REVIEW ARTICLE

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Hemolyzed Specimens: Major Challenge for Identifying and Rejecting Specimens in Clinical Laboratories

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ABSTRACT

Pre-analytical quality in clinical chemistry testing is as important as analytical and post-analytical quality. The most prevalent pre-analytical interference and a major source of error producing unreliable laboratory test results is hemolysis of blood samples. In vitro hemolysis may be due to the blood withdrawal technique or sample handling whereas in vivo hemolysis can originate from acquired, hereditary, or iatrogenic conditions and is not technique dependent. Interpreting in vivo or in vitro hemolysis requires clinicians to supply reliable clinical history and findings. Even then, to reject or release the result with interpretation is still under debate. Thus, hemolyzed specimens are a serious pre-analytical problem calling for well-designed and strictly implemented laboratory guidelines. The aim of this non-systematic review (addressed to healthcare professionals) was to highlight the challenges in identifying and rejecting hemolysis specimens.

Hemolysis is conventionally defined as the release of hemoglobin and other intracellular components of erythrocytes into the extracellular space of blood.[1,2] Hemolysis may occur in vivo and in vitro. In vivo hemolysis is a result of a number of circumstances and diseases (inherited or acquired hemolytic anemias), whereas in vitro is triggered by improper or mishandled procedures during specimen collection. In vitro is the most undesirable precondition that influences the accuracy of the results and dependability of laboratory testing.[1,3] Hemolysis has been recognized as the most frequent pre-analytical artefact encountered in laboratory specimens.[4,5] Though the general incidence of hemolyzed samples in clinical laboratories differs broadly according to the clinical setting, geographical area, and facility type, the prevalence of hemolytic specimens can be as high as 3.3% of all routine samples, thus, accounting for from 40% to 70% of all unsuitable samples identified. This is nearly five-times higher than other causes such as clotted samples, inadequate procedures for collection, insufficient volume, and incorrect samples.[1,5] In vitro hemolysis is the leading cause of specimen rejection for both outpatient and inpatient samples, as well as urgent and routine specimens.[6–9]

In vivo hemolysis

In vivo hemolysis occurs as a result of the premature death of red blood cells (RBCs) within the circulation, which can cause hemolytic anemia when active bone marrow is unable to compensate for the amount of RBC breakdown.[10,11] The etiology of premature RBC death varies and can be due to antigen-antibody reactions, chemical reaction, hemolytic anemias, toxins and poisonous substance, mechanical RBC rupture due to artificial heart valves, and medical intervention such as hemodialysis or the use of the heart-lung bypass machine.[1,12–15] Usually, less than 2% of all samples with detectable hemolysis are due to in vivo hemolysis.[16] Since in vivo hemolysis does not rely on the skills and practice of healthcare providers, preventing its occurrence can be very difficult, and it may not be completely resolved.[17] Rejection of suspected in vivo hemolysis samples is considered malpractice. Further investigations in combination with clinical history, physical examination, and peripheral blood smear are essential for the differentiation of in vivo or in vitro hemolysis.[12,18]

In vitro hemolysis

In vitro hemolysis is a result of pre-analytical causes associated with sample collection, jarring

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transportation methods, extreme temperature, sample handling, delayed processing, and prolonged storage.[19–21] Hemolysis resulting from phlebotomy may be caused by incorrect needle size, improper tube mixing, incorrect filling of tubes, excessive suction, prolonged tourniquet, and difficult collection. As such, hemolysis may occur from the point of venipuncture and then continue downstream of the process up to the time of analysis.[16,22,23]

In vitro hemolysis generates analytical and biological interferences.[11,24–27] The impact of in vitro hemolysis on measured potassium concentrations is well documented. In such cases, reported potassium concentrations are clinically inaccurate, the magnitude of which depends on the degree of hemolysis.[28–30] Many other analytes are dependent on the interference effects of in vitro hemolysis, but perhaps not vigorously studied or reported.

