Hemolysis detection and management of hemolyzed specimens

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Received: March 23, 2010 *Accepted: May 1, 2010*

Abstract

Assay interferences have long been underestimated and unfortunately too often undetected in the daily clinical laboratory practice. The extra-analytical phase of the laboratory testing process has been recognized as the major source of laboratory errors over the past decade. Preanalytical errors are most common errors within the total testing process and hemolysis is recognized as one of the most prevalent preanalytical errors and surely the most prevalent interference in clinical laboratory testing. Visual detection of hemolysis is arbitrary and therefore mostly unreliable since it may over- and underestimate the actual prevalence of hemolyzed serum specimens (i.e., trained observers are unable to accurately rank the degree of interference in serum). Elevated concentration of bilirubin may further impair the ability to detect hemolysis by visual inspection and therefore lead to serious underestimation of hemolysis in neonatal samples where elevated bilirubin concentration is commonplace. The recent advances in laboratory technology have lead to an increasing trend in the automation of various preanalytical processes into large preanalytical modules. Such modules as well as novel automated laboratory analyzers offer the automated detection of serum indices. This is advantageous due to the increased reproducibility and the improvement in detection of mildly hemolyzed specimens (serum hemoglobin < 0.6 g/L). These platforms commonly use the semiquantitative spectrophotometric measurement and grade interfering substances into several categories. However, various analytical platforms may have different decision thresholds for various serum indices. Moreover, different systems might be different in their assay parameters and the degree of the interference of the specific interfering substance. Therefore, more efforts should be focused to standardize the mean of reporting the hemolysis index, especially when this important parameter is used for obtaining meaningful information on the quality of sample collection throughout collection centers and wards. Hemolysis is still one of the biggest challenges to the laboratory specialists. In case of hemolysis, laboratory personnel should always ask for new sample(s). In case new sample(s) can not be obtained, it is the responsibility of the laboratory specialist to communicate the problem with the physician responsible for the patient and seek for the solution to the best of the patient care.

Key words: hemolysis; extra-analytical quality; errors; laboratory testing; interference

Introduction

Although there might be potential substantial detrimental outcomes for patient safety, assay interferences by some common endogenous and exogenous substances have long been underestimated and unfortunately too often undetected in the daily clinical laboratory practice (1,2). Some important changes have occurred over the past decade, facilitating the recognition of the extra-analytical phase of the laboratory testing process as the leading source of laboratory errors (3,4) and the identification of the most successful models for detection, quantification and management of the extra-analytical sources of variability (5-7). Several large surveys have also been performed, with the aim to explore the quality of practices related to pre- and postanalytical procedures. The results of these studies highlight the high degree of heterogeneity and the lack of standardization of laboratory practices (8), the need for introduction of standardized routines and regular staff training (9) as well

as all other relevant actions to improve the quality of extra-analytical phase of the testing process, especially for sample collection (10,11).

As reported by Mario Plebani, the preanalytical errors are most commonly occurring within the total testing process (up to two-third of the total number of errors), followed by the errors from the post-analytical phase (18.5–47.0% of total errors) (12, 13). As in many original reports, hemolysis is recognized as one of the most prevalent preanalytical errors and – surely – the most prevalent interference in clinical laboratory testing (14–16). The aim of this review is to summarize the current knowledge and practices regarding the detection of hemolysis and the management of hemolyzed specimens in the daily laboratory practice.

Visual inspection

It has long been known that visual assessment of the degree of actual concentration of bilirubin, hemolysis and lipids is mostly unreliable. Glick *et al*. explored the frequency of turbid, hemolyzed and icteric specimens (N = 2,599) in one acute-care general hospital (17). Visual assessment was performed using the full-color photographs of serum specimens containing various concentrations of the interferent. In order to assess the accuracy of the visual grading, investigators have determined actual concentrations of bilirubin, hemoglobin and triglycerides in each sample. The main finding of this study was that turbidity, hemolysis and icterus occur quite frequently in concentrations associated with significant interferences in some analytical systems. Furthermore, their results also showed that trained observers are unable to accurately rank the degree of interference in serum even when they had a good standard for comparison.

