Disponible en ligne sur ScienceDirect
www.sciencedirect.com
Elsevier Masson France
EM|consulte
www.em-consulte.com
Médecine et maladies infectieuses
Médecine et maladies infectieuses 39 (2009) 108–115

Original article

Efficacy of a long-term antibiotic treatment in patients with a chronic Tick Associated Poly-organic Syndrome (TAPOS)

Efficacité d'une antibiothérapie au long cours pour un « syndrome poly-organique postmorsure de tiques » (SPOT) chronique

J. Clarissou [a], A. Song [a], C. Bernede [b], D. Guillemot [b], A. Dinh [a], F. Ader [a], C. Perronne [a,*], J. Salomon [a,b]

[a] *Unité des maladies infectieuses, CHU Raymond-Poincaré, AP–HP, 104, boulevard Raymond-Poincaré, 92380 Garches, France*
[b] *Inserm U657, PhEMI, Institut Pasteur, Paris, France*

Received 13 November 2007; accepted 12 November 2008
Available online 4 January 2009

Abstract

Settings. – Despite a now codified antibiotic treatment for Lyme disease, a significant proportion of patients treated according to recommendations complain of persistent signs and symptoms. The pathophysiological mechanisms which underlie this syndrome of post-treatment chronic systemic illness remain unclear. For some physicians post-treatment symptoms indicate a persistent infection requiring prolonged antibiotic therapy. For others, there is no benefit from antimicrobial therapy. The difficulty of assessment encountered in studies is significant because many symptoms are subjective. We think that the term "chronic Lyme disease" is not appropriate and should be replaced by chronic "tick associated poly-organic syndrome" (TAPOS).

Objective. – This open-label prospective study was made on a group of 100 patients having followed a medical treatment for a chronic TAPOS and to evaluate their evolution under prolonged antibiotic treatment.

Results. – The medical management was found to be effective for symptoms, especially for patients with a high probability of chronic TAPOS (NEJM score). Patients with post tick-bite symptoms, which often worsens their quality of life, deserve particular attention.

Conclusion. – This study had methodological limitations but could help in terms of feasibility, choice of inclusion criteria, and design of follow-up for a future randomized, double blind study to test for an optimal management of TAPOS.

Résumé

Contexte. – Malgré un traitement antibiotique désormais consensuel de la maladie de Lyme, une part significative de patients correctement traités présentent des signes chroniques. Les mécanismes physiopathologiques impliqués dans ce « syndrome poly-organique postmorsure de tiques » demeurent mal connus. Pour certains cliniciens, ce syndrome est lié à la persistance d'une infection bactérienne et requiert une antibiothérapie prolongée. Pour d'autres, il n'y a pas de bénéfice à traiter par antibiotiques. L'évaluation de l'efficacité des traitements est rendue difficile du fait du caractère subjectif des symptômes. Nous pensons que le terme de Lyme chronique est inapproprié et proposons donc l'appellation de « syndrome poly-organique associé à une morsure de tiques » (SPOT).

Objectif. – L'objectif de cette étude prospective monocentrique est de décrire les symptômes du SPOT et l'évolution sous traitement antibiotique prolongé de 100 patients.

Résultats. – L'efficacité du traitement antibiotique prolongé semble plus nette chez les patients ayant une forte probabilité de SPOT (score NEJM). Les patients ayant des troubles chroniques subjectifs avec un fort impact sur la vie courante méritent une attention particulière.

* Corresponding author.
E-mail address: c.perronne@rpc.aphp.fr (C. Perronne).

0399-077X/$ – see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.medmal.2008.11.012

Conclusion. – Malgré les biais évidents, cette étude peut aider à choisir les critères de faisabilité et d'inclusion ainsi que les bras d'une étude randomisée double insu permettant d'optimiser la prise en charge thérapeutique des patients traités présentant un SPOT.

Keywords: Antibiotic; Chronic Lyme disease; Tick-bites; Open-label prospective study

Mots clés : Antibiotique ; Lyme chronique ; Morsure de tiques ; Étude ouverte prospective

1. Introduction

Among tick-born infections, Lyme disease is universal and may cause acute, subacute or chronic signs and symptoms. Lyme disease is an initially cutaneous anthropozoonosis that may disseminate secondarily. This disease is caused by a bacterial pathogenic agent, *Borrelia burgdorferi sensu lato,* transmitted by an arthropod vector, the *Ixodes* tick [1]. The *erythema migrans*, which represents the early phase of the disease, was described at the beginning of the 20th century [2]. The secondary disseminating phase allowed to define the disease, following an epidemic of arthritis reported by Burgdorfer, at Lyme in 1982, in Connecticut [3]. When adequate antibiotic treatment is not prescribed in the early phase of the disease, the evolution may lead to chronicity. The diagnosis of chronic Lyme disease must be suggested, if there is a history of a remote tick-bite and/or an *erythema migrans*, and if the patient presents with symptoms involving various organs (central nervous system, joints, myocardium. . .) sometimes objective, often subjective associated with a positive *B. burgdorferi* serology or a prior antibody response. Even if the disease is now well documented, diagnostic and therapeutic issues remain. There is no formal test for the diagnosis, except for isolation of the pathogen *in situ*, which is a complex task [4]. Serology, currently the main diagnostic tool, must be interpreted cautiously, particularly in Europe because of important limitations regarding the test sensitivity and specificity [5–7]. Cases of seronegative Lyme disease proven by isolation of *B. burgdorferi* in patients have been reported [8–11]. In a recent prospective study conducted in patients presenting with a chronic symptomatology suggesting Lyme disease, 45% had a negative *B. burgdorferi* serology [12]. All these seronegative patients had a history of tick-bite and *erythema migrans* skin lesions. Even if the antibiotic treatment is now codified, a significant proportion of patients treated according to the recommendations complain of persistent signs and symptoms. This syndrome of post-treatment chronic Lyme disease (PTCLD) is a systemic illness persisting after adapted treatment, with severe fatigue, myalgia, polyarthralgia, paresthesia, and mood or memory disorders [13–15].

