science [phlebotomy | blood banking/transfusion medicine | coagulation and hematology]

Hemolysis in Serum Samples Drawn by Emergency Department Personnel versus Laboratory Phlebotomists

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- The study examined the rates of hemolysis in 2 sample populations and compared the techniques used to draw blood.
- The frequency of hemolysis was significantly higher in the samples coming from the Emergency Department (ED).

Pre-analytic mechanical hemolysis of blood samples can lead to inaccurate laboratory results. It can cause spuriously elevated levels of potassium, lactate dehydrogenase (LDH), and other analytes. We examined the rates of hemolysis in 2 sample populations, a hospital medical ward and an emergency department, and compared the techniques used to draw blood in order to determine which factors play a true role in causing excess mechanical hemolysis.

We calculated the rate of hemolysis in 4,021 patients. We then examined 202 procedures for various factors involved in the blood drawing process to determine which, if any, have an increased chance of causing mechanical hemolysis. These included needle gauge and material, site of venipuncture, tourniquet compression time, level of tube fill, use of Vacutainer or syringe, and the use of extension tubing. The contributions of the various processes to hemolysis were compared using chi-square analysis and Fisher's exact test. Logistic regression was then used to best model those factors that are most likely to produce mechanical hemolysis.

The rate of hemolysis among emergency department drawn specimens was 12.4% versus 1.6% for those drawn by laboratory personnel ($P<0.0001$). Statistically significant differences in the rate of hemolysis (ie, greater hemolysis) were found when the following parameters were compared: distal arm draw versus antecubital fossa draw ($P=0.0054$), 22 gauge plastic cannula versus 20 gauge cannula ($P=0.0104$), less than half-full tube draw versus greater than or equal half-full tube draw ($P=0.0159$), tourniquet compression time of greater than 2 minutes versus less than or equal 2 minutes ($P=0.016$), and plastic versus metal cannulas ($P=0.0164$). Logistic regression of these data to control for confounding variables demonstrated that the following factors contributed most to causing mechanical hemolysis: drawing from a vein in the distal arm and drawing through a narrower gauge needle. Use of a standardized protocol for blood drawing can reduce the rate of pre-analytic hemolysis by more than 7-fold. Drawing blood from the antecubital fossa using a needle of 20 gauge or more can best reduce hemolysis.

Background

Pre-analytic mechanical hemolysis of blood samples can lead to inaccurate laboratory results and delays in patient care. Hemolysis may result in spuriously elevated serum potassium and bilirubin, as well as acid phosphatase, zinc, magnesium, albumin, and creatine kinase (CK).[1] There are various external factors that may cause hemolysis, including the gauge of the cannula and the pressure exerted on the blood at the time of drawing.[2,3] A syringe can generate enough pressure to cause hemolysis when used with a large bore needle.[2] Syringes are capable of creating a higher pressure differential than a Vacutainer, leading to more hemolysis in vitro.[2] Hemolysis may also result from mechanical trauma occurring during transport to the clinical laboratory. There has been much debate over the role of the pneumatic-tube systems in hemolysis. Studies have shown that parameters such as hemoglobin, potassium, LDH, and acid phosphatase, chosen as markers for hemolysis, rise in samples transported through pneumatic-tube systems, particularly if the specimen tubes are less than half full, or if the specimens were sent though the system repeatedly.[6,8-10] Other studies have found that potassium, LDH, and serum hemoglobin are not altered when blood is sent through pneumatic tubes.[4,5,7] Variability in results may result from system differences in properties such as length, speed, acceleration, and number of turns.

Observations by treating physicians and clinical laboratory staff suggest that the rate of hemolysis in samples drawn in emergency departments exceeds those drawn by professional laboratory phlebotomists. We examined the rates of hemolysis in these 2 sample populations and compared the techniques used to draw blood in order to determine which factors play a role in causing excess pre-analytical hemolysis.

Materials and Methods

The study received approval from the medical center institutional review board. The first part of the study involved a validation of the casual observation of increased frequency of hemolysis observed in specimens drawn in the ED compared to those from a non-emergency department setting. We performed a retrospective analysis of data on 2,992 chemistry panels from the ED and 1,029 from a routine medicine ward in an acute care teaching hospital over a period of 11 weeks. The total number of specimens analyzed was culled from the laboratory

information system. The number of hemolyzed samples was determined by investigating the "Daily Comment Logs" for the "hemolysis" comment in the computer that identifies rejected specimens as analytically unacceptable. These logs include the department from which each of the hemolyzed samples originated.

Prior to beginning the study, the ability of the technologists to properly identify hemolysis was ascertained. A sample of whole blood was hemolyzed with ammonium chloride and the serum was separated. Ten serial dilutions of the clearly hemolyzed serum were made using normal non-hemolyzed serum as the diluent. The samples thus ranged from normal through grossly hemolyzed as ascertained by spectrophotometric analysis at the hemoglobin peak absorption wavelength. Each technologist involved in the study was tested and certified as to their ability to properly identify the presence of hemolysis. The percentages of hemolyzed samples from the medical ward and the ED were compared. Data were analyzed using the chi-squared test.

