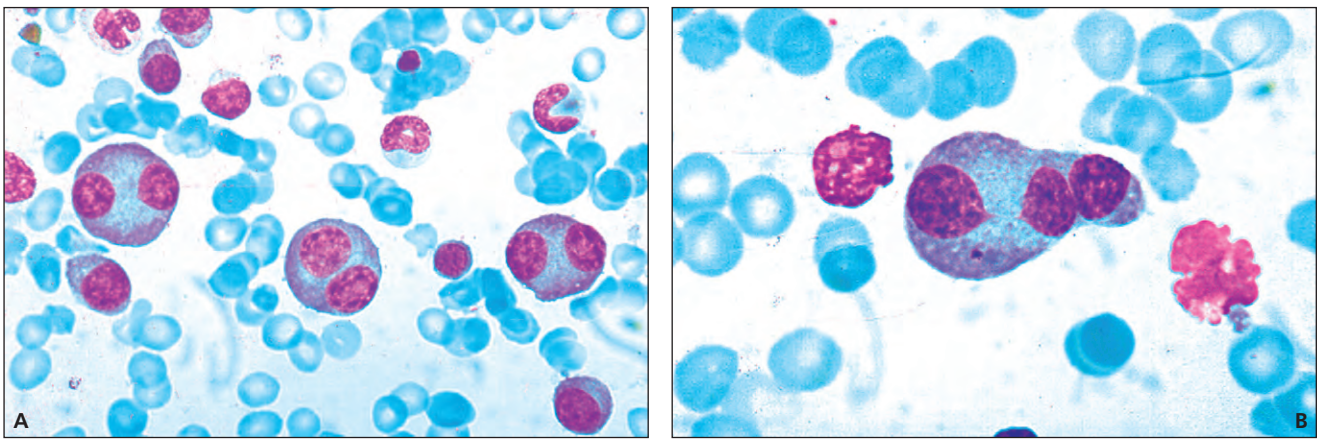



**Fig. 10.25** IgG multiple myeloma with mature plasma cells and frequent multinuclearity; bone marrow aspirate. (A) There are three binucleate plasma cells with plentiful cytoplasm and three small mature plasma cells with a moderate amount of cytoplasm. The morphological features of cytoplasmic maturity are well shown (basophilia and paranuclear Golgi zone). Nuclear chromatin is condensed, in blocks, as shown well in the binucleate cell in the centre. The binucleate plasma cells are blocked in telophase, karyokinesis not having been followed by cytokinesis with division of the cytoplasm. (B) Mature trinucleate plasma cell. The presence of an uneven number of nuclei is the result of asymmetrical mitosis, in which only one nucleus of a binucleate plasma cell divides again. In this cell, the nucleus on the right has mature chromatin in denser blocks than those of the other two nuclei, which have remained joined by a thin filament of chromatin, stretched out between the two nuclei.

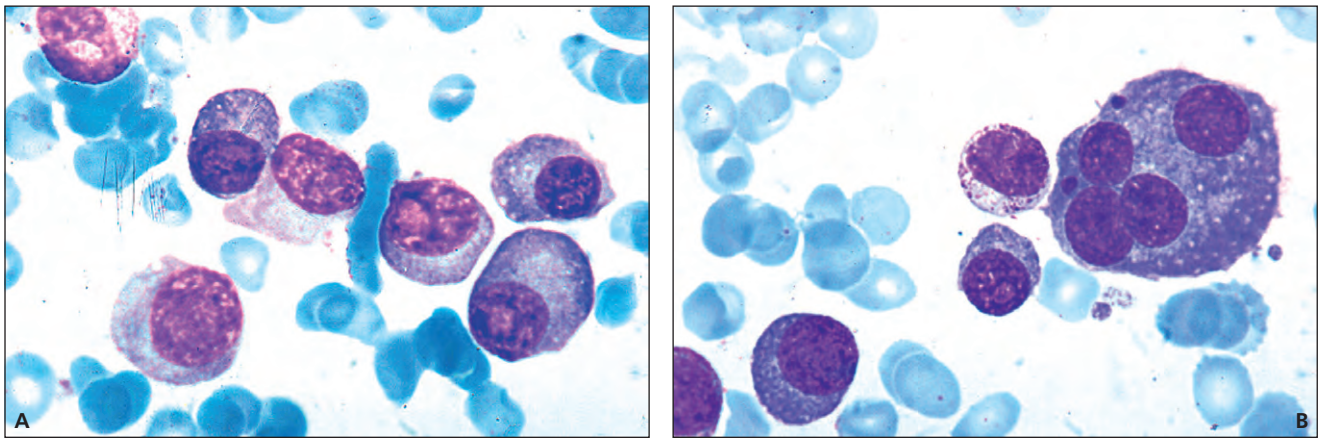



**Fig. 10.26** IgA multiple myeloma; bone marrow aspirate. (A) Massive infiltration of the marrow by mature and immature plasma cells. (B) Large plasma cell with four nuclei and mature chromatin. Adjacent, a small myelocyte and a small mature plasma cell; the cell in the bottom left corner could be classified as an intermediate maturity plasma cell.

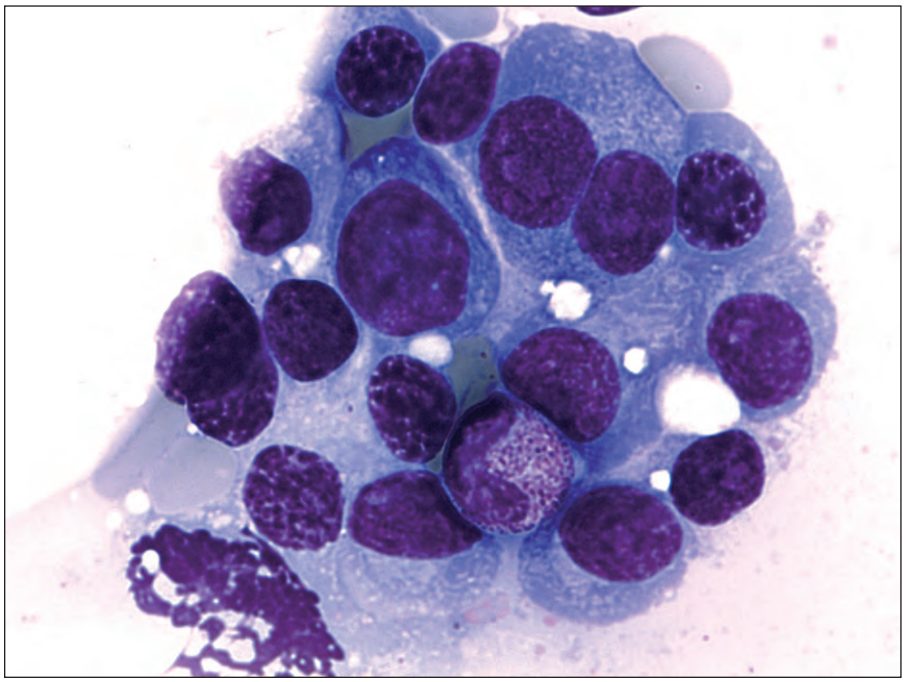


**Fig. 10.27** Heterogeneity in the degree of nuclear maturation in a case of IgG myeloma; bone marrow aspirate. This small fragment is composed of plasma cells that share morphological features (basophilic cytoplasm and eccentric nuclei), but differ in the degree of chromatin condensation. Towards the top, an immature plasma cell with two nuclei with visible nucleoli and chromatin not showing much condensation. On its left, a small mature plasma cell and another large immature plasma cell with a large nucleus and relatively scanty intensely blue cytoplasm. At the bottom, two plasma cells of intermediate maturity. The only cell that is not a plasma cell is an eosinophil metamyelocyte.

The cytoplasm may contain granules, vacuoles or crystals; sometimes it appears uniformly basophilic without a visible Golgi zone; the cytoplasmic membrane may be ragged, resembling that of a hairy cell, or fragmented into large ragged strands that become detached from the cell (see Figs 10.12, 10.13F and 10.31). There may be a predominance, particularly in IgA myeloma, of flaming plasma cells with a very heterogeneous degree of maturation. Sometimes the cellular outlines are indistinct and the myeloma cells are grouped in a pseudosyncytial formation (Fig. 10.32). A peculiar function, although rare, is the ability of some myeloma cells to phagocytose red cells, neutrophils and platelets (Figs 10.33 and 10.34): the myeloma cells with phagocytic properties appear mature, sometimes pseudo-histiocytic, with the cytoplasm containing intact cells or being laden with fragments and detritus of degenerating cells.[37,38]

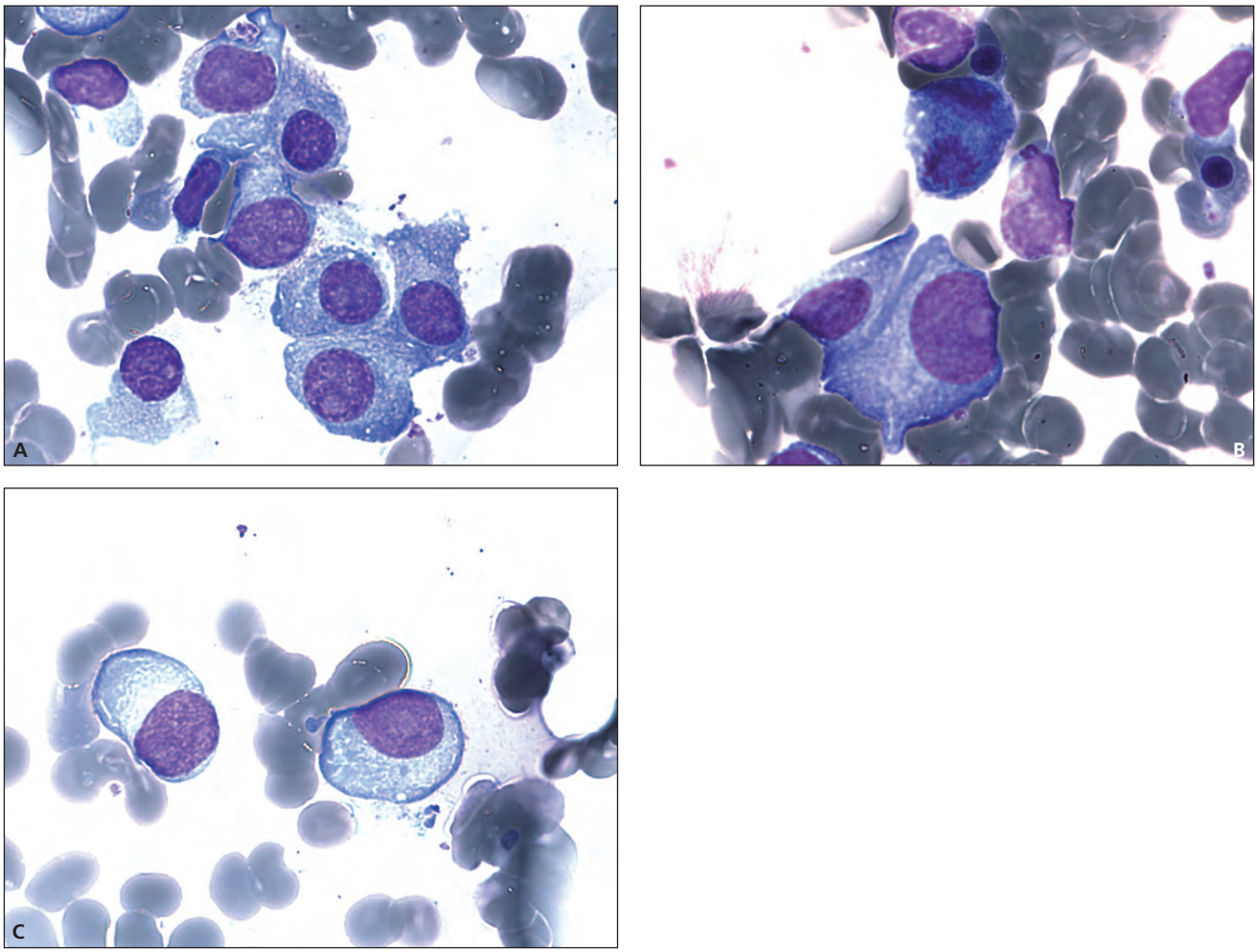



**Fig. 10.28** Morphological features of plasma cells in another case of IgG myeloma; bone marrow aspirate. (A) The immature plasma cells are characterised by eccentric nuclei, with only minor chromatin condensation, without large chromatin clumps and with nucleoli that are often prominent; the cytoplasm is basophilic. Those of intermediate maturity are distinguished particularly by the absence of visible nucleoli and by N:C asynchrony. (B) At the bottom, a large immature plasma cell with a quite prominent nucleolus and dispersed chromatin; the eccentricity of the nucleus and the abundance of the cytoplasm are the main differentiating characteristics in comparison with a plasmablast. At the top, a small hyperbasophilic plasma cell in the final stage of mitosis. On the right, an orthochromatic erythroblast. (C) From the same film, in this better spread area, there are two plasma cells: that on the left can be classified as showing intermediate maturity, because of the lack of visible nucleoli and the large semilunar paranuclear Golgi zone; that on the right is an immature plasma cell.

During the course of therapy, it is useful to evaluate the tumour mass quantitatively and qualitatively to permit the early identification of the inevitable disease evolution. The disappearance of plasma cells from the bone marrow is slow and the persistence of a significant proportion after transplantation should not be taken as indicative of failure of the procedure, since it results from slow plasma cell clearance, which can take as long as 60 days. This morphological observation recalls the slow normalisation of the M component in the serum of transplanted patients, which can be related to the half-life of serum immunoglobulin.

## Morphology and prognosis: plasmablastic myeloma

The possible prognostic significance of plasma cell morphology in patients with multiple myeloma has been emphasised in the literature.[39–42] Various authors, categorising the plasma cells on the basis of predefined morphological criteria such as those shown in Table 10.4, have correlated the extent of infiltration and the degree of immaturity in order to identify successive morphological stages of the disease, characterised by differing survival.[40] A morphological classification of myeloma was proposed in the 1980s[39,40] and applied, after revision, in large series

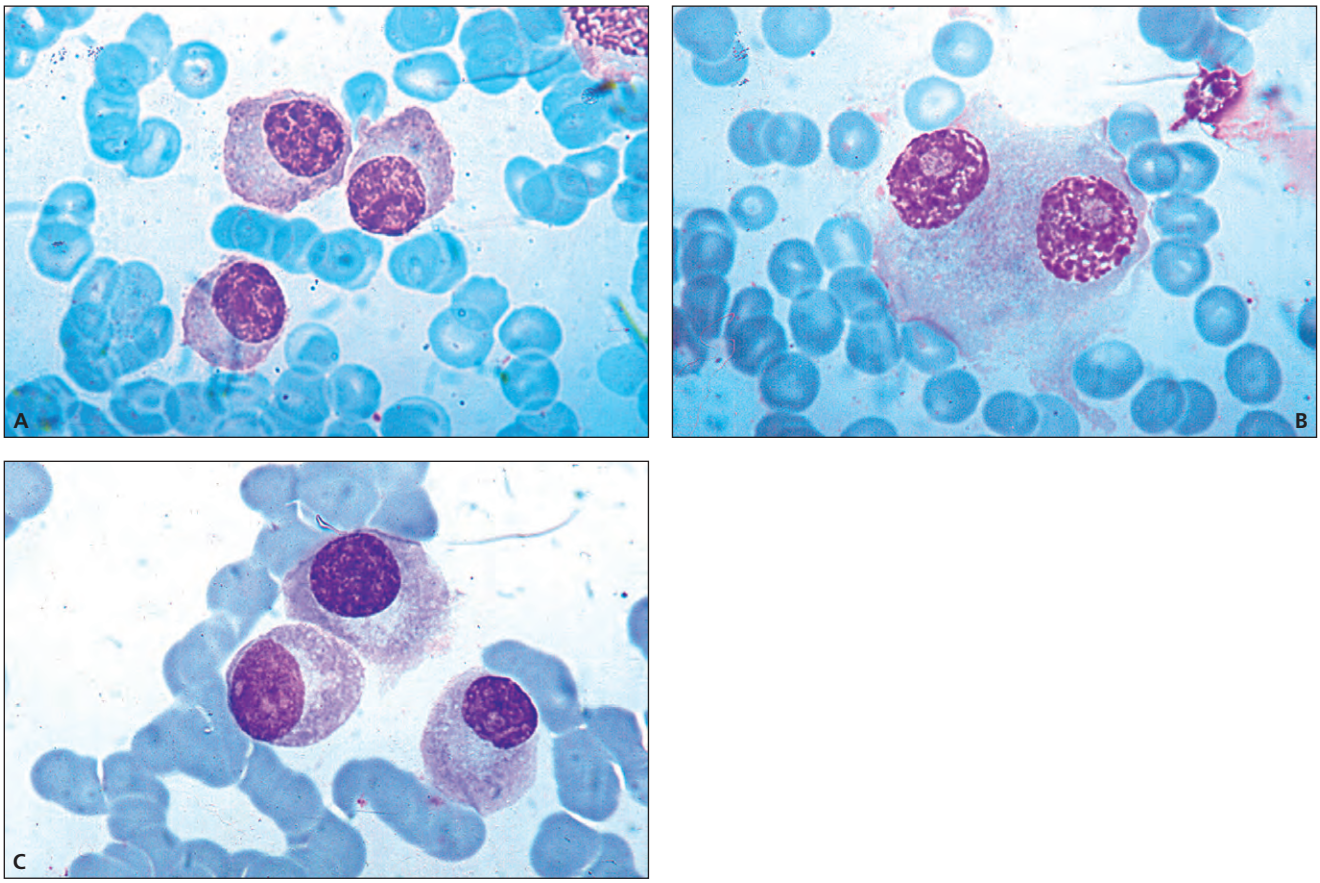



**Fig. 10.29** Multiple myeloma with mainly intermediate and immature plasma cells; bone marrow aspirate. (A) IgA myeloma. Three intermediate plasma cells: the nucleus is eccentric, the chromatin is condensed into blocks and faint arcoplasm is visible. The outline of a large nucleolus remains visible and the N:C ratio is increased. The cytoplasm is pinkish, more intense at the periphery, and appears granular with small vacuoles. (B) IgA myeloma (a different patient); bone marrow aspirate. Intermediate binucleate plasma cell. In this case also the nucleoli are prominent even though the chromatin is condensed into blocks. The nuclei are very large and the cytoplasm is violet and not homogeneous, but there is no visible Golgi zone. (C) IgG myeloma. The cell on the left is an immature plasma cell: the N:C ratio is quite high, the nucleus is large and eccentric with homogeneous chromatin – fine, dispersed, not condensed; there are three not very clear nucleoli; the cytoplasm appears more mature, with a large arcoplasm, but basophilia is weak, with whitish stippling. The other two plasma cells, which would be classified as intermediate, have similar morphological characteristics, but show a greater degree of chromatin condensation.

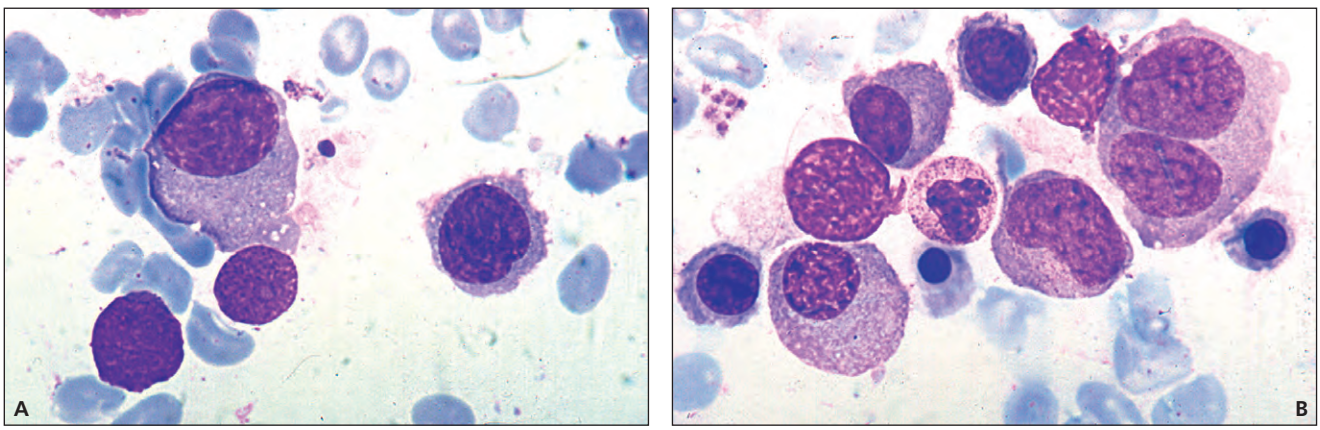



**Fig. 10.30** IgG multiple myeloma: atypia and heterogeneity of the neoplastic population that is invading the marrow; bone marrow aspirate. (A) The cell on the left is a gigantic plasma cell with a large, irregularly condensed nucleus. The nature of the cell on the right is less certain. It has a very high N:C ratio, but its cytoplasmic characteristics look very similar to those of the myeloma cell on its left. It could be either a dysmorphic myeloma cell or a dysplastic erythroblast. (B) On the right, a large plasmablast containing two enormous nuclei with dispersed chromatin and multiple nucleoli; the cytoplasm is scanty, considering the nuclear volume, and is pinkish grey. At the top on the left, a more mature plasma cell with a nucleolus; bottom left, an intermediate plasma cell with incomplete chromatin condensation and plentiful granular cytoplasm. There are also some orthochromatic and polychromatic erythroblasts.

**Table 10.4** Morphological criteria for the classification of plasma cells in multiple myeloma[38–40]

**Mature plasma cells**
Features indistinguishable from normal plasma cells:
- Oval shape
- Eccentric nucleus
- Chromatin condensed into blocks
- Nuclear diameter <8 μm
- Nucleolar diameter <1 μm
- Basophilic cytoplasm
- Pale paranuclear area

**Intermediate plasma cells**
All the cells that do not satisfy the criteria for other categories
Prevalence of features of asynchronous maturation of nucleus and cytoplasm:
- Abundant cytoplasm, intensely basophilic
- Pale paranuclear area
- Eccentric nucleus
- Large nucleus with chromatin less condensed than normal
- In other cells: mature nucleus with condensed chromatin and cytoplasm not very basophilic

**Immature plasma cells**
- Dispersed chromatin
- Nuclear diameter >10 μm
- Nucleolar diameter >2 μm
- Plentiful basophilic cytoplasm, nucleus in eccentric position, pale paranuclear area less developed

**Plasmablasts**
- Nucleus with finely reticular chromatin or with minimal very small areas of condensation
- Large nucleus (estimated at >10 μm) or with a large clearly visible nucleolus (estimated at >2 μm)
- Cytoplasm not very plentiful, less than half of the area occupied by the nucleus, with increased N:C ratio
- Pale paranuclear area absent or barely visible

**Lymphoplasmacytoid cells**
Similar in size to lymphoblasts, but with features of plasma cell differentiation:
- Pale paranuclear area
- Moderately basophilic cytoplasm
- Chromatin arranged in a cartwheel formation
- Nucleus close to the centre or eccentric

of patients[43–45]: cases of multiple myeloma at diagnosis were assigned to four subgroups based on the predominant type of myeloma cells present in the bone marrow aspirate: mature, intermediate, immature or plasmablastic (Table 10.5). Myeloma was defined as plasmablastic when the percentage of bone marrow plasmablasts was more than 2% (Figs 10.35 through to 10.38). The unfavourable prognostic significance of this morphology is, in fact, the only observation that has been confirmed as an independent prognostic factor in more recent studies: patients with more than 2% plasmablasts in the bone marrow aspirate are characterised by a higher concentration of beta-2 microglobulin, a higher percentage of bone marrow plasma cells, a higher frequency of unfavourable cytogenetic aberrations and, above all, shorter survival both after treatment with combination chemotherapy and after autologous stem cell transplantation.[46,47]

## Histology

Histological examination provides useful information on the extent and the pattern of myelomatous infiltration, which can be interstitial (with or without co-existing paratrabecular infiltration) or nodular, as is often observed in the early stages of the disease, or diffuse, particularly in advanced stage disease.[1,48–50] The normal localisation of plasma cells in small groups in proximity to bone marrow sinusoids and arterioles is lost: an irregular distribution of myeloma cells and the tendency to focal aggregation, mainly in a periosteal position, in histological sections is one of the criteria of neoplasia that is most useful in making a distinction from reactive plasmacytosis. The quantification of any fibrosis is important, it being present in less than 10% of cases. Immunohistochemistry for CD138 permits an optimal estimate of the percentage of plasma cells, while staining for kappa and lambda light chains demonstrates clonality.[9] Sometimes an aspirate and a trephine biopsy provide different estimates of the plasma cell percentage, the higher of the two being selected for diagnostic and prognostic purposes.[30] The frequency of recognition of numerous osteoclasts explains the occurrence of osteolysis in multiple myeloma.

## Immunophenotype

Multicolour flow cytometric immunophenotyping permits the detection of plasma cells, which express CD138 and monotypic immunoglobulin in the cytoplasm, but not on the surface membrane; clonality is thus established by the recognition of only a single light chain type in the cytoplasm. However, flow cytometry does not provide a valid measurement of the percentage of myeloma cells in the bone marrow aspirate. The CD56 antigen shows atypical expression in the neoplastic cells of 70–80% of cases of myeloma.

Immunohistochemistry on tissue sections can be important to confirm the clonal nature of plasma cells by demonstration of a single light chain type in the plasma cell cytoplasm.[51]

In addition, immunophenotyping using a combination of markers permits a distinction between normal or reactive plasma cells and neoplastic cells, and can also identify neoplastic plasma cells when they represent only a low proportion of cells. The antigenic profile of normal plasma cells is characterised by intense positivity for CD38 and negativity for CD56; in addition, CD45 is positive, while CD20, CD28, CD33 and CD117 are negative.[52]

Myeloma cells show variable expression of B-lineage antigens: in many cases, numerous membrane markers, such as CD19 and CD20, are not expressed and nor is surface membrane immunoglobulin (SmIg). On the other hand, cytoplasmic immunoglobulin, CD38 and the CD56 adhesion molecule are expressed.[53] CD10 is expressed in 20–50% of cases, particularly in plasmablastic myeloma with an aggressive clinical course. In 10–30% of cases, the myeloma cells express myelomonocytic

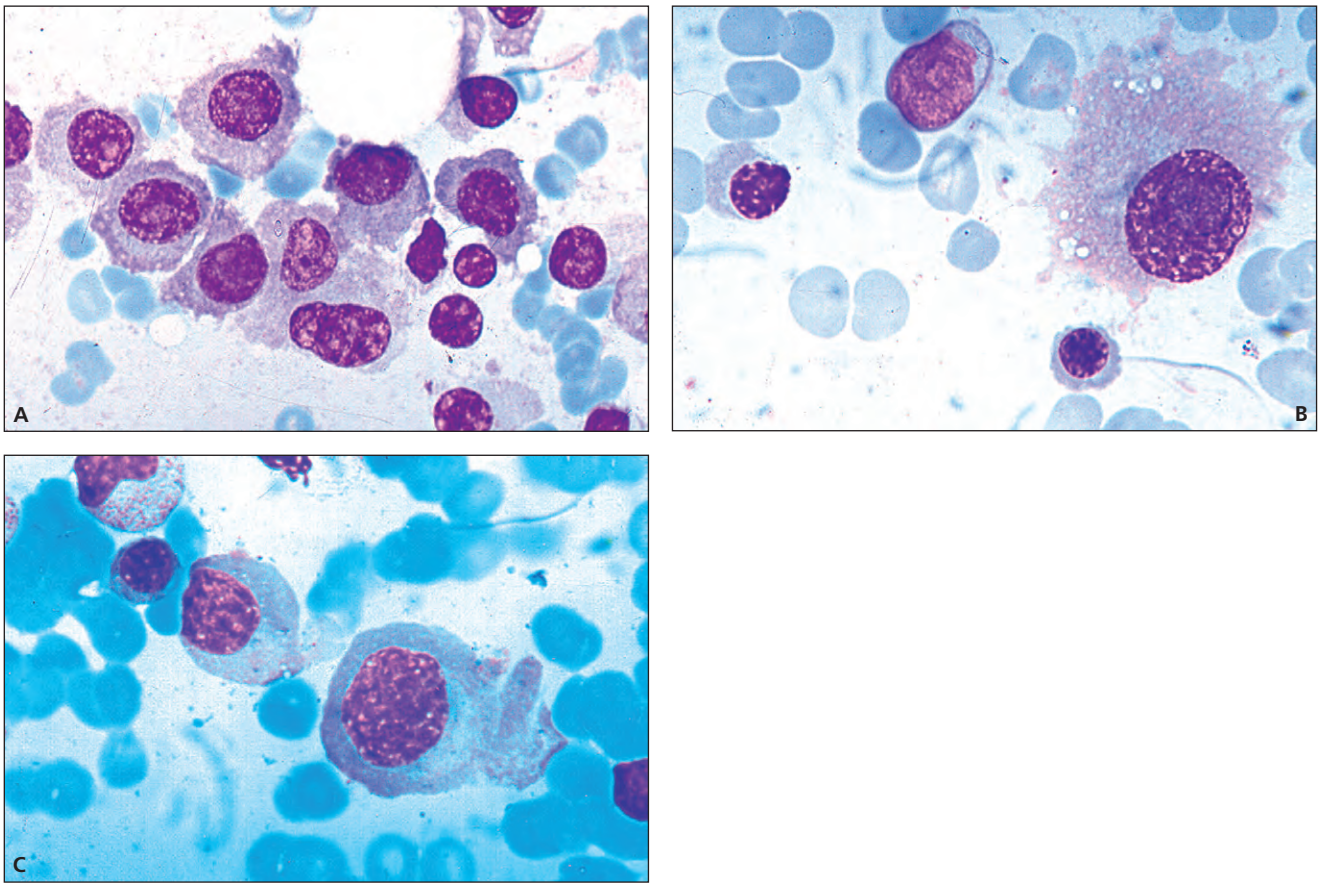



**Fig. 10.31** Multiple myeloma: plasma cells with irregular ragged cytoplasm; bone marrow aspirate. (A) Infiltration by mature, intermediate and immature plasma cells with basophilic cytoplasm and nuclei characterised by different degrees of chromatin condensation. The cytoplasm of some cells is extending into villous projections that resemble those of hairy cells. (B) Another case of IgG myeloma; bone marrow aspirate. Large immature plasma cell with a nucleus that has partly condensed chromatin and a gigantic nucleolus. The cytoplasm is greyish, granular and vacuolated, and has a very ragged periphery. The small cell in the centre at the top is a lymphoblast; the other two cells are polychromatic erythroblasts. (C) From a patient with IgA myeloma; bone marrow aspirate. The large plasma cell in the centre has a large nucleus with an irregular outline and chromatin condensed into heterogeneous clumps; on the right, large ribbons of basophilic cytoplasm that are tending to become detached.

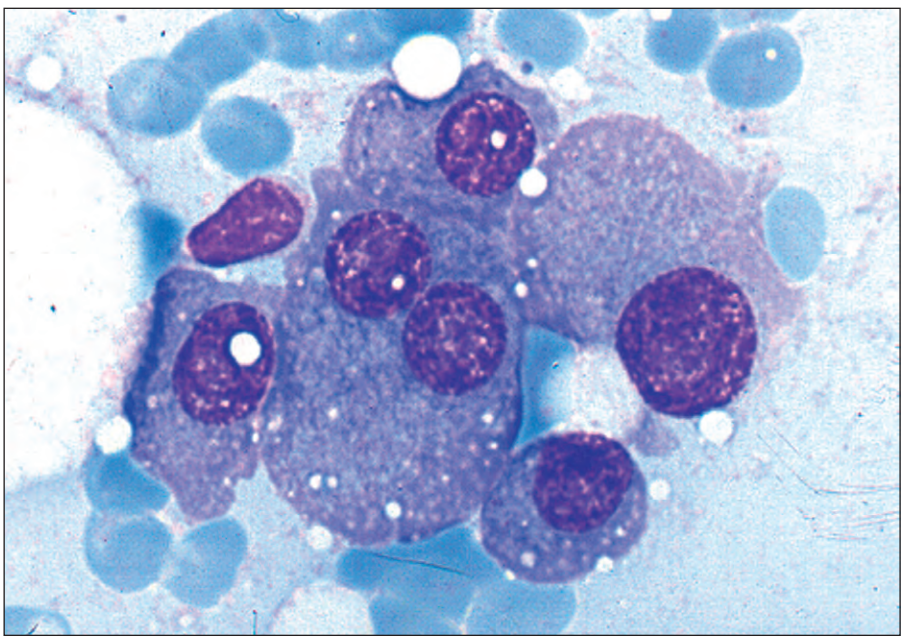


**Fig. 10.32** IgG myeloma; bone marrow aspirate. The plasma cells have plentiful basophilic cytoplasm and are crowding together without clearly distinguishable cell margins, creating a pseudosyncytial appearance.

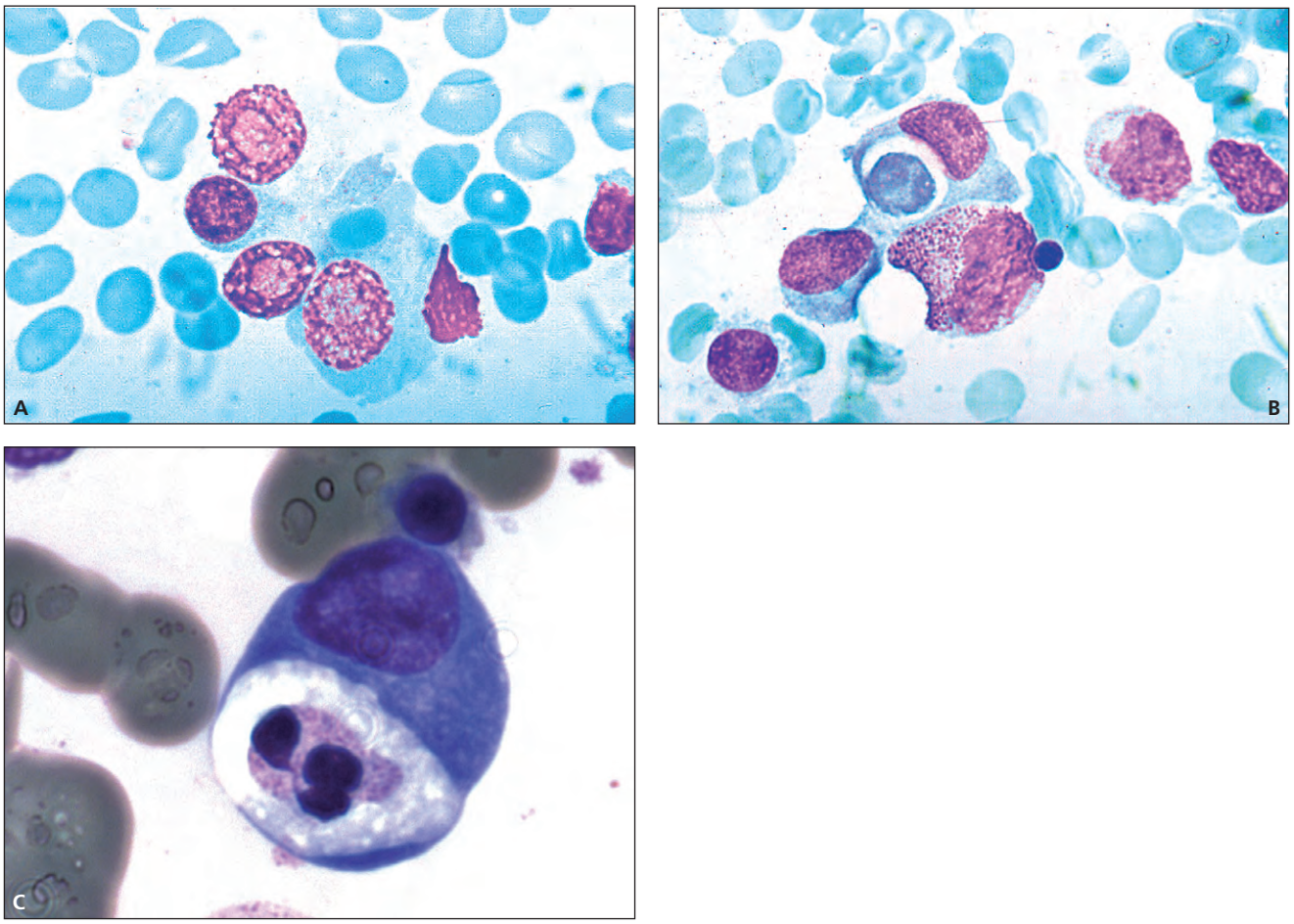



**Fig. 10.33** Haemophagocytosis by myelomatous plasma cells. (A) Erythrophagocytosis in IgG myeloma. A plasma cell with a disintegrating nucleus contains a well-preserved red cell within its plentiful weakly basophilic cytoplasm. Dutcher bodies are visible in the two adjacent nuclei. (B) IgG myeloma; bone marrow aspirate. A cytologically typical plasma cell has a large cytoplasmic vacuole which flattens and deforms the nucleus; it contains a large homogeneous inclusion, which gives it a similar appearance to an LE (lupus erythematosus) cell. (C) A plasma cell with an enormous cytoplasmic vacuole containing a neutrophil granulocyte with pyknotic nuclear lobes.

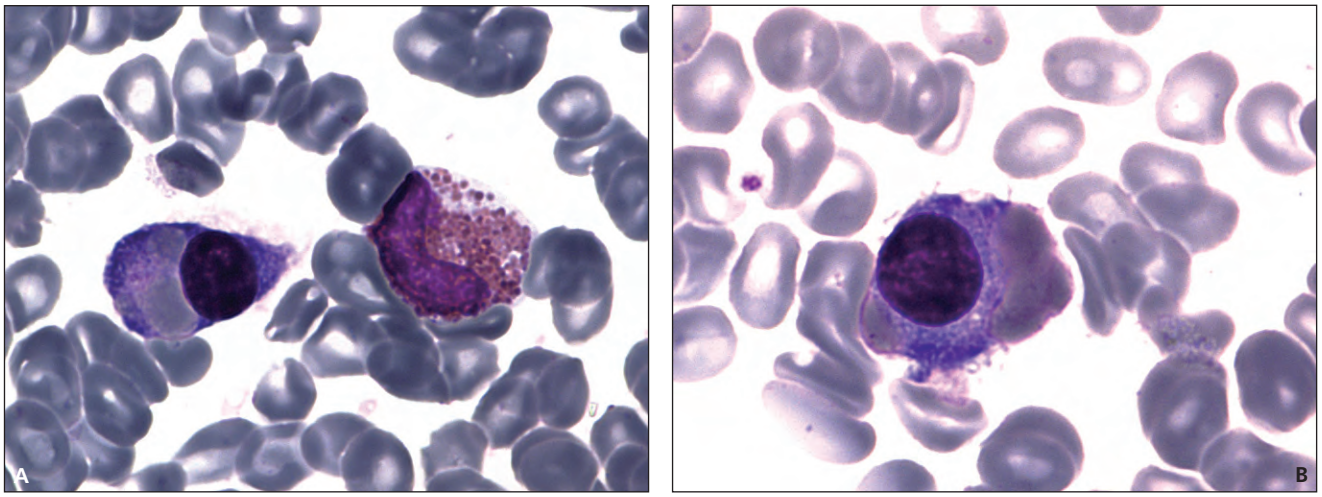



**Fig. 10.34** Erythrophagocytosis in a patient with IgG myeloma; bone marrow aspirate. (A) On the left, a mature pear-shaped plasma cell containing a well-preserved red cell within its basophilic cytoplasm. On the right, a slightly dysmorphic eosinophil metamyelocyte with some dark immature granules and some areas of hypogranular cytoplasm. (B) Mature plasma cell with numerous phagocytosed red cells, both to the right and to the left of the nucleus.

antigens (CD13, CD33, CD15, CD11b and CD11c), often in association with CD10. Using monoclonal antibodies to study a CD38-positive population of plasma cells, it is possible to distinguish polyclonal reactive cells (CD19 positive and CD56 negative) from monoclonal or neoplastic cells (mainly CD19 negative and CD56 positive). Immunophenotypic analysis using paired markers has permitted the identification of immature, intermediate and mature fractions of a plasma cell population.[54]

One of the most useful laboratory investigations from the prognostic point of view is the determination of the proportion of myeloma cells in S phase, which can be performed either by flow cytometry or by using a simple technique on a glass slide, based on the incorporation of bromodeoxyuridine into cells in mitosis that have been labelled simultaneously with a fluorescent antibody[55]: the presence of more than 1% of such cells is associated with a very grave prognosis.[56]

**Table 10.5** Morphological classification of multiple myeloma[40]

**Mature myeloma**
- ≥10% mature plasma cells
- <2% plasmablasts
- <12% immature plasma cells

**Intermediate myeloma**
- All the types that do not fall into one of the other categories

**Immature myeloma**
- ≥12% immature plasma cells
- <2% plasmablasts
- <10% mature plasma cells

**Plasmablastic myeloma**
- ≥2% plasmablasts

## Genetic analysis

Myeloma cells have low proliferative activity. As a result, at the time of diagnosis cytogenetic abnormalities are demonstrable in only a third of patients. The frequency of detection increases with repeated analysis and with the evolution of the illness, which appears to be associated with a very unstable genome.[57,58] The most frequent anomalies are:

- Numerical alterations (hyperdiploid or hypodiploid karyotype);
- Recurrent chromosomal translocations involving the 14q32 locus of the immunoglobulin heavy chain gene, the most frequent of which is t(11;14)(q13;q32), found in 20% of all cases of myeloma[36]; there is dysregulation of specific genes, such as the dysregulation of *CCND1*, encoding cyclin D1, by t(11;14);
- The deletions 13q-, 17p- and 1p-.[59,60]

The presence and type of cytogenetic abnormalities provide important prognostic information, determining the behaviour of the neoplastic cells.[61] Hyperdiploidy is associated with longer survival.