The reliability of visual assessment of the degree of hemolysis was further explored by Hawkins *et al*. (18) in a study aimed to assess the agreement between visual grading and spectrophotometric measurement. This study showed that visual inspection by laboratory personnel is highly unreliable, depends on the sample type and may overestimate the actual prevalence of hemolyzed serum specimens while underestimating it in plasma samples.

In one our recently published study we have compared visual and automated detection of lipemia, icterus and hemolysis in 1,727 routine biochemistry serum samples (19). Visual detection was based on comparison with photographs of samples containing various concentrations of hemoglobin, bilirubin and triglycerides. Automated detection was done using LIH reagent (Olympus, O'Callaghan's Mills, Co. Clare, Ireland) on Olympus AU2700 analyzer. Our key finding was that visual inspection was inferior to automated detection of lipemia, icterus and hemolysis. We have also observed a poor inter-rater agreement in estimating the degree of interference between laboratory personnel (mean kappa coefficient and 95% confidence interval = 0.617 (0.537–0.696). As such, this study provides firm evidence that laboratory staff is unable to accurately and reproducibly detect subtle differences in sample color and turbidity, even when a colored scale is available for comparison.

Elevated concentrations of bilirubin may further impair the ability to detect hemolysis by visual inspection and therefore lead to serious underestimation of hemolysis in neonatal samples where elevated bilirubin concentration is commonplace. In their study on detection of hemolysis and reporting of potassium results in samples from neonates, Jeffery *et al*. compared detection of hemolysis in adult and neonatal samples by inspection by laboratory staff and measurement of hemolysis (H) index (20). They found that the presence of icterus results in underdetection of hemolysis by visual inspection.

Not only bilirubin may have direct influence on the ability to detect hemolysis. Recently, Darby and Broomhead reported that Patent Blue dye used for sentinel lymph node biopsy in breast cancer patients might cause interference with the accurate estimation of the serum indices (21). In this study serum samples were spiked with increasing concentrations of Patent Blue dye and the effect of the dye on the assessment of the degree of hemolysis, lipemia and icterus was explored. Significant

positive interference of Patent Blue dye was observed for the degree of lipemia in serum samples, whereas there was a significant negative bias for the degree of hemolysis and icterus. Although the dye had no direct effect on the routine chemical analyses studied in this work, its presence in serum lead to the failure to reliably detect hemolysis, icterus and lipemia. The effect of the dye on the serum indices was linear, in a dose-response fashion. This study is important because it underlines the complexity of interactions of some more or less common exogenous substances in serum with analytes of interest. Laboratory personnel might not be aware of the presence of such substances in serum, nor of their potential effect on the estimated degree of serum indices. Therefore, such and similar studies are needed to further explore the potential causes of unreliable estimation of serum indices.

However, visual detection of serum interferences by laboratory personnel is unfortunately still being performed in many laboratories.

Automated processing

The recent advances in laboratory technology have lead to an increasing trend in automation of various preanalytical processes into large preanalytical modules. These modules as well as novel automated laboratory analyzers offer a solution to many of the quality requirements, like the ability to systematically detect serum indices. The implementation of systems using the automated detection of serum indices is advantageous for a variety of reasons. Beyond increased productivity, throughput and decreased error rate, its benefits also include increased reproducibility and the improvement in detection of mildly hemolyzed specimens (serum free hemoglobin in the range between 0.3 and 0.6 g/L). Such platforms commonly use the semiquantitative spectrophotometric measurement and grade interfering substances into several categories. The spectrophotometric measurement for hemolysis is usually performed at 400–800 nm wavelengths. Serum index is then calculated using the complex formula and the spectrophotometric measurement data, and is proportional to the concentration of free hemoglobin in serum.

