Rejection of hemolyzed samples can jeopardize patient safety

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\textbf{ABSTRACT}

\textbf{Introduction}

\textit{In vitro} hemolysis is the primary cause of sample/test rejection by the laboratory.

\textbf{Case report}

A 10-year-old, admitted with an asthma attack in the emergency-room, medicated with albuterol sulphate (intravenous bronchodilator that could induce hypokalemia), needed laboratory test monitoring. The physician prescribed the technical-nurse to perform blood sampling for: complete blood count, electrolytes, glucose, and blood gas analysis—within 30min after therapy. Samples were delivered to laboratory with a note “I had difficult to locate an appropriate access to perform the blood collection”.
Laboratory results

Glucose: 4.77 mmol/L. Complete blood count revealed discreet eosinophilia 0.13x10⁹/L, and thrombocytopenia 18x10⁹/L. However, platelet clumps were observed in peripheral blood smear. Blood gas analysis was unreported, laboratory informed that sample had micro clots.

Electrolytes: laboratory did not report the results; sample hemolyzed. 0.9 g/L of free hemoglobin is the cut-off defined by the laboratory; the sample presented 2.3 g/L of free hemoglobin. 3.9 mmol/L of potassium was the unreported result vs 2.1 mmol/L in the new sample. Briefly, the laboratory technician was trained to hide potassium results on hemolyzed sample due to the potential overestimation. Even if the hemolyzed sample presented a potassium value close to the lower reference range value (3.5-5.1 mmol/L), reporting the potassium result could allow the physician starting proper therapy to revert the hypokalemia by albuterol sulfate.

Conclusion
The laboratory should be aware of the clinical patient conditions and of the related physician needs, before hiding results. Therefore, both the laboratory and the clinic personnel should communicate in order to guarantee the patient safety.

INTRODUCTION

Briefly hemolysis is due to leakage of the red blood cells membrane with the release of the erythrocyte-cytoplasm in the fluid (plasma or serum) [1]. We can classify hemolysis in two major categories:

i) in vitro because of improperly blood sample collection procedure [2, 3], venous stasis [4-6], unnecessary or excessive sample mix [7, 8], improper temperature maintenance [9], etc.; and

ii) in vivo due to iatrogenic conditions, acquired, or hereditary; unrelated with any laboratory technique.

Hemolysis is the most frequent pre-analytical source of variability, and in vitro hemolysis is the primary cause of sample/test rejection by laboratory professional [10]. However, Cadamuro et al., properly evidenced that laboratory professionals need deeply understand the pre-analytical interference (i.e., hemolysis) then to establish own laboratory criteria about when and how to report laboratory results in hemolysed samples [11]. The aim of this pre-analytical case report is to show that hemolysis due to inadequate phlebotomy procedure masked hypokalemia by albuterol sulfate (salbutamol).

CASE REPORT

A 10-year-old boy, admitted with asthma attack in the emergency room from a Brazilian general hospital, and properly medicated with intravenous infusion of salbuterol sulfate–4 µg/Kg/min–needed laboratory test monitoring [12, 13]. Briefly, albuterol sulfate (salbutamol - a sympathomimetic amine), is a beta-adrenergic agonist that selectively acts on the beta (2)-adrenergic receptors of intracellular adenyl cyclase, the catalyst for the conversion of adenosine triphosphate (ATP) to cyclic-3’, 5’-adenosine monophosphate (cyclic AMP) [14]. This action increases cyclic AMP levels resulting in bronchial smooth muscle relaxation (bronchial dilation), and inhibition of release of immediate hypersensitivity mediators from mast cells [15]. The physician prescribed the technical nurse to perform blood sampling for laboratory testing–complete blood count, electrolytes (sodium, potassium, chloride, calcium, and magnesium),

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glucose, and blood gas analysis—within half an hour after albuterol sulfate infusion.

Samples were delivered to STAT laboratory by pneumatic tube system with a note in the test-order “I had difficulty to locate an appropriate venous access to perform the blood collection”.

**LABORATORY TESTING**

Complete blood count was performed on Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan); clinical biochemistry testing – glucose, sodium, potassium, chloride, calcium, and magnesium – were performed on cobas 8000 c501 (Roche Diagnostics GmbH, Penzberg, Germany); whereas blood gas analysis was performed on GEM Premier 3000® (Instrumentation Laboratory a Werfen Company, Bedford, USA).