The pathophysiological mechanisms which underlie this syndrome, remain unclear. Several hypotheses have been discussed in the literature. The usual explanation is that it could be a post-infectious autoimmune syndrome or strictly sequels. It could also be due to the persistence of *B. burgdorferi* able to escape the immune response, or to co-infection by other pathogens [16–20]. These co-pathogens (other bacteria, or even parasites such as *Babesia)* could also be inoculated by ticks. There is no consensus on this chronic disease's pathology [21]. For some physicians post-treatment symptoms indicate persistent infection requiring prolonged and intensive antibiotic therapy [22]. For others, there is no benefit from antimicrobial therapy [23–25].

A clear-cut answer to these questions cannot be currently given, due to the difficulty of evaluation encountered in the studies. Many symptoms are subjective, there are often no biological abnormalities, microbiological or serological criteria for diagnosis are often not reliable, and there are no microbiological criteria for cure. Since the etiology is not clear, we think that the term chronic Lyme disease is not appropriate and should be replaced by chronic "tick associated poly-organic syndrome" (TAPOS). The objective of this study was to prospectively describe a group of patients having sought medical treatment for a chronic TAPOS and to evaluate their evolution under long-term antibiotic treatment.

2. Patients and methods

In this open-label prospective study, we analyzed 100 patients having consulted in the Infectious Diseases Unit between January 1999 and July 2002, for chronic TAPOS and answering inclusion criteria. Inclusion criteria were 18 years of age or more, presenting with chronic symptomatology (greater than 6 months) compatible with what already had been described as chronic Lyme disease in NEJM [12] articles and with either a history of *erythema migrans*, positive *B. burgdorferi* serology, or tick-bite. The patients had to have been already treated with an antibiotic course recommended for the primary or the secondary phase of Lyme disease. They should not have previously received a prolonged antibiotic treatment (longer than four weeks), and should not have received antibiotics or anti-inflammatory drugs in the three previous months. Patients presenting with primary forms of Lyme disease with recent tick-bites, and secondary forms (acute meningoradiculitis, myocarditis, arthritis) were not included in the study. Patients had to agree to be followed for at least three months, and if possible six months. All data was collected prospectively, by means of a standardized questionnaire and medical observation. In addition to sex and age, clinical signs and symptoms were described at inclusion (day 0), at month 3 of antibiotic treatment, and when possible at month 6. Suggestive signs and symptoms were classified in nine organ categories (neurological, articular, systemic, psychiatric or cognitive, cutaneous, cardiorespiratory, muscular, gastro-intestinal or endocrinal). We used a clinical score already published in the NEJM [12], which classifies the patients in terms of probability of chronic TAPOS. Points were given according to the presence of each item: *erythema migrans* (+5), positive *B. burgdorferi* serology (+5), tick-bite (+3),

several organ categories of signs and symptoms (+ 2), only one organ category of signs and symptoms (+ 1). The sum of points gave a score allowing classification of patients according to the clinical probability of chronic TAPOS (little probable: less than 5 points, probable: from 5 to 9 points, very probable: more than 9 points). Patients were treated with an antibiotic effective against *B. burgdorferi* for at least three months. When the clinical condition was not clearly improved at month 3, the antibiotic course could be prolonged to six months. All data was collected in the Epi-Info 2000 software and statistical analysis was made by the PhEMI Center (Pharmaco-epidemiology applied to Infectious Diseases, INSERM) at the Pasteur Institute in Paris.

3. Results

3.1. Patients characteristics

One hundred patients were included among those who consulted during the study period for suspicion of chronic Lyme disease. The sex ratio was 0.35 (65 women and 35 men) and the average age was 45.1 years (CI 95: 42–48). The most frequently reported symptoms at the time of inclusion were neurological (94%), articular (91%), systemic (88%), and cutaneous (76%). This clinical pattern corresponds to published clinical data after infection by the *Borrelia* subspecies present in Europe.

3.2. Signs and symptoms at inclusion

The prevalent general signs at inclusion were asthenia (83% of cases), fever (36%), weight gain or loss (35%). They were generally associated with organ involvements, mainly neurological, psychiatric or cognitive, articular or cutaneous. Neurological symptoms were present in 94% of cases:

- paresthesias (83%);
- headaches (63%);
- neuralgias (39%);
- visual disorders (47%);
- auditive disorders (30%);
- ataxia (38%).

Neuropsychological signs or cognitive impairment were:

- mood disorders (39%);
- depression (7%);
- memory disorders (44%);
- concentration disorders (47%);
- sleep disorders (44%).