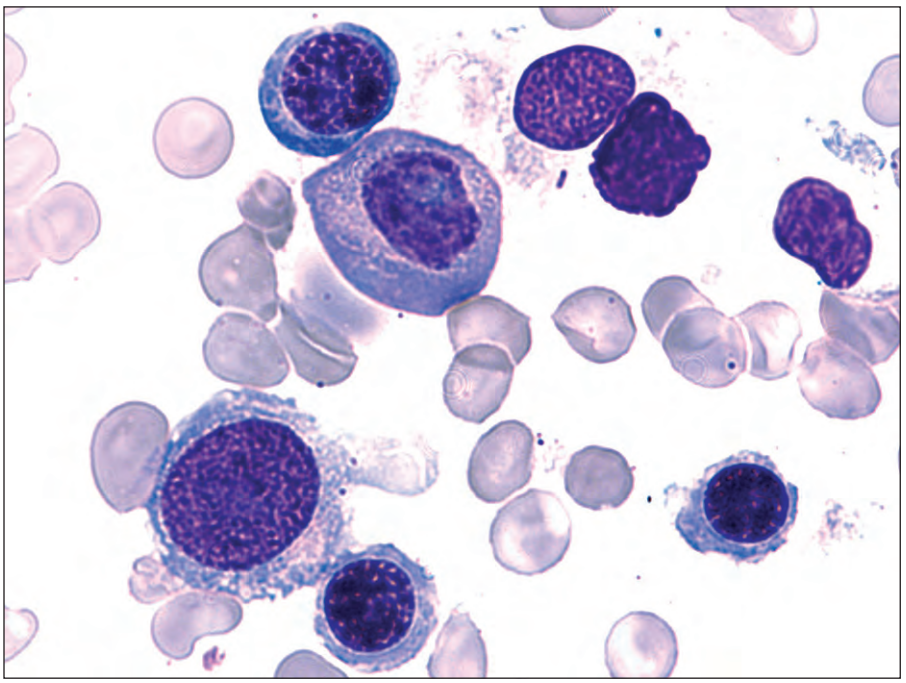
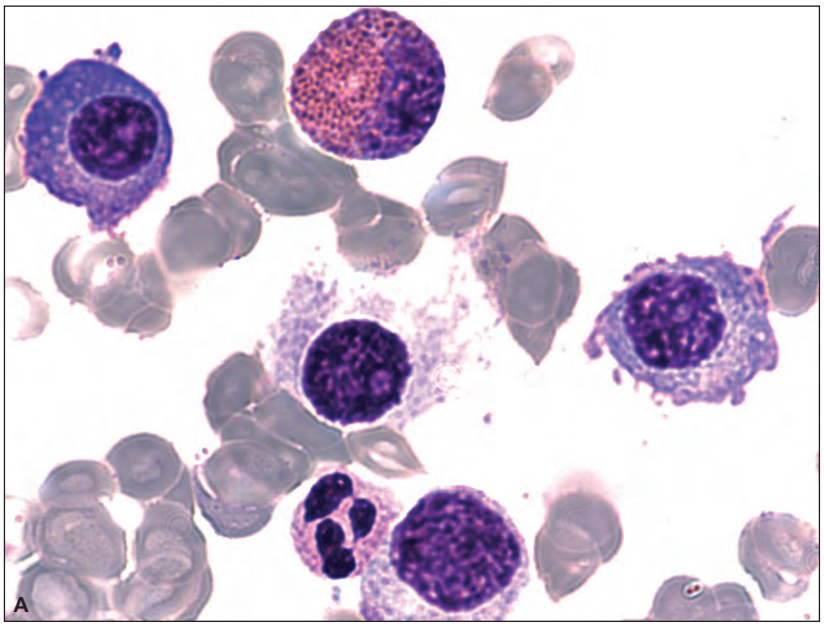
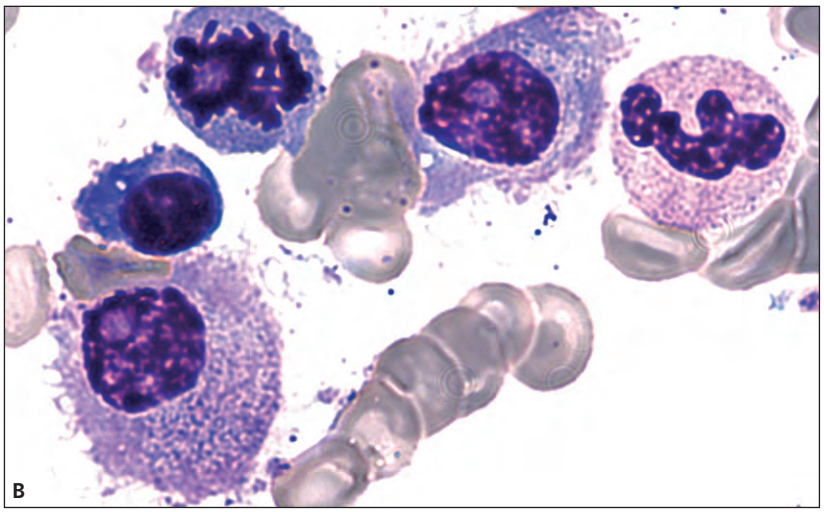


**Fig. 10.35** Plasmablastic myeloma; bone marrow aspirate. Bottom left there is a cell with a large nucleus and a high N:C ratio, that meets the criteria for a plasmablast: the chromatin is reticular with only minimal areas of condensation; the nucleus is large with a visible nucleolus and the cytoplasm is scanty with a poorly developed Golgi zone. If the percentage of these cells is 2% or more, the myeloma is classified as plasmablastic. Above and to its right, an atypical plasma cell with an irregular nuclear outline and some pink, finely vacuolated areas of cytoplasm. At the bottom and to the right, two polychromatic erythroblasts and, given its extensive perinuclear arcoplasm, the cell at the top may also be an erythroblast.



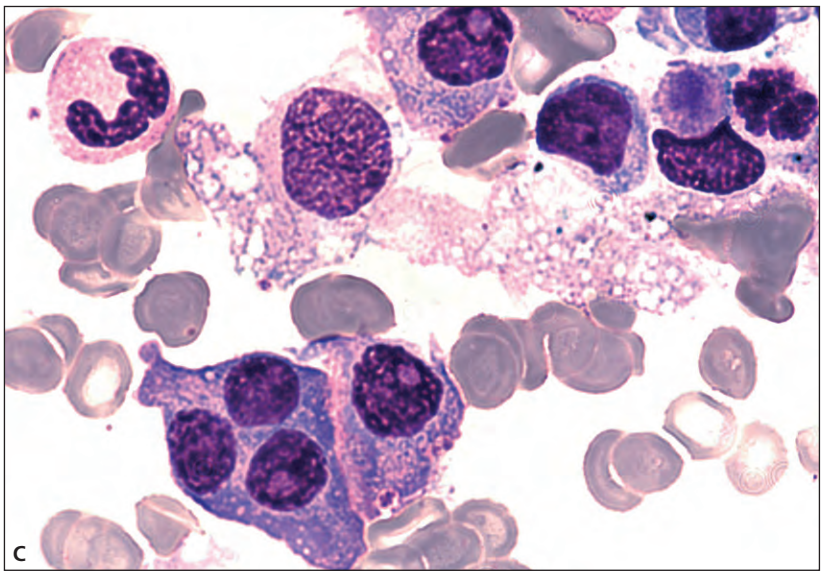




**Fig. 10.36** Cytological heterogeneity in plasmablastic myeloma; same case as Fig. 10.35. (A) In addition to the plasmablasts, there is a population of plasma cells that are often atypical and heterogeneous. Here one observes, in addition to a neutrophil and an eosinophil metamyelocyte, a mature plasma cell, top left, with small acidophilic areas in the cytoplasm and three dysmorphic plasma cells, one of which has damaged cytoplasm. (B) From the bottom left upwards: a plasma cell with granular violaceous cytoplasm and an eccentric nucleus with clumped chromatin and a small Dutcher body; a small mature plasma cell; a mitotic figure; another dysmorphic plasma cell; a band neutrophil. (C) Bottom left, a trinucleated plasma cell with plentiful vacuolated cytoplasm. Towards the top, a macrophage with a large nucleus and spongy chromatin and a vast amount of distended heavily vacuolated cytoplasm.

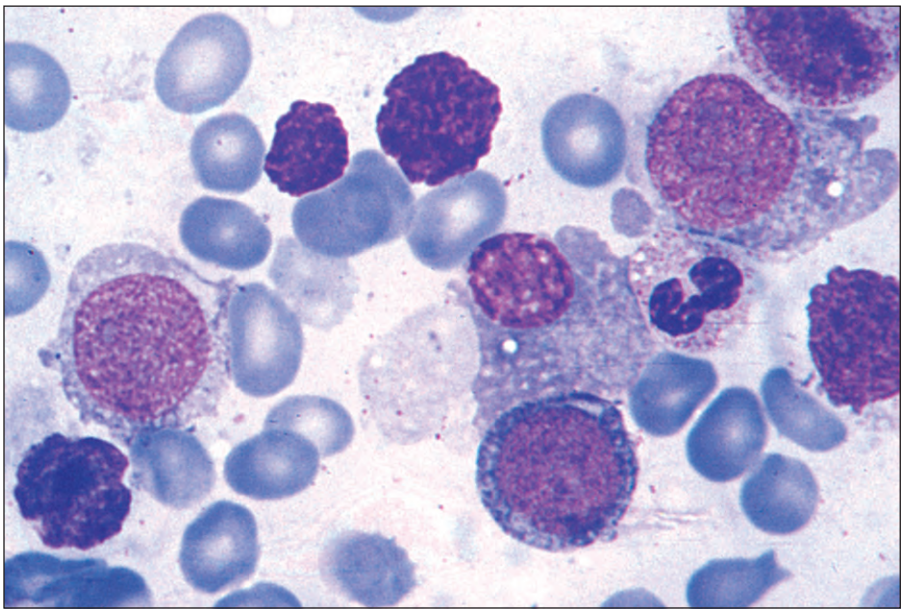


**Fig. 10.37** Plasmablastic myeloma; bone marrow aspirate. In the centre, between a band neutrophil and a proerythroblast with basophilic vacuolated cytoplasm, an almost mature plasma cell with plentiful weakly basophilic cytoplasm and an eccentric nucleus with chromatin condensed into blocks separated by broad pale spaces; it is deformed by the pressure of surrounding cells. On the left and top right, two typical plasmablasts: they have large nuclei with a very high N:C ratio and fine, uniformly dispersed chromatin. There are a number of small stamped out, pale blue nucleoli, which stand out from the violet of the chromatin network. The shape of the nucleus, regular and rounded with a clear outline, is different from that of myeloid blasts. The morphology of these cells could evoke that of monoblasts: the presence of numerous morphologically more typical plasma cells is the key element in the cytological differential diagnosis, which must always take account of the context.

The FISH technique permits the analysis of cells in interphase, so results are not influenced by the infrequency of cells in metaphase. There are now numerous probes available that can identify the aberrations that characterise myeloma, which provide very valuable information on the likely response of individual patients to different forms of treatment[10]: the FISH techniques that are most used are those for the detection of del(17)(p13), t(11;14), t(4;14) and t(14;16).

Finally, investigation of gene expression profile by means of microarray analysis for the detection of amplification and deletion is now being assessed in an attempt to delineate a new molecular classification of myeloma based on biological determinants of the variable clinical progression.[58,62,63]

## Particular forms of myeloma

Myeloma is a heterogeneous disorder from both biological and clinical points of view. It thus presents in a great variety of forms which are being increasingly associated with specific cytological and biological characteristics.[36]

Solitary plasmacytoma of bone and extraosseus solitary plasmacytoma are rare, constituting less than 5% of plasma cell neoplasms. In this form, an M component is not always observed and a radiologically detectable bone lesion may be the only evidence of a clonal plasma cell proliferation. Evolution to multiple myeloma occurs in more than 15% of cases. It is also possible for multiple extramedullary plasmacytomas to develop during the course of progressive disease, as when there is resistance to treatment.

In non-secretory myeloma (1–3% of cases), the M component is lacking and bone marrow plasma cells are above 10%, in the presence of organ damage or insufficiency. There are two types, one in which the plasma cells have lost their capacity to synthesise immunoglobulin or its component parts, the other in which the plasma cells are not capable of transporting the M component out of the cell: in the latter case, the monoclonal immunoglobulin, although absent from the serum, can be recognised in the cytoplasm of the plasma cells by immunohistochemistry. An increase in free light chains with an abnormal kappa : lambda ratio is detectable in the serum in two thirds of patients. The prognosis of non-secretory myeloma seems to be worse than that of classical multiple myeloma, although the data available are controversial because of the rarity of this form of the disease.[64]

Cases of multiple myeloma with osteosclerosis have been reported in which it is hypothesised that there is secretion of an osteoblast-stimulating factor.[65] These rare forms differ in this regard from classical myeloma in which there are osteolytic lesions and osteoporosis and in which some degree of

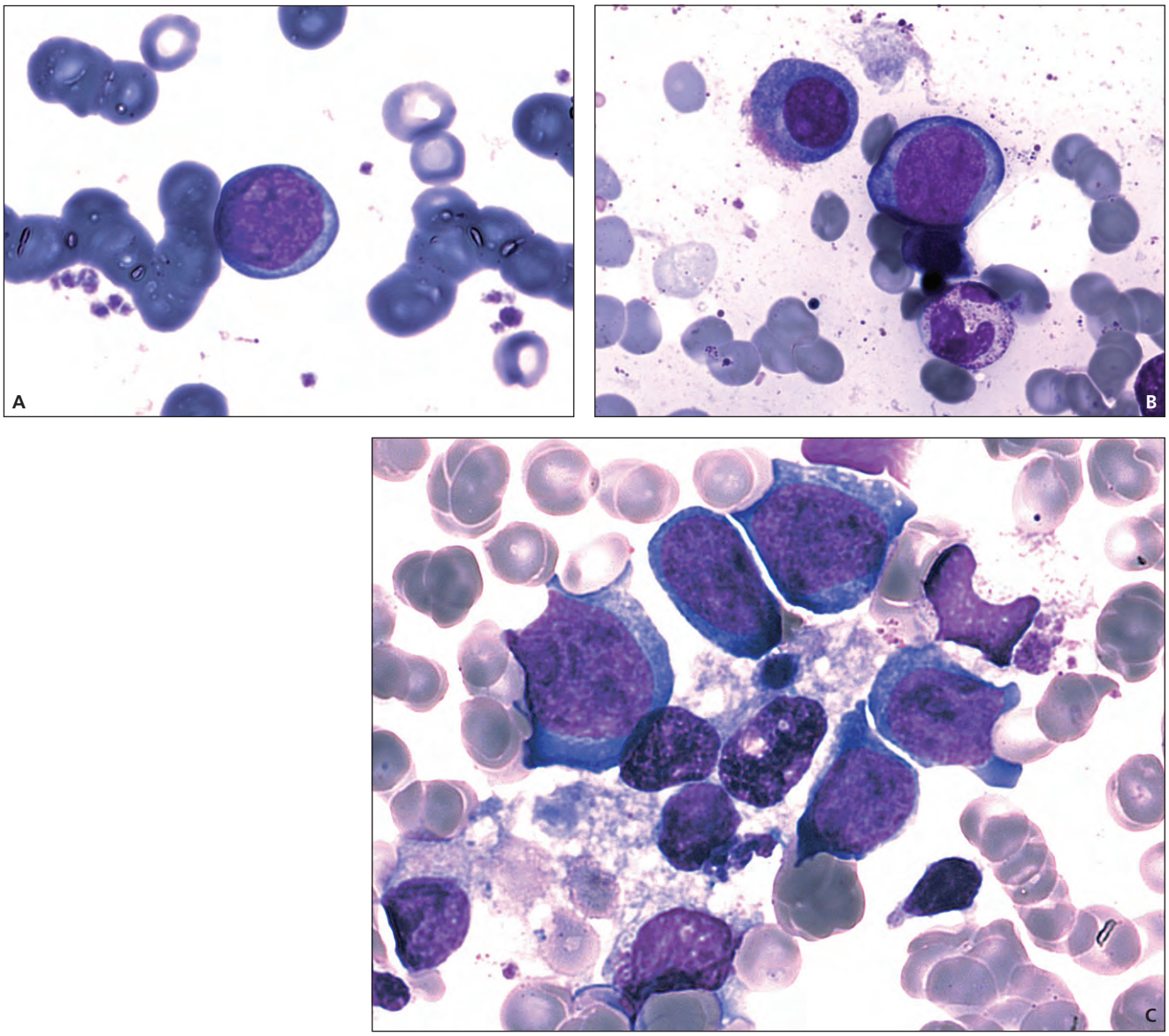



**Fig. 10.38** Plasmablastic myeloma; bone marrow aspirate. (A) This plasmablast does not have cytological characteristics that would permit its being assigned to the plasma cell lineage. The eccentricity of the nucleus, the nucleoli and the basophilia of the scanty cytoplasm could be observed in blast cells of other lineages. One notes, however, the rouleaux formation. (B) A large plasmablast with fine reticular chromatin and visible nucleoli, adjacent to an immature plasma cell. (C) Bone marrow aspirate of the same patient, taken after autologous haemopoietic stem cell transplantation. The cytological picture at relapse is characterised by striking immaturity of the plasma cell population, which shows mainly the cytological features of plasmablasts. In the centre, a macrophage with cytoplasm containing large residues of cells.

osteosclerosis is observed only occasionally in osteolytic areas after a fracture or during the course of treatment. Osteosclerotic myeloma constitutes around 1% of cases and is usually observed in the context of the POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes).[66] This complex array of defining clinical features is attributable to the production of cytokines by the myeloma cells. In 30–40% of these cases, there is polycythaemia or thrombocytosis.

A rare form of myeloma has been described with myelofibrosis, characterised by megakaryocytic hyperplasia with clustering, co-existing with a plasma cell infiltrate.[67]

Rarely, myeloma is associated with histiocytosis; there is reactive hyperplasia of histiocytes containing prominent cytoplasmic inclusions that are immunoglobulin crystals.[68]

The term 'anaplastic myeloma' refers to an acute, rapidly progressive condition in which myeloma cells are dedifferentiated

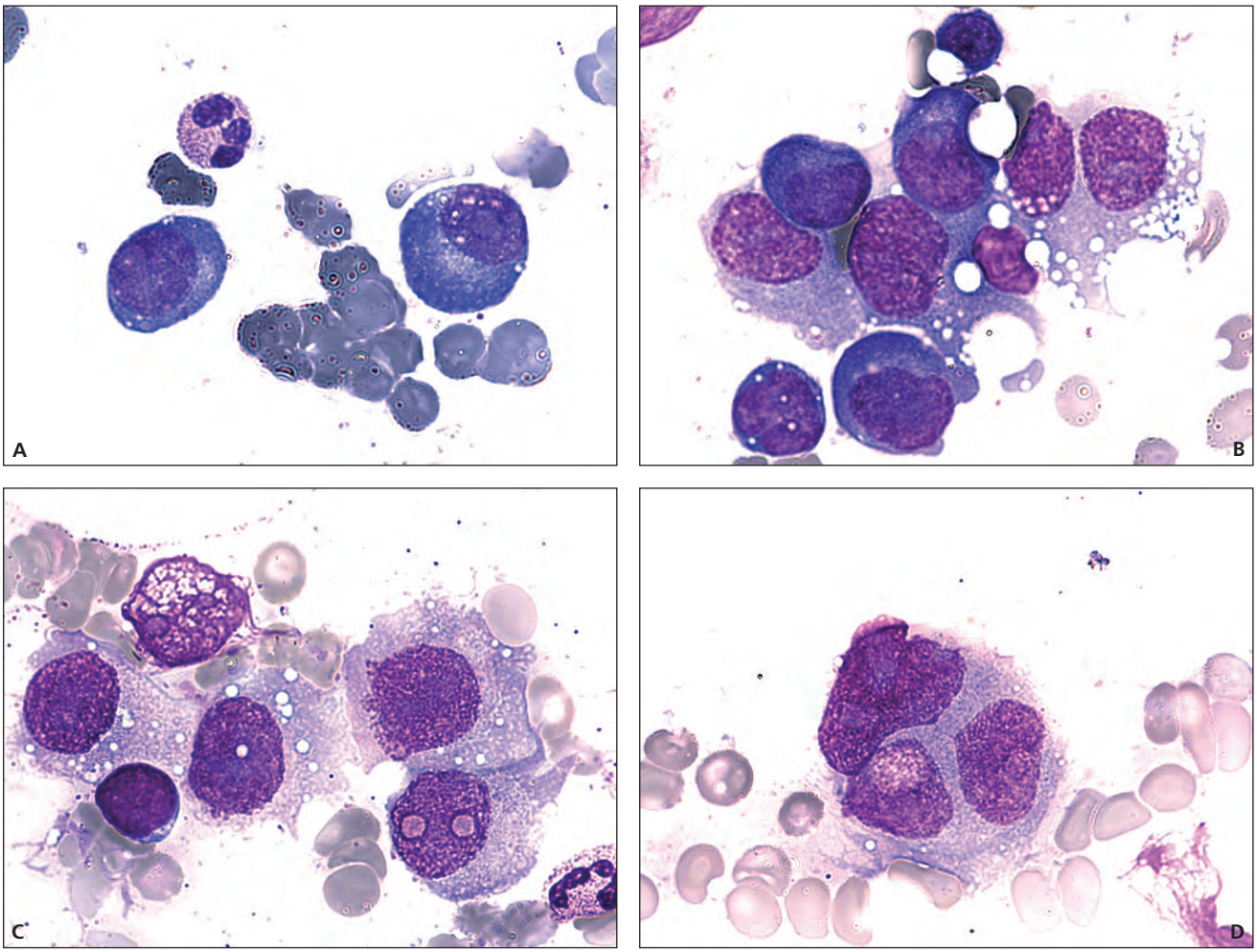



**Fig. 10.39** Anaplastic myeloma, developing during the evolution of treatment-refractory IgG myeloma. Bone marrow cellularity is reduced and the cells present are almost all highly atypical and immature plasma cells. (A) These two large plasma cells can both be classified as immature, even if the cytoplasmic features of plasmacytoid differentiation are evident in the cell on the right. (B) Small group of plasmablasts and dysmorphic plasma cells, which at some points appear as if fused. The nuclei of the more basophilic cells have lost their usual regular outlines and appear indented and convoluted. The chromatin is globally immature and dispersed. (C) Plasmablasts showing a high degree of anaplasia and immaturity. The nuclear outlines appear to be disintegrating and the chromatin is homogeneous and finely granular. The nucleus of the cell at bottom right contains two symmetrical Dutcher bodies. The cytoplasm shows variegated basophilia with a pink tinge and contains a number of small Russell bodies, one of which, in the cell in the centre, is superimposed on the nucleus. (D) Multinucleated plasmablast showing marked dysmorphism and immature chromatin, which in the lower nucleus is largely replaced by a large Dutcher body.

to plasmablasts or immunoblasts in the course of multiple myeloma; it has been reported to have a prevalence of 2–3% in large series of patients. This is very similar to Richter syndrome occurring in lymphoproliferative disorders. The bone marrow is rapidly invaded by a population of atypical immunoblasts or plasmablasts, which are also able to rapidly colonise extramedullary sites (Figs 10.39, 10.40 and 10.41). The morphological transformation can be accompanied by a phenotypic transformation with loss of the ability to secrete the M component: the disease course is rapidly fatal. There have also been rare cases reported with *de novo* presentation (Fig. 10.42), in the absence of a previous diagnosis of multiple myeloma.[69]

## Plasma cell leukaemia

The diagnosis of plasma cell leukaemia requires the demonstration of a significant number of myeloma cells in the peripheral blood. By convention, the absolute value must be more than $20 \times 10^9$/l or the percentage at least 20% of circulating cells.[70–72] Some authors, however, retain that this definition is arbitrary and that the diagnosis would be better based on clinical and haematological features rather than on the number of circulating plasma cells.[73]

Plasma cell leukaemia can be a late complication of myeloma, associated with a rapidly progressive clinical course, little

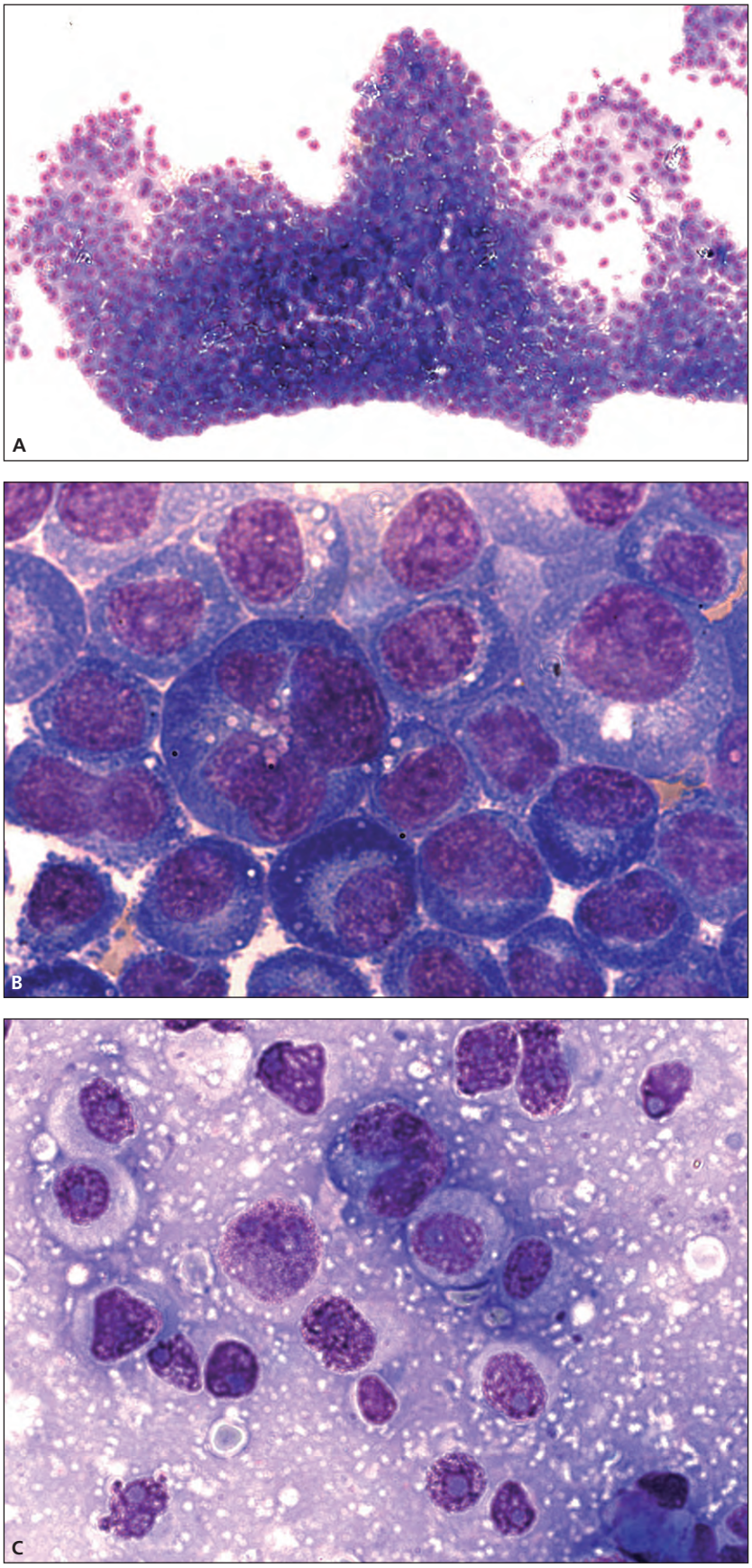



**Fig. 10.40** Extramedullary localisation of anaplastic myeloma; same patient as Fig. 10.39. (A) Pleural effusion. There is a uniform carpet of cells with large nuclei and basophilic cytoplasm. (B) At higher power, the plasma cells in this location retain features of differentiation, which is more evident than in the bone marrow. The large cell with a multilobated nucleus has intensely basophilic cytoplasm and heterogeneous chromatin with areas of condensation. (C) Material from a nodular cutaneous lesion. There are numerous dysmorphic plasma cells with visible nucleoli.

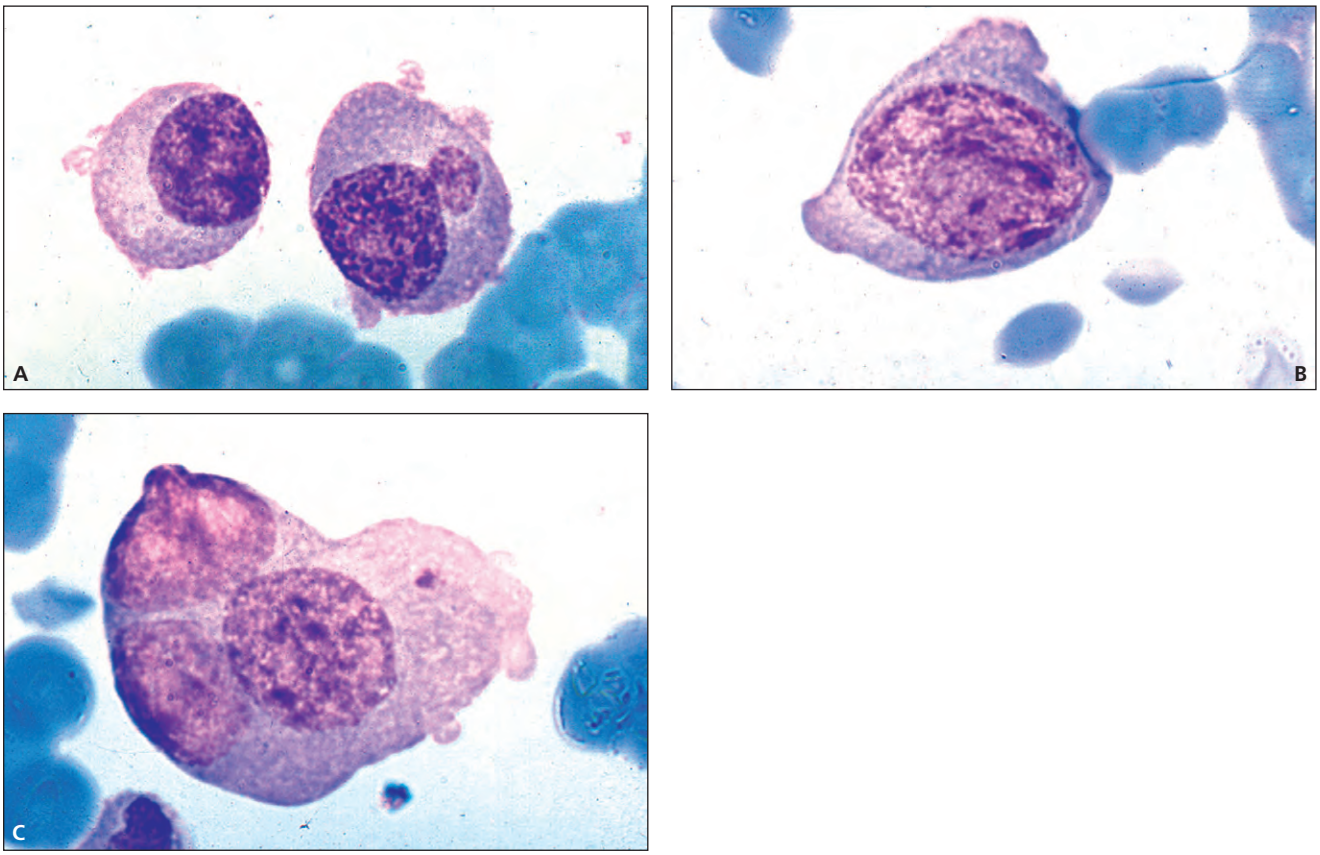



**Fig. 10.41** Anaplastic myeloma; bone marrow aspirate. (A) Two immature plasma cells with large eccentric nuclei and irregularly condensed chromatin; the cell on the right shows anomalous nuclear budding. The cytoplasm is violaceous and flaming; to the left, a semilunar Golgi zone; there are small rounded projections from the surface of both of the cells. (B) Giant plasmablast, from the same patient. The nucleus is very large, central and heterogeneous with alternating areas of dispersed and condensed chromatin. A central nucleolus is barely perceptible. There is only a moderate amount of cytoplasm, which has a violaceous tint, more intense at the periphery. The cytoplasm is tending to form large pseudopodia. (C) Giant trinucleated plasmablast. The chromatin is dispersed in the two eccentric nuclei and partially condensed in the nucleus in the centre. The upper nucleus has a bulge at the top that deforms the outline of the cell. On the right, where the cytoplasm is more abundant, there is a large inclusion of uncertain significance (likely to be nuclear material or precipitated immunoglobulin). Anaplastic transformation correlated in this patient with relapse after eight months of chemotherapy.

response to therapy and a median survival of about two months. More rarely, it presents *de novo* (see Report 10.1), representing 1–3% of all cases of myeloma, with the clinicopathological picture of acute leukaemia and with a very grave prognosis: median survival is less than 10 months from diagnosis.[71] Plasma cell leukaemia is manifest clinically with hepatosplenomegaly and lymphadenopathy, anaemia and thrombocytopenia that are often severe, a leucocyte count generally between 10 and $50 \times 10^9$/l, haemorrhagic manifestations and renal insufficiency. In comparison with multiple myeloma, osteolytic lesions are less frequent and less severe, while extraskeletal lesions are much more common. Less than a half of patients present with a serum calcium above 10 mg/dl (>2.5 mmol/l). The paraprotein concentration is often very high. The relative distribution of immunoglobulin classes is similar to that of multiple myeloma, except that there is a particular increase in cases with Bence-Jones protein as the only paraprotein and of IgD and IgE subtypes: for unknown reasons, more than 25% of cases of the rare IgE myeloma evolve into plasma cell leukaemia. The association with a monoclonal gammopathy is lacking in the non-secretory form, which is not rare, and the differential diagnosis with acute leukaemia can then be morphologically difficult.

Immunophenotyping is always important for the diagnosis[74]: the leukaemic cells are characterised by the expression of CD38 and CD138, by the presence of cytoplasmic monoclonal immunoglobulin, by the lack of aberrant expression of CD56 and by the lack of expression of B-lineage antigens (HLA-DR, CD19 and SmIg), with the exception of CD20, which is more frequent in plasma cell leukaemia than in multiple myeloma.[72] Genetic alterations are frequent, with a prevalence of prognostically unfavourable genotypes.[73]

## Morphology

The circulating plasma cells do not differ from those that can be seen in multiple myeloma: from well-differentiated cells to

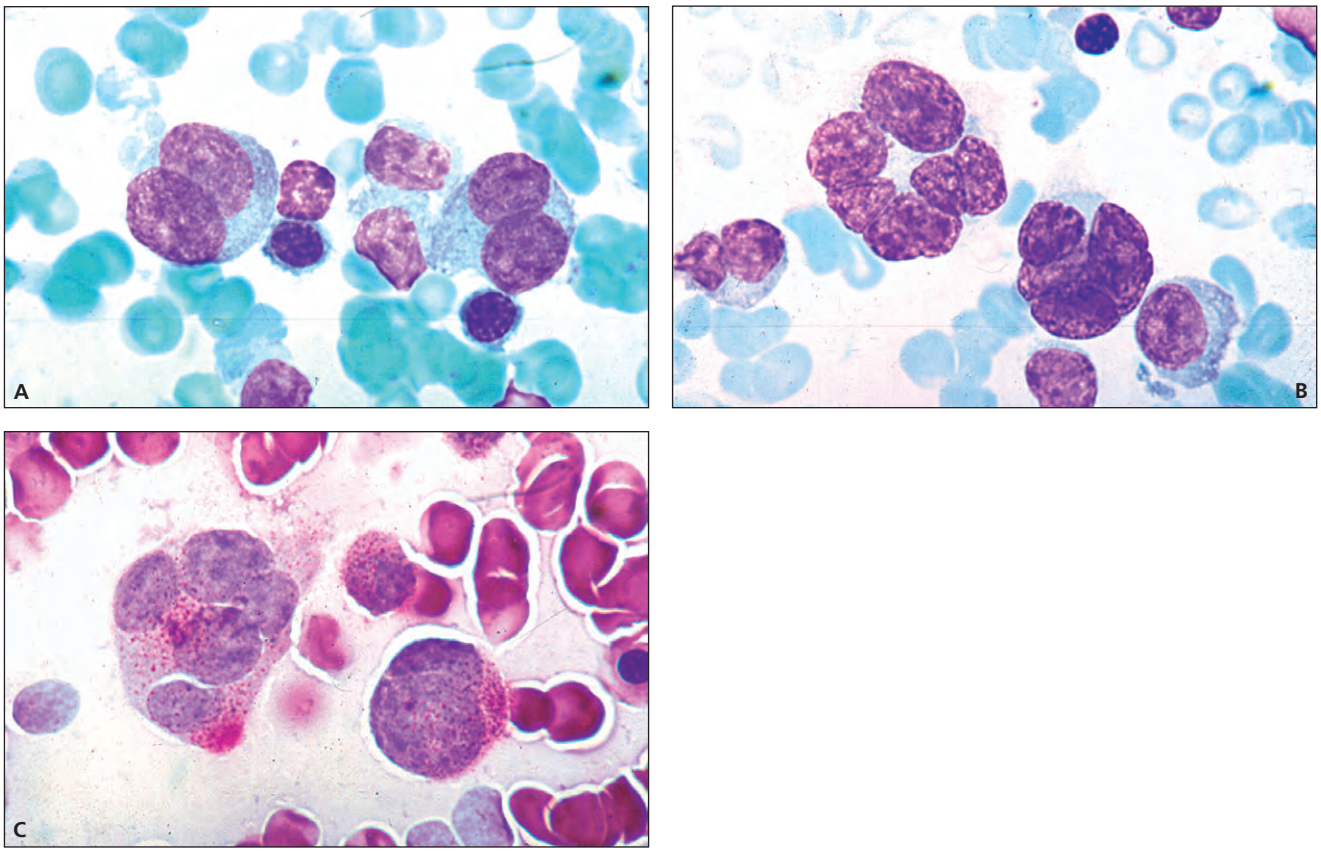



**Fig. 10.42** A woman aged 64 years who presented with marked pancytopenia. There was no monoclonal component in either the serum or the urine. (A) The bone marrow aspirate showed heavy infiltration by highly anaplastic, mainly multinucleated cells, two of which are seen in this field. Their morphology suggests a possible plasma cell nature. The nuclei are very large with partially condensed chromatin, in which there are paler areas corresponding to indistinct nucleoli. The nuclei are superimposed on each other, with the underlying nucleus being partly perceptible. The cytoplasm is moderately basophilic and more plentiful on one side of the cell. There are several lymphocytes and two polychromatic erythroblasts. (B) Two highly atypical multinucleated cells from the same marrow aspirate. In these cells the numerous nuclei are in part superimposed and in part lined up beside each other; the chromatin is condensed into irregular heterogeneous clumps; nucleoli are not seen. The cytoplasm is very scanty and very pale, almost invisible. On the left, a smaller binucleated cell of the same type, while on the right, at the bottom, a plasma cell of intermediate maturity. (C) A stain for acid phosphatase shows intense granular positivity both in the giant multinucleated cells and in the small cells with typical plasma cell cytology, one of which is visible in the centre, just above the two giant cells. Immunophenotyping was performed to confirm the plasma cell nature of these anaplastic cells and was positive for CD38, CD11c, CD56 and cytoplasmic mu chain.

very undifferentiated blast cells identifiable only by immunophenotyping (Figs 10.43 through to 10.47; see also Fig. 10.49). Immature variants are relatively more common with an excess of plasmablasts.

The bone marrow aspirate also shows the full range of morphological variants that characterise the non-leukaemic form of multiple myeloma (Figs 10.45 and 10.46). The infiltration is always massive, more than 90% of bone marrow cells. Two morphological variants have been identified.[1,9,75] The first is characterised by the presence in the circulation of small cells, either similar to lymphocytes, lymphoplasmacytoid cells with intensely basophilic cytoplasm, or typical plasma cells with basophilic cytoplasm, a large pale Golgi zone, an eccentric nucleus and a low N:C ratio. The other form is characterised by the presence of blast cells, often morphologically indistinguishable from those seen in other forms of leukaemia, medium sized to large with a high N:C ratio, delicate reticular chromatin, a prominent nucleolus and little evidence of a Golgi zone. Ultrastructural morphology can be useful for the diagnosis of difficult cases, showing the characteristic profiles of endoplasmic reticulum arranged as parallel tubes.

It is not rare for plasma cell invasion to be recognised also at extramedullary, extraosseus sites, this being particularly frequent in plasma cell leukaemia (Figs 10.48 and 10.49).

## Reactive plasmacytosis

An increase in the proportion of bone marrow plasma cells to 5–20% can be the result of a polyclonal reactive plasmacytosis, which is observed in various different conditions – haematological, infective, neoplastic or autoimmune (Table 10.6). Sometimes

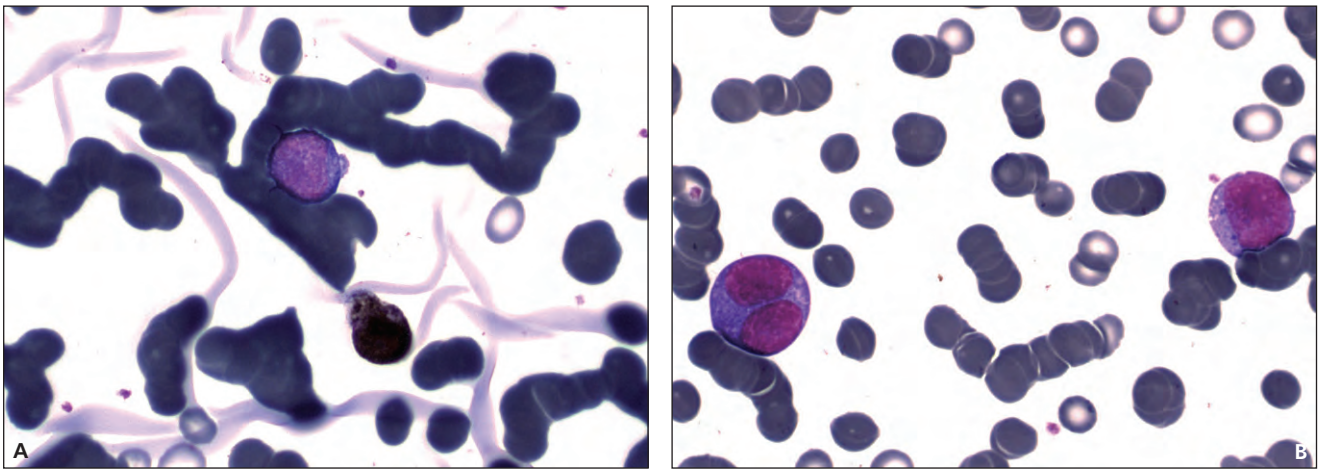



**Fig. 10.43** Plasma cell leukaemia with predominance of undifferentiated plasmablasts; peripheral blood film. (A) Small, apparently undifferentiated blast cell, with a large eccentric nucleus and dispersed chromatin; the cytoplasm is scanty and intensely basophilic with a single protrusion. The rouleaux formation and the presence of numerous comma-shaped proteinaceous deposits suggest the co-existence of a marked abnormality of serum proteins, which in turn suggests a possible plasma cell disorder. (B) Another two blast cells, one undifferentiated and the other with two nuclei and cytological features raising the possibility of a plasma cell nature, requiring immunophenotyping for confirmation.

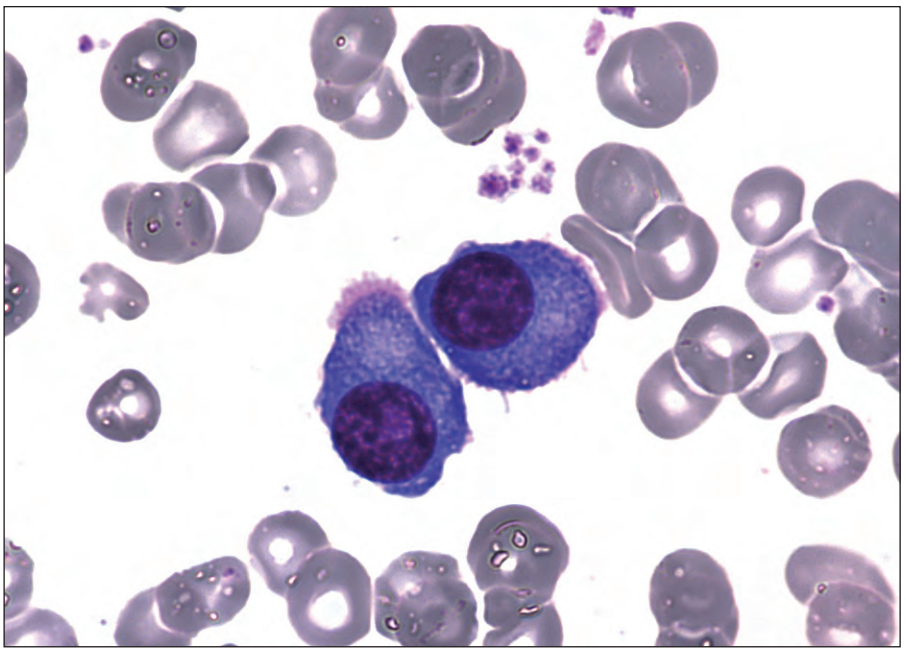


**Fig. 10.44** Plasma cell leukaemia with immature and intermediate plasma cells in the circulation; peripheral blood film. The cytological features of these two cells clearly suggest their origin, particularly the pear shape, the eccentric nuclei with coarse chromatin clumps and the abundant intensely basophilic cytoplasm in which the pale Golgi zone stands out; the Golgi zone is slightly separated from the nuclear membrane. In the cell on the left, there is also a large pale nucleolus and a ragged pink fringe at the top, at the opposite pole to the nucleus. These features can be seen with more difficulty in the cell on the right.

