Mini Review

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Is the hemolysis index always suitable for monitoring phlebotomy performance?

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Abstract: The new generation of clinical chemistry and coagulation analyzers is equipped with technical features allowing a systematic check of sample quality, including an assessment of the so-called HIL ("hemolysis", "icterus", "lipemia") indices. These measures enable an accurate and reproducible assessment of sample hemolysis in serum or plasma, hence the hemolysis index (H-index) is now also increasingly used for monitoring and benchmarking phlebotomy performance. Reliable evidence attests that intravascular hemolysis is not such a rare phenomenon, and its prevalence may be especially higher in geographical areas where congenital hemolytic diseases are endemic, as well as in healthcare settings where patients with acquired hemolytic disorders are more frequently visited or hospitalized. It is hence conceivable that monitoring phlebotomy performance based on the rate of hemolyzed specimens received by the laboratory may not be so straightforward, provided that specimens drawn from patients with intravascular hemolysis can be identified and excluded from the analysis. The aim of this article is to provide an overview of potential drawbacks in using the H-index alone for monitoring phlebotomy performance, and to offer potential solutions to improve its efficiency for this scope. We therefore suggest that the H-index may only be used for purposes of benchmarking phlebotomy performance when the overall number of diagnoses of hemolytic diseases or the haptoglobin values measured by the laboratories are comparable across different healthcare settings or geographic areas.

Keywords: hemolysis; patient safety; phlebotomy; quality.

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Introduction on sample hemolysis

Sample hemolysis is a relatively common occurrence in laboratory diagnostics, whose frequency can be as high as 3–8% of all samples received for routine and urgent testing [1, 2]. Compared to other preanalytical errors, hemolyzed samples are still the leading reason for both rejection of specimens and suppression of test results [3, 4]. The receipt of these samples poses many technical and practical challenges to laboratory professionals, which can be briefly summarized by the compelling need to troubleshoot the underlying source of hemolysis (Table 1). Although the accurate discrimination between in vivo and in vitro hemolysis is of utmost clinical significance, this aspect is often overlooked in routine laboratory practice. Test results measured in samples drawn from patients with intravascular hemolysis should be urgently communicated to the physicians, as many clinical conditions leading to in vivo hemolysis are potentially life-threatening, whereas test results generated using in vitro hemolyzed samples should be managed according to a well-defined balance between safeguarding the quality of testing and the clinical need of releasing important test results to the requesting physicians [5].

According to the underlying sources of spurious hemolysis, the number of hemolyzed samples received by the laboratory may vary widely across hospital settings. For example, the hemolysis rate is constantly higher in the emergency department (ED), where intravenous catheters are also frequently used for drawing blood, a procedure which is known to generate high stress to blood cells and subsequent injury, especially when blood is drawn from catheters directly into high pressure evacuated collection tubes [6, 7].

In vivo hemolysis, also known as intravascular hemolysis or hemolytic anemia, is conventionally defined as a process of red blood cell (RBC) injury and disruption within the bloodstream, typically caused or triggered by a kaleidoscope of congenital or acquired conditions, as summarized in Table 1. Under most circumstances, the diagnosis of intravascular hemolysis requires urgent clinical management, especially when RBC injury is sustained by acute and rapidly

Table 1: Potential causes of in vitro and in vivo hemolysis.