Articular signs were present in 91% of patients:

- poly-arthralgias (83%);
- recurrent localized arthralgia (56%);
- arthritis (12%).

Cutaneous signs were present in 76% of patients:

- a history of chronic *erythema migrans* (53%);
- recurrent *erythema migrans* (16%);
- nonspecific erythematous plaques of chronic evolution (26%);
- aphthosis (16%);
- chronic pain at the tick-bite site (14%).

Cardiorespiratory signs and symptoms were present in 73% of patients:

- arrhythmia (52%);
- thoracic pain (41%);
- effort dyspnea (25%);
- chronic cough (11%);
- recurrent pharyngitis (20%);
- rhythm disorders (13%);
- arterial hypertension (11%).

Muscular signs and symptoms were present in 67% of patients:

- myalgia or flu-like syndrome of cyclic evolution (65%);
- muscle fasciculation (17%).

Gastro-intestinal symptoms were present in 47% of patients:

- epigastric pain (25%);
- or intestinal disorders (diarrhea, constipation or both) (26%).

Thyroid disorders were reported in 4% of patients.
We considered for the analysis that objective signs included:

- heart conduction and rate disorders (registered on electrocardiography);
- arterial hypertension, mono or oligo-arthritis;
- temporal and space disorientation;
- neurological deficit;
- hearing or visual disorders;
- chronic *erythema migrans*;
- galactorrhea;
- dysthyroidism;
- aphthosis;
- adenomegaly;
- fever.

3.3. Clinical score

A history of *erythema migrans* was found in 53% of the patients, *B. burgdorferi* serology was positive in 51%, a history of tick-bite in 69%, and the combination of several organ categories of suggestive symptoms in 82% for more than four organ categories, 10% for four, and 8% for three associated organ categories. Among the 100 patients, 67 were classified as "very probable", 27 patients as "probable", and six patients "little probable".

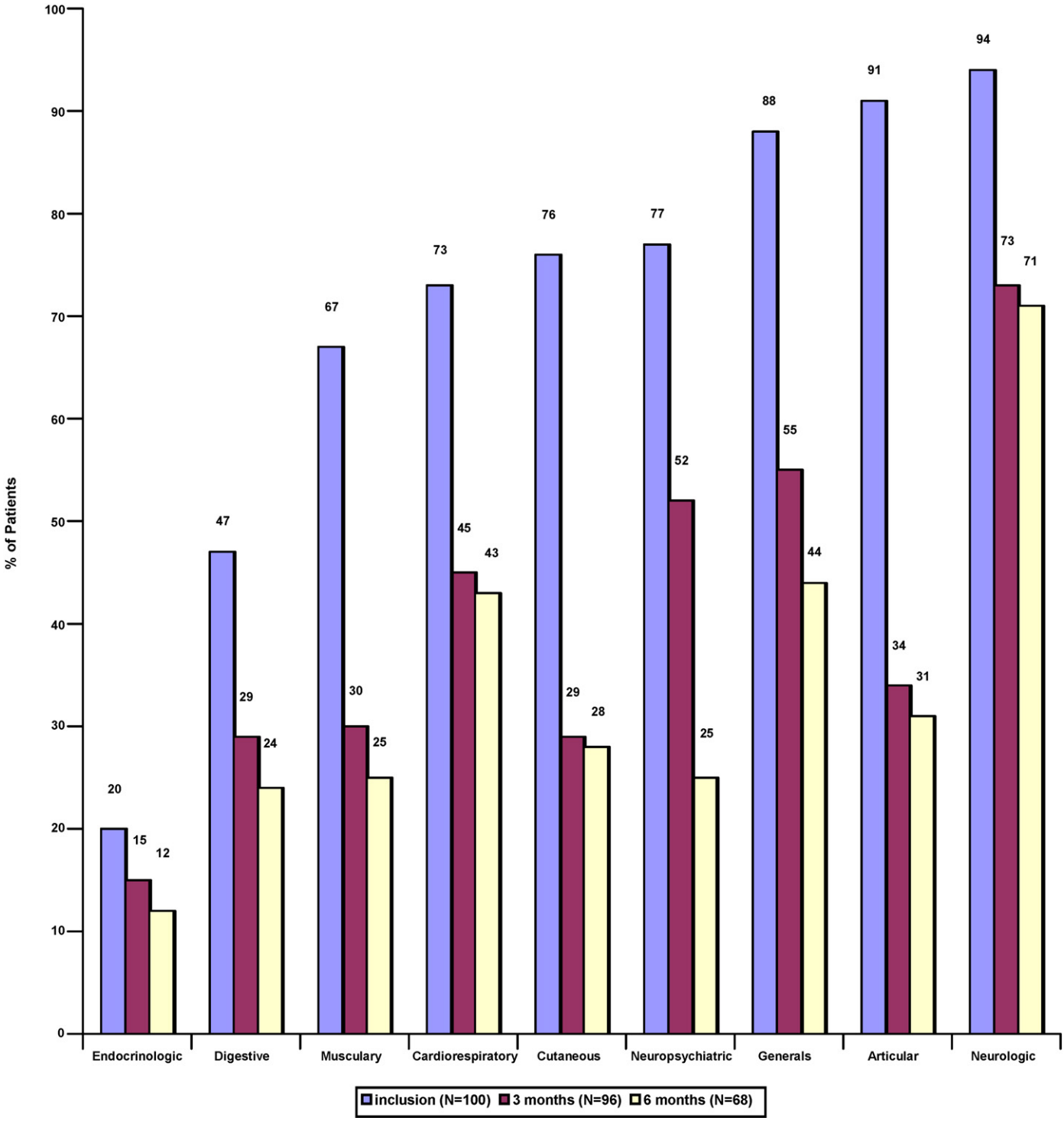

Fig. 1. Evolution of signs and symptoms under treatment, by organ category
Évolution des signes et symptômes sous traitement, par catégorie d'organes.