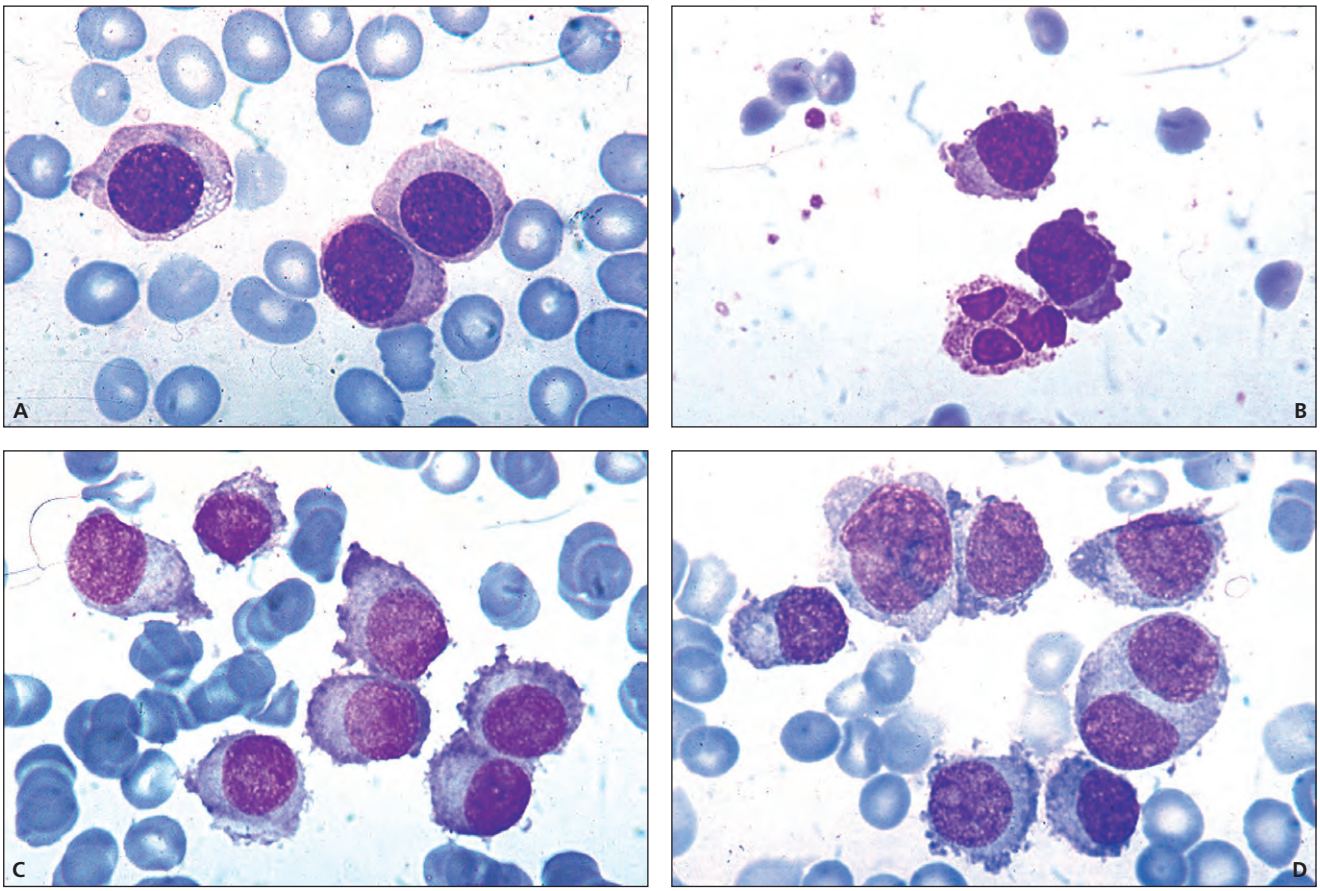



**Fig. 10.45** Plasma cell leukaemia with massive invasion of the peripheral blood at presentation (white cell count 27 × $10^9$/l). (A) Peripheral blood film. Ninety per cent of the circulating cells were blast cells with moderately plentiful greyish cytoplasm and round regular nuclei with homogeneously condensed chromatin and small nucleoli. The plasmablastic nature of these cells was confirmed by immunophenotyping (CD38 positive). (B) The patient in (A) was treated with aggressive chemotherapy, leading to a partial remission with disappearance of the plasmablasts from the peripheral blood. Relapse, which occurred after a few months of treatment, was signalled by the reappearance in the peripheral blood film of atypical plasma cells with scanty, intensely violet cytoplasm, which had numerous protrusions. The chromatin was more condensed than in the blast cells at disease onset. (C) Same case as (A) and (B) with plasma cell leukaemia now in overt relapse; peripheral blood film. The white cell count had increased to 136 × $10^9$/l. The leukaemic cells are immature plasma cells with an eccentric nucleus and dispersed chromatin, indistinct nucleoli and intensely basophilic ragged cytoplasm with a large pale Golgi zone. (D) Same case as in (A–C); bone marrow aspirate at relapse. In comparison with those in the peripheral blood, the myeloma cells in the marrow are more atypical and immature. The largest cell at the top is a typical plasmablast, while the others can be classified as immature plasma cells or, because of the retention of nuclear eccentricity, intermediate plasma cells.

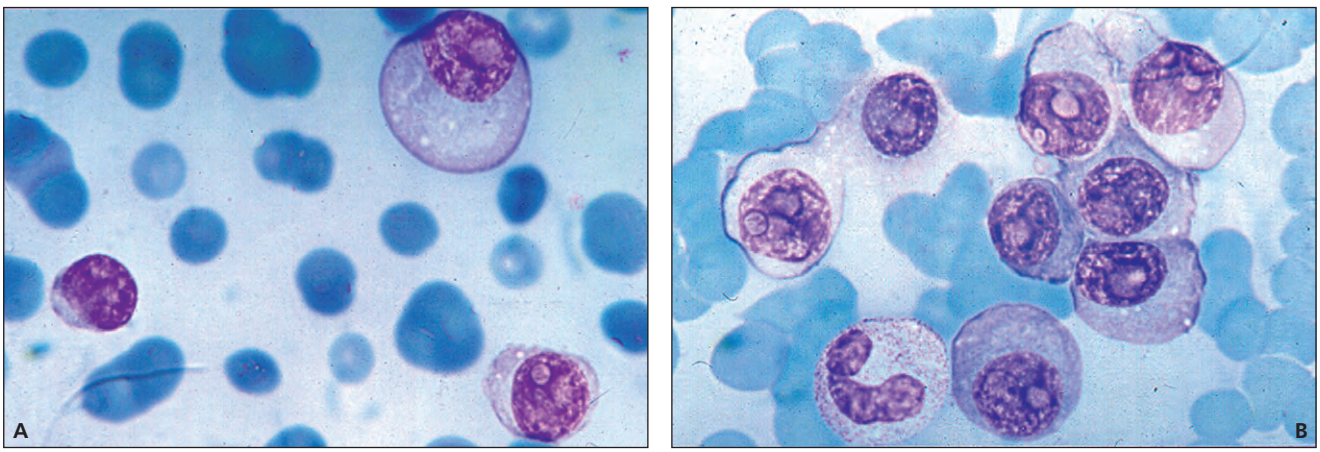



**Fig. 10.46** Plasma cell leukaemia at presentation. (A) Peripheral blood film. On the left, a lymphocyte; on the right, two plasma cells characterised by the presence of large nucleoli. The cell at the top has plentiful cytoplasm with basophilic areas alternating with pale areas; the clear outlining of the cell is the result of the proteinaceous deposit that forms the background of the film. The plasma cell at the bottom differs, having pale, very scanty cytoplasm. The red cells are forming small rouleaux. (B) Bone marrow aspirate of the same patient. Massive infiltration by atypical plasma cells with the same cytological features as those in the peripheral blood. The nuclear chromatin is condensed into small irregular clumps with frequent large nucleoli.

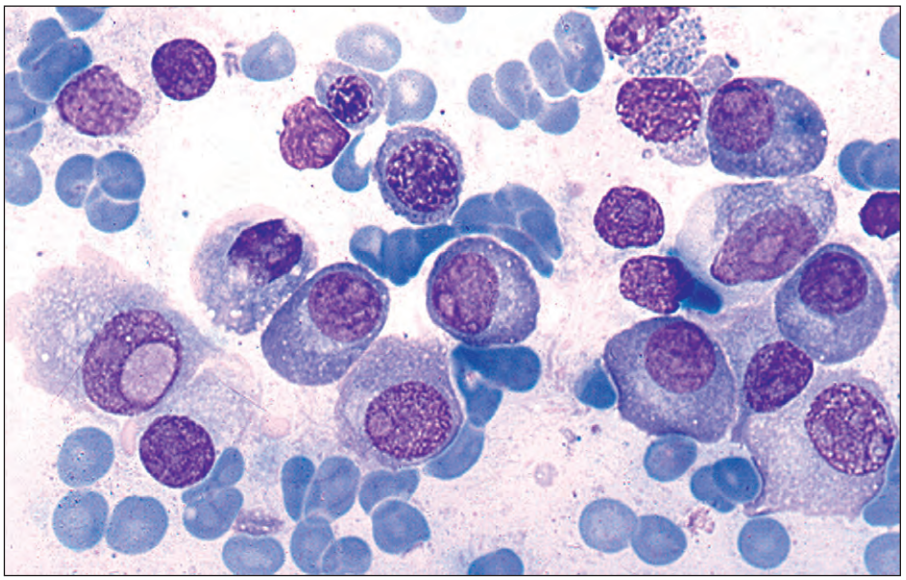


**Fig. 10.47** Plasma cell leukaemia as the terminal phase of IgG multiple myeloma; peripheral blood film. Marked leucocytosis (WBC 89 × $10^9$/l) resulting from the presence of immature plasma cells and plasmablasts, sometimes with considerable atypia. The cell furthest to the left has a gigantic Dutcher body.

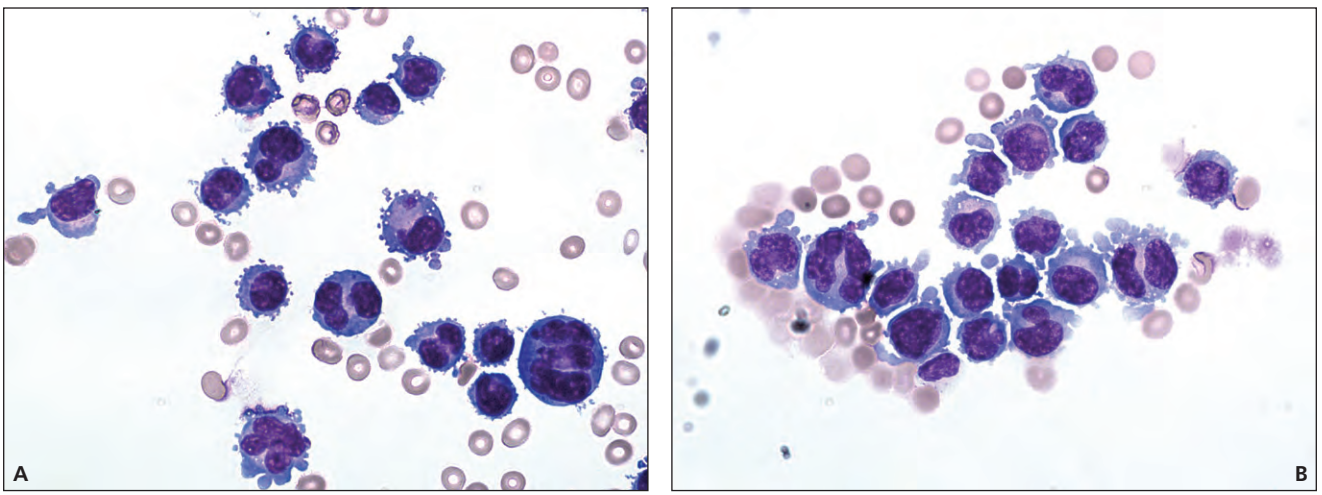



**Fig. 10.48** Plasma cell leukaemia presenting clinically with meningitic features (headache, torpor and vomiting). (A) In this cytospin preparation of cerebrospinal fluid (CSF) there are numerous atypical and dysmorphic cells, often multinucleated, with cytological features suggestive of their plasma cell nature. (B) The nuclear morphology is bizarre with indentations, lobulation and oval and horseshoe-shaped forms. The presence of numerous cytoplasmic projections is a common observation when pathological cells of various types infiltrate the CSF.

there is also peripheral blood plasmacytosis, with circulating plasma cells even reaching levels higher than those suggested for the diagnosis of plasma cell leukaemia.[76] Cases of plasmacytosis simulating plasma cell leukaemia have been described in patients with severe sepsis, pertussis, dengue, infectious mononucleosis, rubella and parvovirus B19 infection.[72] Reactive plasmacytosis is polyclonal or oligoclonal; moreover, it is reversible, being dependent on the proliferation of plasmablasts and other plasma cell precursors in response to growth factors, in particular IL-6.[77] The cells retain their full capacity to differentiate.[77]

In reactive plasmacytosis, the plasma cells are uniformly distributed with aggregates having a perivascular distribution, close to endothelial cells, and in aspirates being in proximity to fragments (Fig. 10.50). The cytomorphology is not always sufficient to suggest the nature of a plasma cell proliferation, since myeloma cells can have an appearance not dissimilar to normal plasma cells, while the plasma cells of reactive plasmacytosis can show atypia, bizarre forms and morphological immaturity (Figs 10.51 and 10.52). It is not exceptional, for example, to find in normal subjects some binucleate forms and indeed some cells with three or more nuclei. In this instance, however, the immunoglobulin appears polyclonal on electrophoresis and the degree of increase in plasma cells shows a good correlation with the degree of hypergammaglobulinaemia. In the differential

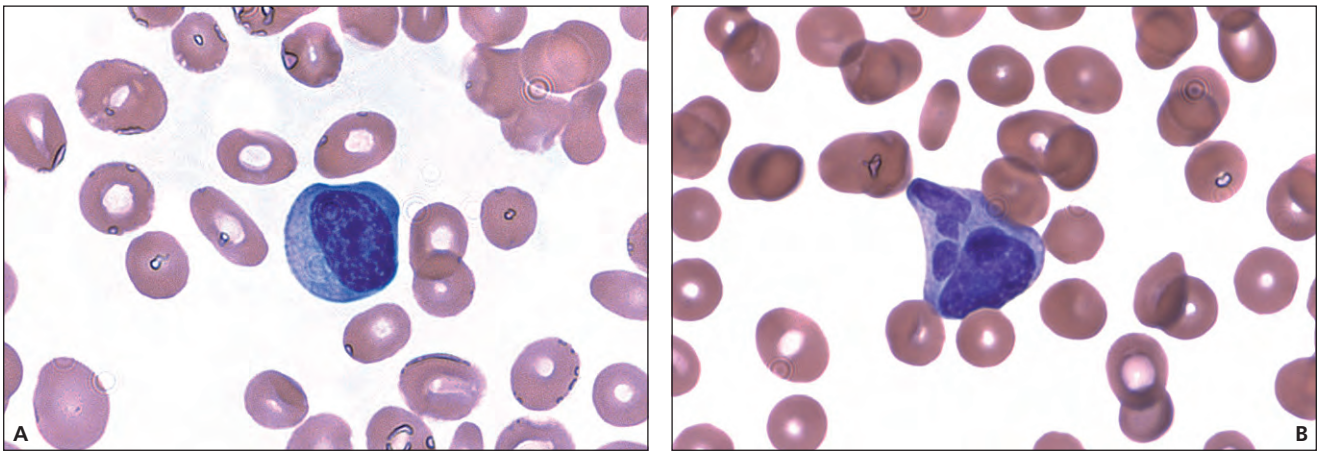



**Fig. 10.49** Same patient as Fig. 10.48; peripheral blood film. (A) Examination of the blood film showed the presence of 4% atypical lymphocytes with plasmacytoid features. (B) Some of these atypical circulating cells have dysmorphic and fragmented nuclei. The bone marrow aspirate showed 12% plasma cells.

**Table 10.6** Principal conditions associated with a reactive (polyclonal) bone marrow plasmacytosis

**Haematological disorders**
- Hodgkin and non-Hodgkin lymphoma
- Myeloproliferative neoplasms
- Acute myeloid leukaemia
- Aplastic anaemia
- Autoimmune haemolytic anaemia
- Iron deficiency and megaloblastic anaemias
- Thrombotic thrombocytopenic purpura
- Immune thrombocytopenic purpura

**Non-haematological disorders**
- Bacterial, viral or protozoan infections
- Chronic inflammatory disorders: cirrhosis, collagen disorders, granulomatous disorders
- Tumours: carcinoma of the lung or prostate; Kaposi sarcoma, bone marrow metastases
- Drug effects (cytotoxic agents, penicillin, sulphonamides)
- Miscellaneous conditions (diabetes mellitus, cardiovascular disorders, exposure to mitogens)

diagnosis, immunophenotyping is very useful to demonstrate that the blood and bone marrow plasma cells are polyclonal and have a normal pattern of expression of markers, with expression of CD19, CD27 and CD45, which are usually negative in myeloma cells, which, conversely, are characterised by aberrant positivity for CD56 and strong expression of CD138.

## Amyloidosis and other disorders relating to immunoglobulin deposition

Amyloidosis is characterised by tissue deposition of an amorphous extracellular material, which can be constituted of altered immunoglobulin. Amyloid can severely compromise the function of the organs involved (kidneys, heart, liver, intestine, peripheral nerves and bone marrow). Amyloidosis has recently been defined as a pathological process due to systemic proteotoxicity, i.e. toxicity due to protein aggregates.[78] The diagnosis continues to be based on tissue analysis, in particular on demonstration of affinity for Congo red, and also on ultrastructural morphology. Investigations for amyloidosis can be done on a needle aspirate of periumbilical fat, or on a biopsy of bone marrow or an organ suspected of being involved. Amyloid is easily seen in histological sections, where it appears hyaline and eosinophilic and is often located in the walls of blood vessels or interstitially; particularly useful is the demonstration of apple-green or yellow-green birefringence when a Congo red-stained section is examined under polarised light (Fig. 10.53). Primary amyloidosis is, in the majority of cases, a plasma cell disorder, which in 20% of cases satisfies the diagnostic criteria for multiple myeloma: amyloid is formed in these cases from immunoglobulin or from immunoglobulin light chains, and there is always a serum or urinary M component. In rare cases, during the clinical course of multiple myeloma, an isolated amyloid tumour develops in non-skeletal tissues.[79] A rarer form of primary amyloidosis, which is not a plasma cell disorder, has a genetic basis and is linked to mutation or variation in the gene encoding transthyretin, a thyroxine-transporting protein produced by the liver. The secondary form of amyloidosis, now rare in developed countries, is associated with chronic infective, neoplastic or inflammatory processes of various aetiologies, e.g. rheumatoid arthritis.[80]

The peripheral blood of patients with amyloidosis often shows moderate anaemia with increased rouleaux formation. Amyloid may be visible in May-Grünwald-Giemsa-stained films of bone marrow in the form of masses or deposits of material that stains pink, deep red, bluish or blue,[81] appearing waxy, hazy or cloud-like, which incorporates degenerating nuclei and is adjacent to fatty tissue and close to fragments (Fig. 10.54);

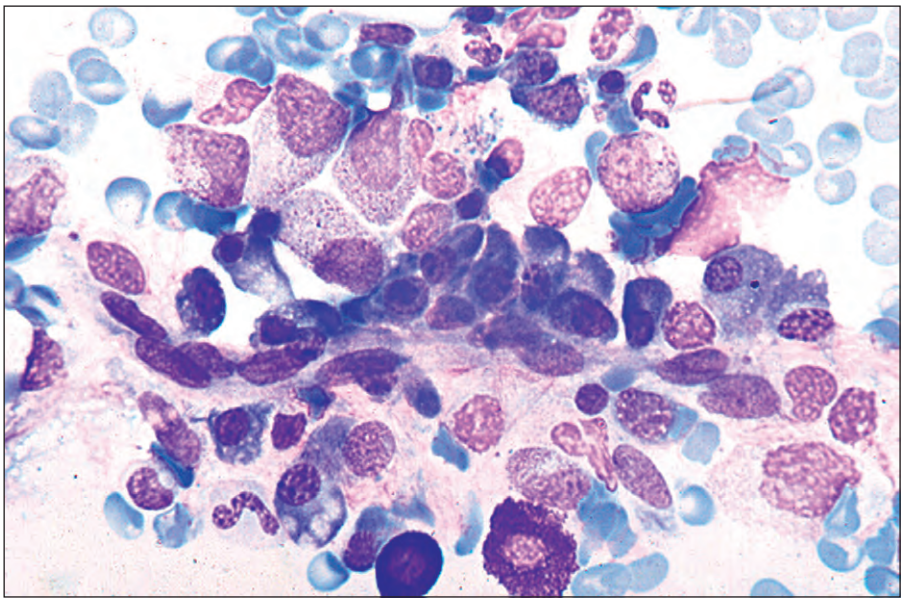


**Fig. 10.50** Perisinusoidal plasma cells in the bone marrow aspirate of a patient with brucellosis. In the centre, a chain of elongated oval nuclei, superimposed on the endothelial cells that line a sinusoid. Above these, a mass of plasma cells, which appear mature, with eccentric nuclei, chromatin in blocks and basophilic cytoplasm with a clearly visible Golgi zone. There are numerous granulocyte precursors and, in the centre at the bottom, two mast cells. The appearances seen here are reactive and not indicative of multiple myeloma. The tendency of plasma cells to gather together in nests must also be considered when performing a differential count of bone marrow cells.

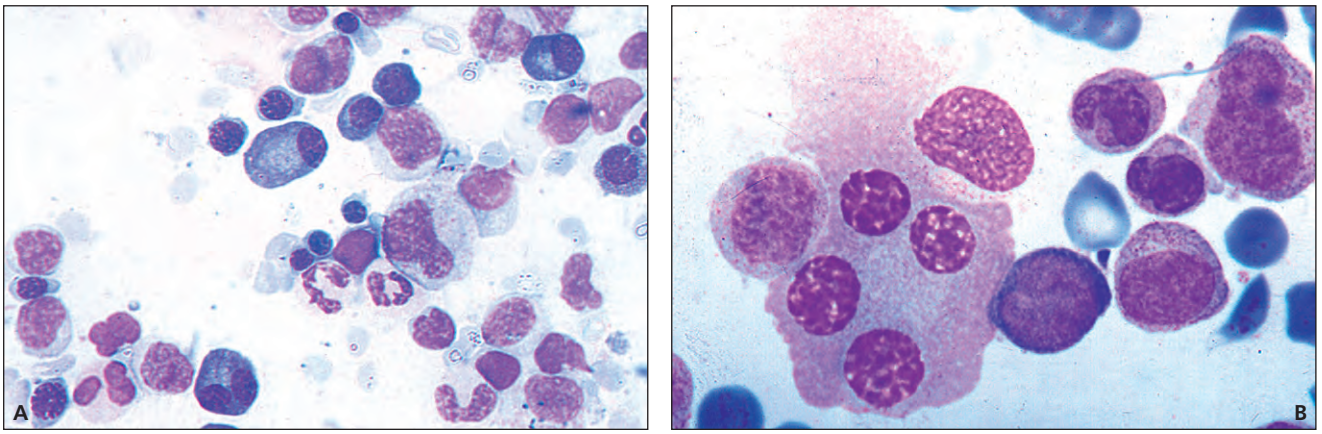



**Fig. 10.51** Reactive plasmacytosis. (A) Increased number of lymphoid cells and plasma cells in a young patient with infectious mononucleosis; the bone marrow aspirate was done for the evaluation of marked thrombocytopenia. In addition to numerous lymphoid cells with plentiful pale cytoplasm, there are a number of plasma cells with hypertrophic, intensely basophilic cytoplasm, one of which is binucleated. (B) Bone marrow aspirate in a case of visceral leishmaniasis. There is a large flaming plasma cell with four mature nuclei with well-condensed blocks of clumped chromatin. The plentiful cytoplasm has a pinkish tinge and a pale central Golgi zone between the nuclei. In this situation of intense and prolonged antigenic stimulation, one can observe all forms of hypertrophy and glycoprotein accumulation in plasma cells, which also tend to increase in number; these plasma cell changes, in addition to increased macrophages and mast cells, characterise this reactive picture. This slight deviation from morphological normality is physiological and must not be considered evidence of malignancy.

deposits may be surrounded by numerous macrophages and sometimes foreign body-type giant cells, the cytoplasm of which is packed with pink proteinaceous material. The plasma cells may be normal or variably increased: particularly in primary amyloidosis, there may be a marked plasmacytosis with the presence of atypical or immature forms (Fig. 10.55). Trilineage haemopoiesis is often well represented. Electron microscopy of amyloid protein shows the typical fibrillar structure (beta pleated sheets).

Other plasma cell disorders, with similar biological and clinical features, are those resulting from deposition of monoclonal heavy or light chain, in which the tissue deposits are constituted of a non-amyloid, non-fibrillar amorphous eosinophilic protein, which is not birefringent with Congo red staining.

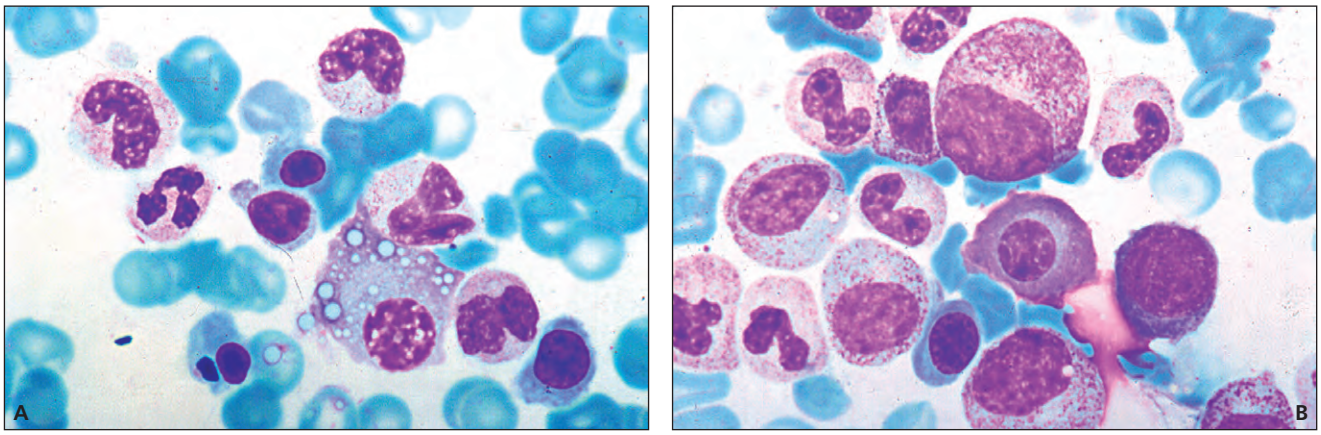



**Fig. 10.52** Reactive plasmacytosis; bone marrow aspirate in a patient with brucellosis. (A) In addition to various granulocytes and erythroblasts and one lymphocyte, there is a plasma cell with a mature nucleus and pale cytoplasm with a violaceous peripheral tinge, containing numerous pale turquoise Russell bodies. Cells with these characteristics correspond to some extent to Mott cells. (B) There are numerous neutrophil granulocytes in all stages of maturation, and two erythroblasts. Just to the right of centre is a small flaming plasma cell. The nucleus is eccentric with chromatin in blocks; the cytoplasm shows plentiful pale arcoplasm with a dark reddish tinge to the rest of the cytoplasm, which is more intense beneath the membrane.

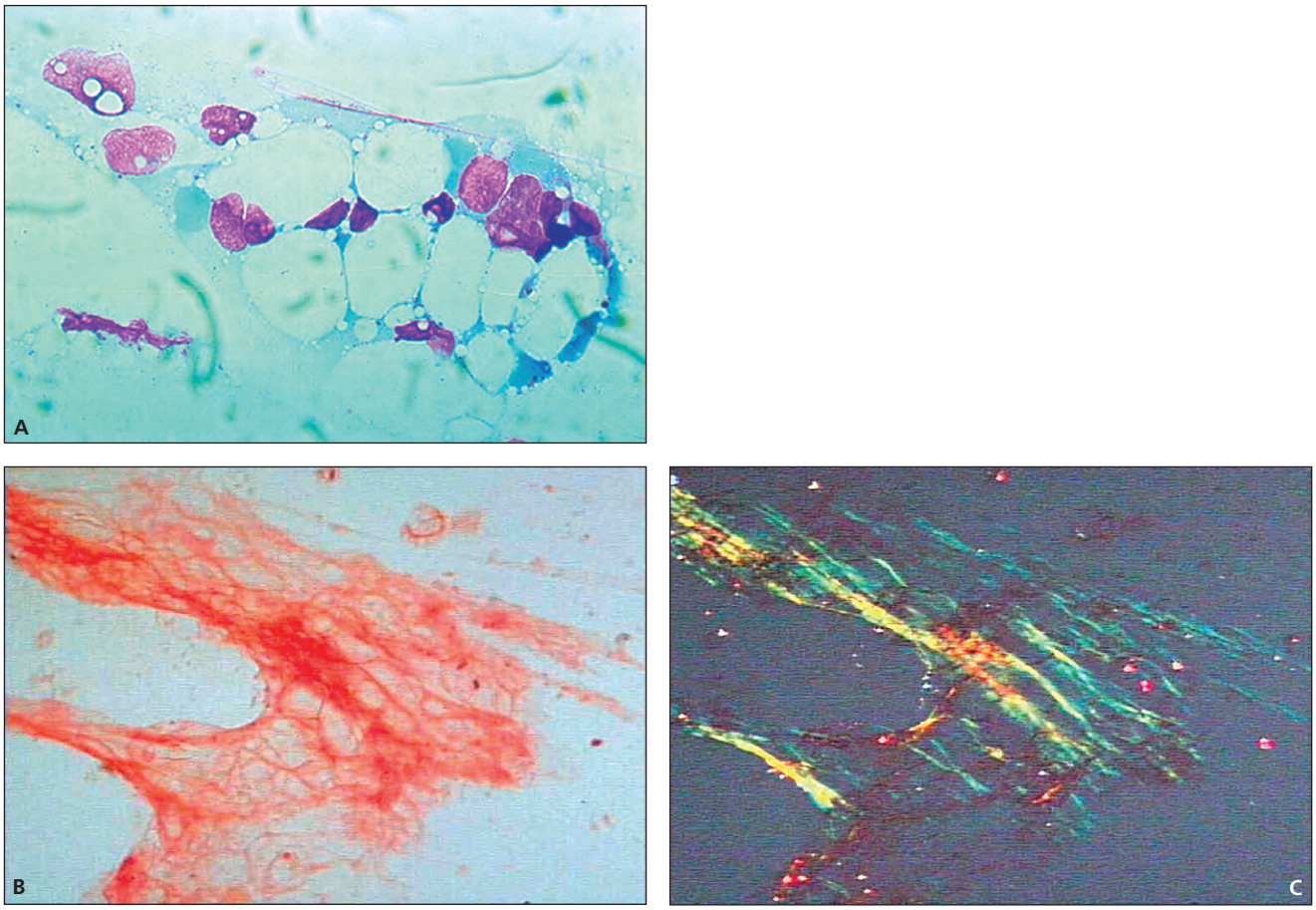



**Fig. 10.53** Primary amyloidosis; aspirate of periumbilical fat. (A) Panoptic staining shows the presence of some immature nuclei plus adipose cells with small eccentric hyperchromatic nuclei and vast swollen cytoplasm, giving the impression of an optically empty ball. (B) With a Congo red stain, the typical red colour of the intercellular matrix is apparent. (C) On examination with polarised light, the characteristic yellow-green birefringence of amyloid is seen.

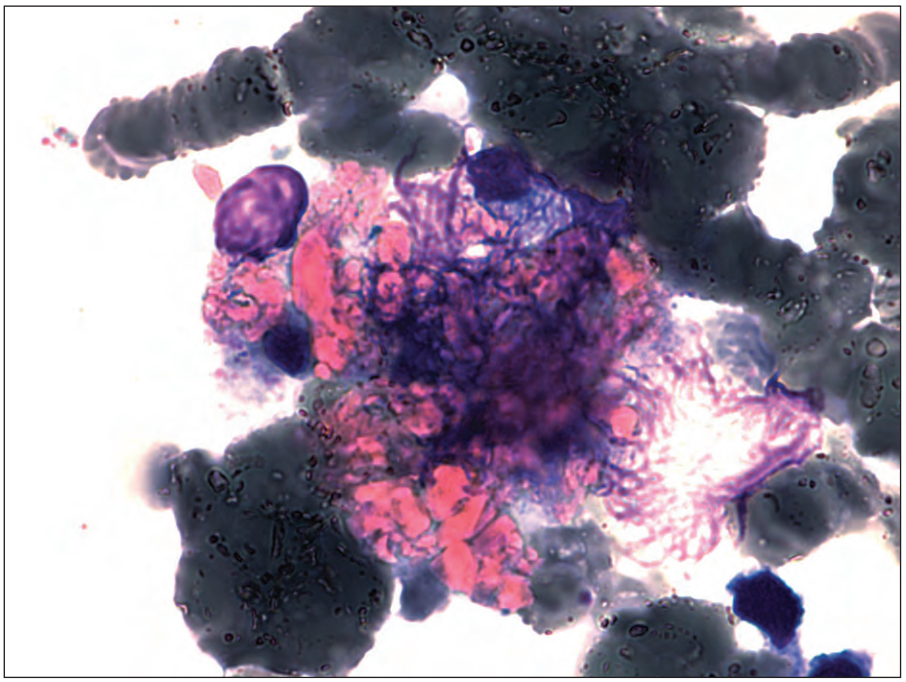


**Fig. 10.54** Accumulation of amyloid within the cytoplasm of a macrophage in a patient with amyloidosis associated with IgG myeloma; bone marrow aspirate, panoptic May-Grünwald-Giemsa stain. The nucleus is flattened, top left in the cytoplasm; the cell is packed with protean red-staining globules.

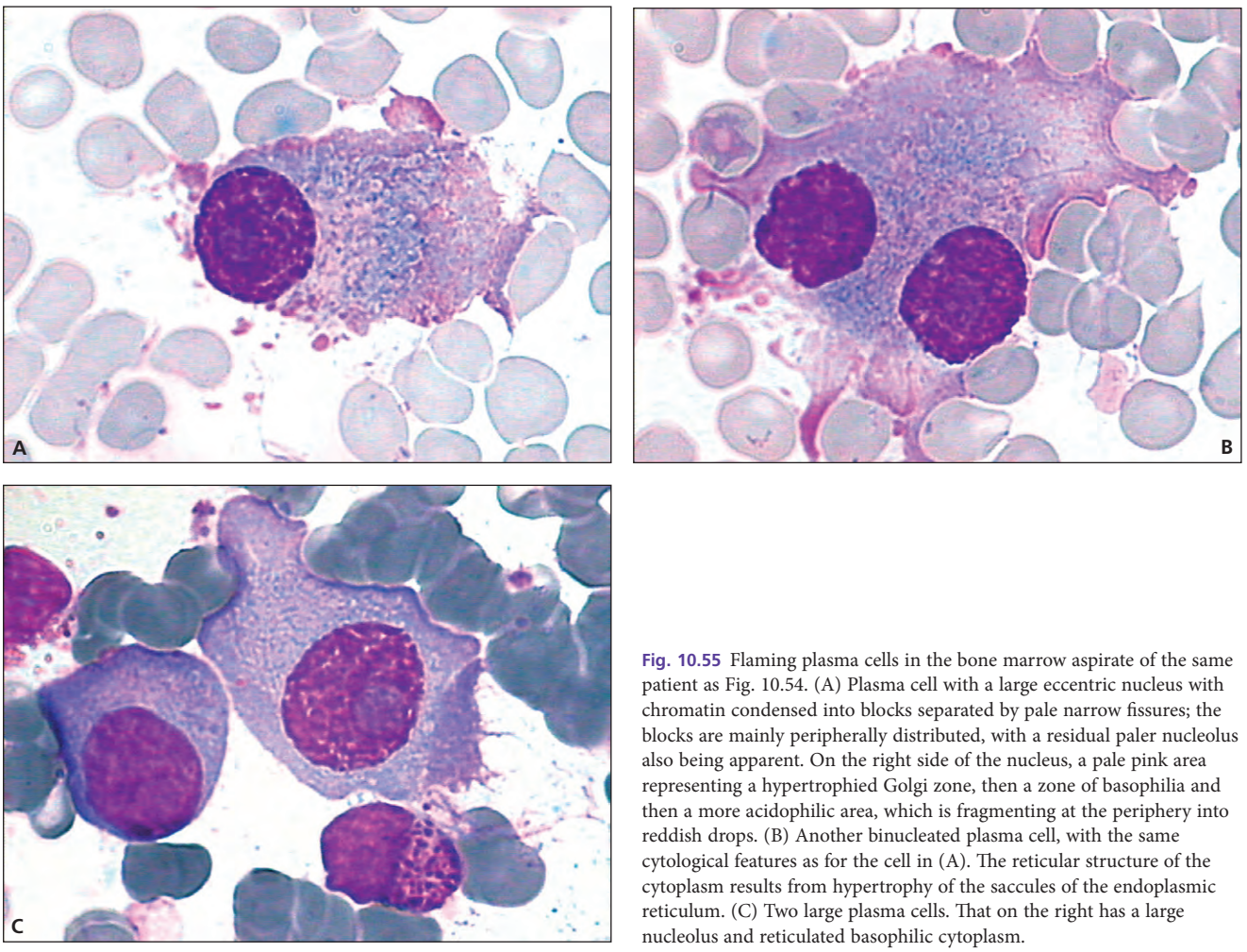
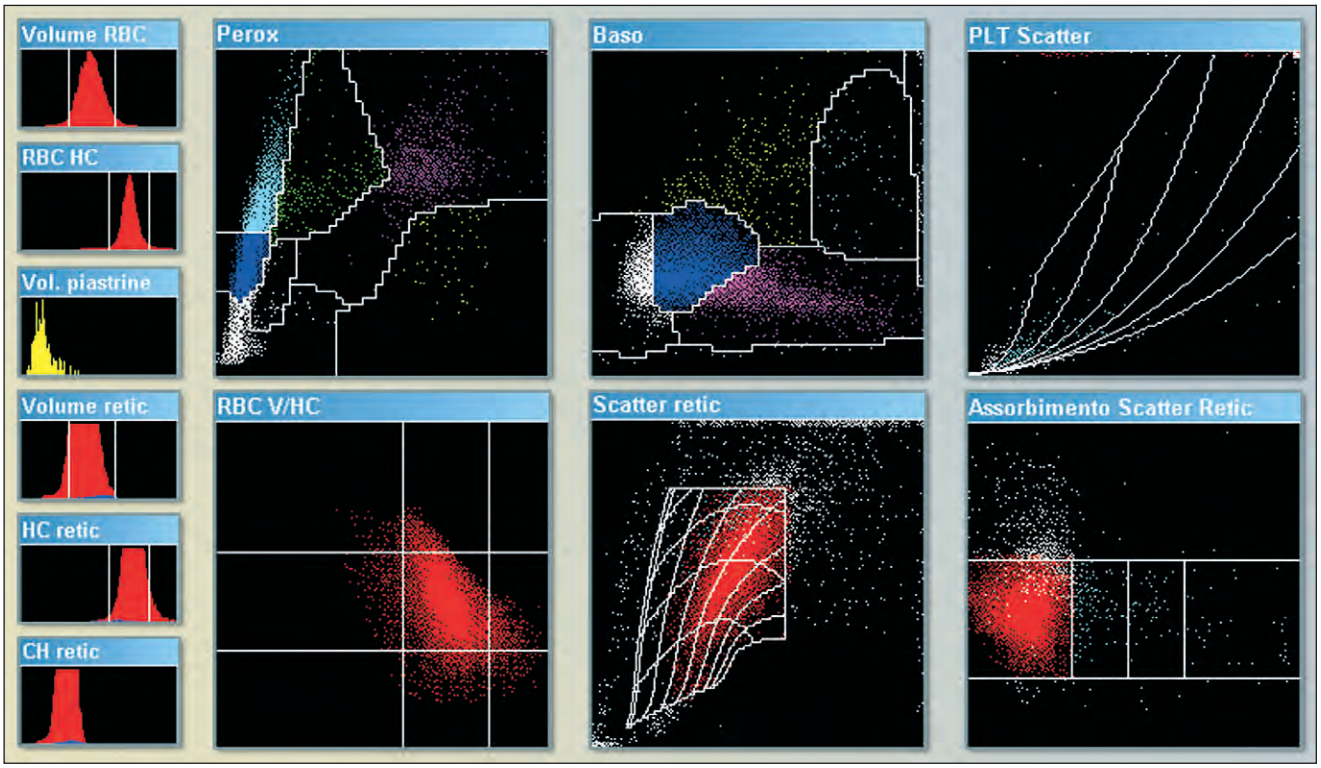



**Fig. 10.55** Flaming plasma cells in the bone marrow aspirate of the same patient as Fig. 10.54. (A) Plasma cell with a large eccentric nucleus with chromatin condensed into blocks separated by pale narrow fissures; the blocks are mainly peripherally distributed, with a residual paler nucleolus also being apparent. On the right side of the nucleus, a pale pink area representing a hypertrophied Golgi zone, then a zone of basophilia and then a more acidophilic area, which is fragmenting at the periphery into reddish drops. (B) Another binucleated plasma cell, with the same cytological features as for the cell in (A). The reticular structure of the cytoplasm results from hypertrophy of the saccules of the endoplasmic reticulum. (C) Two large plasma cells. That on the right has a large nucleolus and reticulated basophilic cytoplasm.

## REPORT 10.1 Plasma cell leukaemia




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 76 | g/l | |
| RBC | 2.43 | $\times 10^{12}$/l | |
| HCT | 0.218 | l/l | |
| MCV | 89.6 | fl | |
| MCH | 31.3 | pg | |
| MCHC | 349 | g/l | |
| CHCM | 351 | g/l | |
| RDW | 17.3 | % | Anisocytosis + |
| HDW | 2.70 | g/dl | |
| HYPO | 0.9 | % | |
| HYPER | 1.9 | % | |
| MICRO | 2.3 | % | |
| MACRO | 2.3 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 0.53 | % | |
| RET | 13.0 | $\times 10^{9}$/l | |
| CHr | 31.9 | pg | |
| IRF (M+H) | 29.3 | % | |
| PLT | 24 | $\times 10^{9}$/l | |
| MPV | 8.5 | fl | |
| PDW | 42.5 | % | |
| Large platelets | 0 | $\times 10^{9}$/l | |
| MPC | 18.5 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 15.92 | | * |
| WBC Perox | 16.40 | | * |
| NEUT | 0.77 | 4.9 | * |
| LYMPH | 8.79 | 55.2 | * |
| MONO | 0.34 | 2.1 | * |
| EOS | 0.07 | 0.5 | |
| BASO | 0.35 | 2.2 | * |
| LUC | 5.60 | 35.2 | * Atyp +++ |
| NRBC | 1.31 | 8.28 | * NRBC +, N-RBC |
| LI | 1.73 | | |
| MPXI | −2.8 | | |
| MN | | 92.5 | |
| PMN | | 4.5 | |
| BLASTS | | 14.1 | Blasts +++ |
| Perox HPX | | 2.4 | |
| Perox SAT | | 0.7 | |

Leucocytosis with severe anaemia and thrombocytopenia in a patient at presentation. In the Perox cytogram there is a pointed fusiform cluster representing a population of peroxidase-negative cells that are extending through the lymphocyte and LUC boxes; the neutrophils, present in reduced numbers, are dispersed. In the Baso cytogram the nuclei of the pathological cells are gathered in a greatly enlarged MN cluster, which is extending upwards, causing a mild pseudobasophilia, and to a large extent is spilling over the blast threshold in channel 8 on the X axis, causing the appearance of the relevant flag with maximum intensity and giving a P0a, D1 PANDA morphology.

**Morphology.** The leukaemic population has the morphological and immunophenotypic features of plasmablasts. No erythroblasts are seen: the flag for NRBC is a false positive due to interference. Quantification of immunoglobulins shows an increase in IgG (79 g/l), with an IgG kappa paraprotein and free kappa light chain in the serum.

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CHAPTER 11

# Reactive Bone Marrow Changes and Non-haemopoietic Neoplasms

The peripheral blood and the bone marrow can be affected by a wide range of conditions that do not primarily involve the haemopoietic or lymphoid systems. Some of these, such as infectious mononucleosis, non-clonal dysplasia in human immunodeficiency virus (HIV) infection and erythroblastopenia associated with parvovirus B19 infection, have already been described in earlier chapters. In this chapter, we will illustrate other pathological processes of particular cytomorphological interest.