In vitro hemolysis
– Blood collection
– Fragile or difficult veins
– Badly performed venipuncture
– Capillary blood collection
– Blood drawing from intravenous lines
– Small gauge needles
– Sample handling
– No mixing or excessive shaking of blood tubes
– Forcing blood into evacuated blood tubes with a syringe
– Sample transportation
– Prolonged transportation time of whole blood samples
– Direct contact with heat or freezing sources
– Mechanical trauma of blood
– Sample preparation
– Excessive centrifugation force
– Inefficient gel barrier
– Re-spun of the specimen
In vivo hemolysis
– Congenital disorders
– Sickle cell disease
– Thalassemias
– Spherocytosis
– Paroxysmal nocturnal hemoglobinuria (PNH)
– Deficiency of glucose-6-phosphate dehydrogenase or pyruvate kinase
– Acquired disorders
– Immune and autoimmune diseases
– Infections
– Cytomegalovirus
– Epstein-Barr virus
– Hepatitis viruses
– Mycoplasma pneumonia
– Malaria
– Reactions to drugs or toxic compounds
– Mismatched blood transfusion
– Hypersplenism
– Burns
– Massive traumas
– Strenuous physical exercise (i.e. foot-strike hemolysis)
– Blood cancers
– Chemotherapy
– Hemodialysis and extracorporeal circulation
– Prosthetic cardiac valves
– Disseminated intravascular coagulation (DIC)
– Hemolytic uremic syndrome (HUS)
– Thrombotic thrombocytopenic purpura (TTP)
– Preeclampsia

evolving disorders [e.g. sickle cell crisis, malarial infection, disseminated intravascular coagulation (DIC)] [8, 9].

Hemolysis assessment in clinical laboratories

Unlike the past century, when sample hemolysis could almost exclusively be identified by visual inspection of serum or plasma after separation from blood cells, the new generation of preanalytical, clinical chemistry and hemostasis analyzers is now equipped with technical features which permit a systematic check of sample hemolysis, carried out by photometric measurements at specific wavelengths corresponding to the peak absorbance of cell-free hemoglobin (i.e. usually between 400 and 600 nm) [10]. These new features, conventionally called HIL ("hemolysis", "icterus", "lipemia") indices enable an accurate and reproducible estimation (or even

quantification) of sample hemolysis, icterus and turbidity in serum or plasma [11, 12], hence they are increasingly used for monitoring and benchmarking phlebotomy performance [13, 14], as also endorsed by the International Federation of Clinical Chemistry (IFCC) Working Group "Laboratory Error and Patient Safety" (WG-LEPS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Task and Finish Group "Performance specifications for the extra-analytical phases" (TFG-PSEP) [15]. This opportunity is supported by recent data published by two separate groups, clearly attesting that these measures display accurate and reproducible performance for the purpose of monitoring the quality of the entire process of blood drawing and management of blood tubes [16, 17].

In order to accurately collect and monitor the hemolysis rates over time, laboratories need to adopt reliable and user-friendly documentation systems. One of these tools has been developed by the IFCC WG-LEPS, which has created a specific online platform where laboratories can enter local hemolysis data on a monthly basis [18]. This system not only allows performing intra-laboratory trend analysis, but also enables benchmarking their data at the national and international levels. In facilities where the laboratory information system (LIS) cannot automatically record hemolysis data, customized software solutions have been developed [19]. Additional software programs have also become available at the national level, such as the German/Austrian hemolysis database project [20].

Notably, harmonization of hemolysis measurements is still regarded as a major hurdle for inter-laboratory benchmark, as the manufacturers of clinical chemistry or coagulation platforms have developed different approaches (i.e. wavelengths and algorithms) for measuring HIL indices and for reporting data, thus making comparison of results challenging or even misleading [21, 22].

Hemolyzed specimens and intravascular hemolysis

Earlier data published by Carraro et al. [23] revealed that approximately 2% of all hemolyzed blood samples referred to a typical clinical chemistry laboratory originate from hospitalized patients with different forms of intravascular hemolysis rather than being caused by preanalytical issues, as those summarized in Table 1. More recently, Ko et al. provided further support to the possibility that intravascular hemolysis may substantially contribute to enhancing the overall number of hemolyzed specimens received by clinical laboratories. Briefly, the authors carried out a retrospective observational study including 171,519 blood specimens collected from outpatients visiting a large medical center [24], and found that the two main causes of intravascular hemolysis, i.e. prosthetic heart valves and chemotherapy, accounted for nearly half (i.e. 48%) of all significantly hemolyzed blood samples (≥1 g/L of cell-free hemoglobin) that could be identified in their survey. Notably, the vast majority (i.e. approximately 90%) of these in vivo hemolyzed blood samples were drawn from patients undergoing chemotherapy, therefore it cannot be thoughtfully established whether hemolysis may have been a direct consequence of cancer, could have been triggered by chemotherapeutic drugs or, rather, was the consequence of cumbersome blood collections, as commonplace in cancer patients (e.g. difficult veins, blood collection from intravenous lines, etc.) [25, 26]. Interestingly, previous data published by Mecozzi et al. [27] provided reliable support to the hypothesis that intravascular hemolysis may be very frequent in patients with prosthetic heart valves, as mild subclinical hemolysis could be identified in 26% of patients bearing a mechanical prosthesis and in 5% of those with a bioprosthesis.