The second part of the study examined possible causes of hemolysis. We directly observed 204 blood drawings in the ED of the hospital. The study population included any patient who came into the ED needing chemistry work. This included pediatric patients. No patient demographics other than sample number were recorded. Only red top tubes drawn for chemistry panels were included in the study. Blood samples were drawn by either ED nurses or technicians. Cannula type and gauge were left to the discretion of the nurse or technician who drew the sample, as was the site of puncture. The type and gauge of the cannula, use of extension piece or syringe, and site of puncture were recorded. The level to which the Vacutainers were filled was determined by comparison to a reference tube that was filled with water and marked in milliliter graduations. All samples were transported to the chemistry laboratory by pneumatic tubes. The endpoint of hemolysis was determined by the chemistry laboratory technician. We reviewed The "Daily Comment Logs" to determine which samples were labeled as "hemolyzed." Results of the site, IV catheter gauge, fullness of the tubes, and use of the extension tubing were analyzed using chi-squared test. Fisher's exact probability test was used for tourniquet time, catheter material, and use of the syringe.

Hemolysis in association with individual parameters. T1

Parameters	n	# hemolyzed	*P*-value/test
Antecubital	94	4	0.0054
distal arm	100	18	Chi-squared
22G-IV	33	10	0.0123
20G-IV	115	12	Chi-squared
<1/2 full	70	13	0.0159
>1/2 full	128	8	Chi-squared
Plastic	163	22	0.0164
Metal	37	0	Fisher's
Extension	77	7	0.68
no extension	125	15	Chi-squared
syringe	10	1	1.0
no syringe	192	21	Fisher's

Results

Comparison of ED to Medical Floor

A total of 4,021 samples were reviewed. Hemolysis was considerably more frequent in the ED samples. Of the 2,992 blood drawings from the ED, 372 (12.4%) were hemolyzed. Of the 1,029 samples from the medical floor, 16 (1.6%) were hemolyzed ($P<0.0001$).

Phlebotomy Technique Analysis

Chi-squared analysis showed that the occurrence of hemolysis in samples drawn through 20 gauge plastic catheters was less [T1] than that from 22 gauge catheters ($P=0.0123$). Hemolysis from plastic catheters versus metal needles (butterflies or straight) was significantly greater ($P=0.0164$). Chi-squared analysis of hemolysis in blood samples drawn from the antecubital fossa (AC) was less than from those drawn distal to the antecubital fossa ($P=0.0054$). The fullness of the sample tubes, less than half versus greater than or equal to half, was also significant ($P=0.0159$); the fuller tubes showing less hemolysis. Use of extension tubing or a syringe during blood drawing did not effect the occurrence of hemolysis ($P=0.68$, $P=1.00$).

Logistic regression of these data was used to control for confounding variables. The site, fullness, and catheter material were included in the analysis. Catheter gauge was excluded because of its relation to the catheter material type, all of the catheters being plastic. Logistic regression by SAS software demonstrated that the following factors contributed most to causing mechanical hemolysis: 1) drawing from a vein in the distal arm ($P=0.034$), and 2) the fullness of the tube ($P=0.026$).

Discussion

ED vs Medical Floor

The frequency of hemolysis was significantly higher in the samples coming from the ED. Blood samples from the medical floor were predominantly drawn by trained phlebotomists who used straight Vacutainer needles and butterflies and transported the blood to the laboratory by hand. These individuals had all undergone formal training and certification in blood drawing techniques. In contrast, the blood samples from the ED were largely drawn through plastic catheters and were transported via pneumatic tube. The ED phlebotomists were trained in the ED and were not certified. The site of puncture in the phlebotomist drawn group was primarily in the antecubital fossa as opposed to the ED group which was more distal. Although inadequately documented, this may be a source of variation between the 2 groups.

Observed Technique Study

Although the chi-squared analysis showed plastic vs metal cannulas as being significantly different, the logistic regression analysis showed this factor to be noncontributory. Logistic regression analysis revealed that the key variables were site of draw and fullness of the tubes. The antecubital fossa may be more favorable than the distal arm because of the faster flow due to its increased diameter and reduced resistance. In addition, smaller cannulas may be used for distal blood draws. The fullness of the tubes may affect hemolysis through the poorly understood effect of pneumatic tube transportation. Further investigation is necessary to elucidate the causes.

The endpoint for hemolysis used in this study, though not quantifiable, is true to life. Samples are rejected not because their spectrophotometer reading indicates excessive hemolysis, but because the technician perceives it to be hemolyzed. Newer clinical chemistry analyzers have built in "hemolysis" flags that reject specimens that exceed a predetermined spectrophotometric standard for hemolysis.

The barriers to reducing hemolysis include personal preference and feasibility. It is common in emergency medicine to draw blood samples from the distal arm when placing IVs to reduce the number of venipunctures. Emergency department personnel avoid placing the IV in the antecubital fossa because of the possibility of the catheter bending and obstructing infusions when the patient moves their arm. They are more likely to draw blood from the distal arm. Likewise, it may be difficult to fill a specimen tube despite one's best efforts.

Use of a standardized protocol for blood drawing can reduce the rate of preanalytic hemolysis by more than 7-fold. Hemolysis can best be reduced by drawing blood from the antecubital fossa and by completely filling the tube

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