## Reactive changes in infection and other non-haematological conditions

### Leukaemoid reactions: peripheral blood

This term, which can be defined as a white blood cell count (WBC) above $50 \times 10^9$/l,[1] indicates a haematological abnormality that is not due to a primary bone marrow disease and that is characterised by a marked neutrophilia with a left shift of the maturation curve and often the presence of immature forms, myelocytes and metamyelocytes, in the circulation (Report 11.1). This picture of granulocyte expansion represents an exaggerated response to severe infective stimuli (*Clostridium difficile* colitis, disseminated tuberculosis or *Shigella dysenteriae* infection), to a neoplasm (particularly carcinoma of the lung), to drugs (corticosteroids, growth factors or minocycline), to toxins (ethylene glycol) or to acute haemolysis or major haemorrhage. The forms associated with non-haematological tumours are not related to bone marrow invasion, but rather to the production of cytokines such as granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), or to other mediators produced by the neoplastic cells,[2–6] as also occurs in multiple myeloma. A leukaemoid reaction may be initially confused with chronic myeloid leukaemia or with other myelodysplastic, myeloproliferative or myelodysplastic/myeloproliferative neoplasms; the differential diagnosis with chronic neutrophilic leukaemia, in particular, can sometimes be quite difficult, since in some cases the morphological features (absence of immature precursors, absence of basophilia, absence of monocytosis) and the biological features (absence of molecular alterations) can be identical.

### Infections: peripheral blood

In the presence of infection, the peripheral blood can show very different features, depending on the infecting organism and the course of the illness. With regard to the white cells, neutrophilia is more often associated with acute bacterial infection: the features seen are non-specific and can include left shift, with an increase of band forms, toxic granulation, Döhle bodies, vacuoles and, quite rarely, phagocytosed bacteria (Fig. 11.1). An increase of neutrophils is also frequent in fungal infections, such as actinomycosis, coccidioidomycosis and systemic candidiasis. Monocytosis is more characteristic of chronic, protracted infection. Neutropenia can be observed in certain infections, including tularaemia, brucellosis, typhoid and paratyphoid fevers, viral infections and helminthic infections. Reactive lymphocytosis is typical of various types of viral infection, pertussis and brucellosis, and can be accompanied by the presence of atypical and plasmacytoid lymphocytes (see Figs 1.40, through to 1.43); conversely, an absolute lymphopenia characterises some acute bacterial infections and also the acquired immune deficiency syndrome (AIDS) due to HIV infection, in which there can also be dysplastic changes in the neutrophils; macrocytosis may also be present, but is seen particularly during zidovudine treatment (see Chapter 3).

Red cells may also show changes in the presence of infection: the 'anaemia of chronic disease' is normocytic or, less often, mildly microcytic; a hypochromic microcytic anaemia can be seen in infection with intestinal parasites as a result of occult intestinal blood loss. An increased platelet count is another non-specific change; thrombocytopenia can have a central cause, such as suppression of the bone marrow by an infective agent, or a peripheral cause, with an immunological mechanism or disseminated intravascular coagulation.

Parasitic infections are often associated with eosinophilia. Malaria parasites (Figs 11.2 through to 11.6) and babesia, when present, can be recognised within red cells; trypanosomes and microfilariae can be observed as free extracellular forms in blood films, while recognition in the peripheral blood of amastigotes of *Leishmania donovani* that have been phagocytosed by circulating monocytes is very rare. In systemic *Candida albicans* infection, fragments of mycelium and fungal spores may be seen within neutrophils and monocytes (Fig. 11.7; see Report 11.2).

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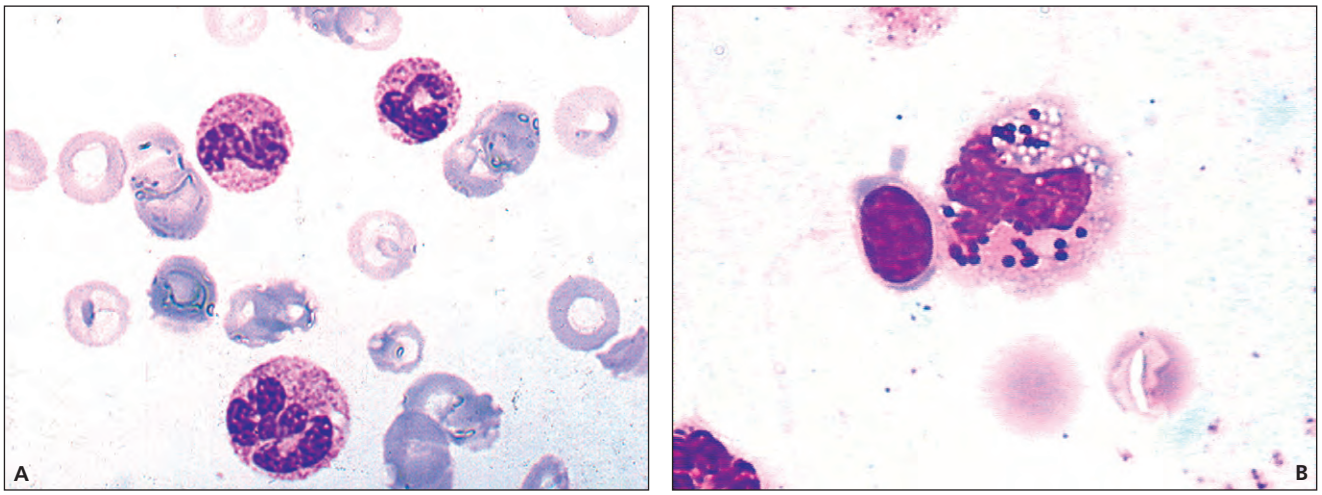



**Fig. 11.1** An example of the alteration in neutrophil granulocytes during the course of infection. (A) Peripheral blood film from a patient with sepsis: neutrophils with toxic granulation; the lower cell is large and has a hypersegmented nucleus. (B) Cytocentrifuge preparation of cerebrospinal fluid: on the right of a small lymphocyte, a neutrophil, which has numerous cocci within its cytoplasm.

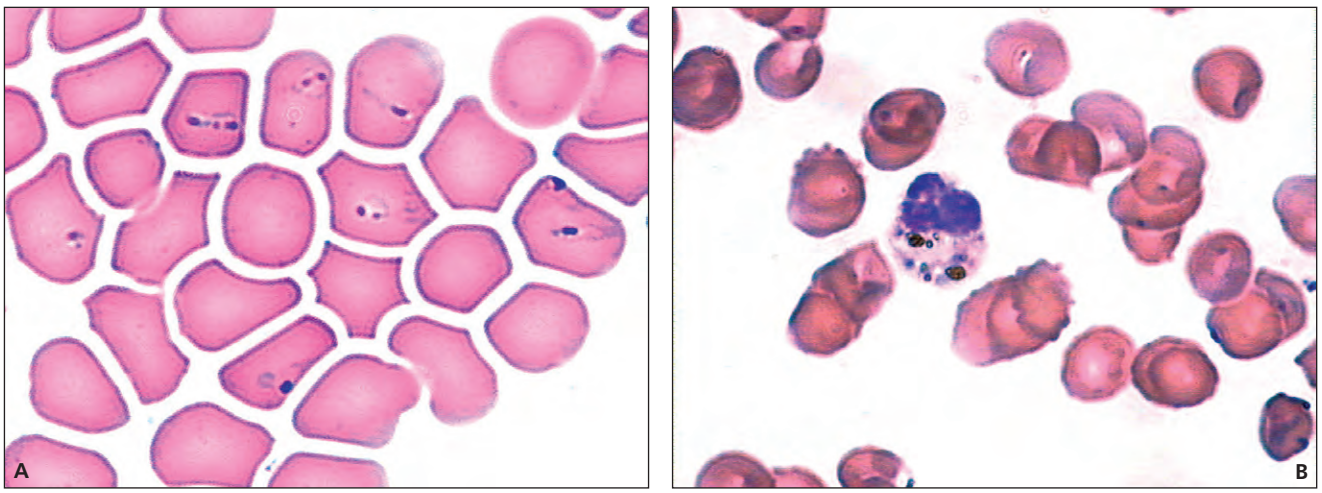



**Fig. 11.2** *Plasmodium falciparum* malaria; peripheral blood film. (A) Young ring-form trophozoites, sometimes with duplication of the small hyperchromatic nucleus, within red cells. (B) In a circulating neutrophil, there are the remains of a phagocytosed parasite plus large inclusions of haemozoin, a golden-black pigment that the parasite has produced by degrading cellular haemoglobin.

Table 11.1 shows the most frequent associations between peripheral blood alterations and infections.

## Bone marrow in infection and in chronic inflammatory states

The bone marrow stroma, in particular its sinusoidal component, can be damaged by toxins of bacterial origin, chemicals or ionising radiation. The damage to the bone marrow microenvironment leads inevitably to interference with the normal mechanisms of proliferation and maturation of haemopoietic precursors.[7–9] If the exposure to a noxious pathogen is brief, in general there is recovery of normal bone marrow function. Bone marrow culture often leads to the identification of the pathogenic agent and thus to the resolution of the problem. In chronic inflammatory conditions, such as severe allergies and autoimmune conditions, bone marrow recovery may not be complete. The microenvironmental damage resulting from irradiation can lead to permanent bone marrow fibrosis. In chronic inflammation involving the bone marrow, the haemopoietic and adipose components are reduced and there is an increase in fibroblasts, reticulin and collagen fibres, associated with neoangiogenesis with a diffuse increase in stromal cells (macrophages, endothelial cells and adventitial reticulum cells). Classically, bone marrow reactions can be classified as follows[10]:

1. Acute necrosis: there are co-existing areas of necrosis (Fig. 11.8) and preserved haemopoiesis; in general, the peripheral

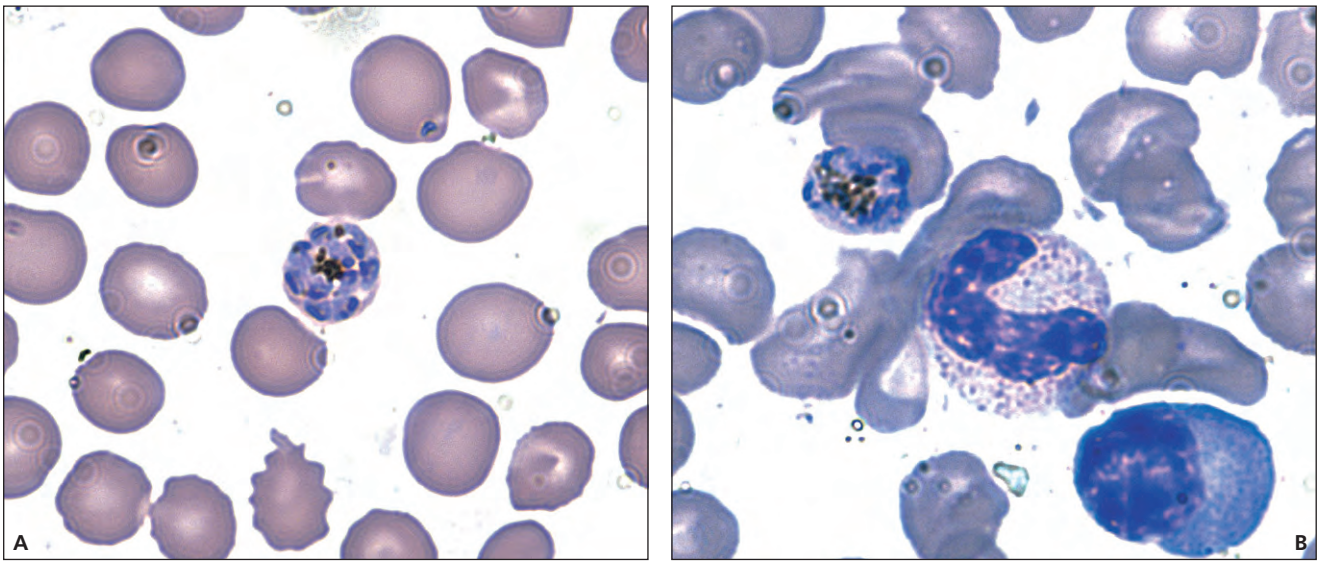



**Fig. 11.3** *Plasmodium falciparum* malaria; peripheral blood film. (A) In the centre, a schizont, which is formed by the division and maturation of a trophozoite. It is composed of numerous merozoites surrounding a central fragmented mass of dark pigment; each merozoite has an an easily recognisable nucleus and cytoplasm. When the schizont ruptures, the merozoites are destined to invade other erythrocytes, thus expanding the number of parasitised cells. This stage of the parasite life cycle is not often observed in the peripheral blood film and is an unfavourable prognostic sign, generally associated with grave disease. The non-parasitised red cells show anisocytosis with some hyperchromic macrocytes and echinocytes. (B) At the top, a schizont with similar features to that in (A); in the centre, a band neutrophil and, at the bottom, a reactive lymphocyte showing advanced plasmacytoid development with an eccentric nucleus, chromatin in blocks, blue cytoplasm and pale arcoplasm.

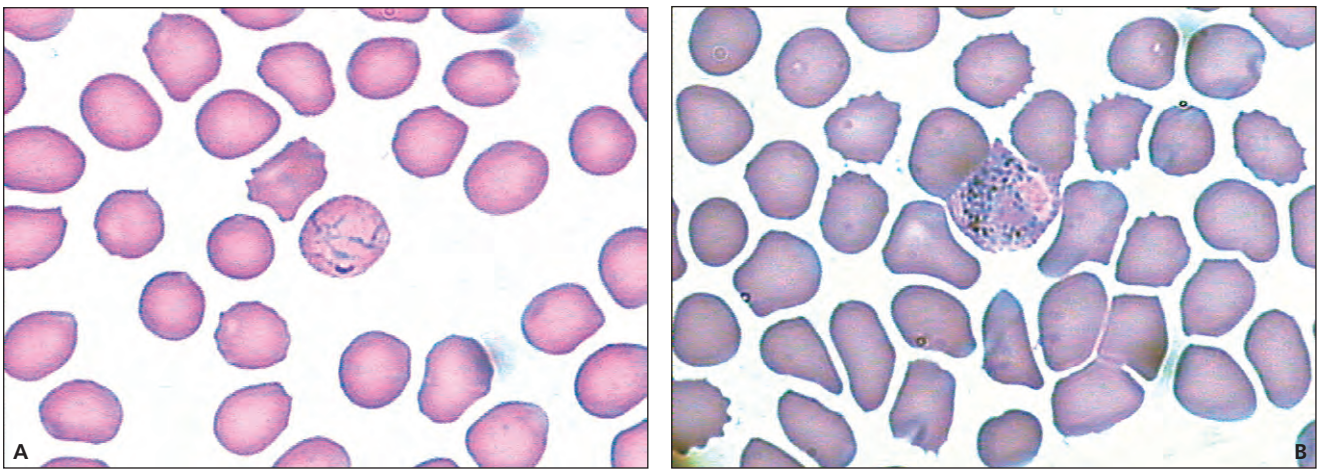



**Fig. 11.4** *Plasmodium vivax* malaria; peripheral blood film. (A) The erythrocyte in the centre is larger than the other cells and contains fine Schüffner's dots, typical of this type of malaria, and a mature trophozoite with amoeboid cytoplasm, ready to divide. Schüffner's dots are present from the stage when the trophozoite starts its maturation until the gametocyte stage: they are smaller, more homogeneous and darker pink than basophilic stippling, with which they should not be confused. (B) The cell in the centre is a female gametocyte: the lilac nucleus is seen on the right of the pale blue cytoplasm, which has many dispersed pigment granules.

blood shows neutrophilia or a leucoerythroblastic picture with erythrocyte fragmentation, thus leading to a differential diagnosis of bone marrow metastasis. Aspiration produces necrotic material with few recognisable cells, mainly stromal cells.[11–13] A similar picture has been observed in patients with neoplastic conditions, particularly haemopoietic neoplasms, tuberculosis, sepsis, sickle cell disease, antiphospholipid syndrome and intoxication or adverse reactions to drugs[14–16];

2. Gelatinous transformation (Fig. 11.9): reduction in the haemopoietic matrix with deposition of mucopolysaccharide and an infiltrate of lymphocytes, plasma cells and mast cells; this is observed in long-lasting chronic infection, in severe

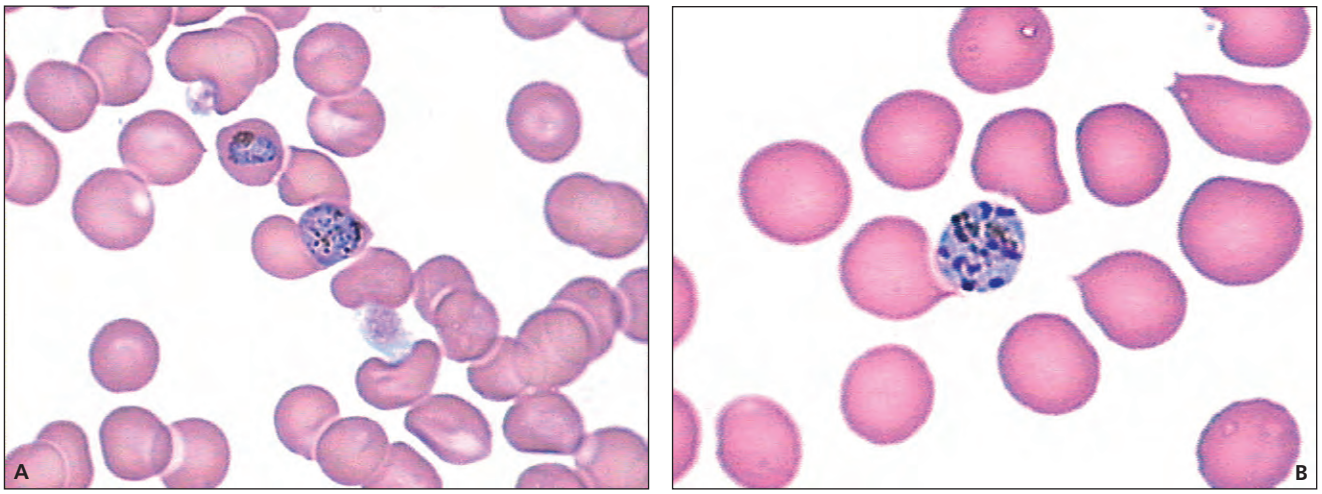



**Fig. 11.5** *Plasmodium malariae* malaria; peripheral blood film. (A) Two female gametocytes, with a compact nucleus, scanty pale blue cytoplasm and dispersed pigment granules. (B) Male gametocyte, which completely fills the infected erythrocyte: the nucleus has a diffuse pattern formed of small fragments of violet chromatin; the pigment granules are large and numerous.

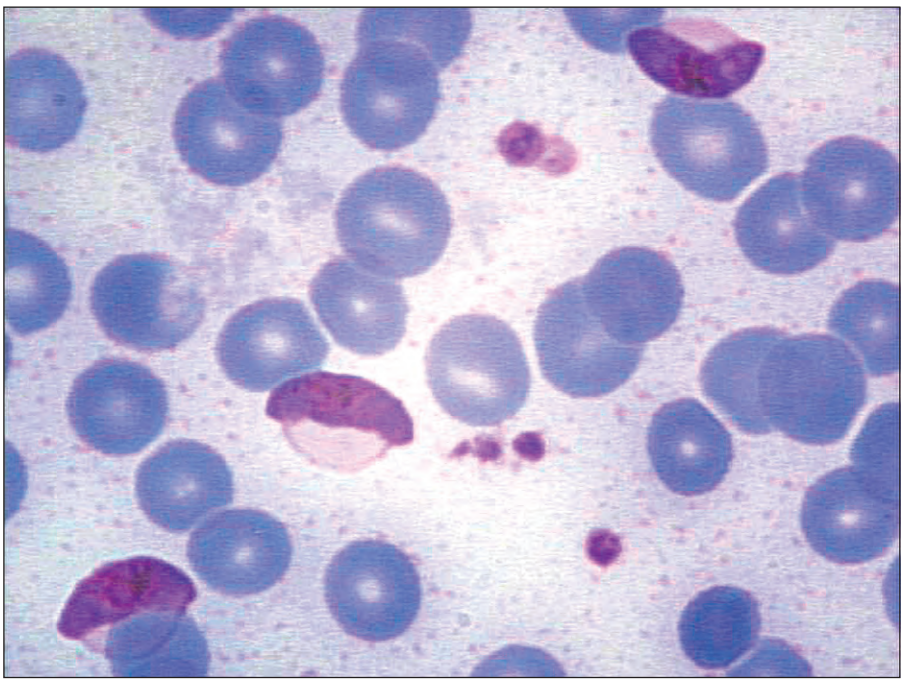


**Fig. 11.6** Three female gametocytes in *Plasmodium falciparum* malaria, with the typical sickle or banana shape. The nucleus is covered by granules that are condensed in the centre of the lilac cytoplasm; on the concave side of the parasite, one can see the membrane of the red cell, which has been emptied of haemoglobin and reduced to a ghost. These sexual forms arise from schizonts in the bone marrow or spleen and are destined to degenerate. However, if they are ingested by a mosquito, they are responsible for the transmission of infection.

malnutrition (including anorexia nervosa) and in neoplastic conditions in the absence of bone marrow metastases;

3. Sclerosing myelitis: reduction in haemopoietic marrow and adipose tissue is associated with fibrosis and new bone formation with groups of osteoblasts and some osteoclasts;
4. Leukaemoid reaction: the marrow is normo- or hypercellular with an increase of reactive cells and neutrophilia, sometimes also eosinophilia and often an increase in megakaryocytes;
5. Granulomatosis: granulomas are recognised in the context of a bone marrow that can vary from hypo- to hyper-cellular (Figs 11.10, 11.11 and 11.12; see also Fig. 1.78); they are compact aggregates of altered macrophages, often including giant multinucleated cells (Fig. 11.13), which can sometimes

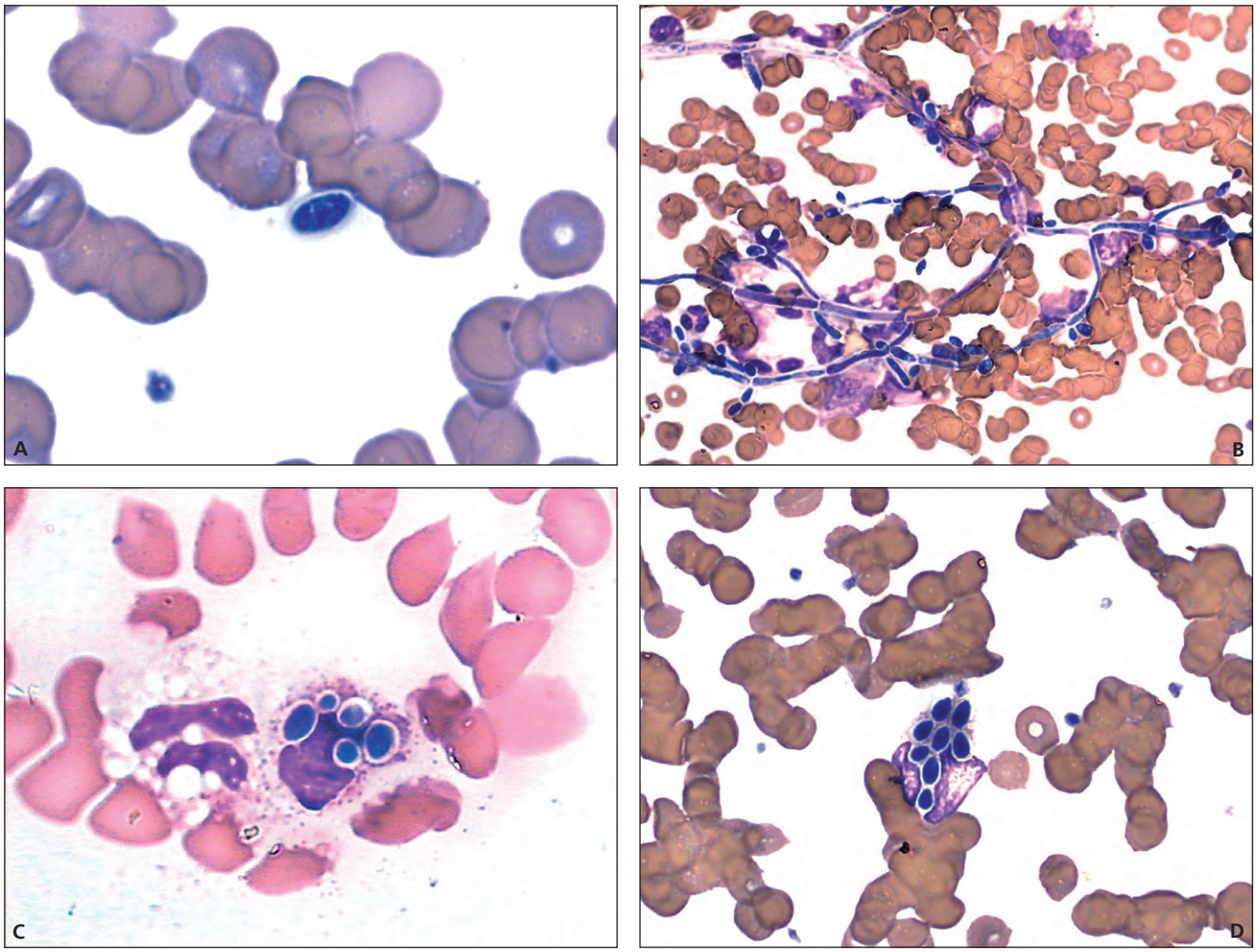



**Fig. 11.7** Peripheral blood film from an immunosuppressed patient who had received chemotherapy for carcinoma of the pancreas, with systemic candidiasis (see Report 11.2). (A) Extracellular spore of *Candida albicans*. (B) Fungal mycelium admixed with spores: the presence of the latter and the thinness of the mycelium avoid diagnostic confusion with microfilariae. (C) Two neutrophil granulocytes: that on the left shows marked cytoplasmic vacuolation; that on the right, in which the nuclear morphology is no longer clearly seen, contains five candida spores of various sizes within its cytoplasm. (D) Neutrophil granulocyte with its cytoplasm packed with a grape-like cluster of fungal spores.

**Table 11.1** Haematological abnormalities that are often recognised in infective conditions

| Cell type | Alteration | Most common causative condition |
|---|---|---|
| Erythrocytes | Anaemia of chronic disease | Prolonged infection or inflammation |
| | Acute cold-antibody-mediated haemolytic anaemia | *Mycoplasma pneumonia* infection |
| | Acute haemolytic anaemia with spherocytosis | *Clostridium perfringens* infection |
| | Acute microangiopathic haemolytic anaemia | Infection with shigella or *Escherichia coli* |
| | Red cell aplasia (with reticulocytopenia) | Parvovirus B19 infection |
| | Rouleaux | Non-specific, various types of bacterial infection |
| Platelets | Thrombocytosis | Non-specific, associated with various types of infection |
| | Thrombocytopenia | Non-specific, can be caused by bone marrow suppression or peripheral destruction |
| Neutrophils | Neutrophilia | Non-specific, acute bacterial infections, fungal infections (phagocytosed fungi may be present) |
| | Neutropenia | Viral infections and certain other infections |
| Eosinophils | Eosinophilia | Parasitic infection |
| | Eosinopenia | Non-specific, occurs in acute infections |
| Monocytes | Monocytosis | Chronic infections |
| Lymphocytes | Lymphocytosis | Bacterial infections (pertussis) in children, brucellosis, rickettsial infections, tuberculosis, syphilis, toxoplasmosis, viral infections |
| | Lymphopenia | Acute bacterial infections, acquired immune deficiency syndrome |
| All cell types | Pancytopenia due to aplastic anaemia | Hepatitis, Epstein-Barr virus infection, virus-induced haemophagocytic syndrome |
| | Other cytopenia | Tuberculosis, other mycobacterial infections, fungal infection, leishmaniasis |

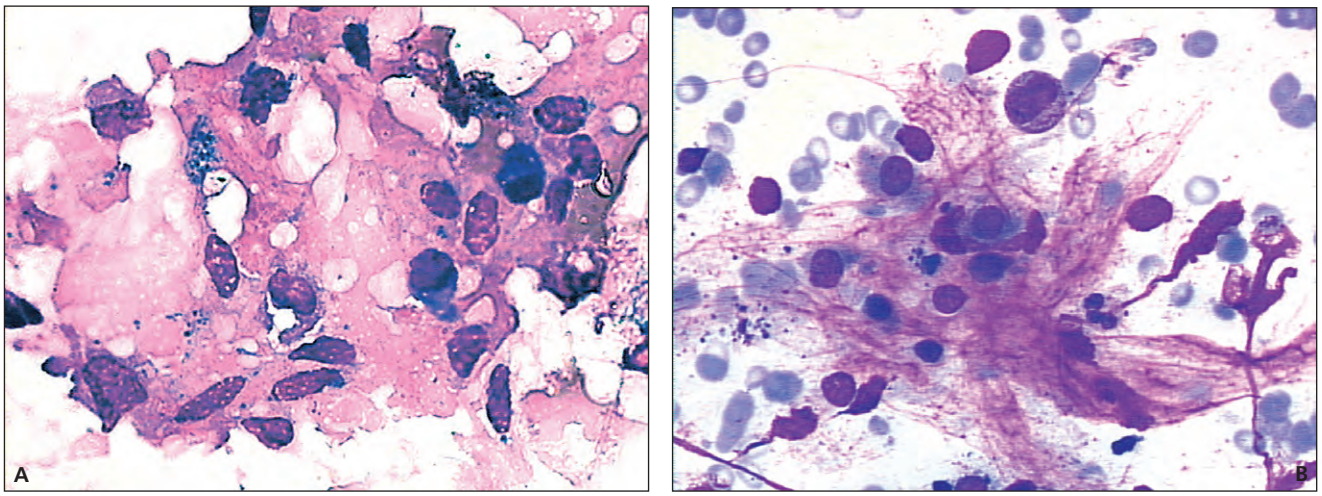



**Fig. 11.8** Bone marrow necrosis and gelatinous transformation. An area of myelitis with necrosis and gelatinous transformation can be identified in this bone marrow film; the cells appear distorted with indistinct outlines, contracted cytoplasm and nuclear pyknosis, and are embedded in a mass of weakly acidophilic hyaline material. (A) Amorphous fibrinoid material and degenerated stromal cells, some with fusiform nuclei; on the right, a plasma cell with intensely basophilic cytoplasm. (B) Some erythroblasts, a myelocyte at the top, various macrophage nuclei and a mass of fibrin fibres and torn nuclei.

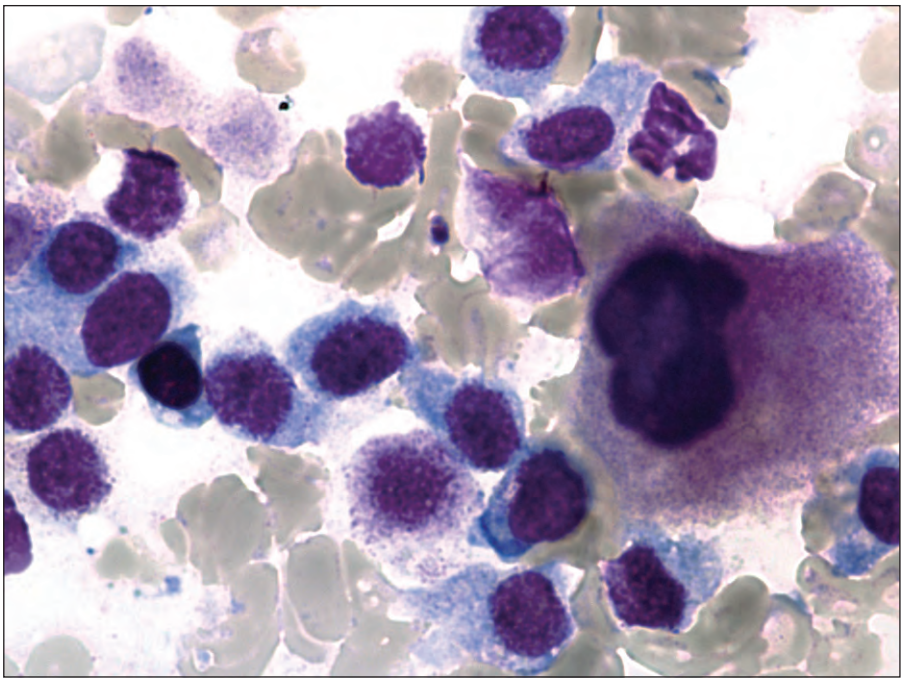


**Fig. 11.9** Gelatinous myelitis in a case of acute myeloid leukaemia at presentation (see also Fig. 4.63). The leukaemic blasts, and also a small megakaryocyte, appear indistinct, with the nuclear structure being particularly altered and difficult to evaluate morphologically. This phenomenon, which was consistently present on repeated aspirates and was associated with immediate coagulation of the material obtained, was not an artefact attributable to technical errors, but resulted from interaction of the acid component of the stain with the gelatinous bone marrow matrix.

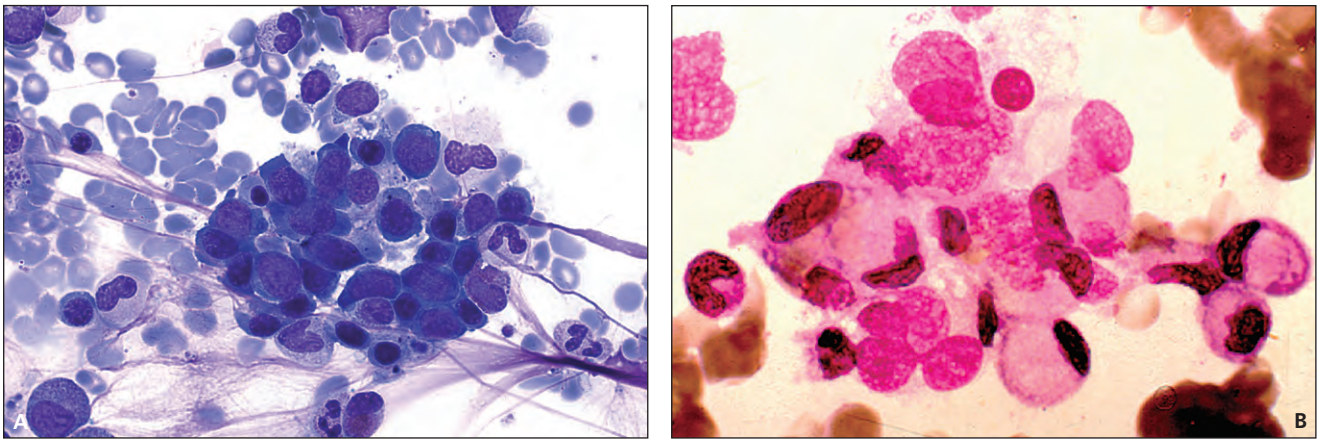


**Fig. 11.10** Granulomas and structures that resemble them; bone marrow aspirate. (A) In this hypocellular fibronecrotic material from a cachectic patient with severe anorexia is a nodule that appears at first sight to be similar to a granuloma or a tumour metastasis. Careful observation, however, discloses that it is a compact island of basophilic erythroblasts; at the periphery, one can recognise a lymphocyte and, at the top, a small blast cell, some granulocytes and various more mature erythroblasts. (B) Small granuloma showing the spongy nuclei of macrophages, sometimes multiple and apparently bare, and epithelioid cells of monocyte-macrophage origin, with hyperchromatic flattened nuclei at the periphery of a large vacuole that occupies the entire cytoplasm, giving a superficial resemblance to a signet-ring cell in a metastasis from adenocarcinoma; from a patient with miliary tuberculosis with bone marrow involvement diagnosed during engraftment of an autologous stem cell transplant.

also be recognised as isolated cells. The number, size and cellular composition of the granulomas can vary. There may be Langhans giant cells, in which the nuclei in general are peripherally placed, or foreign body giant cells, in which nuclei are dispersed throughout the cytoplasm.[17] Other cells in the granulomas can include neutrophils, eosinophils, lymphocytes, plasma cells and fibroblasts.

Granulomas can be a reaction to Hodgkin lymphoma, and are also characteristic of sarcoidosis and tuberculosis: numerous infective agents, however, have the capacity to induce granuloma formation, including not only mycobacteria but also fungi, histoplasma, toxoplasma, leishmania, brucella, *Salmonella typhi* and certain viruses; they can also result from macrophage activation of various origins including autoimmune disorders and drug reactions.[18] In patients with systemic lupus erythematosus, it is sometimes possible to recognise typical LE (lupus erythematosus) cells in the bone marrow (Fig. 11.14), these being neutrophils that have phagocytosed the degenerating nucleus of another cell; this phenomenon depends on the presence in the circulation of antinuclear antibodies.[19] In acute bacterial infections, the bone marrow may show granulocytic hyperplasia and the same changes that are seen in the peripheral blood (toxic granulation, Döhle bodies and cytoplasmic vacuoles).

In addition to the above features, the bone marrow aspirate may show the features of a haemophagocytic syndrome, also known as haemophagocytic lymphohistiocytosis, characterised by a state of activation of T cells, NK cells and macrophages, associated with high levels of cytokines (see also Chapters 1 and 2); this can be a feature of infection, autoimmune disorders and neoplasms, and can occur in patients with immune suppression or following organ transplantation.[20,21] The morphological picture is characterised by bone marrow hypocellularity with an increase of macrophages that show vigorous phagocytosis and contain the remains of haemopoietic cells; within the cytoplasm there are cells in various stages of degeneration, dark granules and detritus that is no longer recognisable (Figs 11.15 and 11.16; see also Figs. 1.71 and 1.72). The infective agents most often responsible are viruses (Epstein–Barr virus, cytomegalovirus, HIV, parvovirus B19), bacteria (*Mycobacterium tuberculosis*, brucella), parasites (particularly leishmania) or fungi. Among the neoplasms that have a specific relationship to haemophagocytic syndromes are T- and NK-cell non-Hodgkin lymphomas, particularly ALK-positive large cell anaplastic T-cell lymphoma derived from dysfunctional cytotoxic T cells that are liberating excessive amounts of cytokines (see Fig. 9.94). Haemophagocytosis is rare in other bacterial infections, while it is often seen with viruses, particularly herpesviruses. In viral infections, the bone marrow can also show the same changes that are recognised in the peripheral blood, with an increase in lymphocytes, generally with the morphology of atypical, reactive cells. Polyclonal lymphocyte aggregates[22] and an increase in polyclonal plasma cells with atypical morphology (see Chapter 10) are often seen in infections, in inflammatory states and in autoimmune disorders (Figs 11.17 and 11.18). In reactive plasmacytosis the plasma cells are usually less than 20%: from the morphological point of view, reactive plasma cells can be indistinguishable from clonal plasma cells.[23] In general, they are mature; it is not infrequent, however, for there to be some multinucleated cells with vacuoles, isolated inclusions (Russell bodies) or multiple inclusions (Mott cells).

In helminth infections, there can be a marked reduction or arrest of granulocyte maturation. An increase in mast cells can

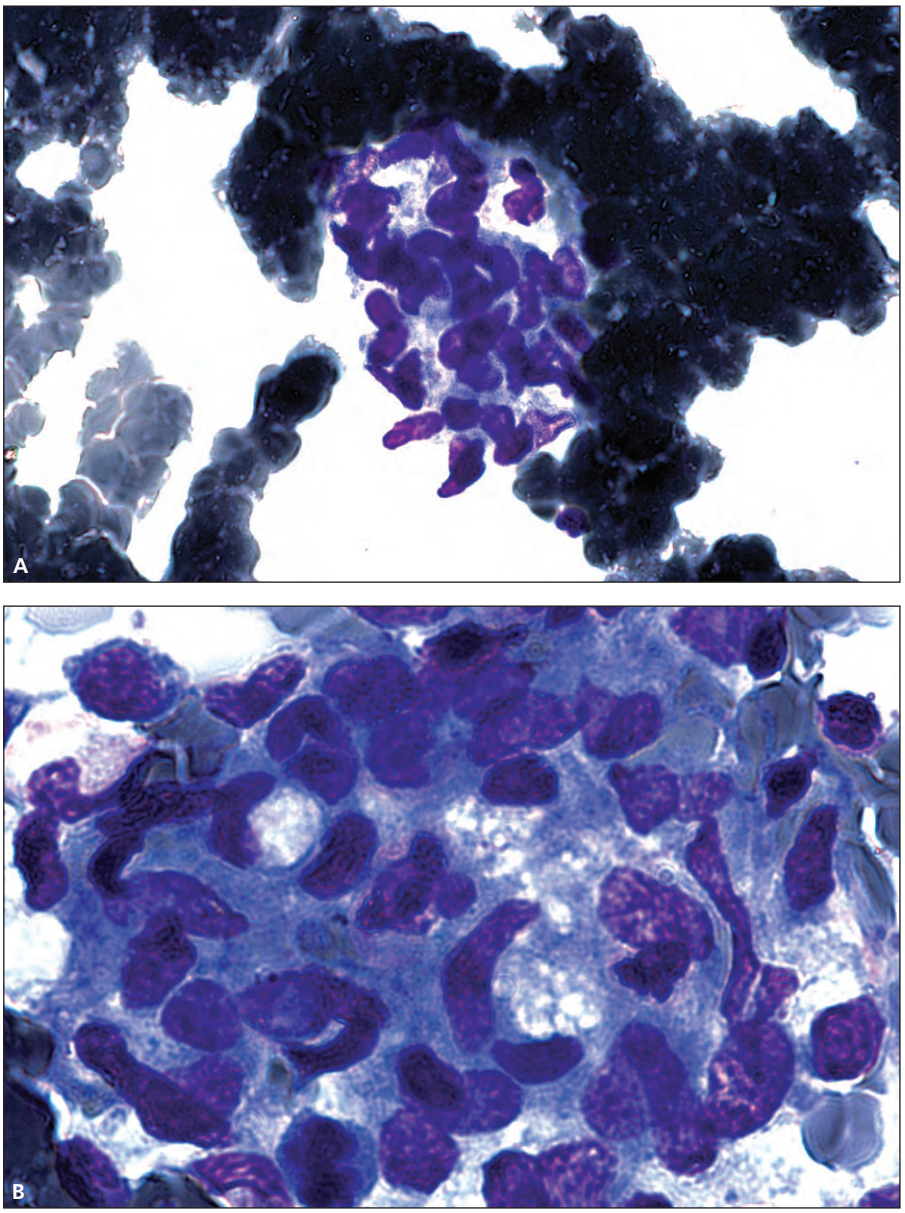



**Fig. 11.11** Granulomas and the differential diagnosis of metastases of solid tumours; bone marrow aspirate. (A) A compact granuloma formed exclusively of small macrophages with the morphology of epithelioid cells with scanty basophilic cytoplasm. Differentiating this appearance from a metastatic lesion can be difficult, but is suggested by the comma shape of the nuclei, by the compactness of the chromatin and by the cytological uniformity. (B) At a higher power, another granuloma in the same patient. In addition to the epithelioid cells with dark, indented nuclei one can note, on the right, the spongy nuclei of macrophages and, top left, a basophilic erythroblast.

occur in association with various infective and inflammatory conditions. Macrophages are generally increased: rarely bacteria are seen within macrophage cytoplasm, particularly in bacterial endocarditis. In leishmaniasis, the amastigotes (a term which means 'without a flagellum'), which are the intracellular forms found in vertebrate hosts, can be seen both within macrophages and free, the latter resulting from the rupture of macrophages[24] (Figs 11.19, 11.20 and 11.21). Amastigotes are most frequent in immunocompromised patients, in whom one can also sometimes observe *Histoplasma capsulatum* or fungi such as *Candida albicans*, aspergillus and *Cryptococcus neoformans*, both free and within macrophage cytoplasm (Figs 11.22 and 11.23). In a bone marrow aspirate, one may also sometimes recognise protozoans such as trypanosomes (Fig. 11.24) or toxoplasma (Fig. 11.25).[25] Malaria parasites are sometimes recognised in the bone marrow (see Fig. 2.52).