3.4. Antibiotic treatment

The most frequently used antibiotics were amoxicillin (39%), ceftriaxone (31%), doxycycline (27%), clarithromycin (4%), and more rarely penicillin G (1%) or tinidazole (2%).

3.4.1. Evolution under antibiotic treatment

Of the 100 patients included in the study, 96 were evaluated at month 3 and 68 at month 6 after the beginning of antibiotic treatment. The evaluation performed at each medical visit (month 3 and month 6) showed a decrease in the number and intensity of signs and symptoms under antibiotic treatment (Fig. 1). The

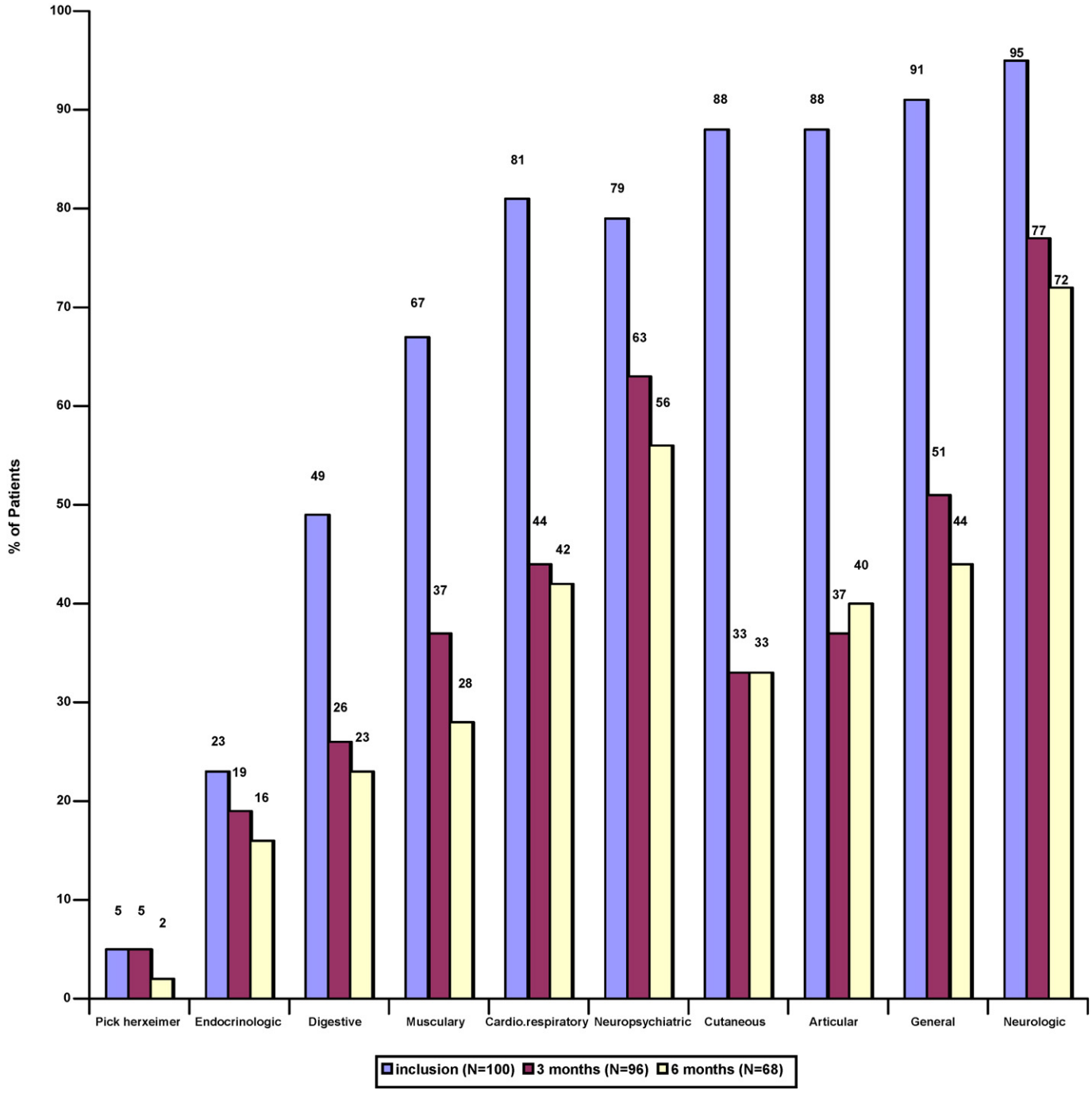

Fig. 2. Evolution of patients classified with a "very probable" diagnosis of chronic "tick associated poly-organic syndrome" (TAPOS).
Évolution des patients classés avec un diagnostic "très probable" de « syndrome poly-organique associé à une morsure de tiques » (SPOT) chronique.

number of organ categories for signs and symptoms presented by the patients declined during the antibiotic treatment period: the percentage of patients presenting with more than four organ categories decreased from 82% at inclusion, to 39% at month 3, and to 31% at month 6.