Hemolysis index and intravascular hemolysis

Albeit the frequency of in vivo hemolyzed specimens seems rather heterogeneous in the available scientific literature (i.e. between 2 and 50% of all hemolyzed samples) due to the different worldwide epidemiological burden of hemolytic diseases [23, 24], an important question arises according to these figures: can the rate of hemolyzed blood samples be considered always suitable for monitoring phlebotomy performance? The rather obvious answer should be no, at least when the hemolysis index (H-index) is used alone. Unlike the findings reported by Ko et al., however, recent statistics attest that the overall prevalence of hemolytic anemia is approximately 0.01–0.02% worldwide [28, 29], which would actually translate into a hypothetical risk that 1:150–400 hemolyzed samples may have been drawn from a patient with intravascular hemolysis. It is hence rather unlikely that this very low frequency of in vivo hemolysis (i.e. between 0.25 and 0.70% of all hemolyzed samples) may introduce a bias in local analyses aimed to assess phlebotomy practice by means of the H-index.

Unlike this conclusion, we believe that the use of the H-index for widespread purposes of benchmarking

phlebotomy practice may be more seriously questioned. Sickle cell disease is a paradigmatic example. According to the Centers for Disease Control and Prevention (CDC), the prevalence of this condition is 1:365 among Blacks and 1:16,300 among Hispanic-Americans [30]. A similar consideration can be made for the prevalence of malaria, wherein 92% of severe cases are recorded in the African region compared to only 2% of severe cases in the Eastern Mediterranean region [31]. Reasonably, the risk of collecting hemolyzed blood specimens from patients with a hemolytic crisis caused by either sickle cell disease or malaria will be nearly 50-fold higher in African than in European laboratories, and this will not necessarily reflect a worse blood collection practice in the former continent. Yet, both these conditions are relatively rare causes of hospital admissions (i.e. around one hospital admission every 1–3 years) [32, 33]. Thus, in the worst scenario, this would translate into a hypothetical risk that 1:33–100 hemolyzed samples (i.e. 0.1–0.3% of all hemolyzed samples) may be collected from patients with an acute hemolytic crisis in endemic areas for sickle cell disease and/or malaria. This would hence only slightly increase the risk of collecting in vivo hemolyzed samples from a percentage comprised between 0.25 and 0.70% of all hemolyzed samples (as in non-endemic geographical areas for hemolytic diseases) [28, 29] to a percentage ranging between 0.35 and 1.0% of all hemolyzed samples in geographical areas where hemolytic diseases are endemic (Figure 1).

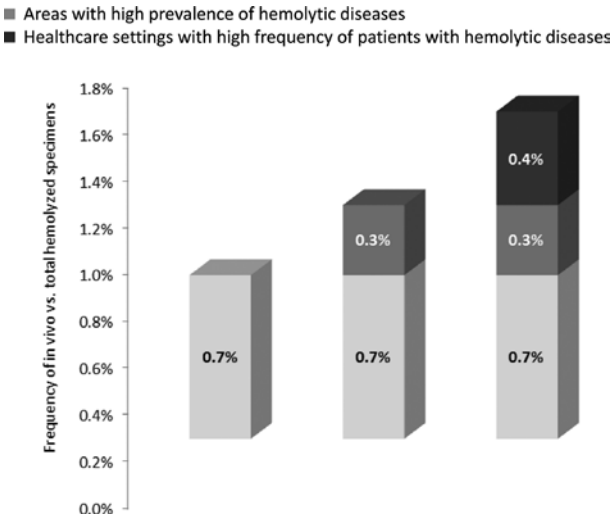

Figure 1: Potential impact of intravascular hemolysis on the overall prevalence of hemolyzed blood samples referred to clinical laboratories.

