Editorial

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Identification and management of spurious hemolysis: controversies, concerns and criticisms

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Although none of us would even argue that laboratory tests are pivotal in healthcare, now being an integral part of clinical reasoning and managed care [1], their real contribution to the clinical decision-making is contingent on safeguarding a high degree of quality throughout the testing process, from collecting samples to test result interpretation [2]. Unlike widespread public perception [3], reliable evidence has accumulated over the past decades supporting the notion that the preanalytical phase is the most vulnerable part of *in vitro* diagnostics, whereby collection of unsuitable specimens – for either quantity or quality – would ultimately represent a substantial threat for data reliability [4].

When artifactual (i.e. spurious), sample hemolysis is certainly the most frequent source of delayed, missed or even wrong diagnoses. The mean frequency of hemolyzed samples received in clinical laboratories can be as high as 3%, accounting to or over 60–70% of unsuitable specimens. Such a paramount incidence, which has not apparently declined in recent times, engages the minds of laboratory professionals, clinicians and nurses, who still struggle for identifying reliable strategies for accurately identifying and appropriately managing spurious sample hemolysis [5]. It is with this important drawback in mind that we have decided to assemble a series of interesting contributions on spurious hemolysis in this issue of *Clinical Chemistry and Laboratory Medicine*.

In the first of such articles, Salvagno et al. have explored the potential impact of hemolysis, hypertriglyceridemia and hyperbilirubinemia on thrombin generation in plasma [6]. The authors demonstrate that hemolysis, either spurious or intravascular, generates a profound impact on blood coagulation, whereby the overall thrombin generation, expressed as endogenous thrombin potential (ETP), constantly increased in parallel with the degree of erythrocyte injury. A potentially clinically significant variation was already noted at cell-free hemoglobin concentrations exceeding 0.7 g/L, a value marginally higher than the conventional hemolysis threshold. These results have important clinical and analytical corollaries, confirming that *in vivo* hemolysis is a trigger of blood coagulation, thus not only enlightening the increased thrombotic burden in patients with hemolytic anemia, but also underlining that thrombin generation shall not be assayed in hemolyzed plasma samples, even when the hemolysis degree seems mild.

The second article, based on the experience of the Nordic cooperation of External Quality Assurance organizers (EQAnord) and involving over 140 Nordic medical biochemistry laboratories [7], provides updated information on the impact of hemolysis on clinical chemistry test results generated with different instrumentation and on how test results obtained on hemolyzed samples will then be reported. The most interesting aspects that emerged from this broad survey are that (a) although manufacturers' hemolysis thresholds varied substantially, satisfactory agreement was observed in the mean hemoglobin value measured by different analytical platforms, that (b) the impact of hemolysis on test results of 15 different analytes, except alkaline phosphatase, total bilirubin and creatine kinase (CK), was overall comparable across various analyzers, and especially that (c) facilities using identical assays undertake rather different actions on equally hemolyzed specimens. This last information reiterates the concept that, although official recommendations for managing hemolyzed samples have been published by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) [8], by the Clinical and Laboratory Standards Institute (CLSI) [9] and even by some national societies of laboratory medicine [10, 11], the lack of harmonization for managing hemolyzed samples remains a controversial and majorly unresolved issue across clinical laboratories worldwide [12–14].

The third and fourth articles in this series are logical sequels of this survey. In their original report, Lindhardt Sæderup et al. have explored the feasibility of using *Staphylococcus aureus* to develop an innovative approach based on hemoglobin binding capacity of iron-regulated surface determinant H (IsdH) protein bound to C Sepharose, for rapidly removing hemoglobin and

hemoglobin-haptoglobin complexes from hemolyzed plasma, thus mitigating hemolysis interference and making hemolyzed samples potentially suitable for laboratory testing [15]. Although this is indeed an intriguing and promising strategy for eliminating spectrophotometric interference from cell-free hemoglobin, and thus allowing performance of tests which may only be biased by this cause, we would all agree that hemoglobin removal is not effective to eliminate other sources of hemolysis-dependent bias. This especially refers to the well-known biological effects of hemolysis, which ultimately lead to enhancement of the plasma or serum concentration of intracellular components released after cell breakdown (e.g. potassium, lactate dehydrogenase), to produce a dilution effect for all other analytes, as well as to generate chemical interference for some tests (e.g. the inhibitory effect of adenylate kinase on CK). Therefore, this interesting method would first need to be externally validated and then only used for measuring those parameters for which the bias is limitedly and theoretically spectrophotometric. A different approach for reporting data on hemolyzed samples has then been proposed by Martínez-Morillo and Álvarez [16]. The use of corrective formulas for adjusting results of potassium (and potentially of other analytes) in hemolyzed samples is a largely debated issue [17–19]. In their original study, the authors have provided additional evidence on the reliability of this approach, showing that inclusion of informative commentaries encompassing corrected potassium results in the laboratory report is highly unadvisable when the hemolysis index is high (e.g. cell-free hemoglobin >5 g/L), as this would then lead to a substantial risk of misinterpretation. Even below such limit, however, the percentage of potential incorrect interpretation is dramatically high, comprised between 18 and 28%. This would actually mean that nearly one fourth of all potassium hemolysis-corrected data would then lead to potentially inappropriate patient management. Can we afford such risk? We will leave the final wisdom to our readers.