3.4.2. Evolution of the signs and symptoms according to the organ category, their objective or subjective nature, and to the clinical score of chronic TAPOS

This evolution was evaluated in the subpopulation of patients whose follow-up was extended to month 6. Among these 64 patients, 43 presented with "very probable", 16 "probable" and five "little probable" chronic TAPOS. The average age and the sex ratio of this subpopulation were not significantly different from those observed in the general population of the study. In the "very probable" group, the reduction of number of signs and symptoms was observed for each organ category. However, neurological signs and symptoms were the least improved during treatment. On the other hand, systemic, articular and cutaneous symptoms appeared more susceptible to antibiotic treatment (Fig. 2). The evolution of these two types of signs and symptoms under antibiotic treatment was evaluated separately. The

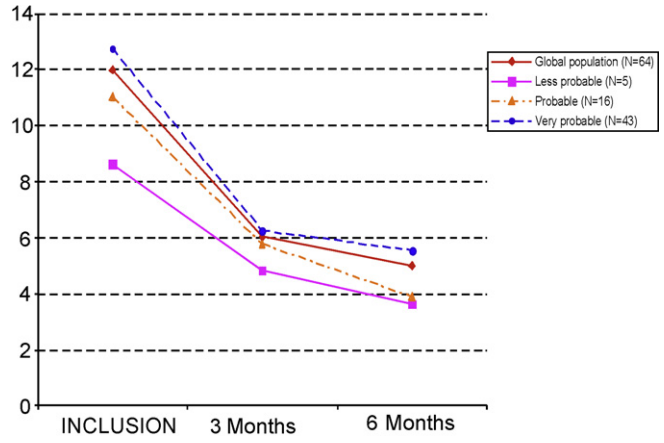

Fig. 3. Evolution of the average number of subjective symptoms according to the clinical score of "tick associated poly-organic syndrome" (TAPOS) at inclusion.
Évolution du nombre moyen de symptômes subjectifs selon le score clinique « syndrome poly-organique associé à une morsure de tiques » (SPOT) à l'inclusion.

"very probable" group had a steeper slope of decrease for subjective symptoms (Fig. 3). The average number of objective signs also decreased in time under antibiotics, with a steeper slope for patients with a "very probable" diagnosis of TAPOS (Fig. 4). We observed a clear decrease of objective signs and subjective symptoms under prolonged treatment with antibiotic, with a stronger effect between inclusion and month 3, especially in the groups of patients classified as "probable" and "very probable". No case of clinical aggravation or serious adverse event was reported in the three groups. Although this data was not prospectively recorded, around three-quarters of our study patients experienced an exacerbation of signs and symptoms during the antibiotic treatment, either early acute reactions typical of Jarish-Herxheimer syndrome, or later subacute reactions. Exacerbation of signs could last several weeks or even several months in some patients, with the possibility of cyclic evolution. The difference between

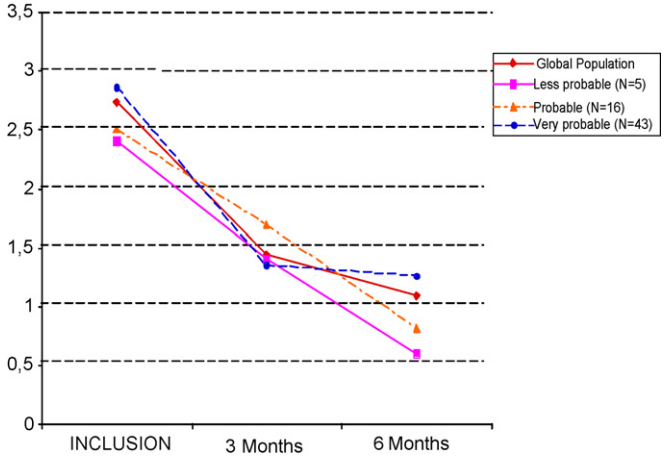

Fig. 4. Evolution of the average number of objective signs according to the clinical score of "tick associated poly-organic syndrome" (TAPOS) at inclusion.
Évolution du nombre moyen des signes objectifs selon le score clinique « syndrome poly-organique associé à une morsure de tiques » (SPOT) à l'inclusion.

slopes of either objective signs or subjective symptoms between inclusion and month 3 was not significant in the "little probable" group, but was highly significant in the "probable" group ($p = 0.002$ and $p < 0.001$, respectively), and in the "very probable" group ($p < 0.001$). The most significant difference was observed in the decrease of objective signs between inclusion and month 3 among patients classified as "very probable".