**RESULTS**

The laboratory present complete blood count and glucose results; whereas unreported results of electrolytes, and blood gas analysis (Table 1).

| Instruments            | Tests                  | Results | Units   |
|------------------------|------------------------|---------|---------|
| Sysmex XN-1000 Sysmex | Red blood cells        | 4.86    | 10^{12}/L |
|                        | Haemoglobin            | 141     | g/L     |
|                        | Hematocrit             | 40.5    | %       |
|                        | Mean corpuscular volume| 83.3    | fL      |
|                        | Red blood cell distribution width | 12.6 | %       |
|                        | White blood cells      | 6.12    | 10^{9}/L|
|                        | Neutrophils            | 3.24    | 10^{9}/L|
|                        | Lymphocytes            | 2.32    | 10^{9}/L|
|                        | Monocytes              | 0.43    | 10^{9}/L|
|                        | Eosinophils            | 0.13    | 10^{9}/L|
|                        | Platelets *1           | 18      | 10^{9}/L|
| Cobas 8000 c501 Roche | Glucose                | 4.77    | mmol/L  |
|                        | Sodium                 |         |         |
|                        | Potassium              |         |         |
|                        | Chloride               |         |         |
|                        | Calcium                |         |         |
|                        | Magnesium              |         |         |
| GEM Premier 3000 Werfen| Blood gas analysis     |         | new sample required *2 |

*Laboratory notes:
1. platelet clumps were observed in peripheral blood smear from the blood sample collected in ethylenediaminetetraacetic acid (K,EDTA) tube.
2. sample hemolyzed +++
3. sample had micro clots being a possible analytical problem for the blood gas analyser.
DISCUSSION

The worried physician called the laboratory about the patient showing signs and symptoms compatible with hypokalemia: therefore, the potassium laboratory result was absolutely needed. In reply, the laboratory technician verbally (by phone) informed that result reporting was not allowed, because the sample was hemolyzed.

Hypokalemia should be expected immediately after salbutamol intravenous infusion for severe asthma treatment, with potassium values mainly between 2.7 mmol/L and 3.4 mmol/L, which generally return to normality within half an hour [16]; whereas, inhalation of salbutamol in children can cause hypokalemia—30 min after inhalation—with potassium levels between 2.5 mmol/L and 4.2 mmol/L [17, 18]. This hypokalemic effect of catecholamines is mediated by the $B_2$-receptor linked to the Na/K ATPase in skeletal muscle [19, 20]. Therefore, the inpatient treatment with salbutamol requires blood gas analysis and potassium monitoring. Measuring potassium in pediatric patients is essential, since these patients frequently have diarrhea, vomiting or are following therapy with diuretics and digitalis too [21].

Cadamuro et al., had shown an impressive heterogeneity from European laboratories on management of hemolyzed samples [22]. Briefly, some laboratories used a color scale for visual hemolysis detection (434/1160); whereas others used hemolysis cut-offs declared by the in vitro diagnostic device-manufacturers’ (624/1160). However, only 246/1160 verified these cut-offs. The general cut-off that defined a sample as hemolytic, lacks harmonization; i.e., same laboratories have rigorous cut-off of 0.1 g/L of free hemoglobin, whereas others have permissive cut-off of 1 g/L of free hemoglobin [22].

**Why did the laboratory not report the results?**

0.9 g/L of free hemoglobin is the cut-off defined and verified by the accredited laboratories by International Organization for Standardization 15189 standard [23] for rejecting samples; the present sample had 2.3 g/L of free hemoglobin. 3.9 mmol/L of potassium was the unreported result on the hemolyzed sample. A newly collected non-hemolyzed sample had shown a potassium of 2.1 mmol/L; with a turnaround time of 1h45min for reporting the proper result, having the whole course considered; whereas the mean turnaround for potassium report (from collection to verification) should be less than 36 min [24]. The reason: the laboratory technician was trained to hide potassium results on hemolyzed sample due to the potential overestimation (release of potassium from red blood cells). Though, the hemolyzed serum sample presented potassium near the lower reference range value (3.5-5.1 mmol/L), reporting the first potassium result could allow the physician starting the proper pharmacological therapy to revert immediately the hypokalemia by salbutamol. Reports support the importance to communicate potassium results with a comment on hemolyzed samples instead of suppressing it [25, 26].

