In this issue: Improving the preanalytical phase in laboratory medicine

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Rejection of hemolyzed samples can jeopardize patient safety

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ABSTRACT

Introduction

In vitro hemolysis is the primary cause of sample/test rejection by the laboratory.

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 0.13x10^9/L, and thrombocytopenia 18x10^9/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.

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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 dilatation), 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),

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).

Table 1 Laboratory testing results*

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	new sample required *2	
	Potassium		
	Chloride		
	Calcium		
	Magnesium		
GEM Premier 3000 Werfen	Blood gas analysis	new sample required *3	

Laboratory notes:
1. platelet clumps were observed in peripheral blood smear from the blood sample collected in ethylenediaminetetraacetic acid (K_2EDTA) 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 2.1mmol/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.

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