4. Discussion

Chronic Lyme disease is often considered in case of long-lasting symptoms after a tick-bite, but there are few descriptive epidemiological studies on the clinical presentation and the evolution in the course of time under prolonged antibiotic treatment [22–25]. This syndrome is not well defined, hence its pathophysiology is unclear and probably not due to only *B. burgdorferi* infection. We think that the denomination chronic Lyme disease is not appropriate and should be replaced by chronic TAPOS. In this prospective study of 100 patients, consulting for chronic TAPOS, the analysis of population epidemiological characteristics and symptoms presented bring new data on some poorly documented concepts. The definition of a clinical diagnostic score permitted the description and comparison of evolution under treatment of three categories of patients. The main objective of this study was to describe a population presenting with TAPOS and to help the clinician improve his diagnosis and therapeutic management by using a probability score. The antibiotics used varied according to the prescribing physician. One can thus regret a lack of uniform choice of medication. The descriptive analysis of our population gave a sex ratio of 0.35 (35 men for 65 women). Classically the sex ratio is close to one, compatible with the mode of vectorial transmission [26,27]. There is no obvious explanation for the high prevalence of women in our study. There may be a genetic or hormonal influence on the occurrence of Lyme disease tertiary phase. Are women more likely to develop post-infectious, autoimmune, or inflammatory symptoms? [28,29]. The average age of this adult population was 45 years, which corresponds to the second peak of Lyme disease. There are two peaks of frequency for the disease, the first between five and nine years and the second between 45 and 60 years. Our patients were young. Inflammatory or autoimmune diseases are frequent at this age range. A positive history of tick-bite was found in 69% of the patients, corresponding to published data [30]. Several explanations can be suggested: an unknown tick-bite (on a invisible zone, or tick-bite at the nymph stage with a small diameter 0.5 to 1 mm), or forgetting the bite. The percentage of positive Lyme serology at the time of inclusion or in the patient's history was 51%. *Borrelia* serology is not fully reliable in Europe [5,6,31]. False-positive results are frequent, and the currently available direct or indirect serologies may present cross-reactions with autoantibodies such as EBV infections, bacterial endocarditis, *Ehrlichia* and *Babesia* infections. False-negative results are also frequent. There is no reliable serological test in Europe and Asia due to heterogeneity of *B. burgdorferi* subspecies. In the early phase, serology is often negative and after a period of positivity, may become negative

(loss of the antibodies, local precipitation, antigen-antibody complexes,). In addition, there may be problems of reproducibility between laboratories (without a reference technique). Moreover, the threshold of positivity of the ELISA test is determined artificially by a consensus of biological experts, in order to avoid more than 5% *B. burgdorferi* seropositive persons in the general population of blood donors. In Europe, the official recommendation for laboratories is to give a negative result for *Borrelia* serological test if the level of antibodies is under the ELISA threshold, even if the Western blot is highly positive.

PCR detection techniques for *Borrelia* DNA in various tissues is currently the most specific technique, but lacks sensitivity [5]. In our study, the most frequently used antibiotics were beta-lactams (mainly amoxicillin and ceftriaxone, penicillin G in a few cases) and doxycycline, which correspond to the drugs currently recommended [32,33]. Clarithromycin was also used [22,34]. A few patients received tinidazole which was proved effective in vitro against cystic forms of *Borrelia* [16,18]. In our study, the frequency of neurological symptoms was more important than that of articular symptoms. In Europe, the known subspecies (*Borrelia garinii and B. afzelii*) have a preferential neurological tropism [4,35]. Contrary to the United States, where the isolated species (*B. burgdorferi sensu stricto*) cause pure articular symptoms, there were no isolated articular presentations in our study [36]. The patients in our cohort presented with various "poly-organic symptoms" and a quasi-constant general impairment (asthenia, anorexia, depressive syndrome). Many symptoms attributed to a late phase of Lyme disease are subjective. Thus, the few signs objectified by the clinician have a great diagnostic value. Some disturbances, seldom described in this disease, were noted in our study. Thyroid disorders were found in 4% of the patients. This frequency could be related to autoimmune mechanisms, with multiplication of *Borrelia* in the thyroid gland [37,38]. In addition, chronic aphthosis was observed in 16% of our patients. Approximately half of the patients of our study presented with memory, sleep, concentration, libido, and mood disorders deteriorating their quality of life. It is difficult to determine whether these disorders were due to a direct *Borrelia* attack in the brain in the primary and secondary phases, or to a depressive syndrome caused by a chronic disease which is painful and invalidating. The frequency of headaches (63%) among patients without history of migraine, associated with neck rigidness (44%) can evoke the existence of a chronic pseudo-meningitis syndrome after a tick-bite. The 100 patients included in the study received from three to six months of antibiotic treatment. No patient experienced severe adverse effects linked to treatment. Only some benign side effects were listed, such as cutaneous rashes, gastro-intestinal disturbances, or taste impairment. The symptoms whatever the diagnostic probability, decreased progressively. The effect of the antibiotic treatment was more important for articular and cutaneous signs, whereas neurological signs were apparently more permanent. The treatment was not fully effective, since only 4% of the patients considered themselves "cured". Improvement was limited in 60% of the patients. This treatment, even if it did not cure all the patients, led to improvement of quality of life, with reinsertion in the family life and often return to work. Various hypotheses can be suggested for the failure frequency of antibiotherapy. It could be due to insufficient dosage (poor intracerebral or intratissue penetration), route of administration or insufficient length of course. The persistence of symptoms after treatment can suggest a secondary autoimmune reaction with antibody over response against *Borrelia* or a general chronic inflammatory disease after bacterial aggression [28,29]. Another hypothesis is the existence of co-infection by one or more microorganisms such as *Babesia, Ehrlichia, Anaplasma, Bartonella, Mycoplasma, Chlamydia, Brucella, Rickettsia, Coxiella*, viruses such as the tick-born encephalitis (TBE) virus or HHV6. Some of these pathogens may be co-transmitted at the time of the tick-bites [39–41]. The partial lack of antibiotic efficacy could also be due to the capacity of *Borrelia* to escape the bactericidal effect of antibiotics as cystic forms. The cyclic conversion of cystic forms in free spirochetes could release new *Borrelia* in tissues [16,18]. The cyclic evolution was noted clinically for 28% of the patients in our study and could correspond to bacterial multiplication cycles. Psychiatric or cognitive symptoms were the most difficult to treat. The central nervous system, altered in an early phase, could be a sanctuary site, quiescent forms less accessible to antibiotic treatment [19,20]. Exacerbation of signs and symptoms during antibiotherapy and the course of this exacerbation has not been well studied in the chronic forms of Lyme disease. These exacerbation phenomena may impede the evaluation of clinical improvement and could be partly responsible for the negative results of antibiotic treatment in chronic Lyme disease reported in several articles. The rather good results observed by Donta in the treatment of chronic Lyme disease when combining hydroxychloroquine to a macrolide antibiotic could be due, to an anti-infectious action of hydroxychloroquine in addition to the possible immunomodulatory effect of hydroxychloroquine [22,34].