It should be noted however that various analytical platforms may have different decision thresholds for the various serum indices. The different systems might also vary in their assay parameters and the degree of interference of the specific interfering substance. Lippi and colleagues have recently published the results of a large multicenter evaluation of the hemolysis index in several automated chemistry systems (22) observing that several different analytical platforms provide highly comparable sensitivity and accuracy of detection of serum indices. Since their study was performed only on a limited number of analyzers, it should be noted that these results may not be valid and applicable to all or at least some other analytical platforms. As there is an obvious lack of standardization of the index decision thresholds and the reporting policies, the authors also concluded that more efforts should be invested into the standardization of the reporting of hemolysis index.

To overcome this lack of standardization, there was one recent initiative in Netherlands aimed to establish uniform use of serum indices and harmonize the management of hemolyzed, lipemic and icteric specimens on the national level (23–25). This Netherlands group of investigators has developed consensus cutoff values for serum indices for a series of analytes on Beckman Coulter LX-20 analytical platform. Based on those cutoffs, they also designed the proposal for nationwide rules for handling the patient samples with clinically significant interferences.

To report or not to report?

There is an ongoing debate as to whether we should or should not report results of the laboratory testing from the hemolyzed sample. Basically, when a hemolyzed sample arrives into the laboratory, we can: i) reject the sample for analysis and ask for the re-collection; ii) perform the analysis and report the results with a comment; iii) do the analysis and mathematically correct the result according to the estimated degree of the hemolysis.

The way hemolyzed specimens are handled varies greatly from one laboratory to another, as well as across country and worldwide.

Recently we have published results from the nationwide Croatian survey on extra-analytical laboratory procedures. Our results showed that 30% of laboratory specialists (43/142) never or rarely ask for a new specimen if a serum is slightly hemolytic even if potassium is requested. Even bigger problem was the fact that approximately 40% of participants determine the degree of hemolysis based on potassium result and are not going to ask for the new specimen if the potassium concentration is not elevated in hemolyzed sample (10).

We all would probably agree that the best option is to get another sample free of interferences. However, sample re-collection is not always possible. Several authors have addressed that question and many formulae have been suggested to correct test results from hemolyzed samples (25). Correction of results should only be performed when intravascular hemolysis has been definitively ruled out. The authors of the previously mentioned study, on the detection of hemolysis and reporting of potassium results in samples from neonate, advocate the use of the automated H index as highly recommended. They furthermore support the use of correction formula for reporting potassium in neonatal and adult specimens, since it might be beneficial to the clinical management of the patient (20). However, it is important to highlight that the practice of correcting results might introduce a certain bias and can therefore lead to inaccurate and misleading results.

Some authors suggest that laboratory reports should be accompanied with appropriate comment pointing to the interference in the sample. Reporting the laboratory result with appropriate comment, as suggested by Carraro, might be helpful in making an early diagnosis and providing appropriate treatment, which is of great interest in the acute care setting (26). Others disagree and strongly argue against the use of such comments (27,28). The main reason is because these results are actually erroneous and might be seriously misleading. It should also be noted that those comments are not easy to interpret and might even not always be noticed by the clinical staff responsible for the patient care. Moreover, adding brief comments to the laboratory reports is of questionable advantage to the patient care, and little if any evidence-base data exist to support interpretation of laboratory test results (29).

Finally, the introduction of unreliable results within the laboratory reports might represent a serious hazard for the longitudinal comparison of patient data, in as much as the variation observed from analytically biased results would not be trustable.

To overcome the problem of the non standardized reporting and harmonize detection and management of unsuitable specimens Italian Inter-society SIBioC-SIMeL-CISMEL (Society of Clinical Biochemistry and Clinical Molecular Biology-Italian Society of Laboratory Medicine-Italian Committee for Standardization of Hematological and Laboratory Methods) Study Group on Extra-analytical Variability has issued the consensus Recommendations for detection and management of unsuitable samples in clinical laboratories (30). Briefly, according to their recommendation, education and responsibility of laboratory staff is pivotal in order to reduce the uncertainties in the preanalytical phase, and objective and standardized systems for detection of unsuitable specimens need to be adopted by each laboratory, depending on the specific needs and context. The laboratory should implement the systematic procedure for detection and monitoring of unsuitable samples. Hemolysed samples should be used for testing only for those analyses not influenced by the specific interference. Laboratory staff should not use samples for testing if requested analyses are significantly influenced by the interfering substances. Laboratory should always request another sample. It is pointed out by the authors of the recommendations that it is always better not to report the result rather than producing spurious data on unsuitable samples. As of the correction of test results, there is a no strong recommendation. Authors state that interference correction is still a matter of debate and suffers from some significant limitations.