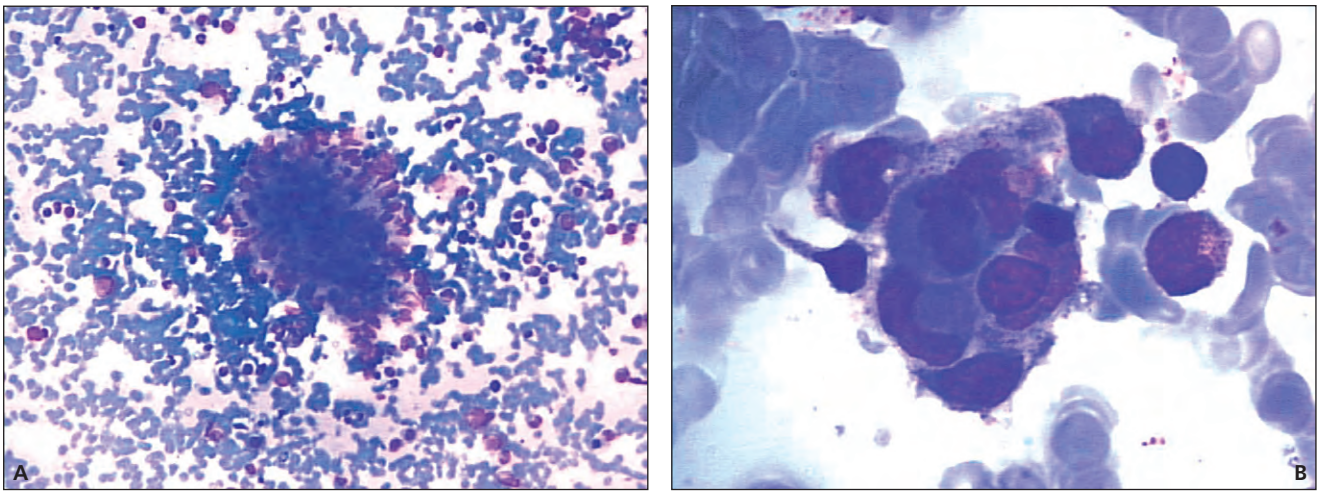



**Fig. 11.12** Granulomas; bone marrow aspirate from a patient with Hodgkin lymphoma (same case as Fig. 9.76). (A) At low power, there are cells radiating and thinning out from a central mass of crowded nuclei: this appearance requires immediate assessment at a higher power, which will show that one is dealing with a granuloma. (B) Small granuloma composed of heterogeneous cells: one can recognise some plasma cells and an epithelioid cell with an indented nucleus.

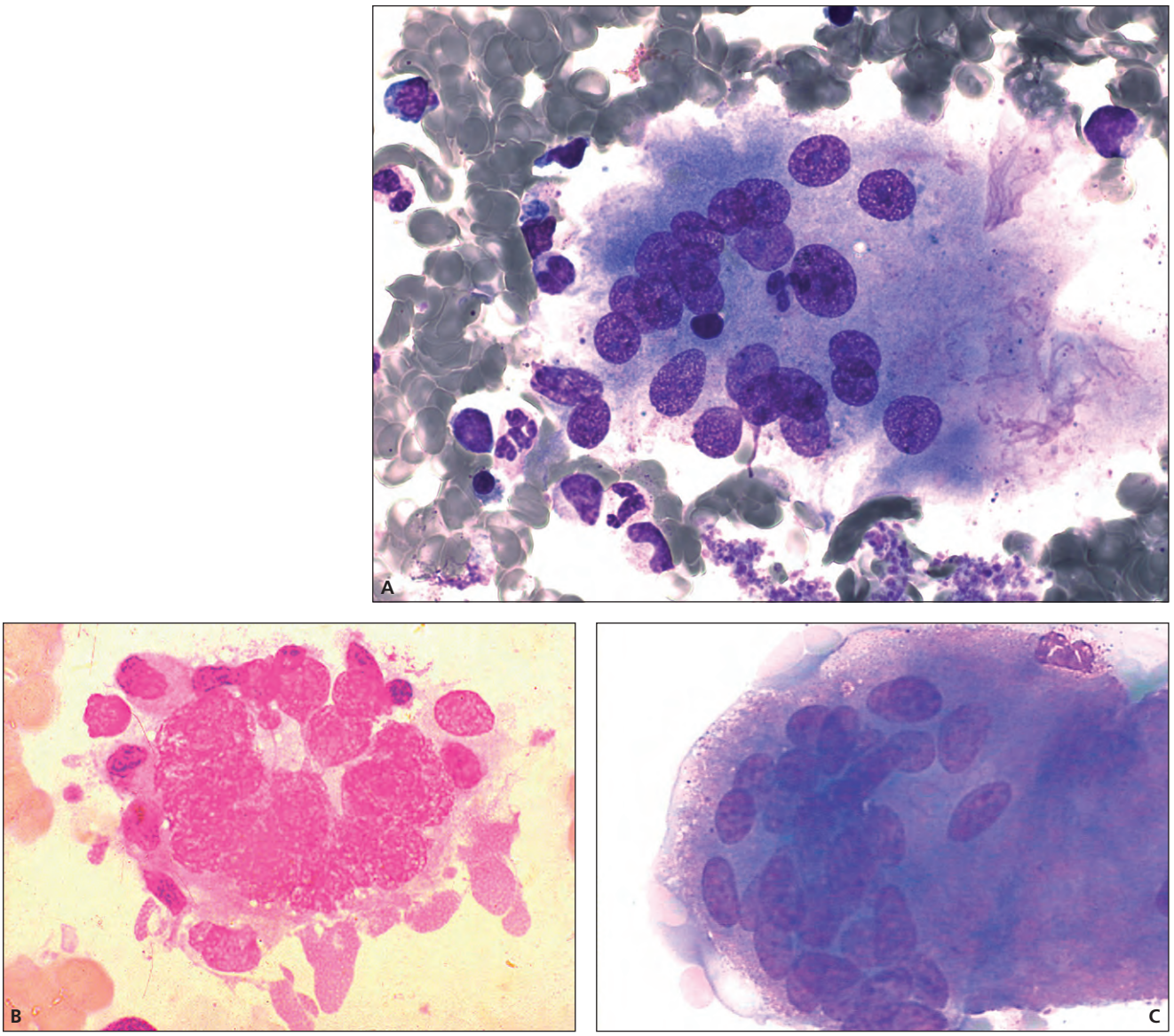



**Fig. 11.13** Giant cells; bone marrow aspirate. (A) Langhans type giant cell. The nuclei, ovoid and of different sizes, tend to crowd together at the periphery of the cell. They are often superimposed on each other, but the underlying nucleus can always be discerned: such transparency emphasises the delicacy of the chromatin network, which has an open spongy structure, similar to the nuclear structure of macrophages. This cell can be distinguished from an osteoclast, particularly because of the lack of cytoplasmic granules. (B) Giant multinucleated cell surrounded by epithelioid cells in a granulomatous formation; same case as Fig. 11.10B. (C) A giant multinucleated cell, similar to that in (A) in the bone marrow aspirate of a patient with bone marrow metastasis from carcinoma of the breast.

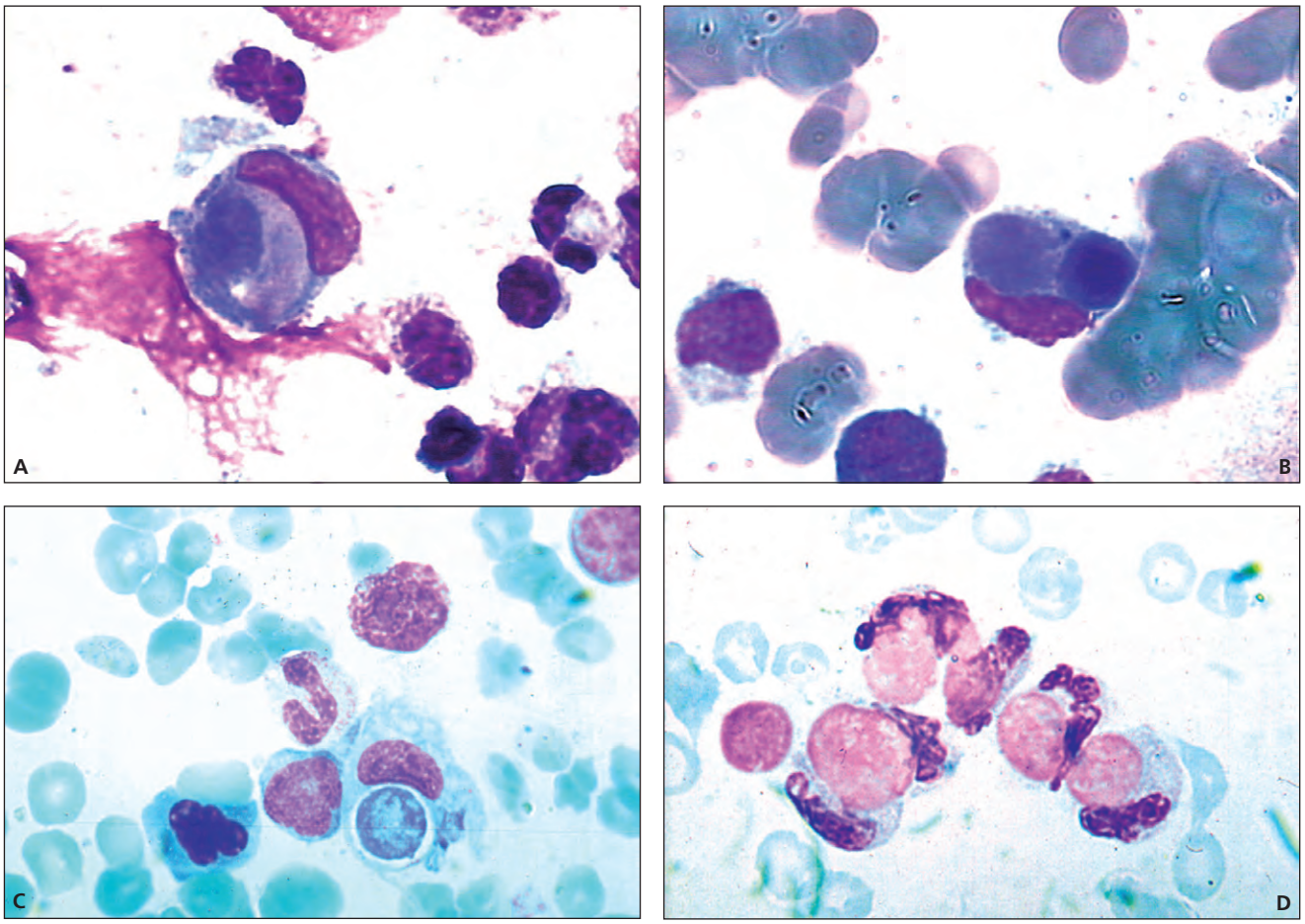



**Fig. 11.14** LE (lupus erythematosus) cells and cells resembling them; bone marrow aspirates. (A–C) Cells resembling LE cells observed incidentally in bone marrow aspirates of patients with different conditions: the typical appearance of an LE cell formed *in vivo* is of a mononuclear cell (monocyte or macrophage) in which the nucleus is flattened at the periphery of the cytoplasm, which contains one or more nuclei of other cells in various stages of degeneration. (D) A positive result of a LE cell test on the peripheral blood of a patient with systemic lupus erythematosus. True LE cells are neutrophil granulocytes that have phagocytosed *in vitro* the nuclei of other buffy coat cells subjected to microtraumatisation and incubated for 30 minutes at 37 °C.

Erythropoiesis shows various types of alteration in severe bacterial infections; in the presence of haemolytic anaemia, there is erythroid hyperplasia; in pure erythroid hypoplasia, erythropoiesis is reduced with maturation arrest at the level of the proerythroblast.

In infective states, megakaryocytes can be normal or increased; when there is peripheral consumption of platelets, they are in general greatly increased. Chronic inflammation is usually associated with an increase in storage iron.

## Bone marrow metastases of non-haematological neoplasms

The first observation of the presence of cancer cells in the bone marrow dates back to 1834[26]; the first systematic study in 75 patients with tumours, published 100 years later, reported that 11 out of 13 patients with bone metastases had bone marrow involvement and they had a worse clinical course than other patients.[27] Since then, bone marrow examination has become an essential investigation in patients with suspected or confirmed cancer, since the skeleton, particularly the axial skeleton and the pelvis, is one of the most frequently involved metastatic sites. The localisation of metastases in the skeleton reflects both the distribution of red marrow and the role of the venous system, in particular retrograde flow in the vertebral plexus of Batson, in transporting metastatic cells towards bones. Pelvic bones and thoracic and lumbar vertebrae represent 70% of all metastatic sites.

The metastasis of a tumour from its primary site is the principal cause of death from cancer, which in turn is the second most common cause of death in western countries. Metastases

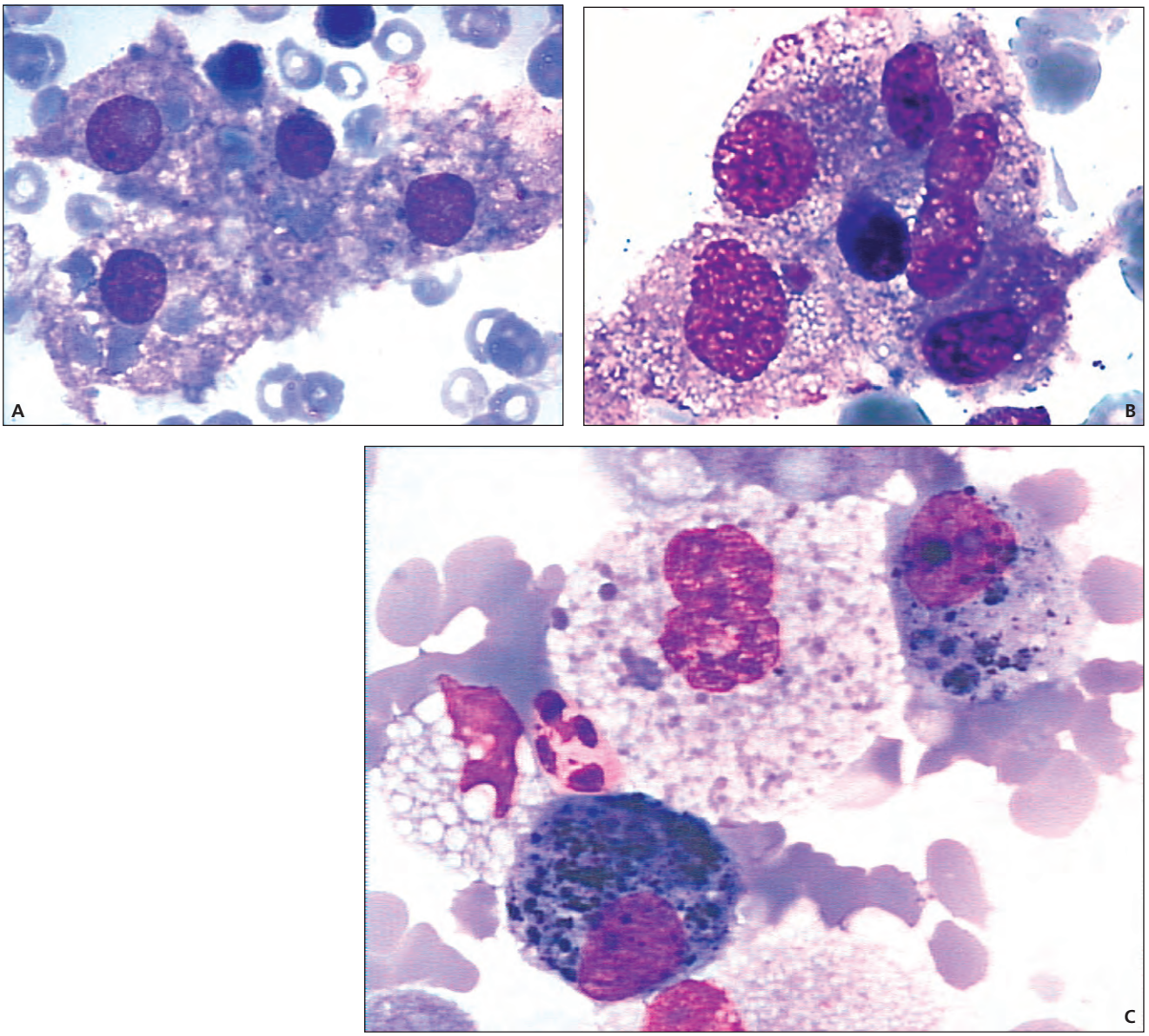



**Fig. 11.15** Activated macrophages in a haemophagocytic syndrome; bone marrow aspirate. (A) Erythrophagocytosis: three macrophages joined in a syncytial formation with vacuolated cytoplasm, which contains violet granules and numerous well-preserved red cells. (B) Syncytium of macrophages with cytoplasm perforated by vacuoles, surrounding a small plasma cell. (C) The largest macrophage appears to be binucleated; the smallest one is foamy, with its cytoplasm full of globular, optically empty vacuoles of very variable size. The other two have small eccentric nuclei and cytoplasm packed with blackish debris: cells of this type resemble the melanocytes of metastatic malignant melanoma and must be differentiated from them on the basis of clinical context and cytological details.

represent the most frequent type of bone tumour, but their true incidence in different types of neoplasm is difficult to estimate. The dissemination of tumour cells is obviously a prerequisite for the formation of metastases, but the two processes are not synonymous: in fact, less than 1% of tumour cells that enter the circulation lead to the establishment of metastases.[28,29] it is evident that there are specific biological characteristics (gene-expression signatures) that permit only some of the tumour cells that enter the circulation to establish metastatic lesions: among the important properties are the ability of the cell to evade the molecular mechanisms of apoptosis or programmed cell death, induced by detachment of the cell from integrins (anoikis), which leads to inadequate or inappropriate interaction with the cellular matrix and the loss of cytoskeletal architecture (amorphosis).[30] The occurrence of bony metastases is related to favourable features of the microenvironment and to its vascularity. This is a complex process that requires a sequence or cascade of events that can be summarised as follows:

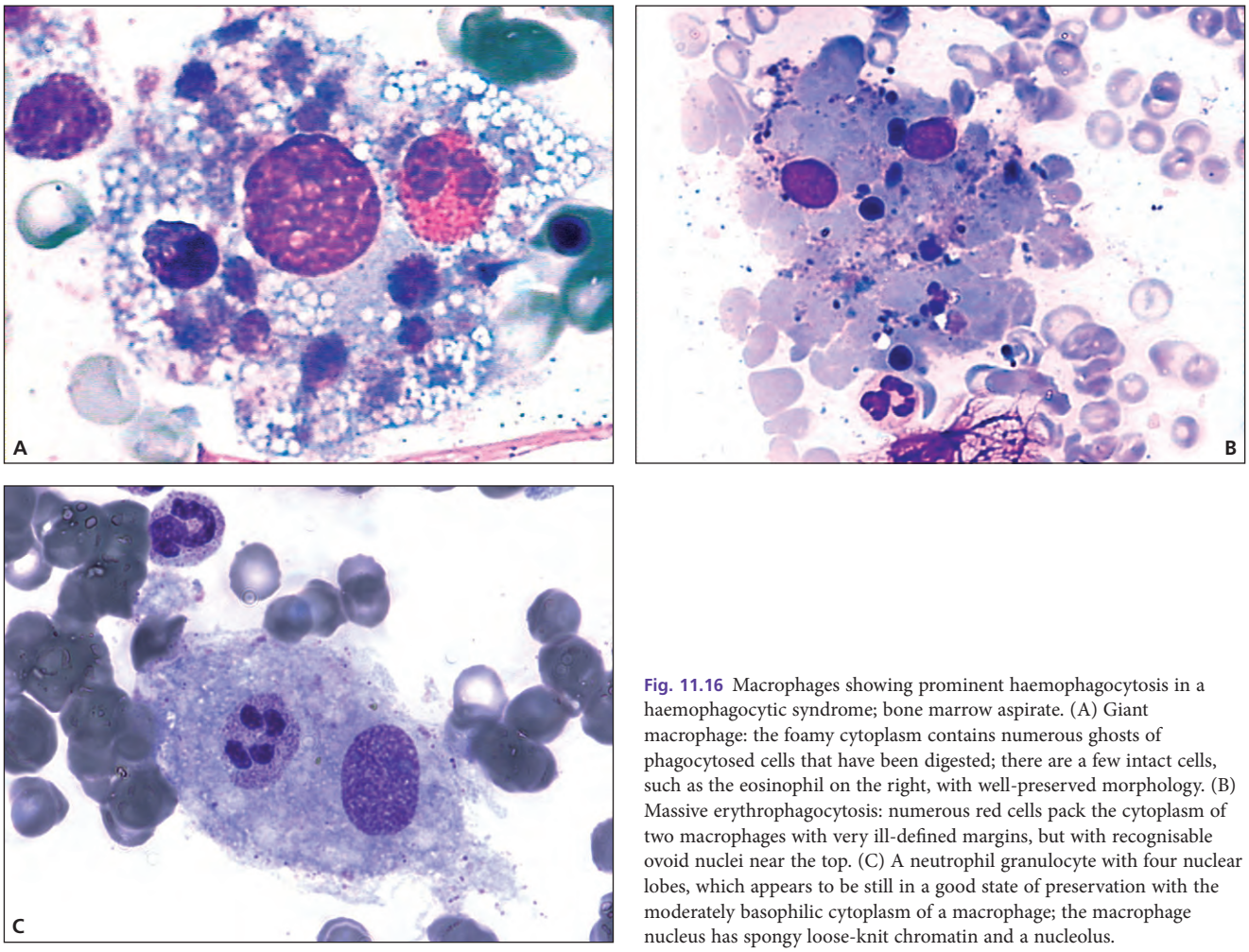



**Fig. 11.16** Macrophages showing prominent haemophagocytosis in a haemophagocytic syndrome; bone marrow aspirate. (A) Giant macrophage: the foamy cytoplasm contains numerous ghosts of phagocytosed cells that have been digested; there are a few intact cells, such as the eosinophil on the right, with well-preserved morphology. (B) Massive erythrophagocytosis: numerous red cells pack the cytoplasm of two macrophages with very ill-defined margins, but with recognisable ovoid nuclei near the top. (C) A neutrophil granulocyte with four nuclear lobes, which appears to be still in a good state of preservation with the moderately basophilic cytoplasm of a macrophage; the macrophage nucleus has spongy loose-knit chromatin and a nucleolus.

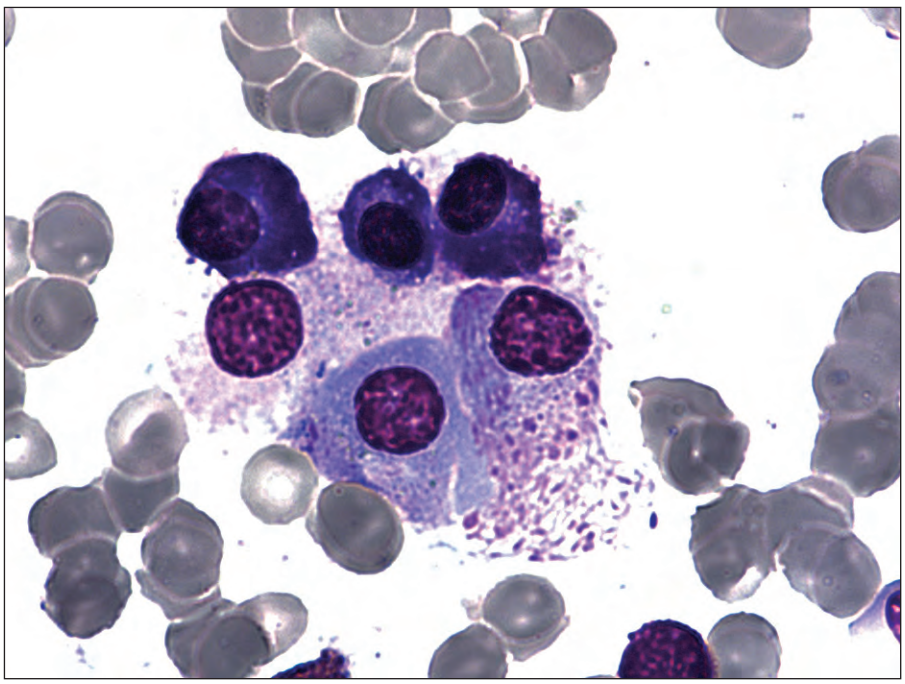
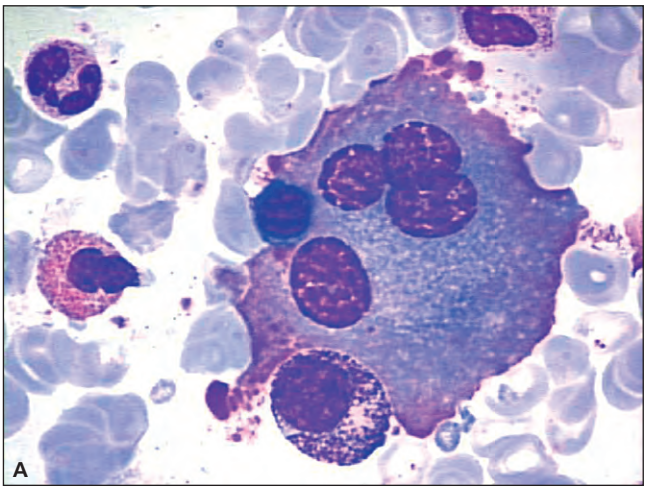
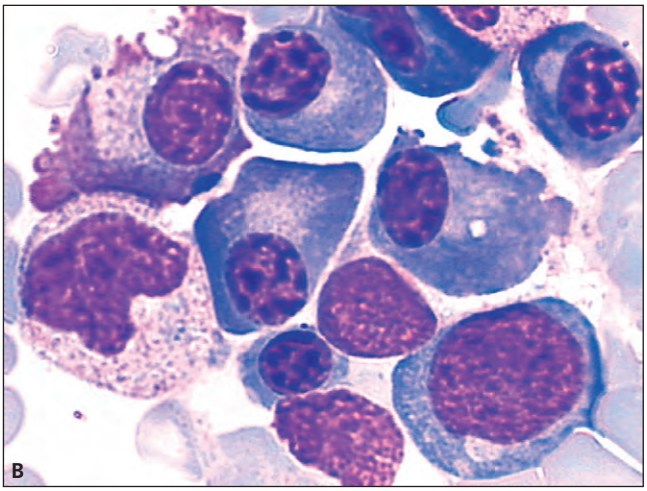


**Fig. 11.17** Morphology of plasma cells in reactive conditions; bone marrow aspirate. At the top, three plasma cells with cytoplasm that is so intensely basophilic that it almost hides the eccentric nucleus. At the bottom, a plasma cell with more ample, pale, distended cytoplasm. On the right, a flaming plasma cell.



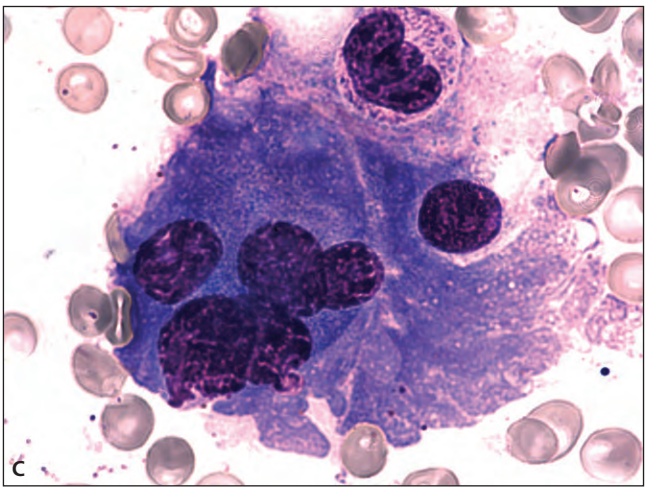
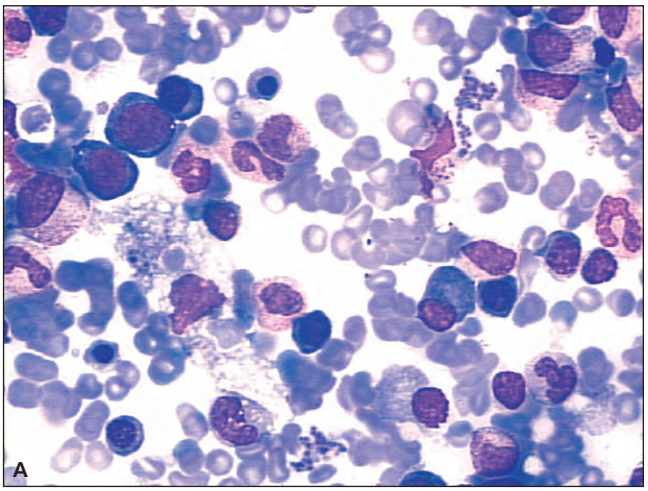
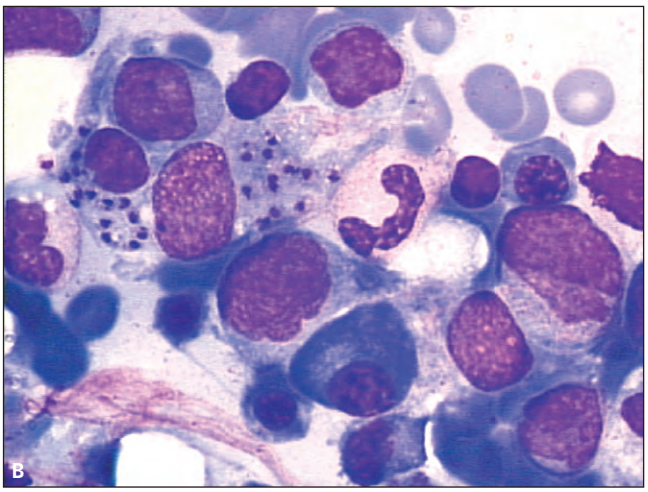




**Fig. 11.18** Morphology of plasma cells in reactive conditions; bone marrow aspirate. (A) A giant multinucleated plasma cell with a flaming cytoplasmic margin; from a patient with Hodgkin lymphoma (same case as Fig. 11.12). (B) A nest of mature plasma cells from a patient with visceral leishmaniasis. At the bottom: on the right, a proerythroblast; in the centre, a small polychromatic erythroblast; on the left, a neutrophil metamyelocyte. (C) Giant multinucleated plasma cell from a patient with metastatic carcinoma of the pancreas, with a hypocellular marrow without any evidence of infiltration. The typical morphology of the nuclei, with chromatin condensed in blocks, avoids any confusion with neoplastic cells.



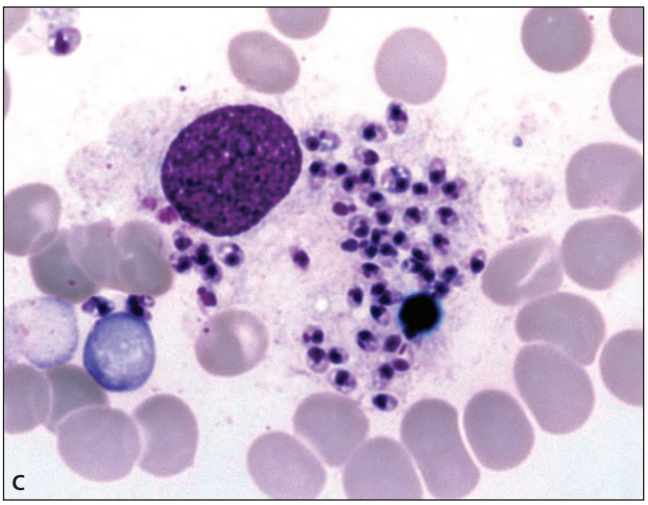




**Fig. 11.19** Leishmaniasis; bone marrow aspirate. (A) The bone marrow is cellular with notable reactive changes. There are some monocytes (at the bottom), a macrophage showing erythrophagocytosis and some plasma cells, together with normal erythroblasts and granulocytes. In febrile patients with reactive marrow changes, it is always necessary to carry out a prolonged search to be sure of not missing leishmaniae. (B) At higher power, the macrophage top left has cytoplasm containing numerous amastigotes. (C) The features of intracellular leishmaniae are readily recognisable because of their preferential localisation within macrophages and their distinctive morphology, that of small organisms with oval cytoplasm and a hyperchromatic, eccentric nucleus: as well as the nucleus, the cytoplasm of leishmaniae has a small rod-shaped paranuclear body, the kinetoplast.

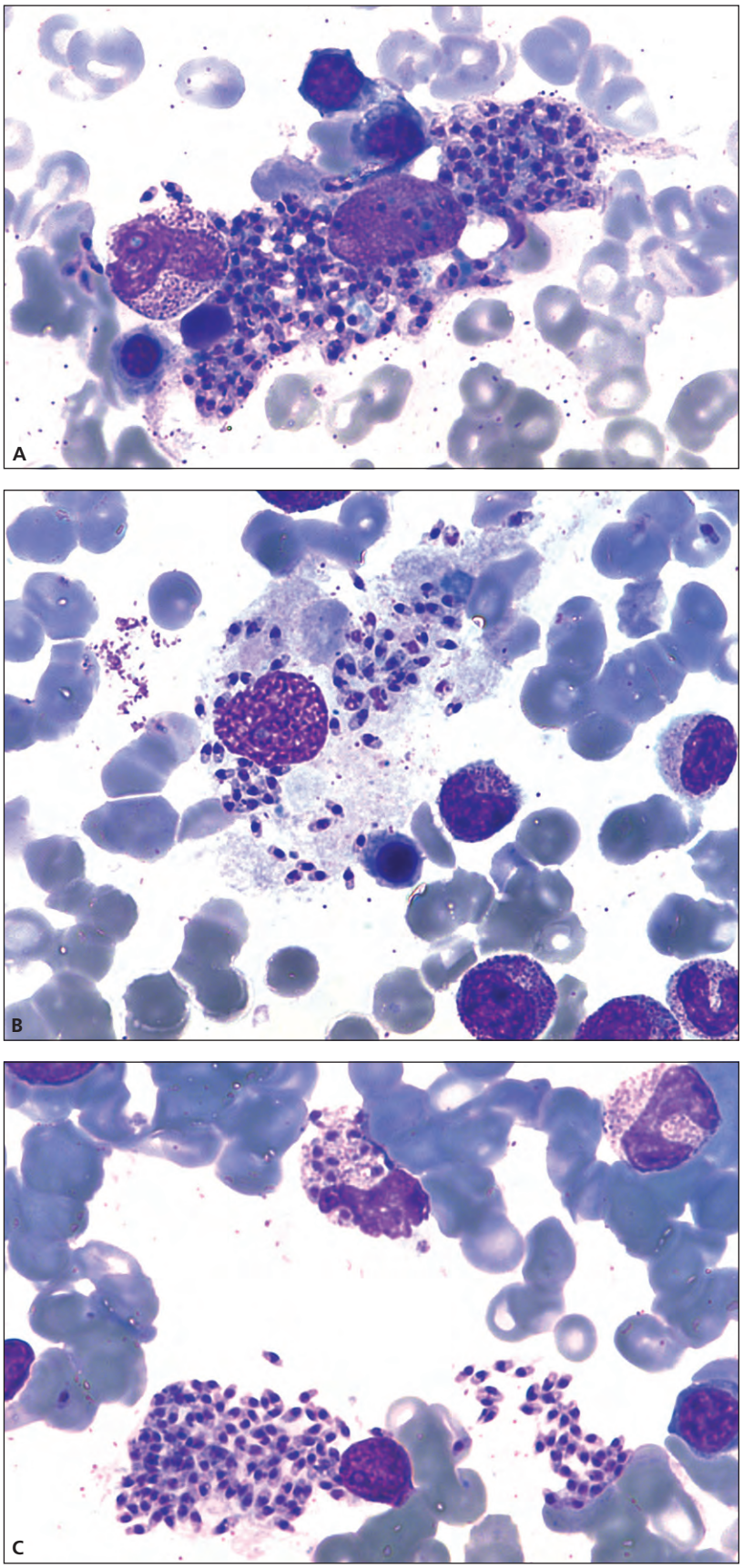



**Fig. 11.20** Leishmaniasis in an HIV-positive patient; bone marrow aspirate. (A) The double dot appearance, attributable to the nucleus and the small kinetoplast, both hyperchromatic but differing in size and orientation, is not always easily appreciated, but is fundamental for the diagnosis of leishmaniasis, because it permits this parasite to be distinguished from non-specific cellular detritus. In immunosuppressed patients, the cytoplasm of macrophages may be packed with parasites, which can also be found free in the background of the films as a result of rupture of the cells that contained them. (B) Macrophage with cytoplasm packed with parasites and one phagocytosed erythrocyte. (C) At the bottom, a parasitised macrophage. At the top, a neutrophil with a degenerating nucleus and a number of parasites in the cytoplasm.

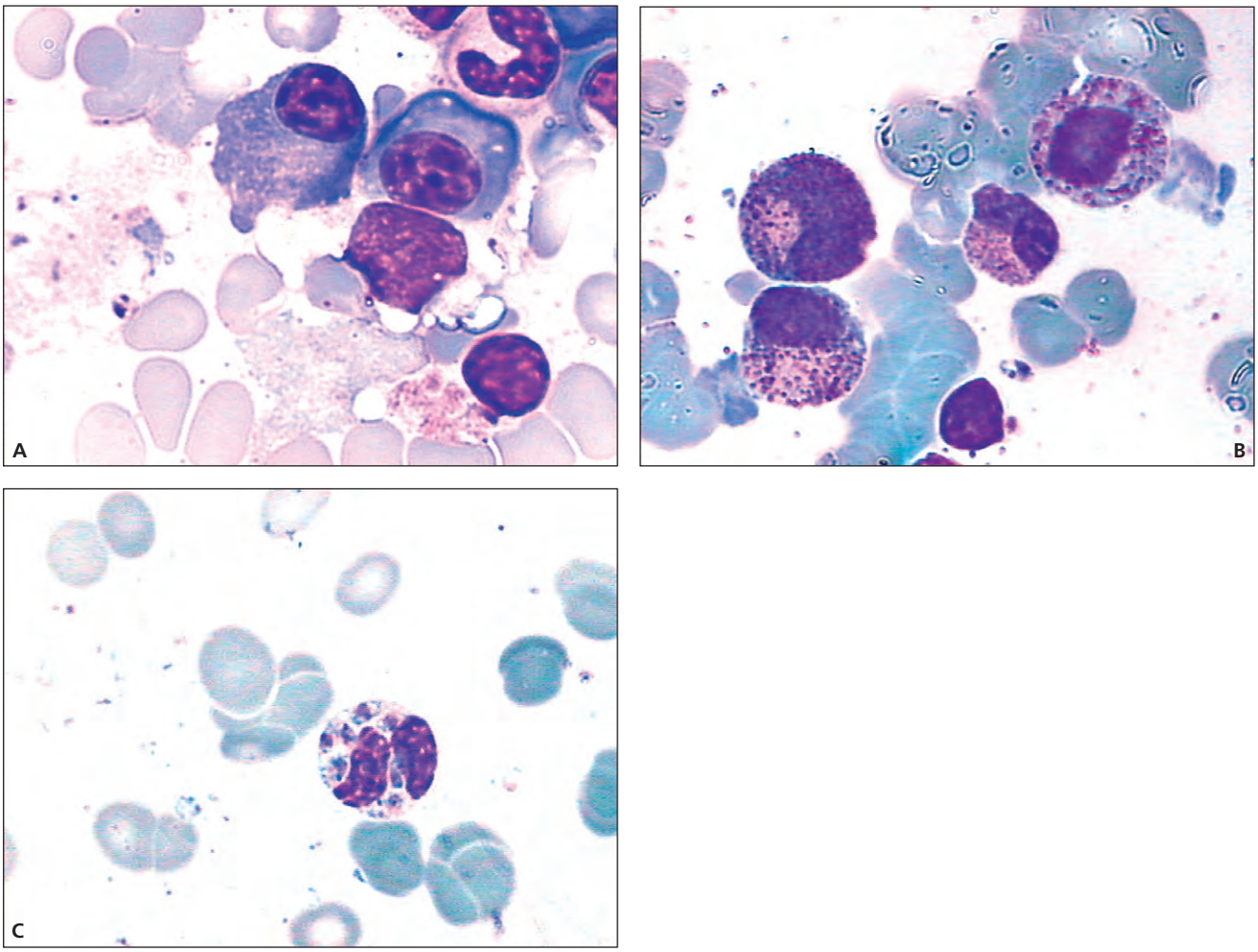



**Fig. 11.21** Leishmaniasis, specific features; bone marrow aspirate. (A) On the left, not far from two plasma cells, another free, extracellular leishmania. (B) In addition to various granulocyte precursors, an isolated extracellular leishmania, recognisable from the double dot appearance of the nucleus and kinetoplast. The finding of elements such as this necessitates the search for more parasites by examining as many bone marrow macrophages as possible. (C) A bilobed neutrophil granulocyte containing a number of leishmaniae. The observation of these parasites within neutrophils is not common.

1. Tumour proliferation;
2. Neoangiogenesis and detachment of tumour cells from the primary site;
3. Release of tumour cell into blood vessels with the resultant embolisation of such cells;
4. Migration and adhesion to the endothelial cells of capillaries in bones at sites distant from the primary tumour;
5. Spread outside the capillaries;
6. Homing to the microenvironment of bone;
7. Growth and colonisation.[31]

Once they have invaded the bony matrix, the tumour cells produce growth factors that can induce, directly or indirectly, the remodelling of bone; there is activation of osteoclasts, osteoblasts or both, with loss of the normal homeostatic mechanisms that operate in the matrix of bone. The release of growth factors by bone cells promotes the survival and proliferation of the tumour cells: there is thus a vicious circle of destruction—bone remodelling—tumour growth. Osteolytic metastases, in which osteoclast activity prevails, are much more frequent than osteoblastic metastases. The process described has some analogies with the physiological mechanisms of migration, homing and regeneration of haemopoietic stem cells: the tumour cells use chemotactic stimuli that act on stem cells to migrate into niches of the bone marrow microenvironment, where they establish the reciprocal relationship with stromal cells that is the basis of this process.[32] This cascade is supplemented by further mechanisms that originate in macrophages and other myeloid cells, designated tumour-associated macrophages and myeloid-derived suppressor cells. Such cells show a progressive accumulation in the interstices between the tumour cells and promote the formation of an inflammatory type of microenvironment that is very favourable to the growth and spread of tumour cells

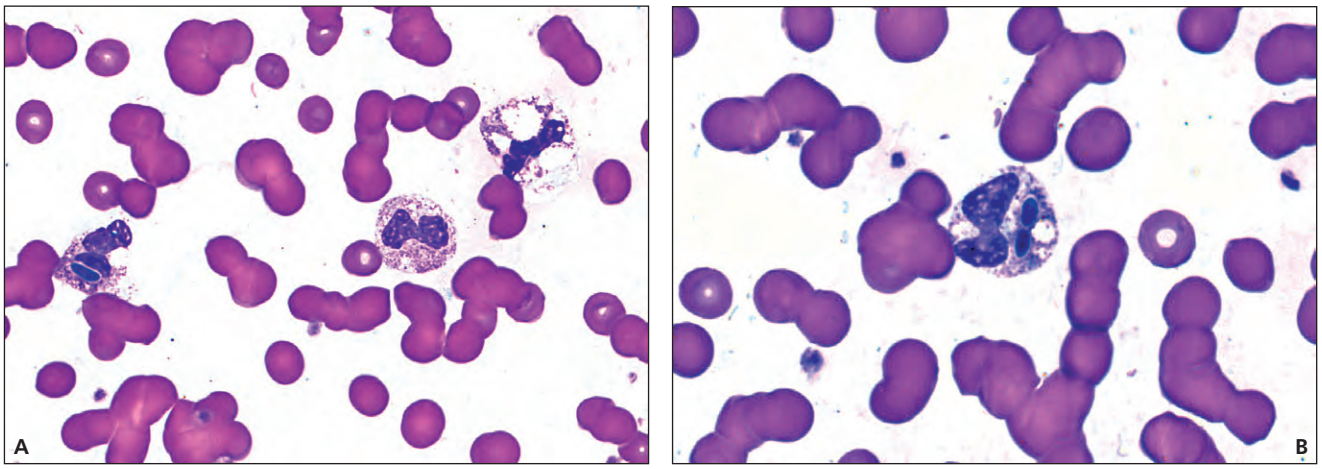



**Fig. 11.22** (A and B) Fungal spores in neutrophil granulocytes in the hypoplastic bone marrow aspirate of a patient who has had a haemopoietic stem cell transplant. One notes cytoplasmic vacuoles in some of the neutrophils, increased rouleaux formation and increased background staining indicating dysproteinaemia.