Hydroxychloroquine is effective against parasites such as *Babesia*, and is also able to enhance the bactericidal activity of antibiotics within leucocytes, as it was shown for *Coxiella burnetii* or *Mycobacterium tuberculosis* [42,43]. Moreover, Brorson and Brorson showed that hydroxychloroquine could exhibit a direct inhibitory effect against *B. Burdorferi* [16,18]. Our patients presented with post tick-bite symptoms, often invalidating and diminishing their quality of life, the cause of frequent medical consultations. The patients, more often female, were sometimes regarded as having disorders of hysterical nature. Nevertheless, these disorders deserve a particular consideration. In our study we tried to demonstrate that medical management was effective on symptoms, especially for patients with a high probability of chronic TAPOS. We did not investigate co-infections. However cases of co-infections by other tick-transmitted diseases are more frequently described, maybe accounting for the limited improvements observed.

5. Conclusion

It was thus appropriate to perform a double-blind, randomized study, taking into account the pitfalls of previous studies, to confirm the antibiotic effect and to rule out a "white blouse effect" on chronic symptoms in the context of chronic TAPOS

[44]. Although the present study may have methodological limits, it will certainly help in terms of feasibility, choice of inclusion criteria, and design of follow-up for a future randomized, double-blind study testing the efficacy of prolonged anti-infectious therapy versus placebo among patients presenting with signs and symptoms of chronic TAPOS.

References

[1] Sternbach G, Dibble CL. Willy Burgdorfer: Lyme disease. J Emerg Med 1996;14:631–4.

[2] Dammin GJ. *Erythema migrans*: a chronicle. Rev Infect Dis 1989;11:142–51.

[3] Burgdorfer W. How the discovery of *Borrelia burgdorferi* came about. Clin Dermatol 1993;11:335–8.

[4] Saint Girons I, Gern L, Gray JS, Guy EC, Korenberg E, Nuttall PA, et al. Identification of *Borrelia burgdorferi sensu lato* species in Europe. Zentralbl Bakteriol 1998;287:190–5.

[5] Wormser GP, Aguero-Rosenfeld ME, Nadelman RB. Lyme disease serology: problems and opportunities. J Am Med Assoc 1999;282:79–80.

[6] Kaiser R. False-negative serology in patients with neuroborreliosis and the value of employing of different *Borrelia* strains in serological assays. J Med Microbiol 2000;49:911–5.

[7] Hauser U, Lehnert G, Lobentanzer R, Wilske B. Interpretation criteria for standardized western blots for three European species of *Borrelia burgdorferi sensu lato*. J Clin Microbiol 1997;35:1433–44.

[8] Berger TG, Schoerner C, Schell H, Simon M, Schuler G, Rollinghoff M, et al. Two unusual cases of diffuse acrodermatitis chronica atrophicans seronegative for Lyme borreliosis. Eur J Clin Microbiol Infect Dis 2003;22:392–5. Epub 2003 May 29.

[9] Dejmkova H, Hulinska D, Tegzova D, Pavelka K, Gatterova J, Vavrik P. Seronegative Lyme arthritis caused by *Borrelia garinii*. Clin Rheumatol 2002;21:330–4.

[10] Brunner M. New method for detection of *Borrelia burgdorferi* antigen complexed to antibody in seronegative Lyme disease. J Immunol Methods 2001;249:185–90.

[11] Hofmann H. Lyme borreliosis-problems of serological diagnosis. Infection 1996;24:470–2.

[12] Klempner MS, Hu LT, Evans J, Schmid CH, Johnson GM, Trevino RP, et al. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 2001;345:85–92.

[13] Asch ES, Bujak DI, Weiss M, Peterson MG, Weinstein A. Lyme disease: an infectious and post infectious syndrome. J Rheumatol 1994;21:454–61.

