

Letter to the Editor

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Suppressing all test results in grossly hemolyzed samples: is this approach appropriate in every case?

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To the Editor,

We read with great interest the article by Lippi et al. [1] reporting practical recommendations for managing hemolyzed samples on behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). We were particularly interested in the application of one of the recommendations, namely, the number 5, i.e. if cell-free hemoglobin concentration (free Hb) in the collected blood sample is >10 g/L, all laboratory results should be suppressed and replaced with a comment, indicating that data cannot be released because the sample is grossly hemolyzed.

Because hemolytic samples are in $>95\%$ of cases due to *in vitro* hemolysis, laboratory results obtained on such specimens and reported to clinical wards would be “erroneous” if hemolysis interferes in analytical measurements [2]. On the other hand, whenever *in vivo* hemolysis is reasonably suspected, test results, except those analytically interfered (for information on the mechanisms of interference, see Lippi et al. [2]), should be released by the laboratory, as the information can be helpful for the patient treatment and the identification of the underlying cause, which is frequently a life-threatening condition.

Sometimes the cause is obvious from the clinical history, but often the diagnosis of intravascular hemolysis requires a synthesis of all clinical information and appropriate laboratory tests [3]. Typical laboratory findings are an increase in reticulocyte count, abnormal bilirubin (unconjugated) and lactate dehydrogenase concentrations in blood and reduced serum haptoglobin, with presence in blood film of spherocytes, agglutination or polychromasia. In case of intravascular hemolysis leading to hemoglobinuria, urinalysis can be positive for hemoglobin, but urine microscopy can be negative for red cells, and urinary hemosiderin can be detected approximately 1 week after the onset.

Result processing in case of gross hemolysis is not a new topic [4], but the harmonization of the management of unreliable samples remains one of the most challenging issues in the integrated project of harmonization of hemolysis assessment [5]. In the era of total laboratory automation, which makes use of the automated measurement of serum interference indices, clinical laboratories can easily handle grossly hemolyzed samples in conformity with recommendations [1]. However, one of the unsolved problems is the identification and handling of samples where gross hemolysis can be due to *in vivo* causes. The percentage of *in vivo* hemolysis among hemolyzed samples of all degrees was found to be 3.2% [4]. However, we were unable to find articles where the rate of *in vivo* hemolysis was estimated only in grossly hemolyzed samples (i.e. with free Hb >10 g/L). Our aim was, therefore, to evaluate how much frequent is *in vivo* hemolysis among grossly hemolyzed samples for which, according to the EFLM WG-PRE recommendations, all laboratory results should be suppressed.

In our clinical laboratory serving a 600-bed metropolitan university hospital, a 400-bed healthcare residence, and with a daily affluence of 650 outpatients, the quality of blood samples is systematically assessed by the automated measurement of interference indices in all incoming serum and plasma samples [6]. Over a

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1-year period (June 2017–May 2018), we retrieved clinical and laboratory data of all patients who had a blood sample (plasma [BD Vacutainer lithium heparin tubes, cat. no. 368884] or serum [BD Vacutainer silica gel tubes, cat. no. 368969]) with a hemolysis index (HI), measured by Abbott Diagnostics Architect c16000 platform, ≥ 1000 , corresponding to a free Hb concentration ≥ 10 g/L [7]. Sixty patients had grossly hemolyzed samples, which accounted to 0.04% of the total ($n=166,752$). The rate of gross hemolysis was significantly higher ($p < 0.0001$, χ^2 -test) in plasma samples (HI ≥ 1000 in 0.13% of all plasma samples, $n=34$) than that in serum samples (HI ≥ 1000 in 0.02% of all serum samples, $n=26$). The higher rate of hemolysis in plasma samples in our setting can be, at least partly, explained by sample transportation with the pneumatic tube system previously shown to promote hemolysis in plasma specimens [8].

Table 1 shows the patient origin and stratification. Out of 60 patients with HI ≥ 1000 , only one was outpatient. Six (10.0%) were newborns in their first 3 days of life, two (3.3%) were < 1 year old and three (5.5%) < 10 years old. No patients had diagnosis of *in vivo* hemolysis by their doctors at admission or during the hospitalization. Retrospectively, two of us (DS and SC) independently further assessed laboratory results and clinical data searching for any evidence of intravascular origin of gross hemolysis. In two pediatric emergency department patients, neither laboratory nor clinical data were sufficient for taking a definitive decision. Both (one infant < 2 months old and a child aged 15 months) were quickly discharged because of a rapid improvement of their clinical condition. Anyway, after data matching and collegial discussion with assisting clinicians, it was

not possible to diagnose an *in vivo* hemolysis condition in any of the recruited patients.