The blood gas analysis revealed normal results with an abnormal flag on potassium results. The arterial blood sample had shown micro clots. Therefore, the laboratory technician supposed that the wrong result derived from the clot presence. D’Orazio accurately reported the impact of clots on blood gas analyses including potassium [27]. The laboratory technician performed the proper maintenance on the blood gas analyzer to eliminate the potential micro clot from the analyzer system; then verified the analyzer performance using a third-party control materials-independent from calibrator materials, as recommended [28]. Thus, laboratory technician required new arterial sample. The laboratory instruments can provide the concentration of potassium in a few seconds, since several blood gas analyzers are incorporating the electrode [29]. However, clinicians should be aware about the
specific reference range for potassium determination on a different sample matrix (relatively lower K⁺ on plasma sample than serum sample) [30]. Hence, divergent potassium results could be reported for the same patient’s samples, respectively collected as lithium heparin anticoagulated plasma for blood gas analysis or as serum for clinical biochemistry.

In conclusion, the laboratory should be aware of the clinical patient conditions and of the related physician needs, before hiding results. Therefore, both the laboratory and the clinic personnel should communicate in order to guarantee the patient safety.

REFERENCES

1. Schaer DJ, Buehler PW, Alayash AI, Belcher JD, Verceilotti GM. Hemolysis and free hemoglobin revisited: exploring hemoglobin and hemin scavengers as a novel class of therapeutic proteins. Blood 2013;121:1276-84.
2. Simundic AM, Bolenius K, Cadamuro J, Church S, Cornes MP, van Dongen-Lases EC, Eker P, Erdeljanovic T, Grankvist K, Guimaraes JT, Hoke R, Ibarz M, Ivanov A, Kovalevskaya S, Kristensen GBB, Lima-Oliveira G, Lippi G, von Meyer A, Nybo M, Salle B, Seipelc T, Sumarac Z, Vermeersch P. Joint EFLM-COLABIOCLI Recommendation for venous blood sampling. Clin Chem Lab Med 2018;56:2015-38.
3. Mrazek C, Simundic AM, Wiedemann H, Krahmer F, Felder TK, Kipman U, Hoppe U, Haschke-Becher E, Cadamuro J. The relationship between vacuum and hemolysis during catheter blood collection: a retrospective analysis of six large cohorts. Clin Chem Lab Med 2017;55:1129-34.
4. Cadamuro J, von Meyer A, Wiedemann H, Klaus Felder T, Moser F, Kipman U, Haschke-Becher E, Mrazek C, Simundic AM. Hemolysis rates in blood samples: differences between blood collected by clinicians and nurses and the effect of phlebotomy training. Clin Chem Lab Med 2016;54:2015-38.
5. Lima-Oliveira G, Lippi G, Salvagno GL, Gaino S, Poli G, Gelati M, Picheth G, Guidi GC. Venous stasis and whole blood platelet aggregometry: a question of data reliability and patient safety. Blood Coagul Fibrinolysis 2015;26:665-8.
6. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Scartezini M, Guidi GC, Picheth G. Transillumination: a new tool to eliminate the impact of venous stasis during the procedure for the collection of diagnostic blood specimens for routine haematological testing. Int J Lab Hematol 2011;33:457-62.
7. Lima-Oliveira G, Lippi G, Salvagno GL, Montagnana M, Gelati M, Volanski W, Boritza K, Picheth G, Guidi GC. Effects of vigorous mixing of blood vacuum tubes on laboratory test results. Clin Biochem 2013;46:250-4.
8. Lima-Oliveira G, Lippi G, Salvagno GL, Brocco G, Gaino S, Dima F, Volaski W, Rego FG, Picheth G, Guidi GC. Processing of diagnostic blood specimens: is it really necessary to mix primary blood tubes after collection with evacuated tube system? Biopreserv Biobank 2014;12:53-9.
9. Lippi G, Lima-Oliveira G, Nazer SC, Moreira ML, Souza RF, Salvagno GL, Montagnana M, Scartezini M, Picheth G, Guidi GC. Suitability of a transport box for blood sample shipment over a long period. Clin Biochem 2011;44:1028-9.
10. Wan Azman WN, Omar J, Koon TS, Tuan Ismail TS. Hemolyzed Specimens: Major Challenge for Identifying and Rejecting Specimens in Clinical Laboratories. Oman Med J 2019;34:94-8.
11. Cadamuro J, Simundic AM, Ajzner E, Sandberg S. A pragmatic approach to sample acceptance and rejection. Clin Biochem 2017;50:579-81.
12. Clayton S, Gilchrist F, Alexander J, Enberby B, Lenney W. An algorithm for the use of intravenous salbutamol in children. Br J Nurs 2012;21:530-4.
13. Babi F, Sheriff N, Borland M, Acworth J, Neutez J, Krieser D, Ng P, Schutz J, Thomson F, Cotterell E, Jamison S, Francis P. Paediatric acute asthma management in Australia and New Zealand: practice patterns in the context of clinical practice guidelines. Arch Dis Child 2008;93:307-12.
14. Johnson M. Beta2-adrenoceptors: mechanisms of action of beta2-agonists. Paediatr Respir Rev 2001;2:57-62.
15. Neame M, Aragon O, Fernandes RM, Sinha I. Salbutamol or aminophylline for acute severe asthma: how to choose which one, when and why? Arch Dis Child Educ Pract Ed 2015;100:215-22.
16. Leitch AG, Clancy LJ, Costello JF, Flenley DC. Effect of intravenous infusion of salbutamol on ventilatory response to carbon dioxide and hypoxia and on heart rate and plasma potassium in normal men. Br Med J 1976;1:365-7.
17. Hung CH, Chu DM, Wang CL, Yang KD. Hypokalemia and salbutamol therapy in asthma. Pediatr Pulmonol 1999;27:27-31.
18. Burgess CD, Flatt A, Siebers R, Crane J, Beasley R, Purdie G. A comparison of the extent and duration of hypokalaemia following three nebulized beta 2-adrenoceptor agonists. Eur J Clin Pharmacol 1989;36:415-7.
19. Gelmont DM, Balmes JR, Yee A. Hypokalemia induced by inhaled bronchodilators. Chest 1988;94:763-6.