[14] Bujak DI, Weinstein A, Dornbush RL. Clinical and neurocognitive features of the post Lyme syndrome. J Rheumatol 1996 Aug;23:1392–7.

[15] Weinstein A, Britchkov M. Lyme arthritis and post-Lyme disease syndrome. Curr Opin Rheumatol 2002;14:383–7.

[16] Brorson O, Brorson SH. In vitro conversion of *Borrelia burgdorferi* to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H medium. Infection 1998;26:144–50.

[17] Murgia R, Cinco M. Induction of cystic forms by different stress conditions in *Borrelia burgdorferi*. APMIS 2004;112:57–62.

[18] Brorson O, Brorson SH. An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to hydroxychloroquine. Int Microbiol 2002;5:25–31.

[19] Aberer E, Koszik F, Silberer M. Why is chronic Lyme borreliosis chronic? Clin Infect Dis 1997;25(Suppl. 1):S64–70.

[20] Liang FT, Jacobs MB, Bowers LC, Philipp MT. An immune evasion mechanism for spirochetal persistence in Lyme borreliosis. J Exp Med 2002;195:415–22.

[21] Edlow JA. Bull's eye: unraveling the mystery of Lyme disease. New Haven, Ct: Yale University Press; 2003.

[22] Donta ST. Tetracycline therapy for chronic Lyme disease. Clin Infect Dis 1997;25(Suppl. 1):S52–6.

[23] Cairns V, Godwin J. Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms. Int J Epidemiol 2005;34:1340–5. Epub 2005 Jul 22.

[24] Fallon BA, Keilp JG, Corbera KM, et al. A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy. Neurology 2008;25:992–1003.

[25] Auwaerter PG. Antibiotic therapy is not the answer for patients with persisting symptoms attributable to Lyme Disease. CID 2007;45:143–8.

[26] Dhote R, Basse-Guerineau AL, Beaumesnil V, Christoforov B, Assous MV. Full spectrum of clinical, serological, and epidemiological features of complicated forms of Lyme borreliosis in the Paris, France, area. J Clin Microbiol Infect Dis 2000;19:809–15.

[27] Bowen GS, Griffin M, Hayne C, Slade J, Schulze TL, Parkin W. Clinical manifestations and descriptive epidemiology of Lyme disease in New Jersey, 1978 to 1982. J Am Med Assoc 1984;251:2236–40.

[28] Bolz DD, Weis JJ. Molecular mimicry to Borrelia burgdorferi: pathway to autoimmunity? Autoimmunity 2004;37:387–92.

[29] Singh SK, Girschick HJ. Lyme borreliosis: from infection to autoimmunity. Clin Microbiol Infect 2004;10:598–614.

[30] Petersen LR, Sweeney AH, Checko PJ, Magnarelli LA, Mshar PA, Gunn RA, et al. Epidemiological and clinical features of 1149 persons with Lyme disease identified by laboratory-based surveillance in Connecticut. Yale J Biol Med 1989;62:253–62.

[31] Reed KD. Laboratory testing for Lyme disease: possibilities and practicalities. J Clin Microbiol 2002;40:319–24.

[32] Wormser GP, Nadelman RB, Dattwyler RJ, Dennis DT, Shapiro ED, Steere AC, et al. Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. Clin Infect Dis 2000;31(Suppl. 1):1–14.

[33] SPILF. 16e Conférence de Consensus en thérapeutique anti-infectieuse. 13 décembre 2006. Borréliose de Lyme : démarches diagnostiques, thérapeutiques et préventives.

[34] Donta ST. Macrolide therapy of chronic Lyme Disease. Med Sci Monit 2003;9:PI136–42.

[35] Baranton G, Marti Ras N, Postic D. *Borrelia burgdorferi*, taxonomy, pathogenicity and spread. Ann Med Int (Paris) 1998;149:455–8.

[36] Baranton G, Marti Ras N, Postic D. Molecular epidemiology of the aetiological agents of Lyme borreliosis. Wien Klin Wochenschr 1998;110:850–5. Review.

[37] Paparone PW. Hypothyroidism with concurrent Lyme disease. J Am Osteopath Assoc 1995;95:435–7.

[38] Becker CB, Trock DH. Thyrotoxicosis resembles Lyme disease. Ann Intern Med 1991;114:914–5.

[39] Buckingham SC. Tick-borne infections in children: epidemiology, clinical manifestations, and optimal management strategies. Paediatr Drugs 2005;7:163–76.

[40] Hermanowska-Szpakowicz T, Skotarczak B, Kondrusik M, Rymaszewska A, Sawczuk M, Maciejewska A, et al. Detecting DNAs of *Anaplasma phagocytophilum* and *Babesia* in the blood of patients suspected of Lyme disease. Ann Agric Environ Med 2004;11:351–4.

[41] Amiel C, Abadia G, Choudat D. Human granulocytic ehrlichiosis in Europe. Med Mal Infect 2004;34:111–22.

[42] Raoult D, Houpikian P, Tissot Dupont H, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Arch Intern Med 1999;159:167–73.

[43] Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infections in the 21st century. Int J Antimicrob Agents 2007;30:297–308.

[44] Feder HM, Johnson BJ, O'Connell S, Shapiro ED, Steere AC, Wormser GP, et al. A Critical Appraisal of "Chronic Lyme Disease". N Engl J Med 2007;357:1422–30.