In conclusion, our data show that *in vivo* factors are involved in a very minority of cases of hemolysis, especially gross hemolysis. Therefore, our experimental findings support the EFLM WG-PRE recommendation advising the suppression of all test results in samples with > 10 g/L of free Hb [1]. In laboratories where the assessment of HI is fully automated, the immediate transmission of hemolysis degree to the laboratory information system permits the test result suppression in grossly hemolyzed samples using computerized predefined rules [9]. We believe that this approach, in addition to being safe for the patient avoiding wrong interpretation of his tests, helps to speed up clinical path and decisions about the possible recollection of another blood sample.

There is an ongoing debate about how laboratories should deal with hemolyzed samples [10]. However, most of the articles focus on the question whether to suppress or report a single test result for analytes interfered by hemolysis. Beyond trying to answer to this question, the EFLM WG-PRE practical recommendations also deal with the issue of full sample rejection in case of gross hemolysis by proposing the suppression of all test results, including those analytes that are not directly interfered by the presence of hemolysis. The experience presented in this paper seems to fully support this approach. However, a dilemma remains unsolved: How to recognize an *in vivo* hemolysis in which the release of test results may become important? In our opinion, given the demonstrated infrequency of the joint presence of intravascular hemolysis and grossly hemolyzed samples, this may be solved only by a specific advice with the requesting clinician.

Table 1: Stratification of recruited patients with a blood sample with hemolysis index (HI) ≥ 1000 according to the probability of suffering of intravascular hemolysis.

| Sample collection site | No. (males) | <i>In vivo</i> hemolysis ruled out by laboratory data, n (%) | <i>In vivo</i> hemolysis ruled out by clinical data, n (%) | <i>In vivo</i> hemolysis ruled out after data matching and collegial discussion, n (%) |
|------------------------|-------------|--|--|--|
| Adults | | | | |
| Emergency department | 30 (15) | 23 (76.7) | 25 (83.3) | 30 (100) |
| Intensive care unit | 1 (0) | 1 (100) | 1 (100) | 1 (100) |
| Internal medicine ward | 16 (7) | 15 (93.8) | 10 (62.5) | 16 (100) |
| Outpatient clinic | 1 (0) | 1 (100) | 1 (100) | 1 (100) |
| Total | 48 (22) | 40 (83.3) | 37 (77.1) | 48 (100) |
| Pediatrics | | | | |
| Emergency department | 4 (2) | 0 (0) | 2 (50.0) | 4 (100) |
| Intensive care unit | 1 (1) | 0 (0) | 1 (100) | 1 (100) |
| Internal medicine ward | 7 (3) | 7 (100) | 1 (14.3) | 7 (100) |
| Total | 12 (6) | 7 (58.3) | 4 (33.3) | 12 (100) |
| Grand total | 60 (28) | 47 (78.3) | 41 (68.3) | 60 (100) |

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References

1. Lippi G, Cadamuro J, von Meyer A, Simundic AM, on behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE). Practical recommendations for managing hemolyzed samples in clinical chemistry testing. *Clin Chem Lab Med* 2018;56:718–27.
2. Lippi G, Blanckaert N, Bonini P, Green S, Kitchen S, Palicka V, et al. Haemolysis: an overview of the leading cause of unsuitable specimens in clinical laboratories. *Clin Chem Lab Med* 2008;46:764–72.
3. Barcellini W, Fattizzo B. Clinical applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. *Dis Markers* 2015;2015:635670.
4. Carraro P, Servidio G, Plebani M. Hemolyzed specimens: a reason for rejection or a clinical challenge? *Clin Chem* 2000;46:306–7.
5. Dolci A, Panteghini M. Harmonization of automated hemolysis index assessment and use: is it possible? *Clin Chim Acta* 2014;432:38–43.
6. Dolci A, Giavarina D, Pasqualetti S, Szóke D, Panteghini M. Total laboratory automation: do stat tests still matter? *Clin Biochem* 2017;50:605–11.
7. Aloisio E, Carnevale A, Pasqualetti S, Birindelli S, Dolci A, Panteghini M. Random uncertainty of photometric determination of hemolysis index on the Abbott Architect c16000 platform. *Clin Biochem* 2018;57:62–4.
8. Pasqualetti S, Szóke D, Panteghini M. Heparinate but not serum tubes are susceptible to hemolysis by pneumatic tube transportation. *Clin Chem Lab Med* 2016;54:785–9.
9. Clinical and Laboratory Standards Institute [CLSI]. Hemolysis, icterus, and lipemia/turbidity indices as indicators of interference in clinical laboratory analysis: approved guideline. CLSI document C56-A. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
10. 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.