Challenges in identifying of in vivo and in vitro hemolysis

An emerging challenge for clinical laboratories is to differentiate between in vivo and in vitro hemolysis. The laboratory only sees the samples and not the patients. When multiple samples are received by the laboratory, comparing a hemolytic sample of a patient with other samples from the same patient received at the same time may help in differentiating in vitro from in vivo hemolysis.[31] For example, if the first sample is hemolyzed but the second or previous sample is not, the suspicion of in vitro hemolysis is high. On the other hand, if the first sample is hemolyzed and the subsequent sample is also hemolyzed, the likely cause would be in vivo hemolysis, and a clinical history is required to support this hypothesis. Theoretically, in cases of in vivo hemolysis, the contents of the erythrocyte circulate throughout the vascular volume, and some components equilibrate with the interstitial fluid. It is a true increase of analytes from the hemolyzed sample and not an artefact of methodologic interference. Hence, in this form of hemolysis, the potassium values are correct. Attaining another blood sample does not resolve the problem because the hemolysis occurs before the sampling.[32] Whereas in in vitro hemolysis, there is a parallel increase of erythrocytes content including potassium, lactate dehydrogenase (LDH), and aspartate aminotransferase (AST) corresponding to the hemoglobin concentration in serum or plasma.[31,33,34] The reduction of plasma haptoglobin is considered a reliable marker for the rapid identification of accelerated in vivo RBC damage irrespective of the site of hemolysis. Compared with other prospective markers, haptoglobin levels are not affected by in vitro hemolysis because the haptoglobin-hemoglobin complexes formed during RBC breakdown are promptly cleared from the circulation upon uptake by monocytes and tissue macrophages via CD163 receptors.[35] This parameter is available in most chemistry analyzers and the most appropriate tool to distinguish between in vivo and in vitro hemolysis. However, the diagnostic value of haptoglobin is restricted by impaired liver function test with the decrease of haptoglobin synthesis.[36] The presence of concomitant infection or chronic hemolysis should be ruled out given haptoglobin is also an acute phase reactant, and the diagnostic reliability of haptoglobin has been questioned as a marker of hemolysis. This is because the synthesis of haptoglobin is increased during an acute phase reactant and could compensate free hemoglobin-mediated haptoglobin reduction.[37] Typical signs of in vivo hemolysis are an elevation of indirect bilirubin level and reticulocyte count, which is an indicator of marrow compensatory response.[38,39] In in vitro hemolysis, the reticulocyte counts remaining normal. An elevation of LDH activity is typical of intravascular hemolysis.[40,41] Yet, because of low diagnostic sensitivity and specificity, a change in the LDH isoenzyme pattern seems less appropriate for the identification of in vivo hemolysis.[31]

Hemolysis (either in vivo or in vitro) is traditionally detected by visual inspection of the specimen after centrifugation and comparing it with the hemolytic chart, which shows the color of samples with increasing concentrations of free hemoglobin.[42–44] Visual inspection is time-consuming, thus, causing a delay in reporting.[1,45] Visual detection of hemolysis may also vary from one person to the other and may lead to inaccuracy in estimating the actual prevalence of hemolyzed serum samples (i.e., experienced technologists are incapable to exactly rank the various concentration of hemolysis in serum).[42,43] In neonatal samples where elevated bilirubin concentration is common, the ability to detect hemolysis by visual inspection may further be impaired causing gross underestimation of hemolysis.[2] Visual assessment is highly inaccurate and almost impossible to standardized, enabling only gross classification of hemolysis.[40,43] Currently,

chemistry analyzers are capable of performing automated assessments of serum indices including hemolysis index (HI). They provide quantitative measurement with high repeatability, thereby standardizing the identification process of hemolyzed specimens. Besides the advantages of automated HI, a recent online international survey with 338 respondents revealed that 56% of clinical laboratories still performed visual assessment to detect hemolysis, and 43% used automated HI quantification. Sadly, 1% of laboratories did not perform any pre-analytical check.[46] Although automated HI assessment had been well documented in national and international guidelines, it has not been strictly accepted and followed. Reasons accounted include unease of increased specimen rejection rate, lack of standardize units of measure, differences in instrument-specific cutoff, negative impact on throughput, poor harmonization of analytical techniques, organization and laboratory economics, and lack of a reliable quality control systems. Many of these concerns, however, have been addressed and evidence now supports automated HI in enlightening quality and patient safety.[47] Since there is no consensus on how to help clinicians distinguish between the two hemolysis types (in vivo and in vitro), clinical surveillance is essential. These laboratories should have standard operating procedures on how to detect, analyze and report hemolysis, and possible results interference for both in vivo and in vitro hemolysis. Cases suspected of in vivo hemolysis should include certain analytes such as potassium, which provides clinicians with vital information and identification of clinical situations that require urgent intervention.

Reject or not to reject?