Conclusion

Hemolysis is still one of the biggest challenges to the laboratory specialists. As for the current knowledge, automated platforms are the most suitable solution for continuous successful and standardized detection and management of hemolyzed specimens, as well as for obtaining meaningful information on the quality of sample collection throughout collection centers and wards. Visual detection should be abandoned, due to the low sensitivity and low reproducibility. If hemolyzed sample is referred to the laboratory, the personnel should always ask for new sample(s). In case new sample(s) can not be obtained, it is the responsibility of the laboratory specialist to communicate the problem with the physician responsible for the patient and seek for the best solution for the best of the patient care.

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Detekcija hemolize i postupanje s hemolitičnim uzorcima

Sažetak

Dugo vremena se interferencijama nije pridavao dovoljan značaj te su nažalost, prečesto u dnevnoj kliničkoj laboratorijskoj rutini ostale neotkrivene. Tijekom zadnjih desetak godina izvananalitička faza procesa laboratorijskog ispitivanja prepoznata je kao velik izvor laboratorijskih pogrešaka, a upravo su prijeanalitičke pogreške najučestalije u cjelokupnom procesu laboratorijske analize. Zna se da je hemoliza najčešća prijeanalitička pogreška, što je čini i najčešćom interferencijom u medicinsko-biokemijskom laboratoriju. Vizualno određivanje stupnja hemolize je individualna prosudba i samim time najnepouzdanija metoda, budući da se na taj način stupanj hemolize u uzorku može precijeniti ili podcijeniti (odnosno, čak niti obučeni promatrači ne mogu točno odrediti stupanj interferencije u serumu). Povišena koncentracija bilirubina mogla bi narušiti sposobnost vizualnog određivanja stupnja hemolize i time dovesti do otežane detekcije hemolize u neonatalnim uzorcima gdje je povišena koncentracija bilirubina uobičajena.

Nedavna postignuća u laboratorijskoj tehnologiji dovela su do automatizacije prijeanalitičkih procesa u velike prijeanalitičke module. Takvi moduli, kao i novi automatizirani laboratorijski analizatori, nude automatizirano određivanje serumskih interferencija pa time i hemolize. Velika prednost ovog pristupa leži u većoj reproducibilnosti i uspješnijoj detekciji lagano hemolitičnih uzoraka (koncentracija hemoglobina u serumu < 0,6 g/L). Te platforme obično rabe semikvantitativna spektrofotometrijska mjerenja te svrstavaju interferirajuće tvari prema stupnjevima u kategorije. Međutim, različite analitičke platforme mogu imati različite granične vrijednosti za različite serumske pokazatelje. Štoviše, različiti bi se sustavi mogli razlikovati u parametrima svojih analiza i u stupnju interferencije pojedine interferirajuće tvari. Stoga treba usmjeriti više truda i napora u standardizaciju izvještavanja o stupnju hemolize, pogotovo zato što je taj parametar važan za dobivanje korisnih informacija o kvaliteti uzorkovanja na bolničkim odjelima i mjestima centralnog vađenja uzoraka. Hemoliza je još uvijek jedan od najvećih izazova za laboratorijske stručnjake. U slučaju hemolize, osoblje laboratorija bi uvijek trebalo zatražiti novi uzorak. Ukoliko to nije moguće, odgovornost je laboratorijskog stručnjaka prenijeti problem liječniku odgovornom za bolesnika te pronaći najbolje moguće rješenje za bolesnika.

Ključne riječi: hemoliza; izvananalitička kvaliteta; pogreške; laboratorijsko ispitivanje; interferencije