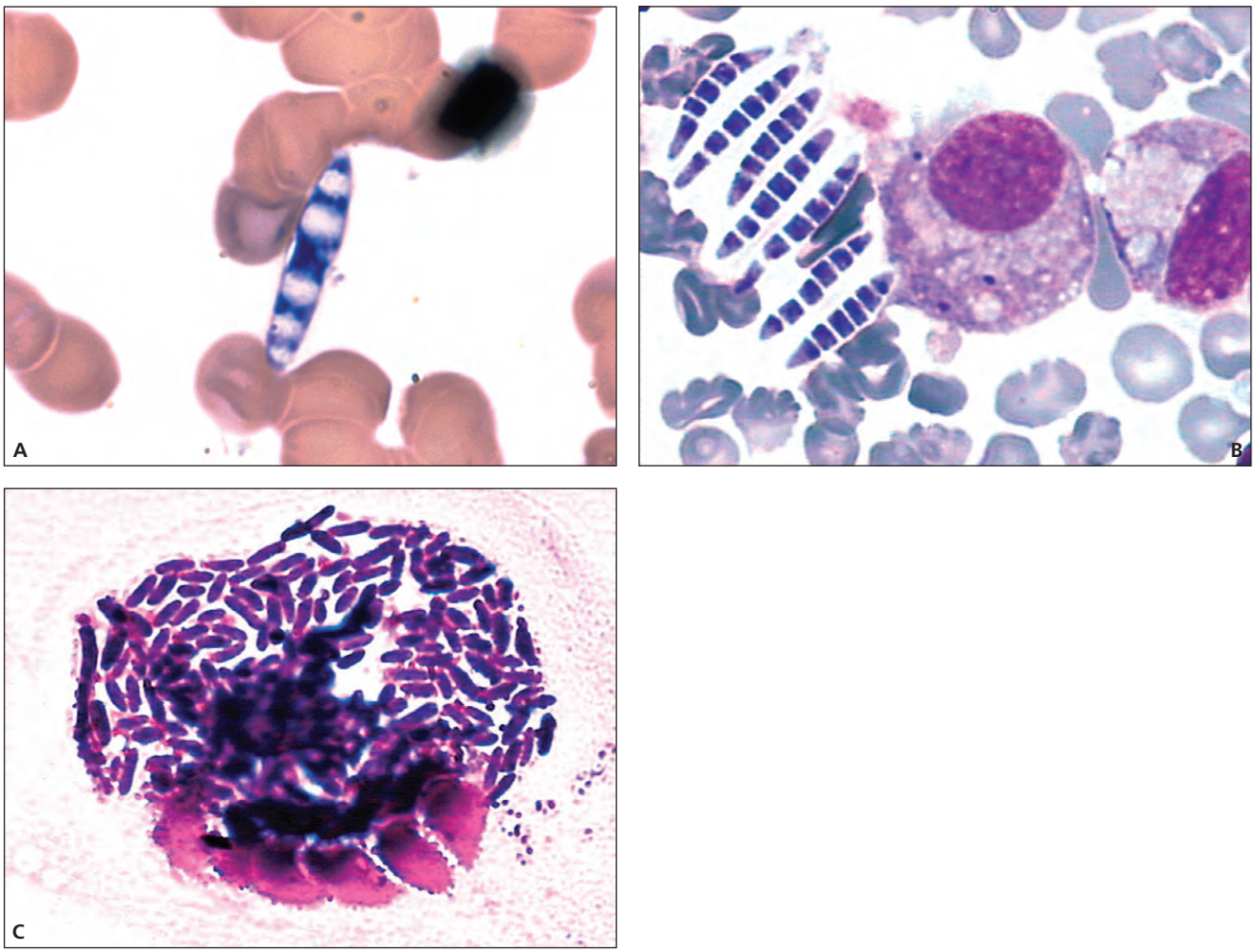



**Fig. 11.23** Fungi in haematological preparations. (A) Aspergillus in the peripheral blood of a patient with nasopharyngeal carcinoma. (B) Fusarium spores, probably an exogenous contaminant, in a bone marrow film. (C) Spores of unknown origin in the torn cytoplasm of a cell in a bone marrow aspirate.

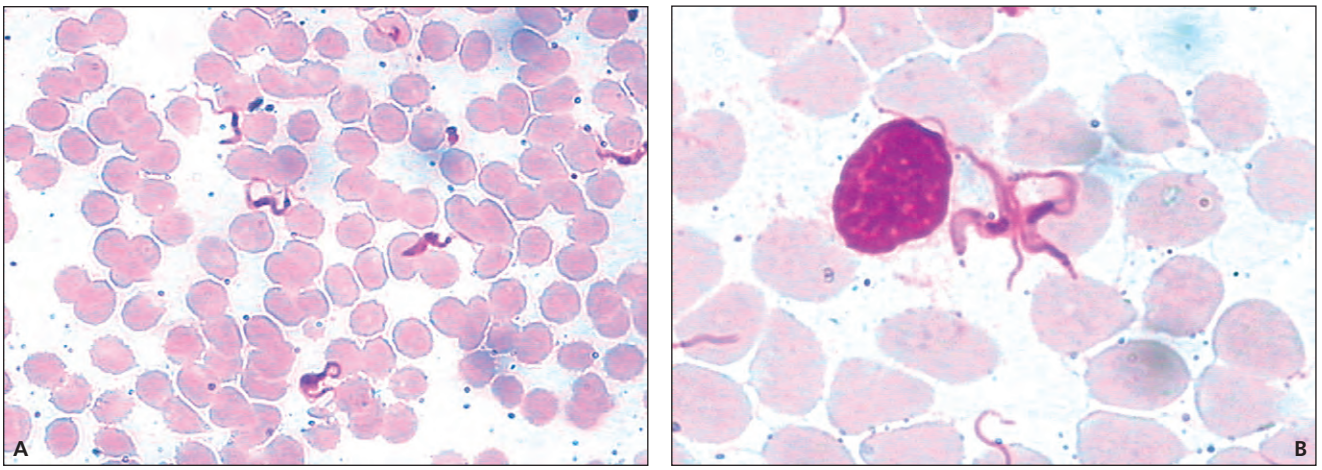



**Fig. 11.24** (A and B) *Trypanosoma gambiense*; peripheral blood film. Trypanosomes are thin and pointed, shaped like a comma or a snake, with a small amount of pink cytoplasm that contains some azurophilic granules. The nuclei appear like small oblong hyperchromatic bodies in a central position. The kinetoplast can be seen in some parasites at one of the extremities. In bone marrow aspirates, trypanosomes are seen mainly within macrophages.

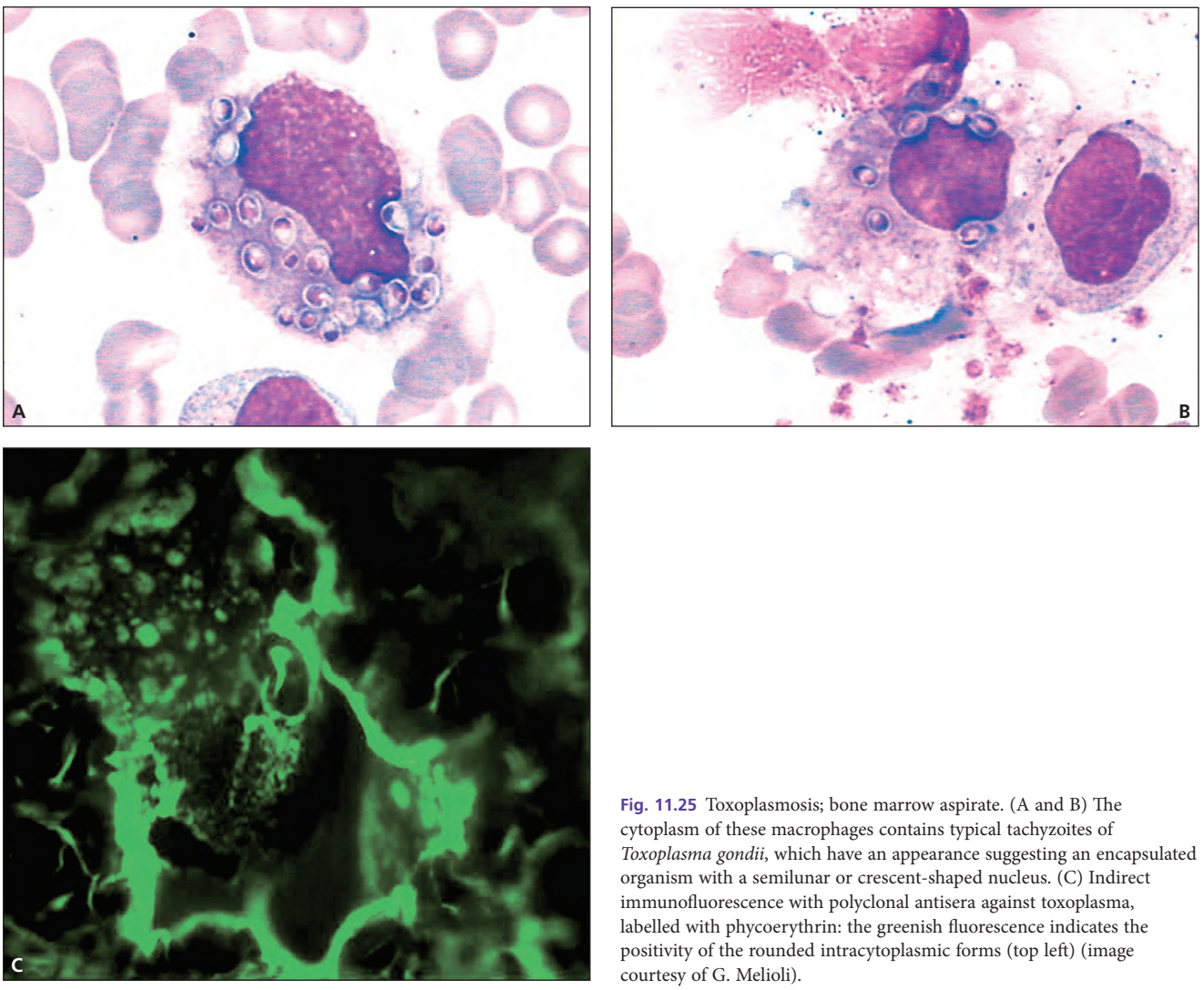



**Fig. 11.25** Toxoplasmosis; bone marrow aspirate. (A and B) The cytoplasm of these macrophages contains typical tachyzoites of *Toxoplasma gondii*, which have an appearance suggesting an encapsulated organism with a semilunar or crescent-shaped nucleus. (C) Indirect immunofluorescence with polyclonal antisera against toxoplasma, labelled with phycoerythrin: the greenish fluorescence indicates the positivity of the rounded intracytoplasmic forms (top left) (image courtesy of G. Melioli).

through mechanisms such as immunosuppression, neovascularisation and resistance to antitumour therapy.[33,34]

Not all solid tumours show the same capacity to produce bone metastases: around 80% of such metastases originate in tumours of the breast, prostate, lung, kidneys and thyroid; conversely, gliomas and basal cell carcinomas of the skin, although being highly invasive, do not tend to form metastases. Among the various components of the skeleton, the bone marrow is the site of localisation of blood-borne metastases of non-haematological tumours with an incidence between 10% and 50% in different series.[35,36] Any type of non-haematological tumour capable of metastasising can infiltrate the bone marrow: those that most often do so in adults are, in order of frequency, carcinomas of the prostate, breast, lung, thyroid, kidneys and gastrointestinal tract, and melanoma; in the paediatric age range there is a prevalence of neuroblastoma, rhabdomyosarcoma, Ewing tumour, retinoblastoma and osteosarcoma.[36–38]

In the literature from the 1970s and 1980s, numerous studies compared the diagnostic sensitivity of a bone marrow aspirate and a trephine biopsy. In an analysis based on post-mortem aspiration and needle sampling (equivalent to a biopsy), in comparison with autopsy findings, the sensitivity was respectively 28% and 45%.[39] The diagnostic superiority of a trephine biopsy was confirmed in almost all studies, but with very variable sensitivity depending not only on the competence of the morphologist but also on the number of preparations examined and the degree of tumour infiltration; for example:

- 44% of positive aspirate results in comparison with 79% of positive results by trephine biopsy in a series of 22 cases of carcinoma of the breast[40];
- 92% of positive aspirate results out of 39 positive biopsies performed simultaneously[41];
- A sensitivity of 72% for aspiration and 92% for needle biopsy in a retrospective study[42];
- The same diagnostic sensitivity[43];
- 75% concordance between aspiration and needle biopsy in an extensive series of simultaneous evaluations.[44]

In all the published studies, and also in our personal experience of 104 patients with suspected bone marrow metastases in whom simultaneous bone marrow aspiration and trephine biopsy were performed, there are always a small number of false-negative trephine biopsies in patients in whom the aspirate shows metastatic cells. In addition, adenocarcinoma cells can be observed unexpectedly in a bone marrow aspirate obtained for another indication in a patient not known to have carcinoma. For these reasons, the two procedures should be considered as complementary[43,45,46] and both should be performed when a solid tumour is being staged.

In between 18% and 49% of patients, depending on the selection criteria in case series and the techniques used, bone marrow infiltration represents the first sign of neoplasia, while in 15–50% of patients the recognition of bone marrow metastases occurs at the same time, or subsequent to, the diagnosis of the primary neoplasm . As a result, it is important that even in the absence of any specific suspicion, bone marrow films are carefully searched for metastatic cells, in a systematic manner and examining an adequate number of slides.[47,48]

## Peripheral blood changes

The invasion of the bone marrow by metastatic cells can cause a profound alteration in haemopoiesis, with suppression of haemopoiesis being a fundamental change. Erythropoiesis and thrombopoiesis tend to be more compromised than granulopoiesis, but peripheral cytopenia can be trilineage, of various degrees of severity.[49] There is almost always a normocytic normochromic anaemia of the type that is associated with chronic disease; the presence of bone marrow metastases has also been noted to be associated with an increased red cell distribution width (RDW).[50] Bone marrow involvement can be suspected when there is a leucoerythroblastic picture, i.e. the presence in the peripheral blood of erythrocytes and granulocyte precursors, often associated with schistocytes (Fig. 11.26 and 11.27). A leucoerythroblastic blood film, a highly predictive abnormality, is observed in around 50% of patients with bone marrow metastases of carcinoma.[51] These alterations in peripheral blood correlate with reactive bone marrow fibrosis induced by the tumour, rather than with the presence of metastases *per se*.[52] The combination of a leucoerythroblastic picture, an increase of lactate dehydrogenase (LDH) to above 500 IU/l, a platelet count below $100 \times 10^9$/l and bone pain has been reported to be highly predictive of bone marrow metastases; further features strengthening this suspicion are an increase in serum alkaline phosphatase, hypercalcaemia and leucopenia.[53,54] These manifestations are related to the destruction and local replacement of the haemopoietic marrow and the development of fibrosis, which are recognised with a different frequency in different primary tumours: anaemia is the most frequent and least specific abnormality; other cytopenias, also, can have multiple origins. The presence of intracellular melanin has been described in metastatic melanoma. The identification of mucin, either intracellular in neutrophils or extracellular, has been reported in Wilms tumour and in carcinoma of the breast and the ovary; when it is abundant, it can also interfere with automated cell counts.

Tumours can cause paraneoplastic syndromes resulting from the secretion of cytokines: among those that involve the haemopoietic system are thrombosis, which is often associated with carcinoma of the pancreas, disseminated intravascular coagulation associated with carcinoma of the stomach, microangiopathic haemolytic anaemia associated with carcinoma of the prostate, polycythaemia associated with renal cell carcinoma and marked leucocytosis and neutrophilia associated with small cell carcinoma of the lung. These associations are neither absolute nor specific, being recognised also in a variety of other neoplasms of various types. The recognition of tumour cells in the blood film, carcinocythaemia, is fairly rare, occurring at higher frequency in small cell tumours of children (Figs 11.28 and 11.29; see Report 11.3).[55–59] Carcinocythaemia is an important phenomenon to recognise, since it can be confused with

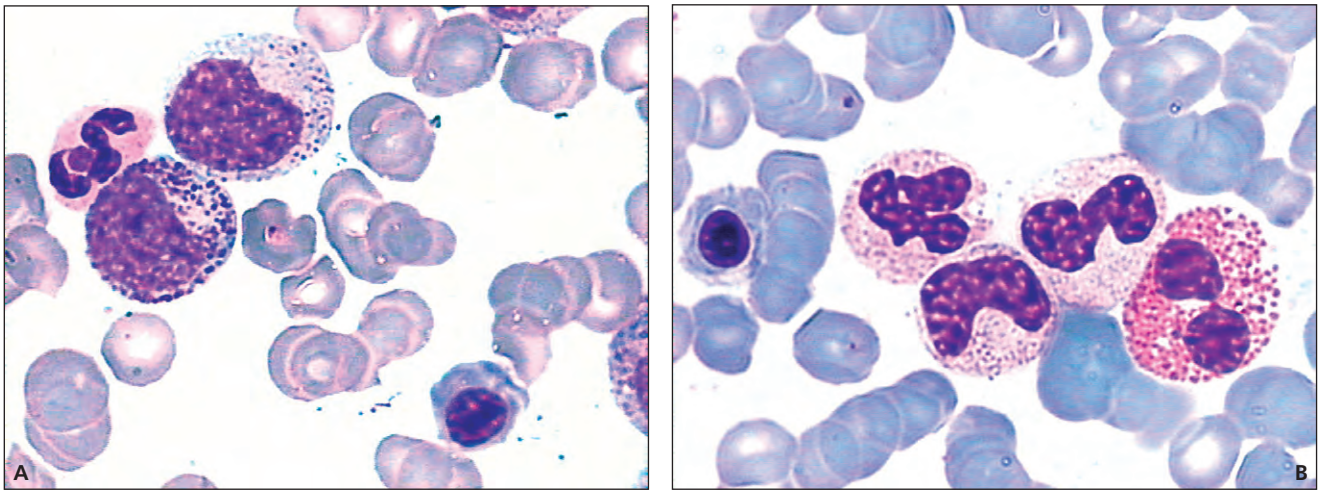



**Fig. 11.26** Leucoerythroblastic picture; peripheral blood film. (A) A neutrophil, two myelocytes differing in granularity and a polychromatic erythroblast in the film of a patient with bone marrow metastases from carcinoma of the prostate. (B) From the same patient, an almost orthochromatic erythroblast, a segmented neutrophil, a small metamyelocyte, a band neutrophil and a mature eosinophil.

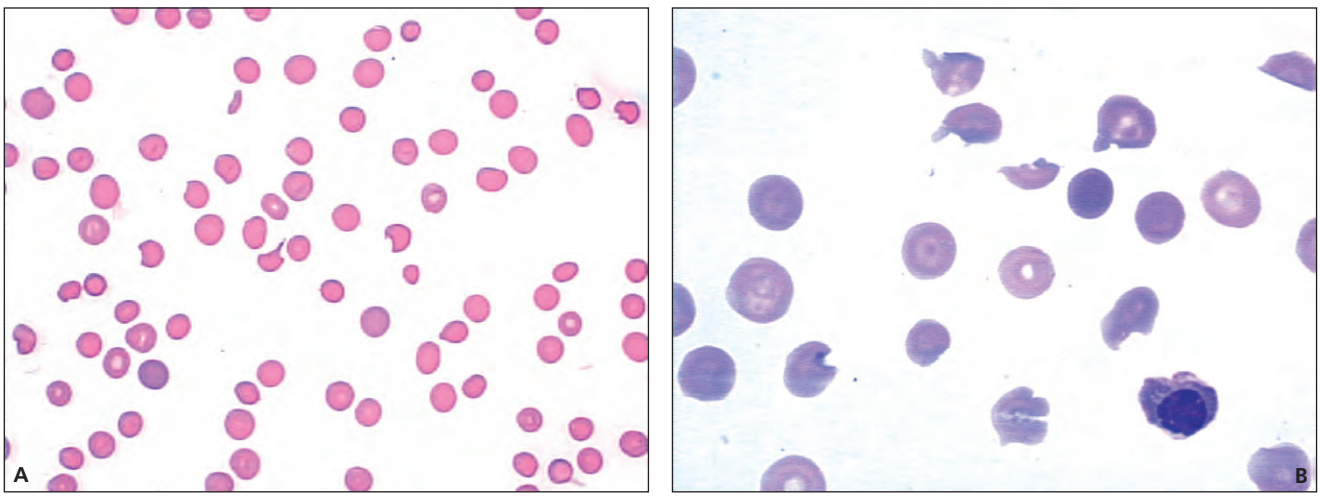



**Fig. 11.27** Microangiopathic haemolytic anaemia in patients with metastatic malignancies; peripheral blood films. (A) Schistocytes and polychromatic erythrocytes from a patient with carcinoma of the uterine cervix (same case as Fig. 11.32). (B) Schistocytes, microspherocytes and an orthochromatic erythroblast (same patient with gastric carcinoma as Fig. 11.41).

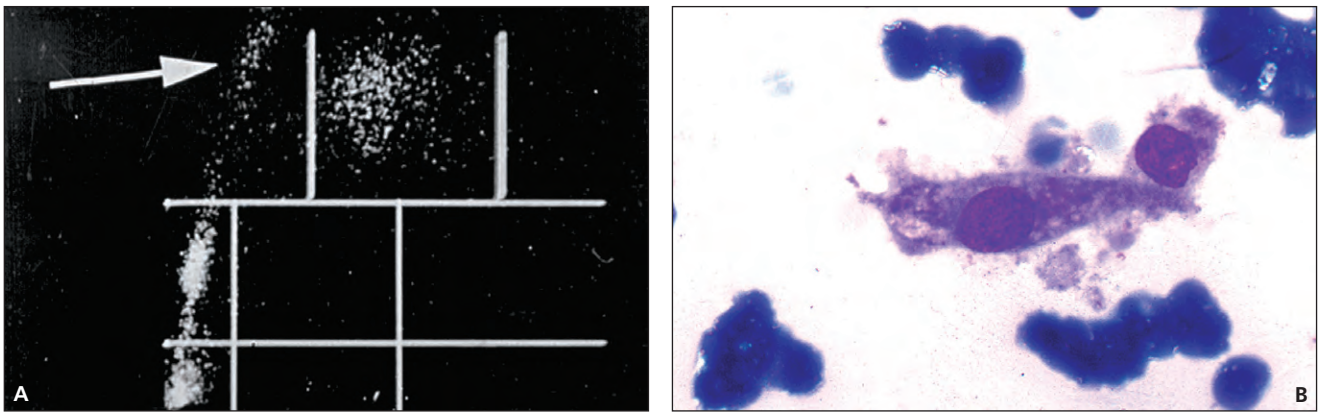



**Fig. 11.28** Carcinocythaemia. (A) Perox cytogram of the Hemalog D system, the first system providing an automated differential white cell count based in part on cytochemistry (taken in 1979). Peripheral blood with marked leucopenia. The arrow indicates a population of atypical signals, produced by very large cells with no peroxidase activity (LUC) (see Report 11.3). (B) Buffy coat preparation from the same peripheral blood specimen as (A). Two large atypical cells, probably joined at one pole. The cytoplasm of the larger cell is fusiform, violet-blue with an irregular ragged outline. The nucleus is oval with a regular, reinforced membrane. The chromatin is spongy, like that of a histiocyte. The bone marrow aspiration has yielded scanty hypocellular material with a few small groups of cells with the same characteristics. This case represents bone marrow metastases with the occasional entry of tumour cells into the circulation; the patient had carcinoma of the ovary that had already metastasised to multiple sites and had been under treatment for six months.

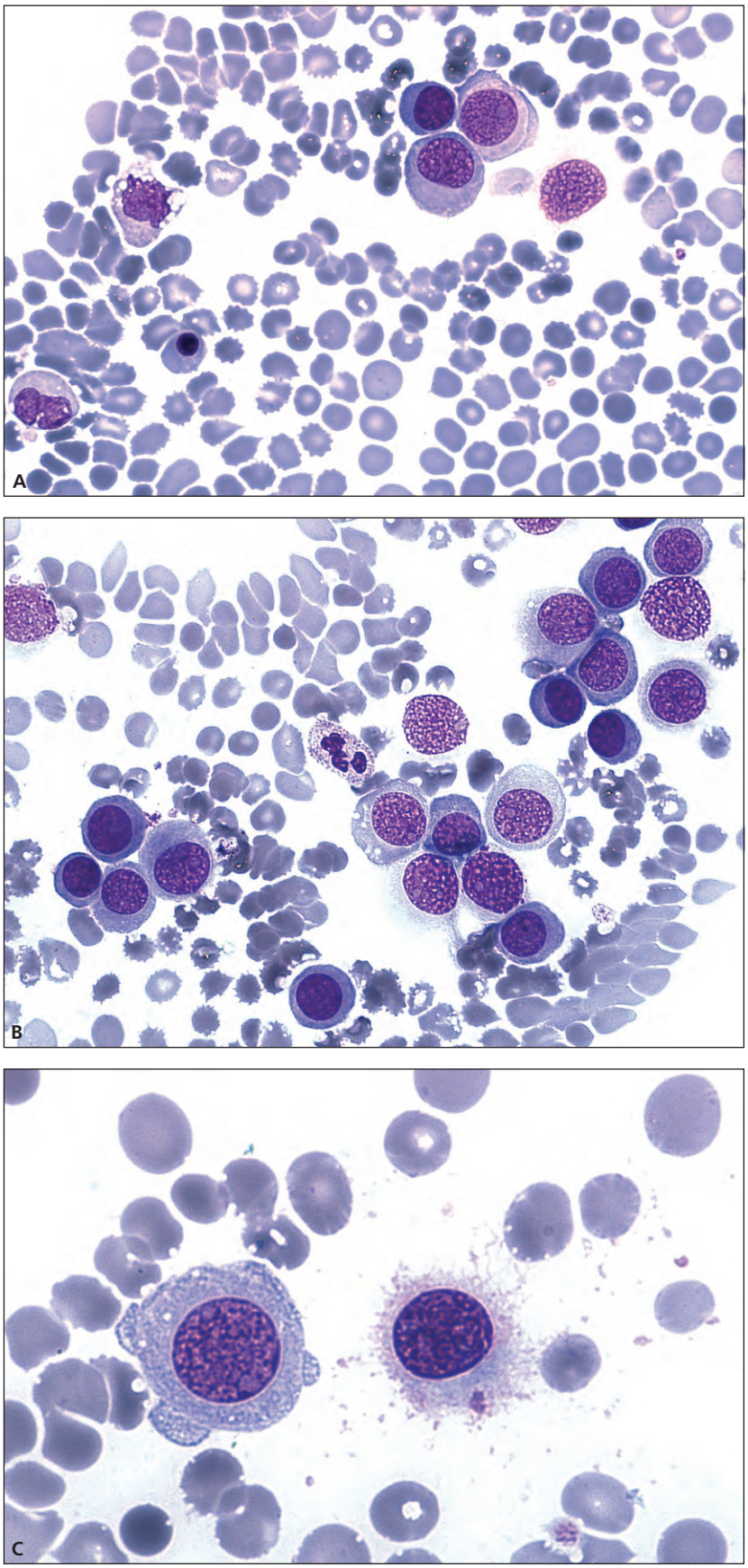



**Fig. 11.29** Carcinocythaemia: peripheral blood film from a patient suspected of having a haematological disorder (images courtesy of V. Rocco). The primary neoplasm was subsequently identified as carcinoma of the ovary. (A) On the left, two monocytes and an orthochromatic erythroblast. On the right, three cells that resemble blast cells, two of which are large with plentiful greyish cytoplasm and, in the upper right cell, a double membrane. The nucleus of this cell is rounded and has immature, loose-knit chromatin with a prominent nucleolus. The lower cell has an eccentric nucleus with denser chromatin. The third is smaller and more compact. (B) There are numerous large cells that resemble blast cells with a range of cytological features analogous to those in (A): round nuclei with chromatin of variable density, from very open to compact, relating to the size of the cell; prominent nucleoli in the more immature cells; cytoplasm that generally has a rounded outline and is plentiful and basophilic. Flow cytometric immunophenotyping showed the cells to be negative for CD45 and for all the markers used for immunophenotyping cases of leukaemia. (C) One notes, as in (A) and (B), the presence of tumour cells and a number of microspherocytes. (D) Marked leucocytosis (WBC 19.9 $\times$ $10^9$/l) with neutrophilia; the neoplastic cells are clustered in a tall solid peak of LUC (24%) in the Perox cytogram and are overflowing the left side of the MN cluster in the Baso cytogram.

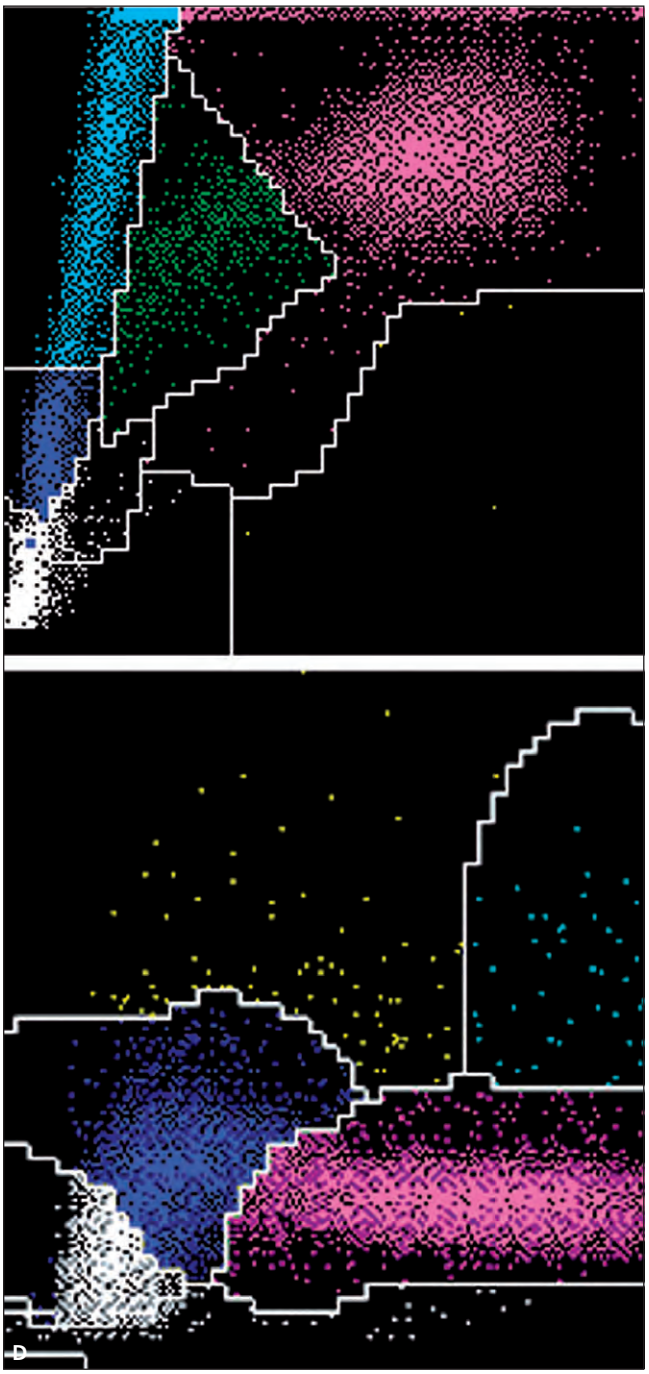



**Fig. 11.29** (*Continued*)

leukaemia and other haematological disorders, such as the leukaemic form of Burkitt lymphoma.[60–62]

## Bone marrow

The presence of fibrosis is not a specific feature of metastases, since it can be found in a number of haematological neoplasms, both lymphoid and myeloid, and both acute and chronic. Acute conditions in which fibrosis can be a feature include: acute megakaryoblastic leukaemia; acute panmyelosis with fibrosis; certain forms of acute lymphoblastic leukaemia (ALL). Chronic conditions include: hairy cell leukaemia; primary myelofibrosis and other myeloproliferative neoplasms including chronic myelogenous leukaemia; lymphoplasmacytic lymphoma and multiple myeloma. In the presence of metastases, the bone marrow aspirate picture is often of marked hypocellularity with reactive fibrosis, fibronecrosis and increased osteoblasts, osteoclasts and reactive cells, such as plasma cells, eosinophils and mast cells (Fig. 11.30).[17] Gelatinous transformation is uncommon.

Depending on the type of tumour, the neoplastic cells may infiltrate as single cells, admixed with normal haemopoietic cells, or as more or less cohesive, isolated infiltrates. Tumour cells in the bone marrow must be distinguished from lymphoma and leukaemia cells and from other cells that are normally present in the bone marrow that are large, have multiple nuclei or occur in small groups, such as osteoblasts, reticulum cells, osteoclasts, endothelial cells, dysplastic megakaryocytes, granulomas and erythroid and lymphoid aggregates (Figs 11.11, 11.12, 11.15, 11.31 and 11.32).

### General morphological characteristics

The tumour cell is a morphological and functional monstrosity as a result of major alterations in its cellular metabolism and in its capacity for autonomous development.[63] In general, tumour cells are larger than haemopoietic cells other than megakaryocytes; there are, however, tumours such as small cell carcinoma of the lung and small cell tumours or other tumours of infancy in which bone marrow metastases can pose serious diagnostic problems, the differential diagnosis being acute leukaemia, particularly ALL, acute undifferentiated leukaemia, acute monoblastic leukaemias and blastic plasmacytoid dendritic cell neoplasm. A useful morphological criterion, which at least suggests the right diagnosis, is the recognition in tumour metastases of cells that vary in size, although they are of the same lineage, and of polymorphic features such as multinuclearity or nuclear multilobation, which are dependent on the biological phase of the tumour cells, whether they are growing or degenerating; in comparison, the picture in acute leukaemia is usually more homogeneous. In addition, tumour cells often have a tendency to adhere to each other, thus forming compact groups, which sometimes reproduce an abortive version of the tissue of origin.

The nuclei of tumour cells observed in the bone marrow are usually large and sometimes very voluminous, with atypical and bizarre forms, sometimes hyperlobated, hyperchromatic on histological sections and in cytological preparations, as a result of an increased DNA content; the chromatin distribution is heterogeneous and irregular with chromocentres that are rich in nucleic acids. Nucleoli are numerous and large, relating to the biological phase of the cell; they often appear intensely basophilic because of the high RNA content and they characteristically persist even during the course of mitosis. The cytoplasmic features are variable, being dependent on the type of tumour; often

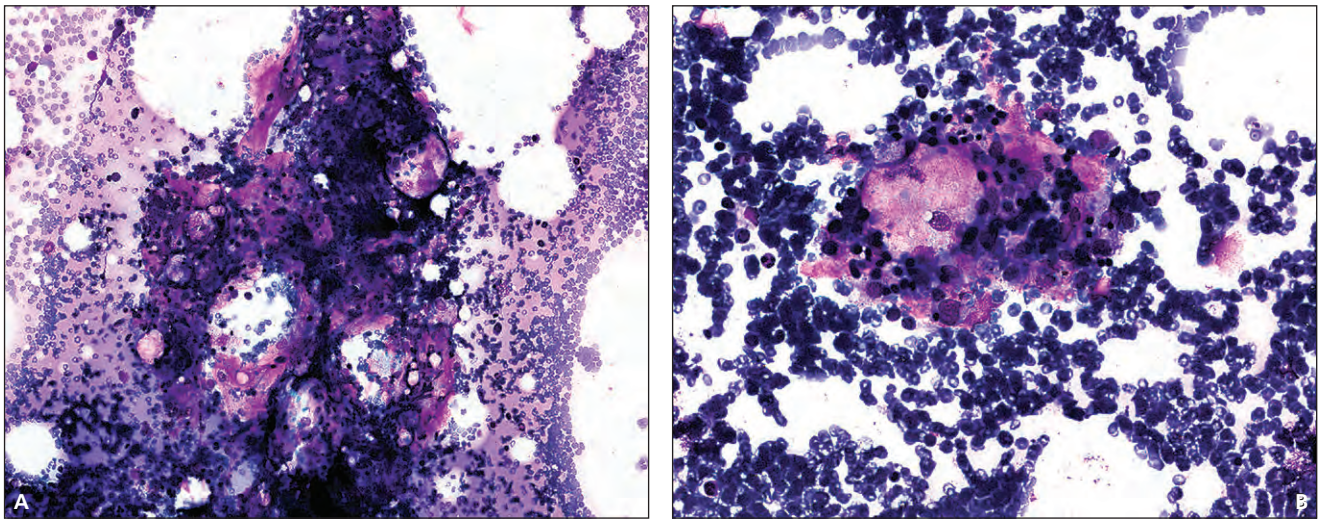



**Fig. 11.30** (A and B) Non-diagnostic bone marrow aspirate in a patient with bone metastases from carcinoma of the colon. The aspirate was hypocellular and the film shows some fibrohyaline fragments containing mainly stromal cells. A long and careful search for neoplastic cells was negative.

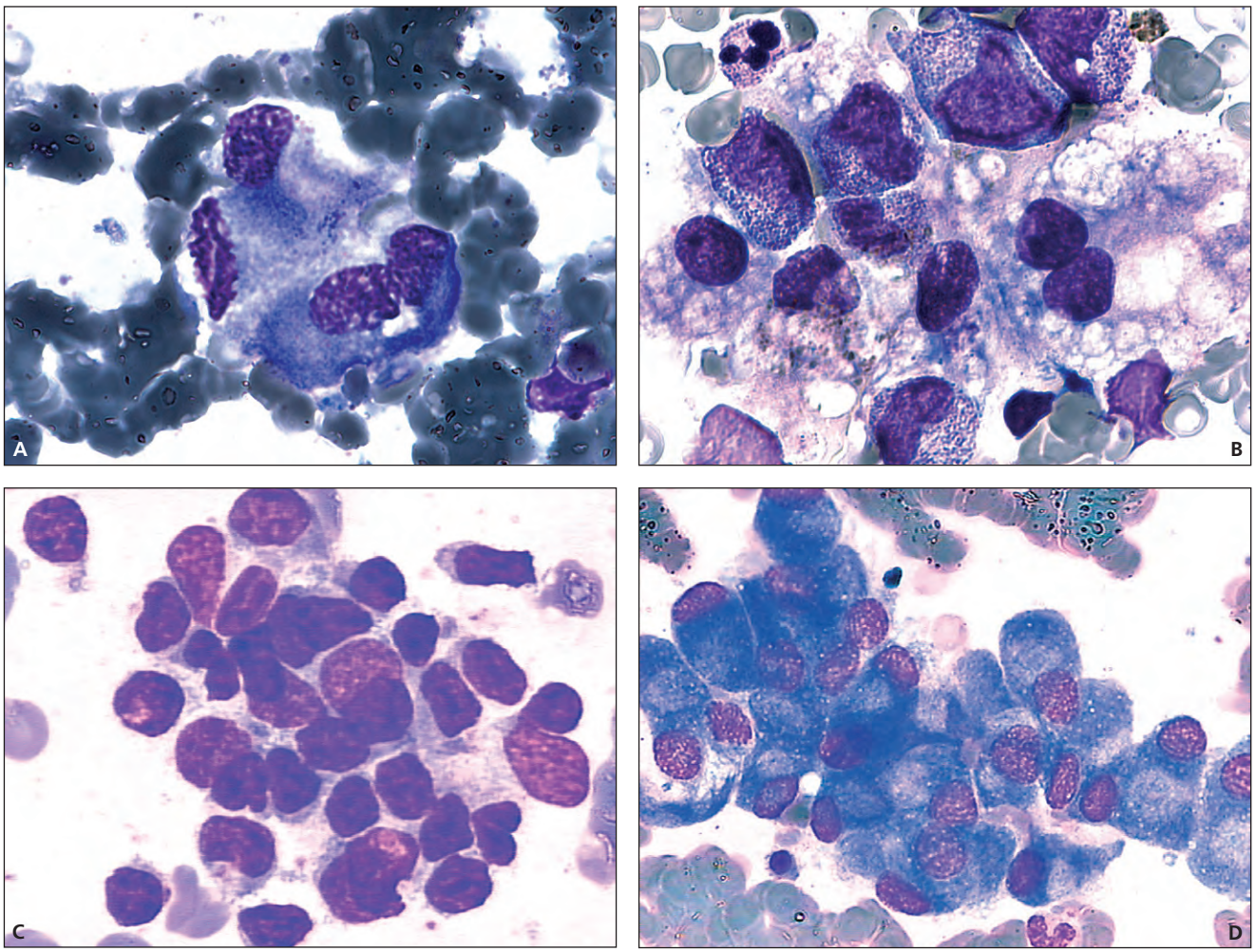



**Fig. 11.31** Non-diagnostic bone marrow aspirate, tumour cells not being found, in a patient with bone metastases from a solid tumour. Small cohesive groups of non-tumour cells in bone marrow aspirates can simulate metastases with the risk of a false-positive diagnosis. (A) From a patient with a pulmonary neoplasm: reticulum cells with basophilic cytoplasm. (B) From a patient with carcinoma of the pancreas: macrophages and granulocyte precursors. (C) Lymphoid nodules of no pathological significance: the presence of some spongy macrophage nuclei can make this small cluster seem suspicious. (D) Nest of osteoblasts from another patient with carcinoma of the colon. Observations such as this are very frequent in patients with skeletal metastases, in whom they indicate remodelling of bone.