There is an ongoing debate as to whether laboratories should or should not report results from samples affected by hemolysis.[22,48] This issue is not easy to answer or resolve since both choices could affect the treatment or management of patients especially in acute or emergency care. In the absence of sufficient relevant and specific clinical data and guidelines, there is understandably substantial heterogeneity among the laboratories, statewide, nationwide, and globally in the way they handle and manage hemolyzed specimens.[3,49] Most clinical laboratories will reject hemolyzed samples and request recollection. This practice has consequences since repeating sample collection is not always possible and rejecting the sample means subjecting patients to another invasive test and may delay diagnosis. Rejecting the sample may cause other potential harm to the patient should it lead to an incorrect clinical decision, treatment option, or patient monitoring following the lack of laboratory results.[50,51] For laboratory specialist dealing with analytes (such as potassium) measurement is another challenge.

By not reporting the result, the laboratory is suggesting to the clinical team that the potassium concentration analytically unmeasurable, and this is appropriate for in vitro hemolysis. But the result may be of clinical use in cases of in vivo hemolysis whereby the availability of potassium result, which had perhaps mounted acutely in a relatively short time, could have led to earlier dialysis treatment.[28] The other option would be to perform the test and analysis but to report the result with a remark on the clinical interpretation of the values. However, the lack of standardization with this option has led to reporting inconsistencies that seriously affect the true significance of the measured value following the wide variation of the attached remarks.[50] Even among laboratory specialists, there has been disagreement and intense debate on the use of such comments or remarks. The addition of a brief comment to the laboratory report with very little evidence-based data support cast doubts on the interpretation of test results and its benefit to patient care.[1,52,53] The final option in managing hemolyzed samples would be to perform the analysis, but the final result is then mathematically adjusted based on the estimated degree of sample hemolysis.[54] Correcting and reporting results may be essential in making a primary diagnosis, but it should be performed after intravascular hemolysis has been ruled out. Due care must be taken since this practice might introduce bias and depending on the multiplier used may cause inaccurate or false results.[22] It would also be of paramount importance to discuss and educate clinicians of the method applied so that the result would not be mistakenly interpreted or ignored by the respective clinicians.[22]

The management of hemolyzed samples remains an unsettled dilemma given the available options. Many opinions reject hemolyzed samples and favor rapidly informing clinicians about the need to redraw the samples as the best option (clinically and analytically) for safe practice.[55] In situations where

new samples are not obtainable, communication between the clinician and laboratory specialist is important to seek a resolution tailored to the individual patient (e.g., patients in critical condition, intensive care unit, or emergency and trauma centers). Excellent two-way communication between clinicians and laboratory specialists is of paramount importance since good information regarding a patients' status might provide laboratory specialists with some possible lead to whether it was an in vitro or in vivo hemolysis. The laboratory specialist in return would then be able to guide clinicians on the most appropriate use of test results, for example, the use of potassium analyte results in lieu of the possible in vivo hemolysis, which would be crucial to the management of patients as opposed to in cases of in vitro hemolyzed samples.[28,50] There is a need to stress the importance of precautions to avoid in vitro hemolysis, especially for repeat samples. This can be accomplished by transporting samples to the laboratory without mechanical agitation (for example, avoiding pneumatic tube transport), taking blood in a lithium heparin container, and immediate separation of plasma from cells. In the laboratory, HI report generated automatically through laboratory information system can save human resources and reduces turnaround time.[50] However, the system must be able to over-ride the suppression of potassium results in cautiously taken but persistently hemolyzed repeat samples and alert clinicians to the likelihood of in vivo hemolysis in such cases.[28]

CONCLUSION

Hemolytic specimen is still a major concern to laboratory specialists worldwide. Satisfactory skills and a relevant and good level of knowledge and experience are essential to collect a quality specimen that produces anticipated and accurate results. There is a need to have or develop an effective laboratory guideline with emphasis on standardizations of procedures for identification of hemolyzed clinical specimens, measurement, and immediate communication of laboratory results, which can provide clinicians with essential information for immediate or subsequent management of patients. The automated platforms are considered the most appropriate solution for continuous, standardized and effective detection, and management of hemolyzed specimens. It is perceived as a more objective option when deciding to reject hemolyzed specimens and requesting recollection. Automated HI may also reduce laboratory expenditure of performing unnecessary blood tests, shorten the turnaround time to run the test, and avoid inaccurate test results that can affect patient care.

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