20. Brown MJ, Brown DC, Murphy MB. Hypokalemia from beta2-receptor stimulation by circulating epinephrine. N Engl J Med 1983;309:1414-9.

21. Kerem E, Levison H, Schuh S, O’Brodovich H, Reisman J, Bentur L, Canny GJ. Efficacy of albuterol administered by nebulizer versus spacer device in children with acute asthma. J Pediatr 1993;123:313-7.

22. Cadamuro J, Lippi G, von Meyer A, Ibarz M, van Dongen E, Lases, Cornes M, Nybo M, Vermeersch P, Granqvist K, Guimaraes JT, Kristensen GBB, de la Salle B, Simundic AM. European survey on preanalytical sample handling - Part 2: Practices of European laboratories on monitoring and processing haemolytic, icteric and lipemic samples. On behalf of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for the Preanalytical Phase (WG-PRE). Biochem Med (Zagreb) 2019;29:020705.

23. International Organization for Standardization. Medical laboratories - Requirements for quality and competence ISO document 15189. Geneva, Switzerland 2012;International Organization for Standardization.

24. Valenstein P. Laboratory turnaround time. Am J Clin Pathol 1996;105:676-88.

25. Carraro P. Potassium report of hemolyzed serum samples. Clin Chem Lab Med 2008;46:425-6.

26. Ismail A, Shingler W, Seneviratne J, Burrows G. In vitro and in vivo haemolysis and potassium measurement. BMJ 2005;330:949.

27. D’Orazio P. Effects of Blood Clots on Measurements of pH and Blood Gases in Critical Care Analyzers. Point of Care: The Journal of Near-Patient Testing & Technology 2011;10:186-8.

28. Lima-Oliveira G, Lippi G, Salvagno GL, Brocco G, Guidi GC. In vitro diagnostic company recalls and medical laboratory practices: an Italian case. Biochem Med (Zagreb) 2015;25:273-8.

29. Beneteau-Burnat B, Pernet P, Pilon A, Latour D, Goujon S, Feuillu A, Vaubourdolle M. Evaluation of the GEM Premier 4000: a compact blood gas CO-Oximeter and electrolyte analyzer for point-of-care and laboratory testing. Clin Chem Lab Med 2008;46:271-9.

30. Lima-Oliveira G, Monneret D, Guerber F, Guidi GC. Sample management for clinical biochemistry assays: are serum and plasma interchangeable specimens? Crit Rev Clin Lab Sci 2018;55:480-500.