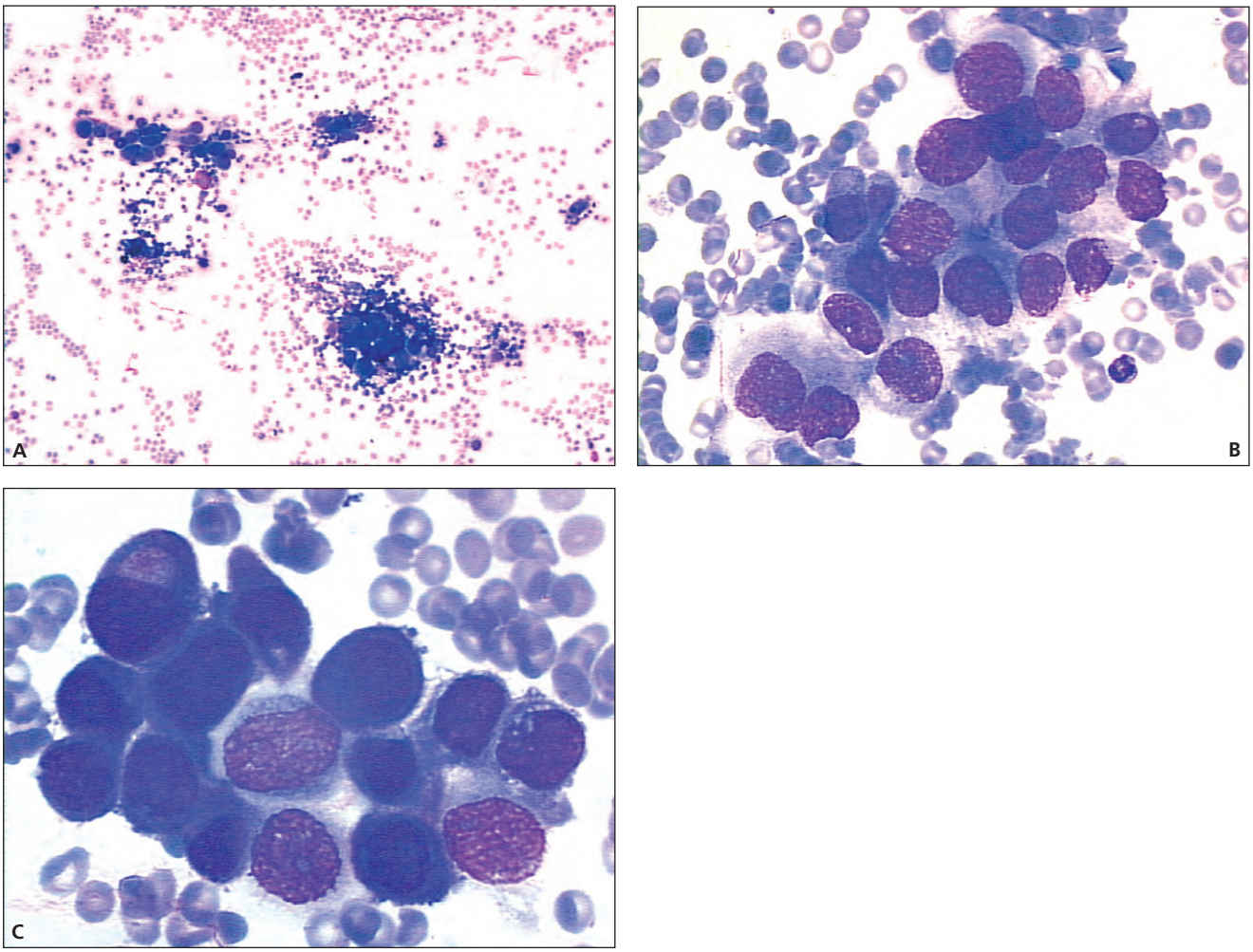



**Fig. 11.32** Bone marrow metastases from undifferentiated carcinoma of the uterine cervix; bone marrow aspirate (same case as Fig. 11.27A). (A) Observing this very hypocellular preparation at low power, it is very important to identify cohesive groups of cells, which are suspicious of malignancy, both because of the size of the individual cells and the overall appearance, which differs from that of clusters of haemopoietic cells. (B) Small compact mass of undifferentiated neoplastic cells with a high nucleocytoplasmic (N:C) ratio. The cytology is relatively monomorphic with large nuclei with spongy or granular cytoplasm, with some nucleoli, sometimes damaged and with a tendency to superimpose on each other. The cytoplasm is scanty with indistinct margins, sufficient to give an almost syncytial appearance. (C) The nuclear chromatin is pale only in some of the better spread cells. Top left, a neoplastic cell with plentiful cytoplasm and a pale paranuclear zone that could suggest a secretory cell.

the cytoplasm is very abundant and frayed, but it can also be almost totally absent; the colour varies from pale grey to intensely basophilic, depending on the quantity of RNA. In certain tumour types there are vacuoles full of glycogen or sudanophilic substances, granular inclusions or the nuclear residues of phagocytosed neutrophils. The main cytochemical characteristics of tumour cells are positivity for periodic acid-Schiff (PAS), indicating glycogen or mucopolysaccharide, for nucleic acids (Feulgen for DNA, percloric acid for RNA) and for cellular enzymes, such as acid phosphatase. The cytoplasmic margins are often indistinct and the cells show a tendency to cohesiveness and the formation of syncytia, with loss of the individuality of single cells. In some tumour types there are isolated cells, but in general there are groups of cells, often large, containing 20 to more than 100 cells, with central zones of necrobiosis and pale areas in the nuclei due to distension of the chromatin. Mitoses are frequent and often abnormal, tetrapolar or multipolar, with bizarre mitotic figures, hyperploidy or loss of chromosomes. The presence of short thick chromosomes suggests a possible epithelial derivation, while long thin chromosomes may indicate a mesenchymal origin. Problems can also arise with the differential diagnosis with haematological neoplasms when the haemopoietic matrix is totally replaced by tumour cells that have lost their typical cohesiveness that could have suggested the right diagnosis. In these cases the immunophenotype of the infiltrating cells, by flow cytometry or immunohistochemistry,

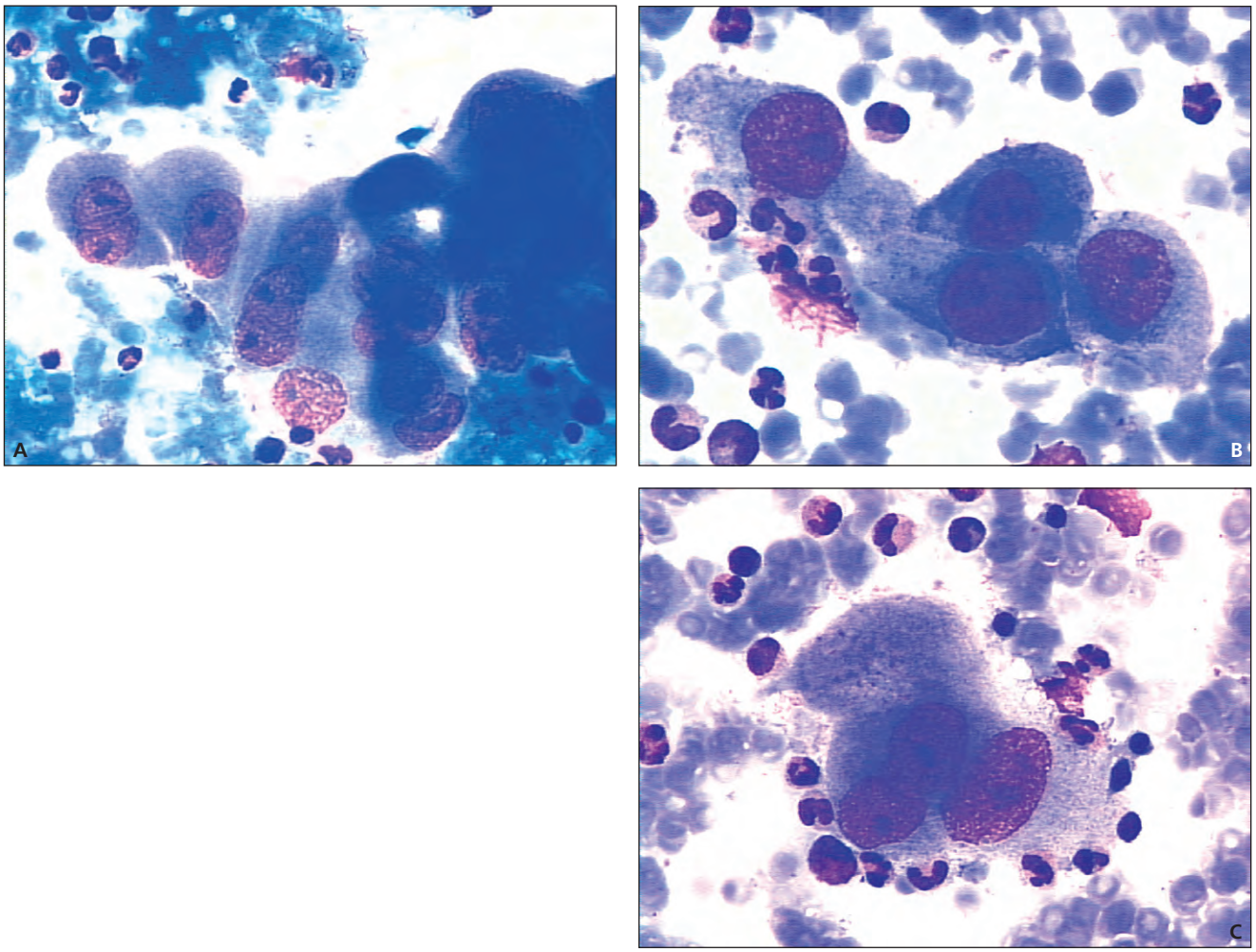



**Fig. 11.33** (A–C) Metastases of undifferentiated giant cell carcinoma of the lung. The tumour cells are anaplastic, polymorphic and very voluminous; they form more of less compact masses, from which some cells detach to form small groups with cellular margins that remain adherent to each other (B) or small syncytia (C). The nuclei have thick, open, loose-knit chromatin and can be multiple or multilobated, with single prominent nucleoli. The cytoplasm is plentiful, blurred and intensely basophilic. The considerable size of the tumour cells can be appreciated by comparing them with the surrounding neutrophils.

can provide the diagnosis, e.g. by indicating an epithelial origin using antibodies to cytokeratin.[64,65]

## Morphological characteristics according to tumour type

### Undifferentiated carcinoma

This type of tumour can originate in many organs and tissues (e.g. neuroendocrine, thyroid, renal or uterine cervix). The tumour cells, in general, are aggregated into small syncytia and have a high nucleocytoplasmic (N:C) ratio with nuclei that can be either low density or hyperchromatic and pleomorphic with well-demarcated outlines and dense reticular chromatin, reminiscent of the pattern of macrophages: the nucleoli are blue, numerous and prominent, while the cytoplasm is moderately abundant with a variable degree of basophilia (Figs 11.32 and 11.33). Sometimes they are phagocytic.

### Undifferentiated small cell carcinoma of the lung

In this tumour, the marrow is a preferential site for localisation of metastases and it can be the only site. The tumour cells are smaller than in other carcinomas, with an increased N:C ratio (Figs 11.34 through to 11.38). The nuclei have condensed chromatin and the nucleoli are barely visible: a very characteristic feature is the manner in which cells mould themselves around adjoining cells; these cells have very little cytoplasm so that the reciprocal moulding of the nuclei creates an appearance rather like a stick of bamboo. It is frequent to see inclusions, known as paranuclear blue bodies, which are sited adjacent to the nucleus of the tumour cells that are very close to the centre of the aggre-

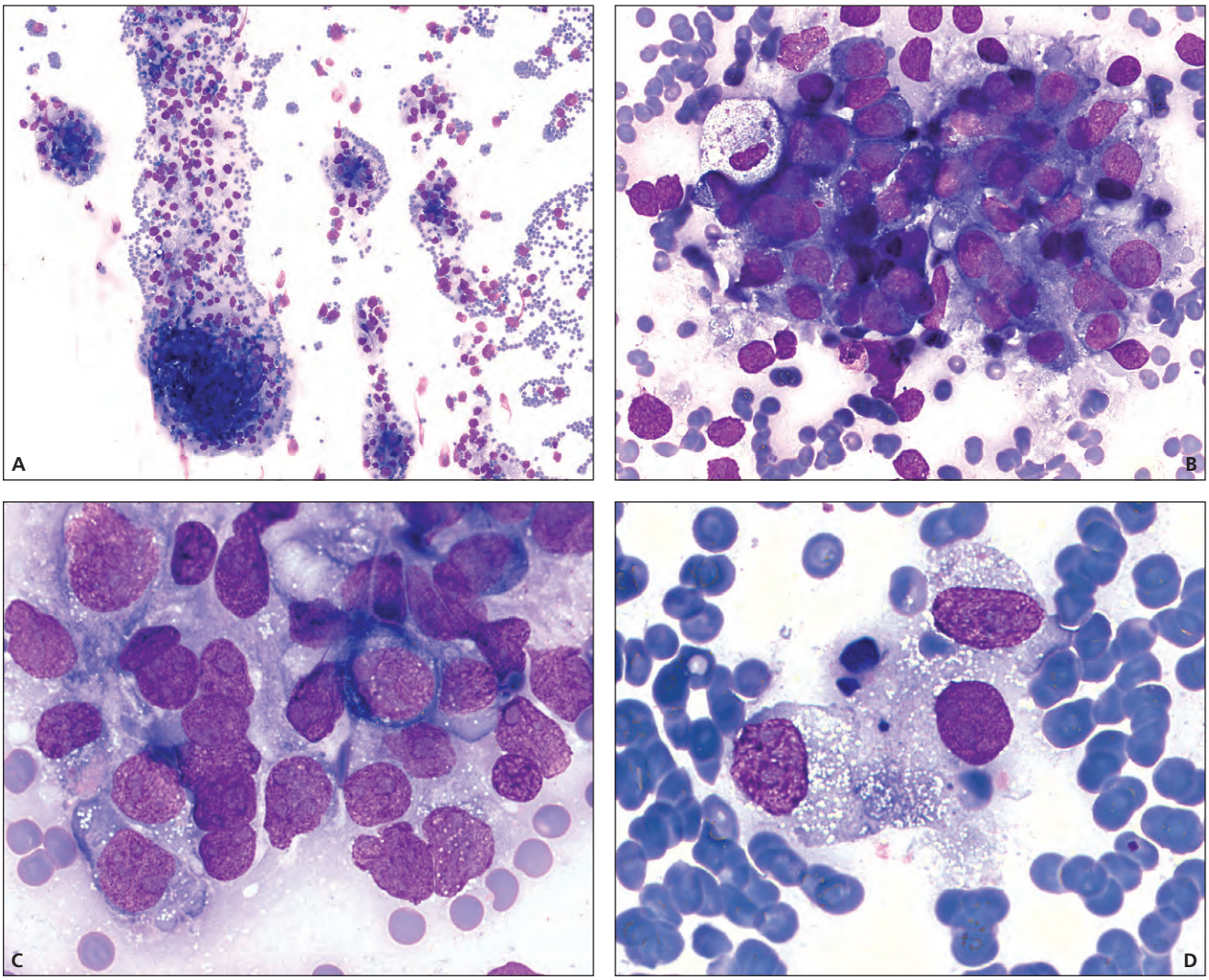



**Fig. 11.34** Small cell undifferentiated carcinoma of the lung; bone marrow aspirate. (A) The haemopoietic marrow appears completely replaced by a monomorphic population of medium-sized cells with a blastic appearance, which are forming masses of variable size that can be found both in the body of the film and, as in this patient, in the tail, thus simulating the appearance of bone marrow fragments. (B) A dense mass of cells with basophilic cytoplasm and round nuclei with delicate chromatin. One notes the presence of numerous small hyperchromatic bodies that correspond to apoptotic nuclei. On the left, a macrophage. At the periphery of the mass, nuclei that are torn and lacking in cytoplasm are detaching from the mass. (C) At a higher power, in a better spread part of the film, the metastatic cells have worm-like chromatin, prominent nucleoli and irregular outlines; the cytoplasm is weakly basophilic and vacuolated. To the left of the centre, a vertical column of nuclei that reproduce the appearance of a stick of bamboo or a vertebral column, which is a cytological marker of this tumour. (D) In contrast with the designation of this tumour as 'small cell carcinoma of the lung', the cell size in this case (and in general in relation to the size of haemopoietic cells in bone marrow aspirates) is quite large. One notes in these three tumour cells, the heterogeneity and irregularity of the chromatin and the presence of numerous small vacuoles in the cytoplasm.

gate. On electron microscopy, the inclusions are seen to be composed of red cells and other cellular material, including nuclei that have been phagocytosed. These inclusions are seen with maximum frequency in air-dried films stained with a Wright stain or a May-Grünwald-Giemsa stain, whereas they are not visualised in alcohol-fixed films prepared for cytology (Papanicolau stain) or in films stained with haematoxylin and eosin. These structures were first described in bone marrow aspirates of small cell undifferentiated carcinoma[66] and were subsequently found to be typically, although not exclusively, associated with small cell carcinoma of the lung[67,68]: their presence in cancer cells can help to signpost the diagnostic pathway.

It is important to remember that, because of their cytological features with scanty, weakly basophilic cytoplasm and no strong tendency to aggregation, metastases of small cell carcinoma of the lung can be difficult to distinguish from ALL with homogeneous cellular features and some undifferentiated myeloid leukaemias. Immunophenotyping is a valuable tool in these cases.

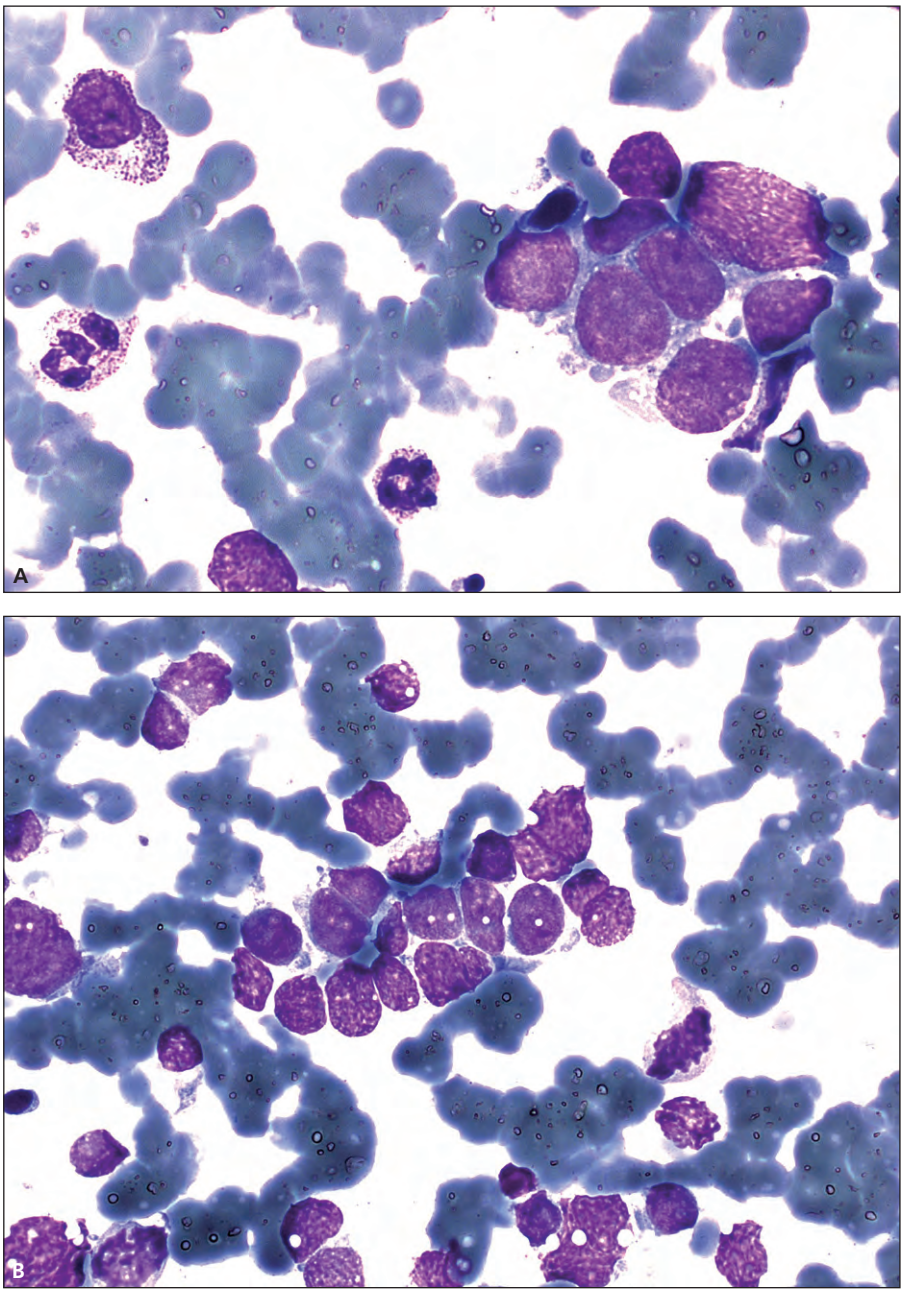



**Fig. 11.35** Small cell undifferentiated carcinoma of the lung; bone marrow aspirate. (A) Sometimes the cells that invade the bone marrow in this neoplasm are very aggressive and have morphological features that can be confused with those of undifferentiated leukaemic blast cells. The tendency to cohesion and the very high N:C ratio are, however, distinctive features of this small group of neoplastic cells. (B) The neoplastic cells are diffusely invading the bone marrow and often appear to be lacking in cytoplasm, thus resembling lymphoid blast cells. However, the alignment of the nuclei in this small neoplastic cluster shows the typical feature of this tumour with cells forming a column, reciprocally moulded around each other. (C) The compact solid structure and the clear, stamped-out outlines of this mass of monomorphic cells is very suggestive of their solid tumour, rather than haemopoietic, origin. (D) One notes again, at the top, the 'stick of bamboo' formation with partial superimposition of the nuclei, which appear to be lacking in cytoplasm; however, the tendency to superimposition is not marked; rather the cells appear like a vertebral column with each cell flattened where it is in contact with another, the phenomenon of moulding.

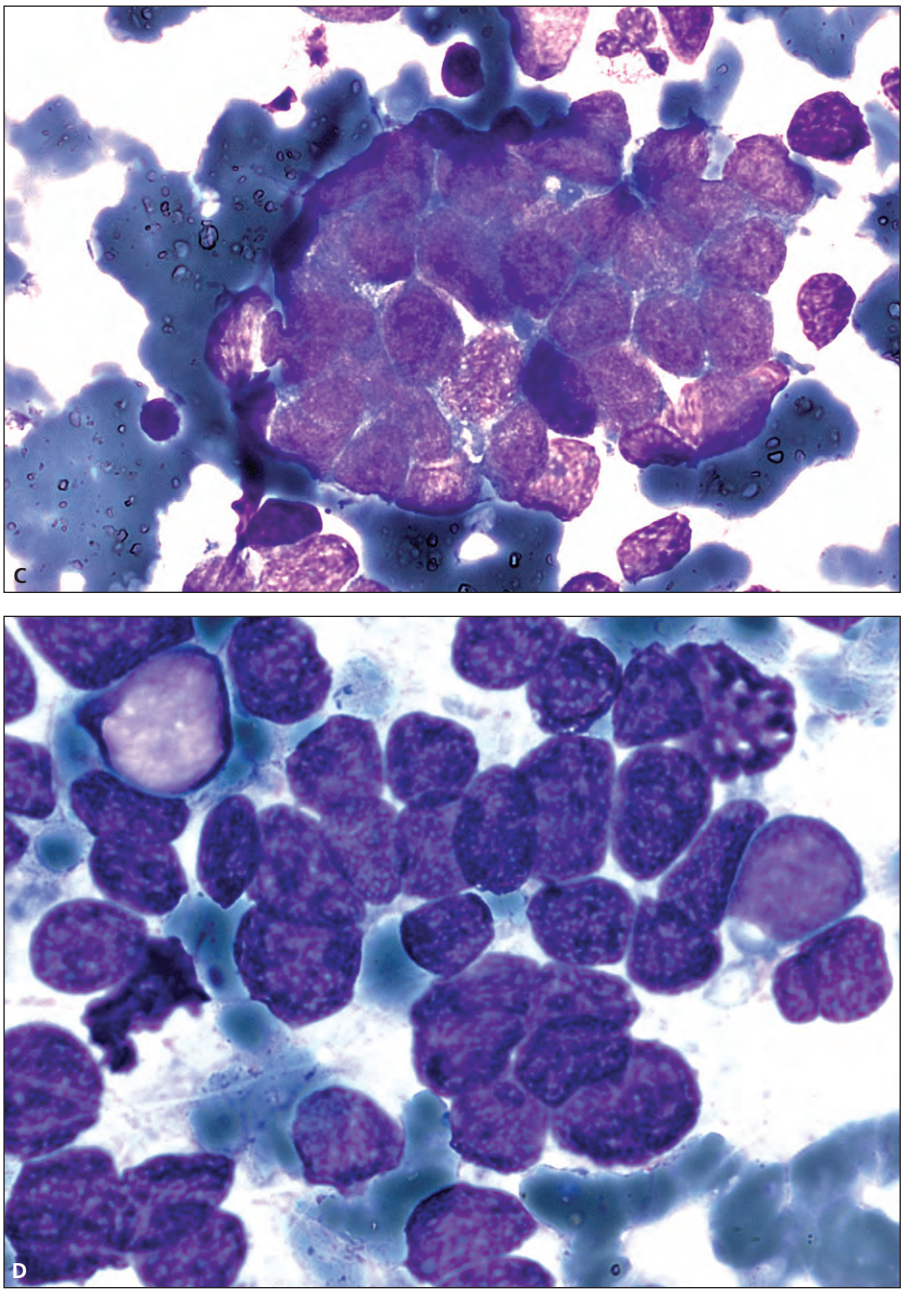



**Fig. 11.35** (*Continued*)

### Adenocarcinoma

These metastatic cells usually occur in clusters, sometimes in part structured so as to suggest the original glandular structure. The cells have large, dense, hyperchromatic nuclei and contain a number of basophilic nucleoli of variable size[69]; the cytoplasm is often vacuolated, sometimes foamy, with the nucleus pushed to one side by an accumulation of mucin: this appearance is referred to as a 'signet-ring cell'. Some of these neoplasms (breast, prostate or stomach) have distinctive morphological features, which will be described below.

### Carcinoma of the breast

The morphology of these metastases is relatively uniform: there are clusters of medium-sized to large cells (15–30 μm) with a high N:C ratio, which can have some features suggesting the glandular structure of the tissue of origin (Fig. 11.39). In addition to the presence of these aggregates, it is not infrequent to see single tumour cells with abundant basophilic vacuolated cytoplasm and nuclei with a distinct outline and sometimes a signet-ring appearance.[70] The presence of aggregates of tumour cells is often associated with fibrosis, the fibrous tissue

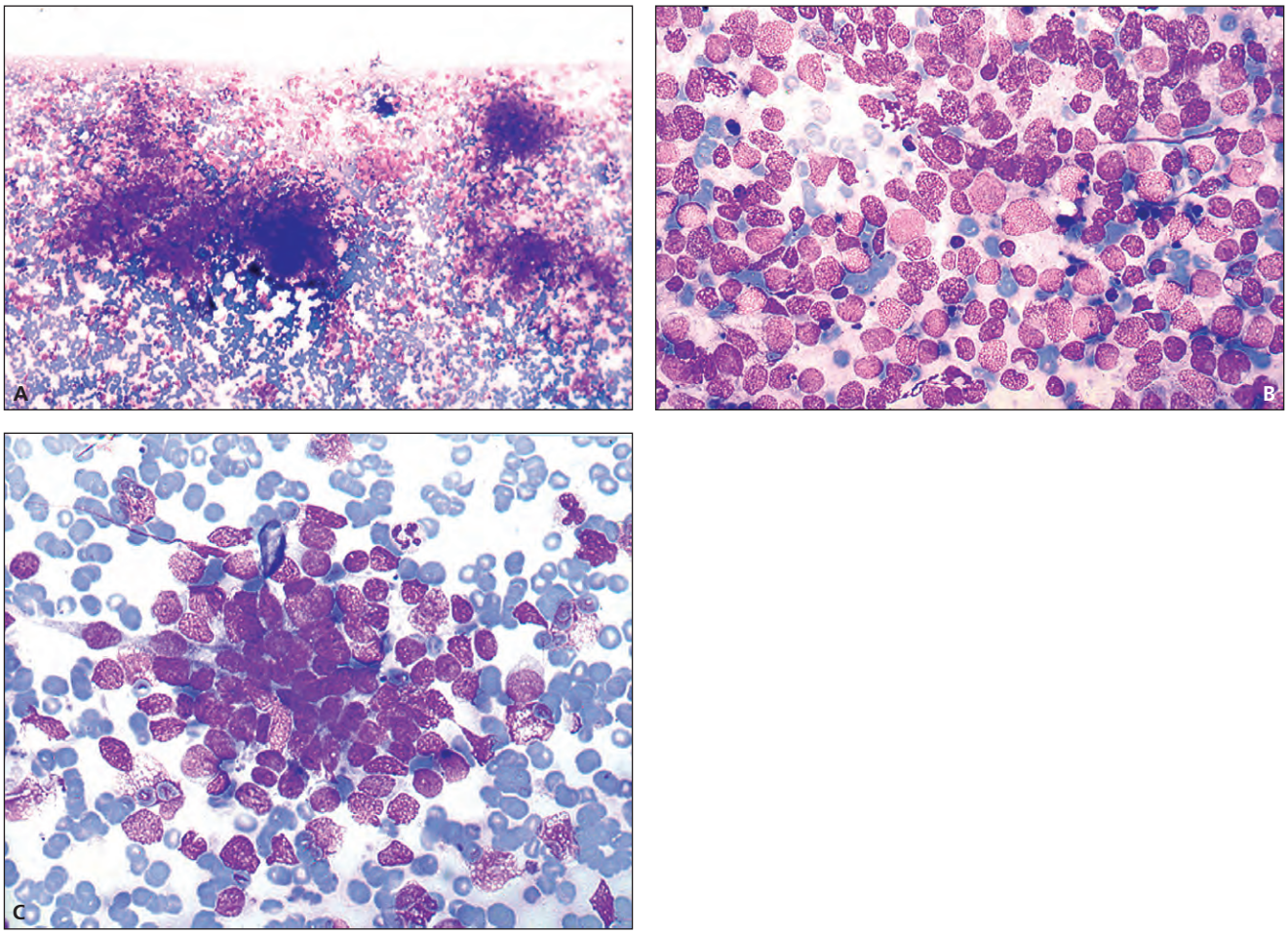



**Fig. 11.36** Small cell carcinoma of the lung, massive invasion of the bone marrow with complete replacement by a diffuse infiltrate plus solid masses of cells. (A) At low power the metastases appear as numerous voluminous masses, which could be confused with bone marrow fragments, both because they are so cohesive and because of the uniform morphology of the constituent cells. (B) The bone marrow space is occupied by a thick carpet of small and medium-sized cells, with cytoplasm that is scanty or invisible, and nuclei of variable density: the general appearance is barely distinguishable from that of an acute leukaemia. (C) The presence of some cohesive groups with nuclei lined up like a stick of bamboo is a useful feature, directing one towards a correct assessment of the aspirate as indicating metastases of small cell carcinoma.

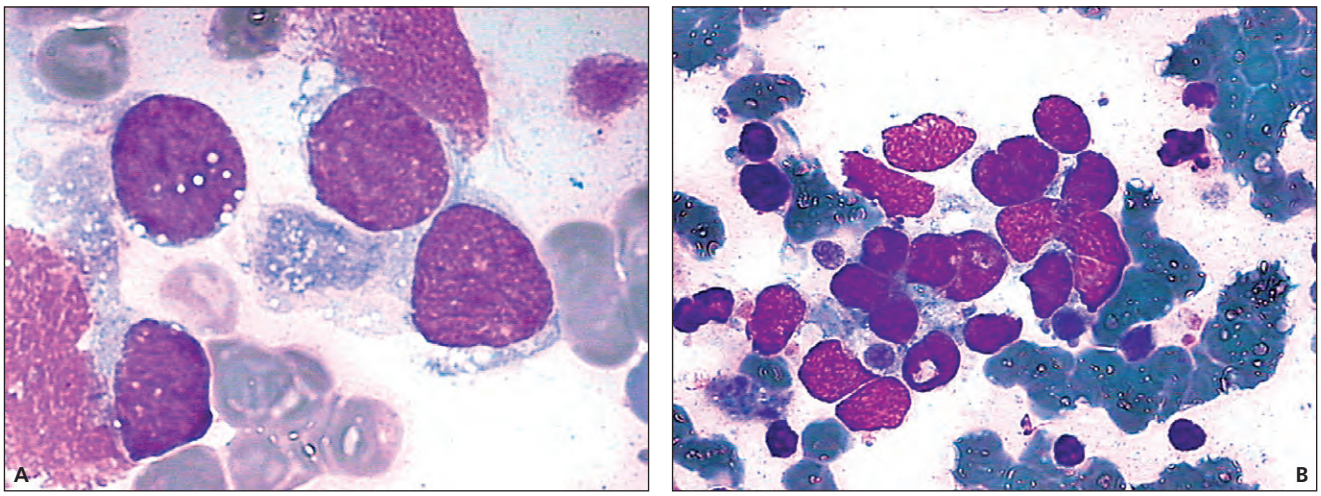



**Fig. 11.37** Cytological analogies between the undifferentiated cells of small cell carcinoma of the lung and leukaemic blasts. (A) These tumour cells have rounded nuclei and homogeneous, fairly compact chromatin; the cytoplasm is very scanty, basophilic and extended into projections that create a hand-mirror appearance. There are vacuoles both in the cytoplasm and in the nuclei. (B) The formation of cohesive clusters is a useful clue, but sometimes undifferentiated leukaemic lymphoid and myeloid blast cells can have a similar tendency to form clusters.

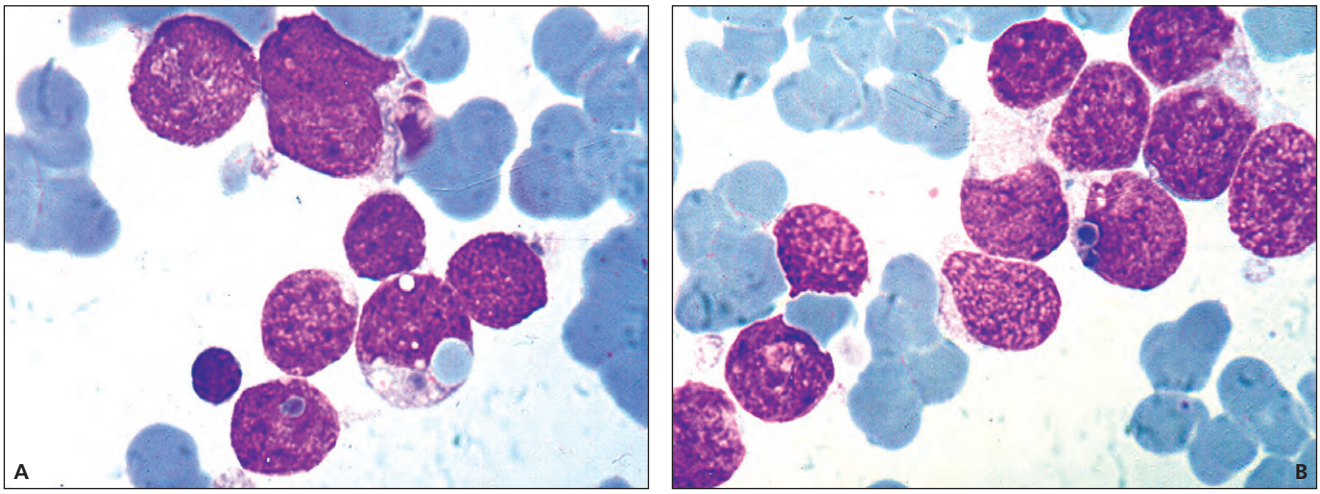



**Fig. 11.38** Bone marrow metastases of small cell carcinoma of the lung; bone marrow aspirate. (A) Erythrophagocytosis: the presence of red cells within the scanty cytoplasm of neoplastic cells is not a rare phenomenon. Superimposed on the nucleus lower down is a small blue paranuclear body. (B) Blue paranuclear bodies can also be observed in this cell; this type of inclusion is very characteristic of neoplastic cells. In fact, it represents structural degradation of phagocytosed cells.

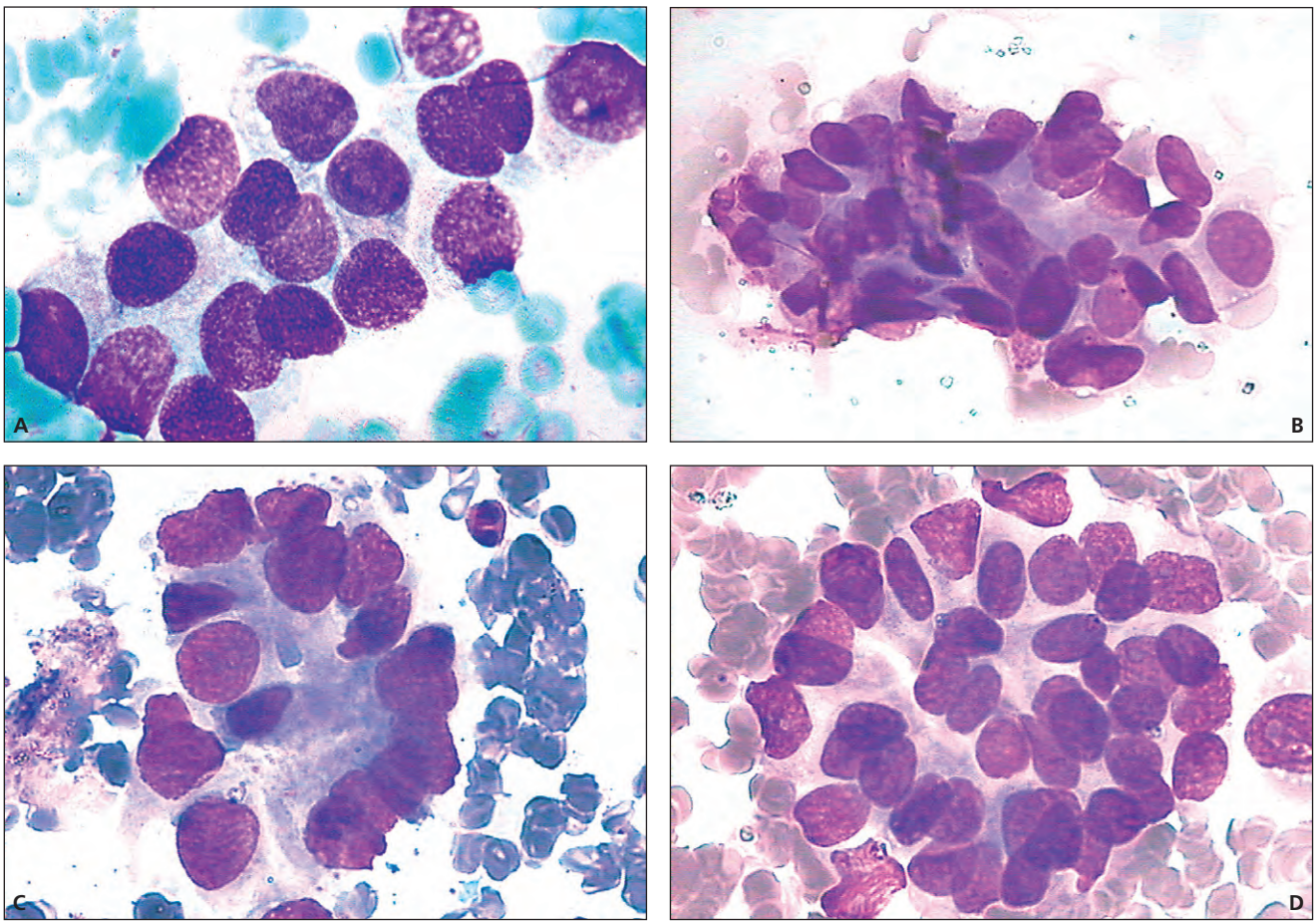



**Fig. 11.39** Metastases of carcinoma of the breast in different patients; bone marrow aspirates. In the context of an often hypocellular aspirate, the presence of cohesive masses of cells that are superimposed on each other, almost as if reproducing acinar and tubular structures, suggests metastatic cells originating from glandular epithelium . (A) In this patient the neoplastic cells that infiltrated the marrow showed a limited tendency to reproduce tubuloglandular structures. This type of picture can be confused with acute leukaemia. One notes the transparency of the superimposed nuclei. (B) A tightly packed mass of neoplastic cells with elongated, hyperchromatic nuclei. In particular, the clear outline and the compactness of this mass of cells permit a distinction from an epithelioid granuloma. (C) In this metastatic clump, the nuclei are towards the periphery as if to reproduce the acinar structure around cytoplasm that is joined centrally as a syncytium. The nuclei on the right seem to be lined up like a stick of bamboo, but in fact they are superimposed and there is no moulding. (D) These carcinoma cells have scanty cytoplasm, with a centripetal arrangement. The nuclei are side by side and arranged in a pseudoacinar formation. There is no transparency in the areas of superimposition.

surrounding the pseudoglandular structures. In this carcinoma, the presence of early micrometastases, not morphologically identifiable, correlates with a worse prognosis and should be carefully sought in order to choose an appropriate therapeutic approach. In the past, this was done by collecting 20–60 ml of bone marrow from different sites, separating the cells on a density gradient and, applying immunocytochemical techniques, examining ten or more films of cells spread on glass slides.[71] Today, fortunately, there are easier, specific and sensitive methods such as the polymerase chain reaction (PCR).

**Carcinoma of the prostate**

This is the most common cancer in men in the developed world. There are metastases at presentation in around 50–80% of cases. The most typical observation is the presence, in a hypocellular aspirate, of masses of large pleomorphic cells, often with ragged cytoplasm and with prominent nucleoli (Fig. 11.40). The cytoplasm can be positive for acid phosphatase. Bone marrow fibrosis is common and, particularly in the presence of splenomegaly, the differential diagnosis includes primary myelofibrosis and other secondary myelofibrosis.

**Gastric carcinoma**

Metastases can be isolated large cells or masses of cells. The nuclei are eccentric with condensed chromatin, sometimes packing the cell; the nucleoli are barely visible. The cytoplasm is abundant and finely granular with varying degrees of basophilia and is positive for PAS and acid phosphatase. It is not rare to recognise signet-ring forms (Fig. 11.41). These cells must be distinguished from plasma cells and osteoblasts.

**Clear cell carcinoma of the kidney**

This is the most common renal tumour. The neoplastic cells have a small, hyperchromatic nucleus, the nucleoli are barely visible and the cytoplasm is abundant and weakly basophilic.

**Squamous cell carcinoma**

This is the most common tumour, not only of the skin, but also of the larynx, the buccal cavity, the oesophagus, the bronchi and the uterus (Figs 11.42 and 11.43). A distinctive morphological feature is the presence in the usually abundant cytoplasm of a peripheral reddish region that contrasts with the perinuclear basophilia.[70]

**Neuroblastoma**

The pattern of growth of this tumour in the bone marrow is fairly characteristic. With rare exceptions, there is formation of groups of cells, often in the form of a rosette (known as a Homer-Wright rosette), in which the tumour cells are arranged radially around a central mass of neurofibrils (Fig. 11.44). A similar picture can be observed in retinoblastoma and medulloblastoma. The nuclei are round or oval, with the chromatin being dense, granular and hyperchromatic; the cytoplasm is scanty with frequent projections; sometimes there is differentiation into ganglion cells.[72] In the presence of a diffuse infiltrate of small cells, the distinction from other tumours of infancy, including ALL, is not always easy: the presence of extracellular, basophilic, amorphous or fibrillar material at the centre of a rosette is strongly suggestive of neuroblastoma. Also in this tumour, cytochemical stains may identify glycogen.

**Retinoblastoma**

Metastases take the form of syncytia or pseudorosettes of medium-sized to large cells with pleomorphic nuclei, visible weakly basophilic nucleoli and basophilic vacuolated cytoplasm: in some cases, the cytological differential diagnosis includes the leukaemic form of Burkitt lymphoma.

**Medulloblastoma**

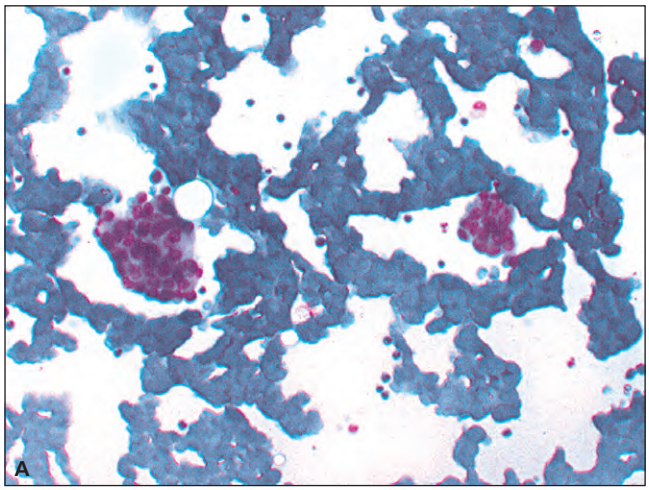
The pattern of growth of this neoplasm has similar morphological features to that of retinoblastoma.

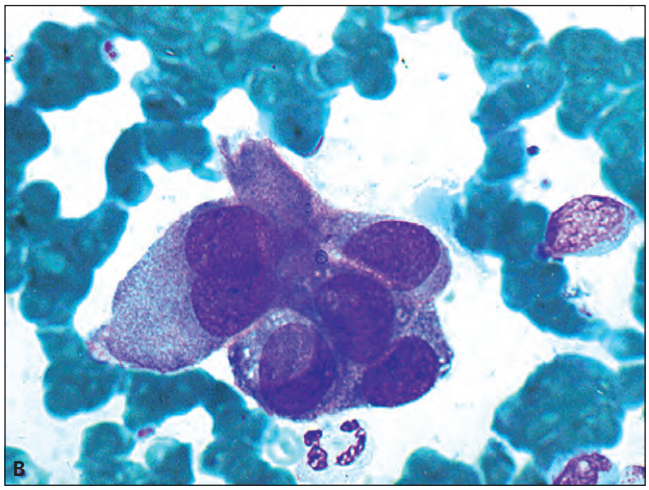
**Rhabdomyosarcoma**

The not rare medullary metastases of this neoplasm take the form of giant cells that are multinucleated or have bizarrely multilobated nuclei. The plentiful pale cytoplasm is quite often heavily vacuolated (Figs 11.45 and 11.46): this appearance creates problems of cytological differential diagnosis with acute monoblastic leukaemia, aggravated by the possible positivity for non-specific esterase.[73–76] In the most differentiated cases, it is possible to identify characteristic binucleated fusiform cells, the rhabdomyoblasts.