Regardless of the heterogeneous epidemiology of congenital hemolytic disorders, a similar consideration can be made for benchmarking phlebotomy practices across hospital settings. Hemolytic anemia is often a life-threatening condition. It is hence unlikely that patients with intravascular hemolysis may have their blood collected at an outpatient phlebotomy center, whilst the vast majority of these patients will be visited in the ED or will be immediately admitted to the intensive care unit (ICU) [28, 34]. It cannot hence be discounted that the higher prevalence of hemolyzed samples recorded in emergency healthcare settings (i.e. emergency rooms, ICUs) or in dialysis units, for example, may be at least partially attributable to blood collections from patients with some forms of intravascular hemolysis, which can be as high as 0.4% of all blood collections [35].

Conclusions

Although no comprehensive statistics have been published so far on the overall epidemiological burden of patients visited in the ED or hospitalized for acute hemolytic crises besides the data reported in the study by Lemke et al. (i.e. around 0.4% of all ED visits, leading to hospitalization in ~17% of cases) [35], it seems reasonable to conclude that the impact of intravascular hemolysis may be somehow meaningful when the H-index and the hemolysis rate are used for monitoring and benchmarking phlebotomy practice. Overall, the potential bias due to inclusion of in vivo hemolyzed blood samples in the statistical analysis is seemingly higher across different hospital settings than across separate geographical areas, as previously discussed (Figure 1). In particular, a larger bias is predictable when the hemolysis rate is used for comparing phlebotomy practices between outpatient phlebotomy clinics and emergency, critical or hemodialysis units. The benchmark may only be reasonable and useful when the major confounding factors (thus including the risk of collecting blood from patients with intravascular hemolysis) can be minimized. Ideally, all samples collected from patients in whom a diagnosis of acute hemolytic diseases can be made should be excluded from the calculation of spurious hemolysis rate, or else phlebotomy performance monitoring by the H-index should be limited to longitudinal trend analysis, within the same healthcare or hospital setting. We clearly acknowledge that accessing patients' diagnoses is not always (or everywhere) a simple and straightforward enterprise, as some laboratory medicine services have no access to clinical data. Indeed, routine assessment of reticulocyte count, haptoglobin or even direct antiglobulin test (DAT) in all samples poses an economic

burden for routine identification of in vivo hemolysis. Nevertheless, the discrimination between in vivo and spuriously hemolyzed samples seems unavoidable for obtaining more accurate statistical data. A possible and seemingly feasible solution is accessing the hospital database for retrieving International Classification of Diseases (ICD) codes or Diagnostic Related Group (DRG) of all patients. This would enable identifying the vast majority of patients who have been visited or hospitalized for hemolytic anemia (e.g. 2018 ICD-10-CM Codes D55-D59), who should then be excluded from the calculation of the hemolysis rate for purposes of monitoring phlebotomy performance across different hospital or healthcare settings. Once the data have been cleaned, the hemolysis rate can hence be used more accurately for performing root cause analysis and planning specific actions to improve phlebotomy performance.

Alternatively, a statistical analysis of all haptoglobin values generated by the laboratory would provide valuable information for both intra- and inter-facility benchmarking. Therefore, the impact of intravenous hemolysis on the H-index would be less likely when the frequency of decreased haptoglobin levels is globally comparable across different healthcare settings or geographic areas, whilst a major bias may be predictable when a considerable shift of haptoglobin levels can be observed.

In conclusion, we suggest that the H-index may only be used for purposes of benchmarking phlebotomy performance when (i) the overall number of diagnoses of hemolytic diseases or (ii) the haptoglobin values measured by the laboratories are comparable across different healthcare settings or geographic areas.

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