In conclusion, we are thankful to the authors who have provided these interesting contributions and we sincerely hope that our readers will appreciate this collection of articles on sample hemolysis.

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References

1. Lippi G. The irreplaceable value of laboratory diagnostics: four recent tests that have revolutionized clinical practice. EJIFCC 2019;30:7–13.
2. Plebani M, Lippi G. Closing the brain-to-brain loop in laboratory testing. Clin Chem Lab Med 2011;49:1131–3.
3. Lippi G, Salvagno GL, Mattiuzzi C. Public perception of diagnostic and laboratory errors among Internet users. Diagnosis (Berl) 2019;Feb 7. doi: 10.1515/dx-2018-0103 [Epub ahead of print].
4. Lippi G, Simundic AM. The EFLM strategy for harmonization of the preanalytical phase. Clin Chem Lab Med 2018;56: 1660–6.
5. Carraro P, Servidio G, Plebani M. Hemolyzed specimens: a reason for rejection or a clinical challenge? Clin Chem 2000;46:306–7.
6. Salvagno GL, Favaloro EJ, Demonte D, Gelati M, Poli G, Targher G, et al. Influence of hypertriglyceridemia, hyperbilirubinemia and hemolysis on thrombin generation in human plasma. Clin Chem Lab Med 2019;57:1784–9.
7. Gidske G, Moberg Aakre K, Rustad P, Sandberg S, Norling A, Pelanti J, et al. Handling of hemolyzed serum samples in clinical chemistry laboratories: the Nordic hemolysis project. Clin Chem Lab Med 2019;57:1699–711.
8. Lippi G, Cadamuro J, von Meyer A, Simundic AM. Practical recommendations for managing hemolyzed samples in clinical chemistry testing. Clin Chem Lab Med 2018;56:718–27.
9. Clinical and Laboratory Standards Institute (CLSI). Haemolysis, icterus, and lipemia/turbidity indices as indicators of interference in clinical laboratory analysis: approved guideline. First edition. Wayne, PA, USA: CLSI; 2012. CLSI C56-A.
10. Lippi G, Caputo M, Banfi G, Daves M, Dolci A, Montagnana M, et al. SIBioC-SIMeL consensus recommendations for the identification and management of hemolysed specimens and the implementation of hemolysis index. Biochim Clin 2011;35:481–90.
11. Pineda-Tenor D, Prada de Medio E, Belinchón Torres PM, Gascón Luna F, Morales García LJ, Lorenzo Lozano MD, et al. Handling the altered test results of hemolyzed samples. Recommendations of the Quality, Management, Safety and Evidence Committee (CCGSE) of the Spanish Association of Medical Biopathology and Laboratory Medicine (AEBM-ML). Clin Chem Lab Med 2017;56:e1–4.
12. Cadamuro J, Mrazek C, Haschke-Becher E, Sandberg S. To report or not to report: a proposal on how to deal with altered test results in hemolytic samples. Clin Chem Lab Med 2017;55:1109–11.
13. Li L, Vecellio E, Gay S, Lake R, Mackay M, Burnett L, et al. Making sense of a haemolysis monitoring and reporting system: a nationwide longitudinal multimethod study of 68 Australian laboratory participant organisations. Clin Chem Lab Med 2018;56:565–73.
14. Szoke D, Caruso S, Pasqualetti S, Aloisio E, Falvella FS, Dolci A, et al. Suppressing all test results in grossly hemolyzed samples: is this approach appropriate in every case? Clin Chem Lab Med 2019;57:e118–20.
15. Lindhardt Sæderup K, Revsholm J, Bjork Richardt P, Hill Christiansen S, Hennig D, Kragh Moestrup S, et al. Using the hemoglobin-binding Staphylococcus aureus protein IsdH to

enable plasma analysis of hemolyzed blood samples. Clin Chem Lab Med 2019;57:1690–8.
16. Martínez-Morillo E, Álvarez FV. Management of potassium results in haemolysed plasma samples at the emergency department laboratory. Clin Chem Lab Med 2019;57:e271–3.
17. Simundic AM, Topic E, Nikolac N, Lippi G. Hemolysis detection and management of hemolyzed specimens. Biochem Med (Zagreb) 2010;20:154–9.
18. Lippi G, Avanzini P, Pavesi F, Bardi M, Ippolito L, Aloe R, et al. Studies on in vitro hemolysis and utility of corrective formulas for reporting results on hemolyzed specimens. Biochem Med (Zagreb) 2011;21:297–305.
19. Hawkins RC. Repeat requesting on hemolysed plasma potassium requests. Clin Chem Lab Med 2018;56:e129–31.

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