**Melanoma**

Bone marrow metastases take the form of single mononuclear cells, with variability of nuclear size; the nucleoli are large and pale with indistinct margins and the cytoplasm contains black melanin granules, which are sometimes quite abundant. The cytological differential diagnosis includes macrophages that have phagocytosed melanin or cellular detritus. The absence of dark granules does not exclude a diagnosis of melanoma since the case may be of amelanotic melanoma.[6]



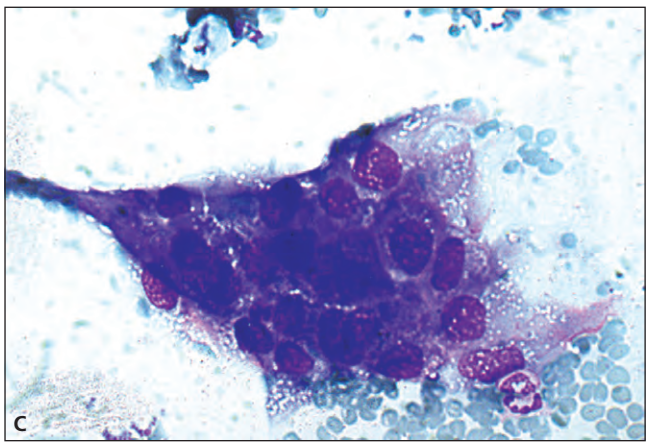
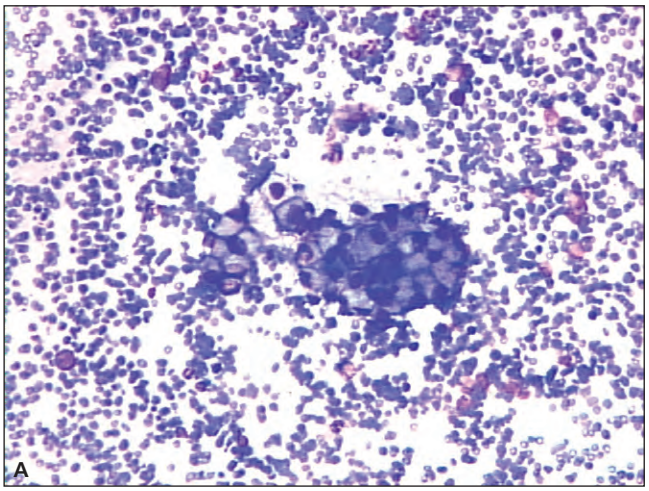
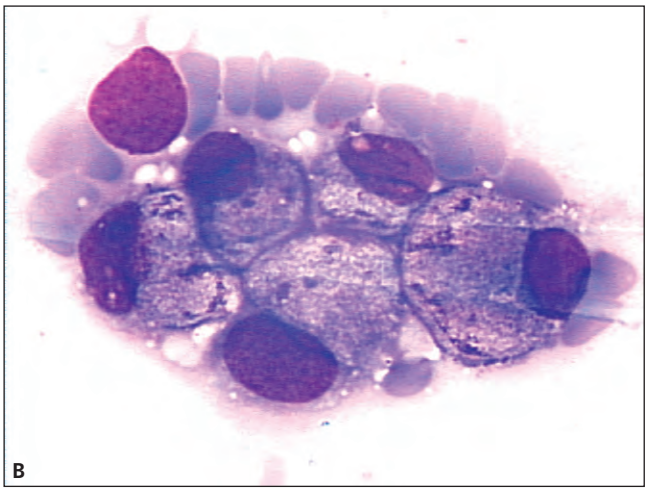




**Fig. 11.40** (A–C) Metastases of carcinoma of the prostate; bone marrow aspirates from different patients. In these films, all characterised by hypocellularity, there are compact, clearly defined masses of tumour cells that vaguely reproduce the structure of glandular tissue.



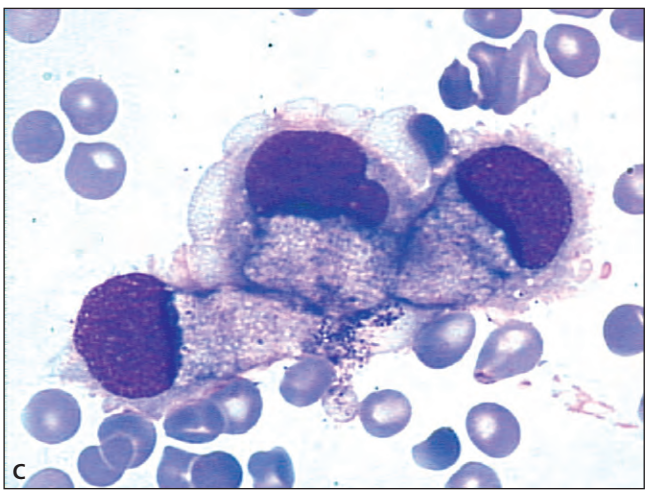




**Fig. 11.41** Metastases of gastric carcinoma, signet-ring cells; bone marrow aspirate, same case as Fig. 11.27B, presenting with a picture of thrombotic microangiopathy. (A) In this small compact group, the monomorphic cells have eccentric nuclei and plentiful pale cytoplasm. (B) The signet-ring appearance, with nuclei flattened by the globular cytoplasmic contents, is typical of mucous-secreting adenocarcinoma. One notes that individual cells are still distinctly outlined, separated by their cytoplasmic membranes. (C) Particularly in the upper cells, there is a clear difference between the free cytoplasm, which has pseudopodial projections extending upwards, and the pink mucinous material that is accumulating in the paranuclear area that corresponds to the Golgi zone.

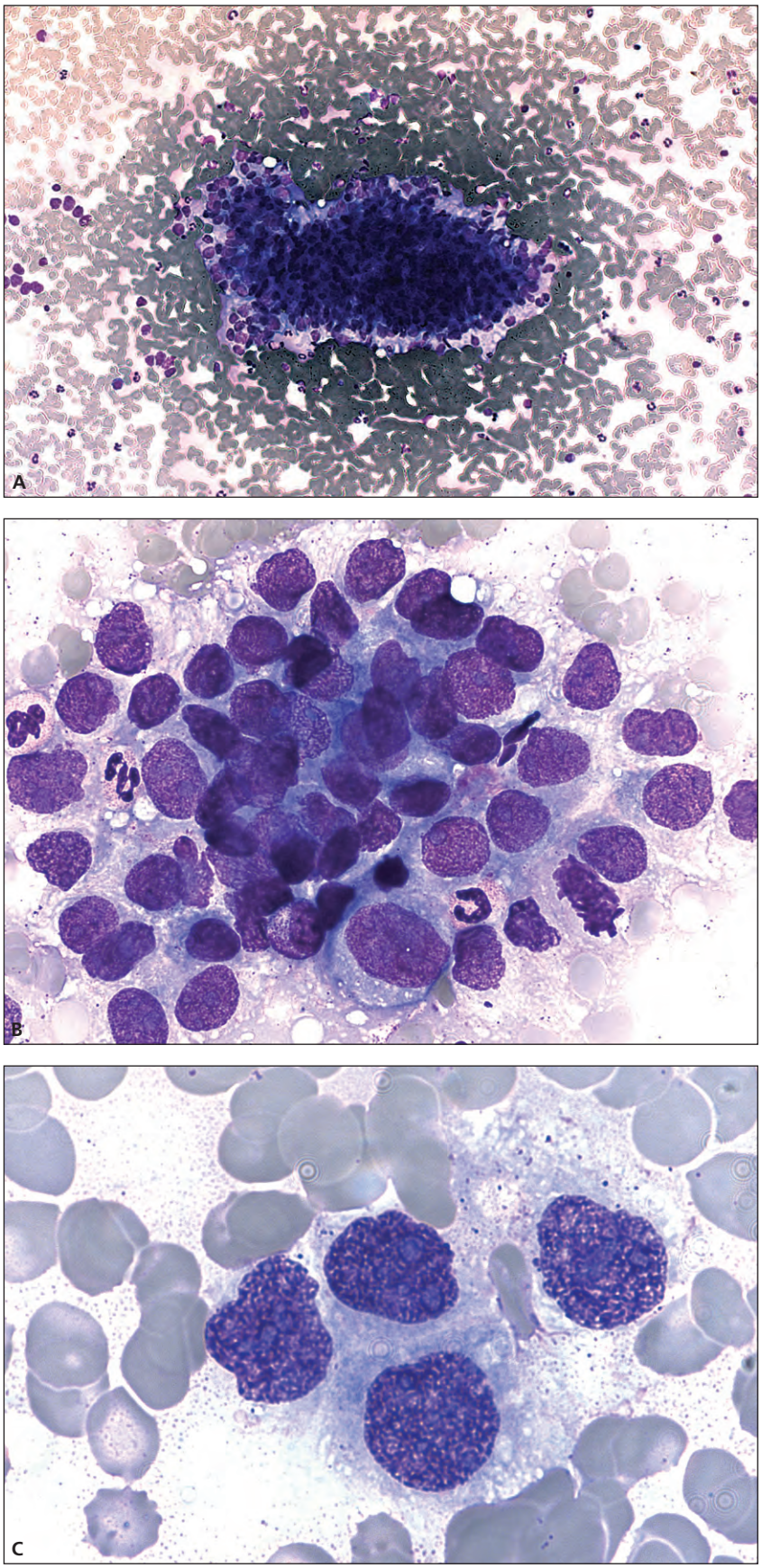



**Fig. 11.42** Squamous cell carcinoma of the oesophagus; bone marrow aspirate. (A) A large solid mass of apparently monomorphic cells can be seen at low power in this otherwise hypocellular film. (B) The cells in this mass are medium-sized to large, as can be appreciated by comparing them with the three neutrophils. The often superimposed nuclei have spongy chromatin with prominent pale nucleoli; the cytoplasmic margins are generally indistinct. Some nuclei towards the centre are hyperchromatic as a result of apoptosis. Bottom right, a mitotic figure. (C) In this small isolated syncytium, the heterogeneous chromatin structure is clearly seen; it is thickly interwoven and there are multiple lilac nucleoli. The cytoplasm is well represented and basophilic with spread out margins. It is the nuclear morphology, as well as the cytological context, that excludes the possibility of these cells being normal macrophages.

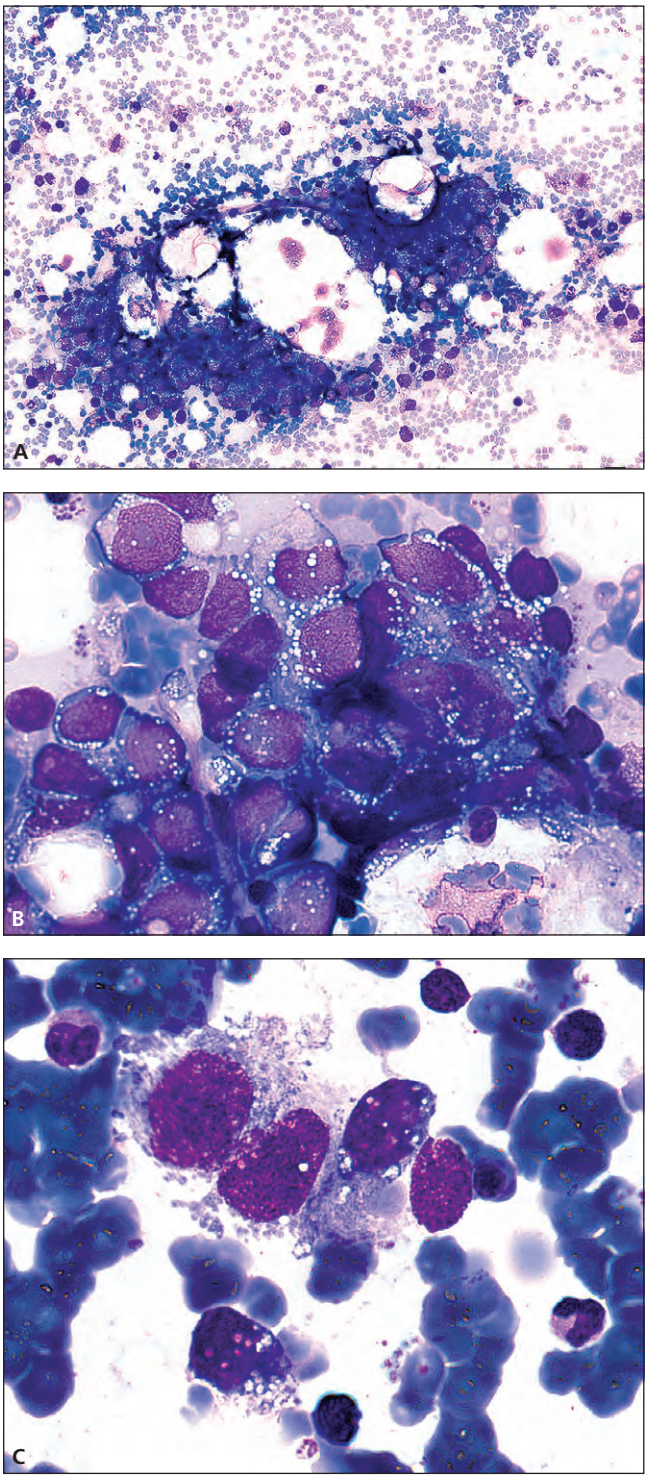



**Fig. 11.43** Squamous cell carcinoma of the larynx; bone marrow aspirate. (A) Hypocellular marrow: what appears on first impression to be a bone marrow fragment is in fact a metastatic nodule. (B) At higher power, the monomorphism of the tumour cells is apparent; they have nuclei with loose homogeneous chromatin and basophilic vacuolated cytoplasm, an appearance that simulates that of Burkitt lymphoma. (C) The squamous epithelial origin can in part be recognised in some anaplastic cells with paler cytoplasm that is extending into long filamentous projections.

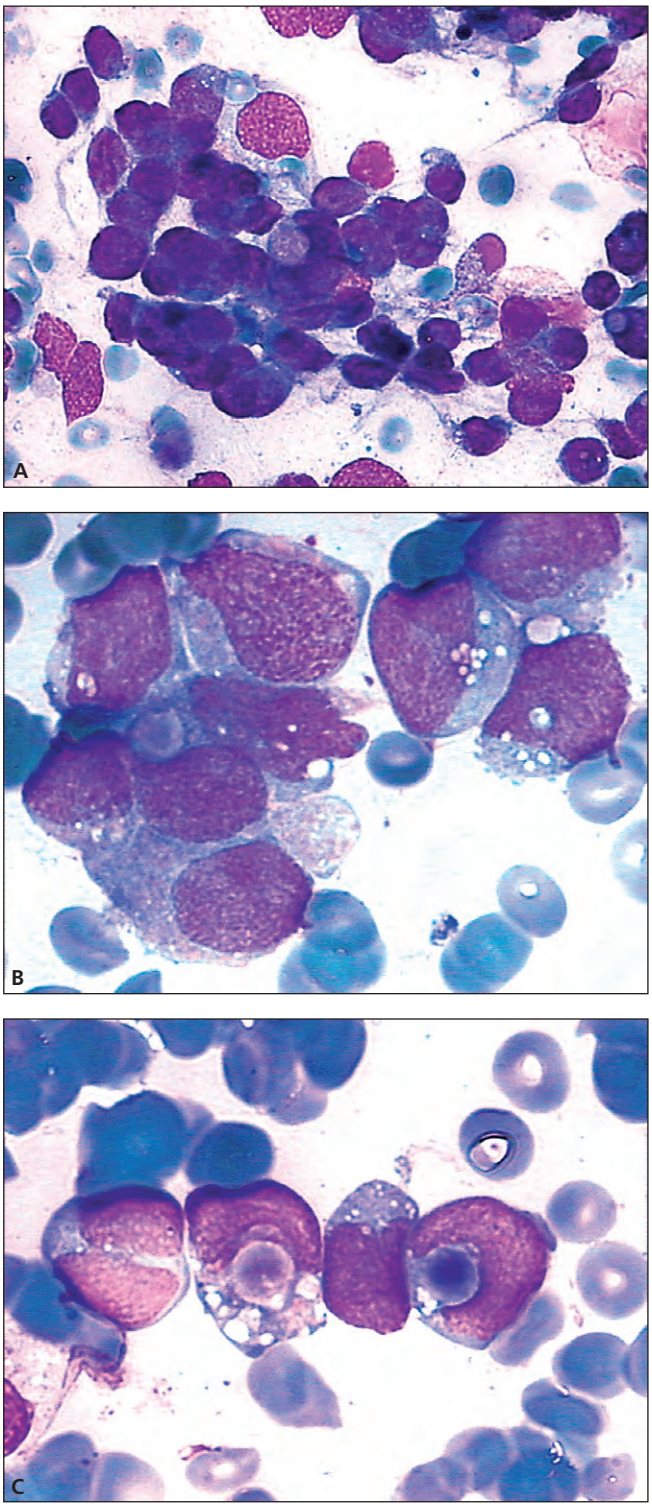



**Fig. 11.44** Metastases of neuroblastoma; bone marrow aspirate. (A) A mass composed of compact cords of small tumour cells with dense nuclei. There are short neurofibrils extending from some cells (e.g. top right). (B) The cells on the left are forming a small rosette around a central amorphous globule. Granular pink material stands out in the basophilic cytoplasm. (C) Two of the tumour cells (on the right) contain a phagocytosed erythrocyte and a blue paranuclear body, similar to those already described in relation to small cell carcinoma of the lung. A pseudopod with a fibrillar extremity is extending from the smallest cell, crushed between two others.

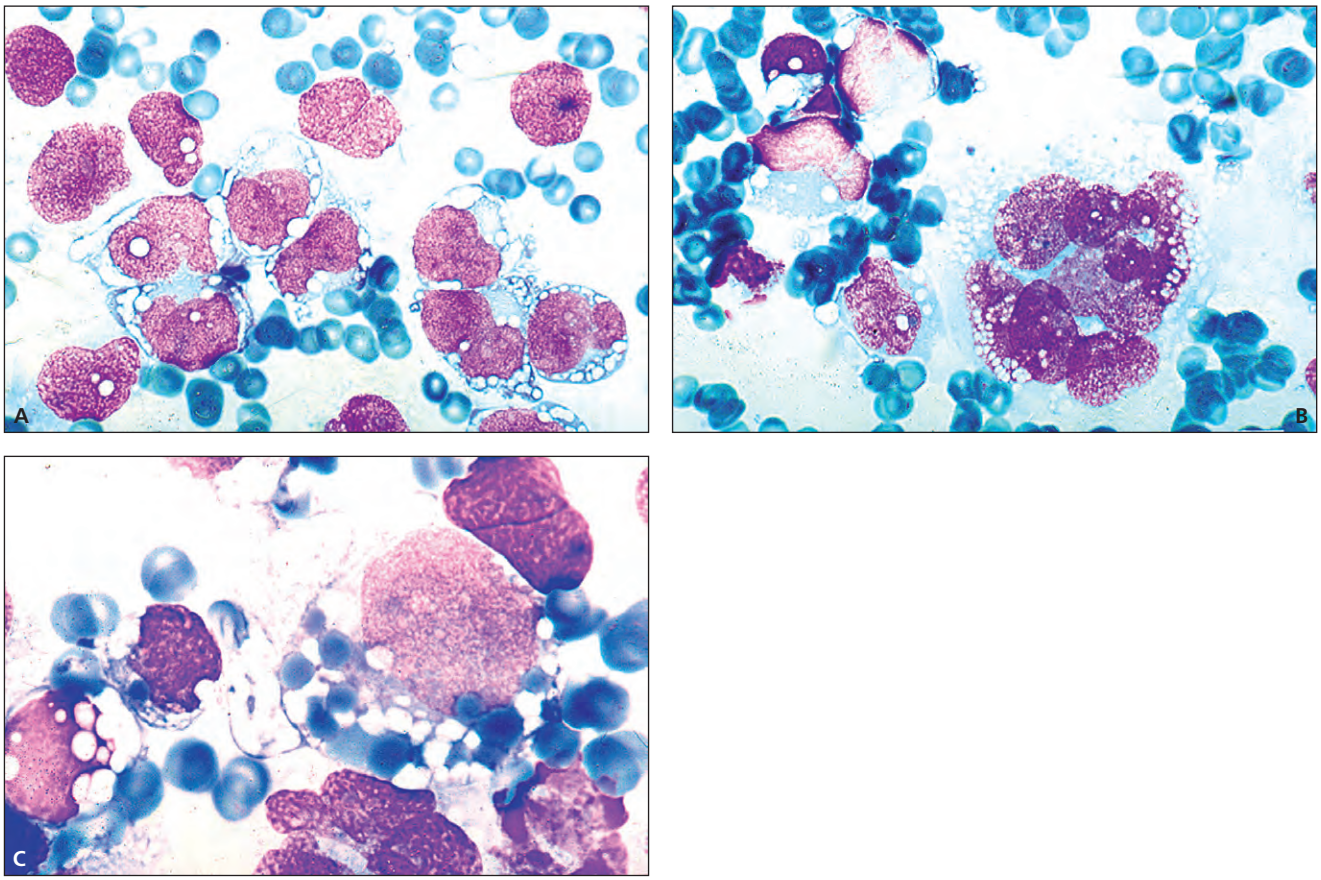



**Fig. 11.45** Bone marrow aspirate from a young adult hospitalised with sepsis. The aspirate is cellular, but the haemopoietic precursors have disappeared. (A) The only cells present are blast cells of very variable size, but mainly large with a low N:C ratio. The nuclei are voluminous and irregular with deep incisions that are dividing them into two lobes. The chromatin is delicate, reticular and loose knit, formed by interweaving of fine fibrils. Many cells have numerous not very distinct nucleoli of variable size. The cytoplasm is plentiful and very pale with cell margins that are often indefinite, perforated by numerous vacuoles that are extremely variable in size; some of the latter are merging to form optically empty lakes beneath the cytoplasm. The initial diagnostic suspicions are: acute lymphoblastic leukaemia of FAB L3 type, atypical monoblastic leukaemia or malignant histiocytosis. (B) Numerous giant multinucleated cells are admixed with the blastic cells. The chromatin is delicate and the superimposed nuclei appear transparent. There are numerous vacuoles in both the cytoplasm and the nuclei. A peroxidase stain was negative. (C) A large polyploid blast shows vigorous phagocytic activity, with its plentiful cytoplasm being loaded with partially digested red cells. The definitive diagnosis of metastatic rhabdomyosarcoma was made by immunocytochemistry with anti-actin and antimyosin antibodies. The primary site of the tumour was subsequently identified by magnetic resonance imaging and histological examination of tissue from the iliopsoas muscle.

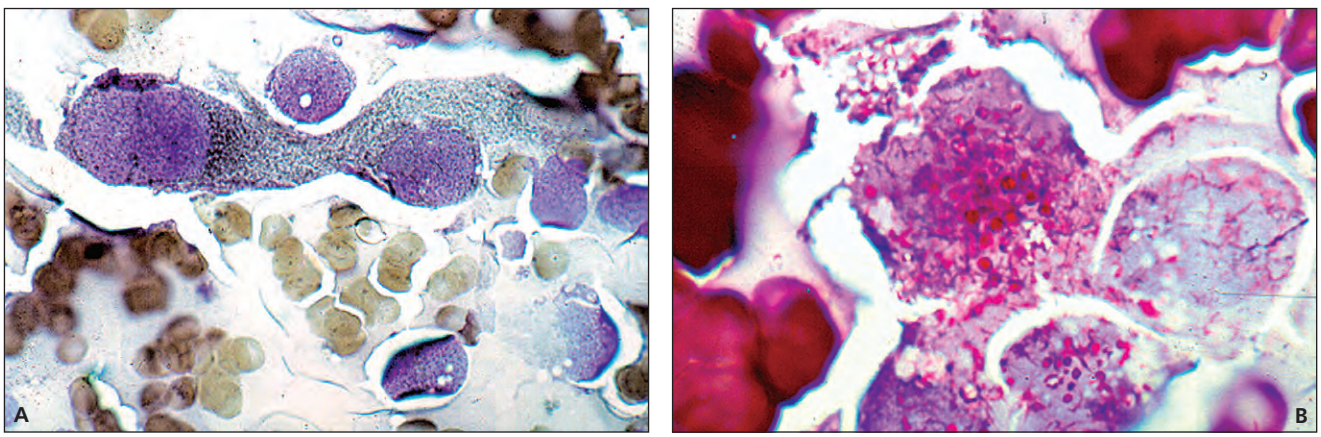
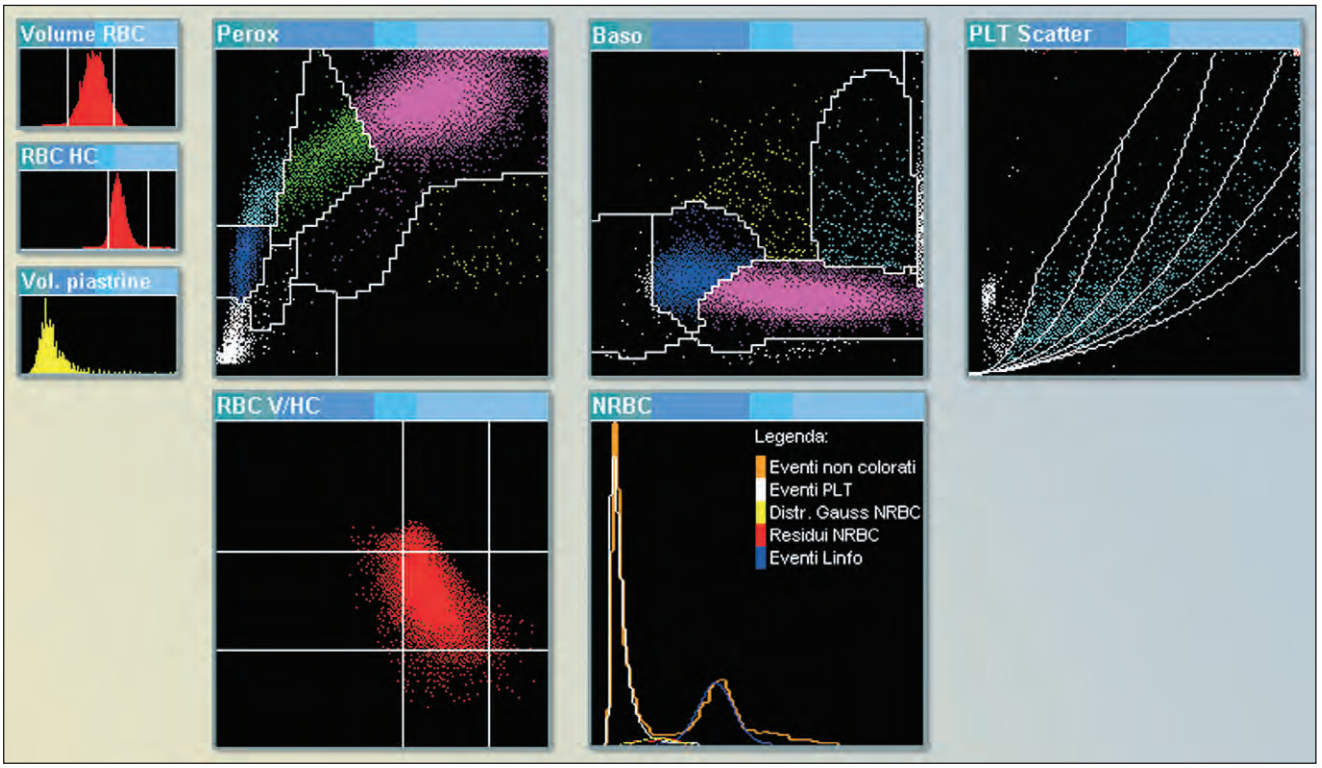



**Fig. 11.46** Metastatic rhabdomyosarcoma in a bone marrow aspirate; cytochemical stain (same case as Fig. 11.45). (A) There is granular positivity for alpha-naphthyl acetate esterase in the cytoplasm of two tumour cells that are fused at one pole. (B) The precipitate of large red granules indicates acid phosphatase activity in the rhabdomyosarcoma cells.

## REPORT 11.1 Paraneoplastic neutrophil hyperleucocytosis in carcinoma of the breast with multiple lymph node metastases



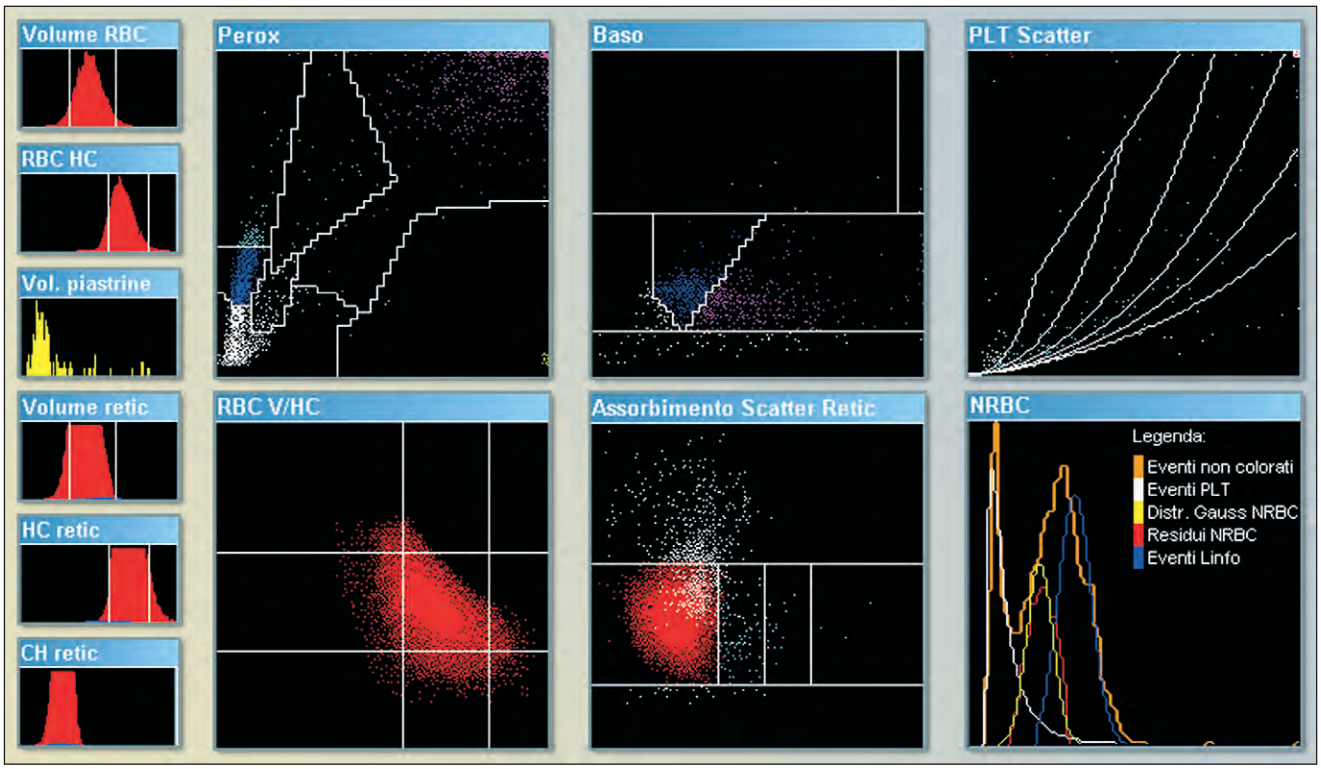

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 117 | g/l | |
| RBC | 3.77 | $\times 10^{12}$/l | |
| HCT | 0.362 | % | |
| MCV | 95.9 | fl | |
| MCH | 31.1 | pg | |
| MCHC | 324 | g/l | |
| CHCM | 318 | g/l | |
| RDW | 15.6 | % | |
| HDW | 2.48 | g/dl | |
| HYPO | 3.8 | % | |
| HYPER | 0.3 | % | |
| MICRO | 0.8 | % | |
| MACRO | 4.2 | % | Macrocytosis + |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.06 | $\times 10^{12}$/l | |
| RET | 1.1 | % | |
| RET | 41.5 | $\times 10^{9}$/l | |
| CHr | 30.1 | pg | |
| IRF (M+H) | 8.3 | % | |
| PLT | 167 | $\times 10^{9}$/l | |
| MPV | 12.2 | fl | |
| PDW | 48.1 | % | |
| Large platelets | 11 | $\times 10^{9}$/l | |
| MPC | 23.1 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 45.81 | | |
| WBC Perox | 44.67 | | |
| NEUT | 41.44 | 90.5 | |
| LYMPH | 1.76 | 3.8 | |
| MONO | 1.,67 | 3.6 | |
| EOS | 0.12 | 0.3 | |
| BASO | 0.30 | 0.6 | |
| LUC | 0.53 | 1.2 | |
| NRBC | 0.00 | 0.0 | |
| LI | 2.03 | LSH + | |
| MPXI | 0.3 | | |
| MN | | 16.8 | IG +++ |
| PMN | | 80.5 | |
| BLASTS | | 0.2 | |
| Perox HPX | | 3.6 | |
| Perox SAT | | 2.4 | |

The haemoglobin concentration is just below normal for a woman; the platelet count is within normal limits. The only blood count abnormality is the increased leucocyte count, which raises the suspicion of leukaemia. The full blood count shows marked neutrophilia and inspection of the cytograms shows correctly positioned, clearly defined clusters of neutrophils in the Perox channel and of PMN in the Baso channel. A proportion of the peroxidase-positive cells, however, overflow the PMN area, causing a correlation error with an IG flag. Some of the signals derived from leucocytes can be seen as a small white cluster, at the bottom, to the left, in the platelet cytogram, in the area of red cell ghosts, which appear to be increased in number.

**Morphology.** White cell differential count from a peripheral blood film: neutrophils 94% (with around 10% being band forms), metamyelocytes 2.5%, lymphocytes 3%, monocytes 0.5%. The picture is not dissimilar to that of chronic neutrophilic leukaemia, but it is certainly the result of the patient's disseminated neoplasm. The automated monocyte percentage is slightly overestimated because the monocyte area is invaded by the apex of the exuberant neutrophil cluster.

## REPORT 11.2 Disseminated candidiasis



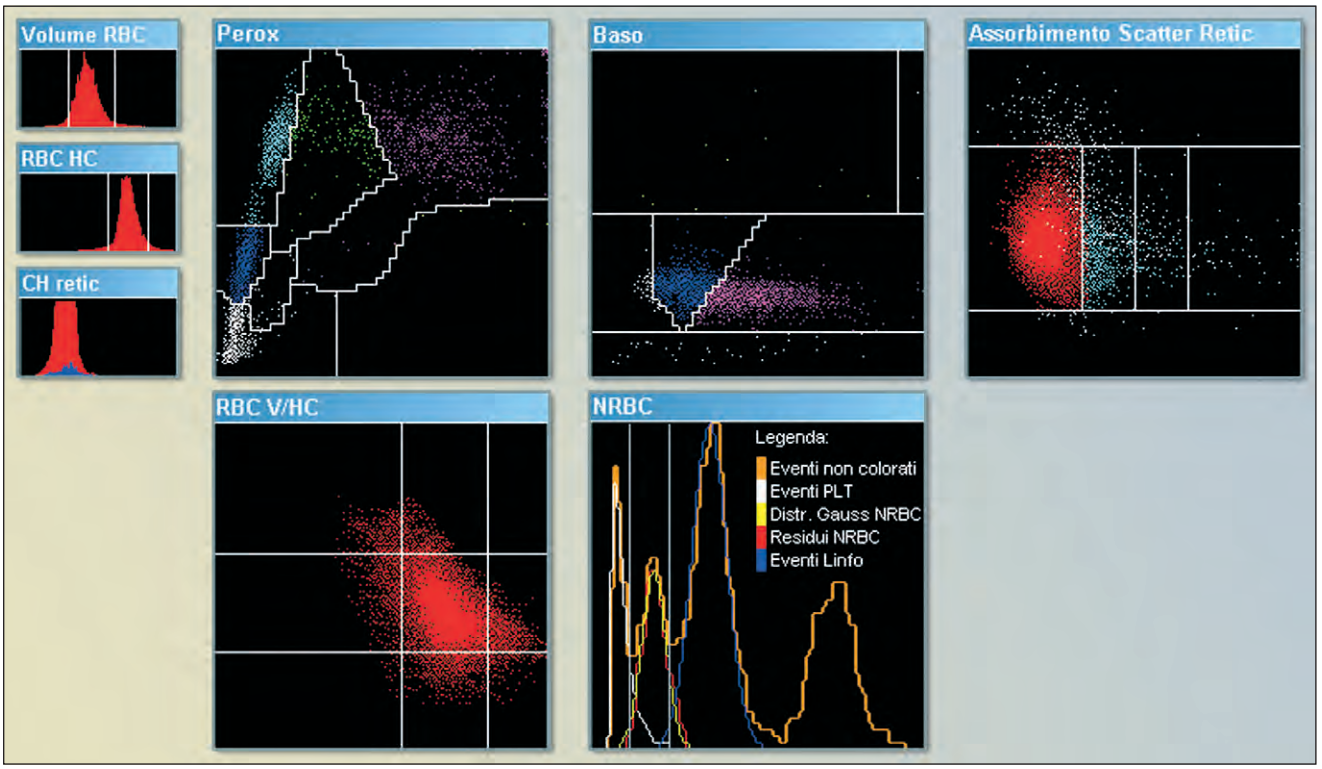

| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 83 | g/l | |
| RBC | 2.95 | $\times 10^{12}$/l | |
| HCT | 0.255 | % | |
| MCV | 86.6 | fl | |
| MCH | 28.2 | pg | |
| MCHC | 325 | g/l | |
| CHCM | 327 | g/l | |
| RDW | 19.1 | % | Anisocytosis ++ |
| HDW | 3.67 | g/dl | Anisochromia + |
| HYPO | 8.9 | % | Hypochromia ++ |
| HYPER | 1.4 | % | |
| MICRO | 5.0 | % | Microcytosis + |
| MACRO | 2.0 | % | |
| RBC fragments | 0.00 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 0.4 | % | |
| RET | 11.8 | $\times 10^{9}$/l | |
| CHr | 29.6 | pg | |
| IRF (M+H) | 8.3 | % | |
| PLT | 15 | $\times 10^{9}$/l | * Platelet aggregates |
| MPV | 11.8 | fl | * PLT-NO |
| PDW | 88.5 | % | * |
| Large platelets | 2 | $\times 10^{9}$/l | * NRLPLT |
| MPC | 23.8 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 0.82 | * | |
| WBC Perox | 2.12 | * | |
| NEUT | 0.22 | 26.6 | * |
| LYMPH | 0.52 | 63.9 | * |
| MONO | 0.01 | 1.4 | * |
| EOS | 0.01 | 1.1 | * |
| BASO | 0.01 | 0.9 | * |
| LUC | 0.05 | 6.1 | * |
| NRBC | 0.00 | 0.0 | * |
| LI | – | * NV-lobularity | |
| MPXI | 24.6 | | |
| MN | | 59.6 | |
| PMN | | 36.7 | |
| BLASTS | | 3.2 | |
| Perox HPX | | 9.5 | |
| Perox SAT | | 7.5 | |

Significant anaemia with a normal MCV and marked heterogeneity of size and density of the red cells. Severe thrombocytopenia and leucopenia, with particularly marked absolute neutropenia. The Perox cytogram is characterised by an anomalous distribution of signals, particularly in two opposite corners: bottom left, there is a marked increase in white signals indicting background noise, corresponding to small particles, which are also responsible for the platelet flag (very high PDW and large platelets) and the anomalies in the platelet histogram; top right, the few neutrophils present are very large and are absorbing a lot of light, pushing into the channel of saturation at the top (with high values of HPX and SAT). The leucocyte count in the Perox channel is more than double that in the Baso channel.

**Morphology.** Examination of a blood film from this patient with AIDS and disseminated candidiasis showed that the neutrophils contained, within their cytoplasm, numerous spores of *Candida albicans* (see Fig. 11.7). These organisms, present also in large numbers outside cells, had invaded the Noise and Lymphocyte areas in the Perox channel (causing a falsely high count, which was not reported) and interfered also with the counting of platelets. In the Baso cytogram also there were abnormal signals scattered beneath the horizontal threshold.

## REPORT 11.3 Carcinocythaemia from metastatic colon cancer




| Parameter | Value | Unit | Flag |
|---|---|---|---|
| HGB | 77 | g/l | |
| RBC | 0.266 | $\times 10^{12}$/l | |
| HCT | 22.8 | % | |
| MCV | 85.6 | fl | |
| MCH | 29.1 | pg | |
| MCHC | 340 | g/l | |
| CHCM | 348 | g/l | |
| RDW | 17.7 | % | Anisocytosis + |
| HDW | 3.34 | g/dl | |
| HYPO | 2.7 | % | |
| HYPER | 3.3 | % | |
| MICRO | 3.1 | % | Microcytosis + |
| MACRO | 2.1 | % | |
| RBC fragments | 0.01 | $\times 10^{12}$/l | |
| RBC ghosts | 0.00 | $\times 10^{12}$/l | |
| RET | 2.61 | % | |
| RET | 69.3 | $\times 10^{9}$/l | |
| CHr | 29.8 | pg | |
| IRF (M+H) | 23.1 | % | |
| PLT | 14 | $\times 10^{9}$/l | |
| MPV | 8.1 | fl | |
| PDW | 51.4 | % | |
| Large platelets | 4 | $\times 10^{9}$/l | |
| MPC | 23.9 | g/dl | |

| Parameter | $\times 10^{9}$/l | % | Flag |
|---|---|---|---|
| WBC Baso | 2.36 | | |
| WBC Perox | 2.31 | | |
| NEUT | 0.76 | 32.4 | |
| LYMPH | 0.81 | 34.3 | |
| MONO | 0.18 | 7.9 | |
| EOS | 0.01 | 0.3 | |
| BASO | 0.01 | 0.5 | |
| LUC | 0.58 | 24.6 | Atyp +++ |
| NRBC | 0.29 | 12.4 | NRBC + |
| LI | 1.37 | LSH +++ | |
| MPXI | 2.6 | | |
| MN | | 54.7 | |
| PMN | | 44.7 | |
| BLASTS | | 2.2 | Blasts + |
| Perox HPX | | 2.4 | |
| Perox SAT | | 1.9 | |

Severe anaemia with anisocytosis and anisochromasia, a mildly increased reticulocyte percentage (but with a normal absolute count) and very severe thrombocytopenia. The leucocyte count is reduced with an absolute neutropenia, increased LUC, and Blasts and Atyp flags. In the Perox cytogram the peroxidase-positive neutrophil population is very spread out, as is seen in patients with myelodysplasia, and is partly invading the monocyte area; the most important abnormality, however, is the presence of an isolated group of large cells in the LUC area, completely separate from the lymphocyte population. The Blast flag is activated, together with the Atyp flag, because the relevant signals are more than 1.5% and the LUC are more than 4%. The representation of the NRBC shows a well-defined intermediate peak in the erythroblast area and an unusual fourth peak on the right, produced by the LUC population, which is clearly distinct from the lymphocytes.

**Morphology.** The blood film shows large cells, in a similar percentage to the LUC, with a round nucleus, which has spongy chromatin and stamped out nucleoli, and cytoplasm that is not very abundant and has a polygonal outline. Flow cytometric analysis showed that these were negative for CD45 and for all the other markers used for phenotyping haematological neoplasms, thus confirming in an indirect manner their origin from an undifferentiated carcinoma of the thyroid. The neutrophils have large toxic granules. There are orthochromatic and polychromatic erythroblasts in a proportion of 10 to every 100 white cells.

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  AMML – acute myelomonocytic leukaemia
  MDS – myelodysplastic syndromes
  MGUS – monoclonal gammopathy of undetermined significance

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