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Original Research Article

Influence of spurious dilution and hyperglycemia on erythrocytes and platelets evaluated with two different hematological analyzers

Giuseppe Lippi [a,*], *Ruggero Buonocore* [a], *Roberta Musa* [a], *Rosalia Aloe* [a], *Alessandra Picanza* [a], *Silvia Pipitone* [a], *Fabian Sanchis-Gomar* [b]

[a] *Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy*
[b] *Department of Physiology, Faculty of Medicine, University of Valencia, Fundación Investigación Hospital Clínico Universitario/INCLIVA, Spain*

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ABSTRACT

The integrity of whole blood samples may be compromised by suboptimal collection practices. Therefore, we investigated the influence of spurious hyperglycemia on erythrocytes and platelets, assessed with two hematological analyzers using optical or impedance technique. Three K_2EDTA blood specimens were collected from 12 healthy subjects, pooled and divided into four aliquots. The first aliquot was left untreated (glucose concentration 4.4 mmol/L), whereas scalar amounts of standard 5% glucose solution were added to the remaining, generating glucose contamination of 5% (19.2 mmol/L), 10% (33 mmol/L) and 20% (62 mmol/L). Hematological testing was then performed using Siemens Advia 2120 and Sysmex XE-2100, whereas glucose and cell-free hemoglobin were measured in centrifuged plasma. Hemolysis did not occur in contaminated aliquots, whereas hemoglobin, erythrocyte count, hematocrit, mean corpuscular hemoglobin concentration and platelet count progressively decreased with glucose concentration. No clinically significant variation was found for red blood cell distribution width and mean corpuscular hemoglobin. The erythrocyte size increased in parallel with glucose, exhibiting larger variation with Advia 2120 than with XE-2100. The mean platelet volume increased on Advia 2120 but not on XE-2100. These results suggest that hyperglycemia induces alterations of erythrocyte and platelet properties, which differ according to the technology used for assessment.

* *Corresponding author at*: U.O. Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci, 14, 43126 Parma, Italy. Tel.: +39 0521 703050/703791.
E-mail addresses: glippi@ao.pr.it, ulippi@tin.it (G. Lippi).

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Introduction

Osmosis is conventionally defined as the process by which water moves across a semipermeable membrane from an area of low solute concentration to another of high solute concentration. As any other human cell, blood cells are essentially composed by water, for up to 90% of their volume (Gallagher, 2013). Since the plasma membrane does not effectively contrast its diffusion, water flows into the cell by osmosis. When this process is greatly amplified as a consequence of high solute concentration in the extracellular fluid (i.e., blood), the cell swells and the membrane looses integrity, up to eventual rupture (Vander et al., 2001). The osmotic swelling and possible lysis of red blood cells (RBCs) as a consequence of very high glucose concentrations in the extracellular fluid is a physiological phenomenon (Losche et al., 1989), which is also currently employed (after replacing glucose with glycerol 135 mmol/L) for diagnosing abnormalities of RBC membrane such as hereditary spherocytosis (Vettore et al., 1984).

Glucose-rich plasma may produce glycosylation of RBC proteins and, therefore, significant hemorheological changes (Shin et al., 2008). Moreover, high levels of plasma glucose also cause important damage in RBC membrane, which are mainly attributable to oxidative stress (Resmi et al., 2005; Jain et al., 2006; Korol et al., 2011; Yang et al., 2012).

Hyperglycemia also activates platelets via osmotic stress and specifically the nitric oxide (NO)/cyclic nucleotide pathway (Massucco et al., 2005). Extracellular glucose is used by platelets for calcium homeostasis and maintenance of mitochondrial integrity (Hartley et al., 2007). The induction of acute hyperglycemia can lead to increased platelet reactivity and platelet activation in healthy adults (Yngen et al., 2001; Vaidyula et al., 2006; Undas et al., 2008). Finally, exposure of platelets to hyperosmolar solutions also causes increased reactivity (Keating et al., 2003), whereas hyperglycemia may trigger morphologic changes and proaggregatory effects on blood platelets (Assert et al., 2001).

Despite these evidences, no information is available about the effect of different degrees of hyperglycemia on erythrocytes and platelets using modern hematological analyzers to the best of our knowledge. This is an important drawback, since the identification of significant sources of biological variation is a crucial aspect when establishing the role of erythrocytes and platelets in health and disease, as well as for establishing the analytical and clinical impact of spurious hyperglycemia (i.e., the exogenous contamination of blood samples) on physical and morphological properties of these blood elements. In particular, the collection of blood through vascular access devices is commonplace in several healthcare settings, especially in intensive care units and in emergency rooms. In this condition, blood contamination with glucose solutions may occur as a result of insufficient volume of blood drawn to clear the line, the use of glucose solutions rather than saline to "flush" the line, or collection of blood samples from a site proximal to the placement of a vascular access devices in a patient receiving intravenous glucose (Leslie et al., 2013).

As such, this study was planned to evaluate the influence of different degrees of blood contamination with a standard glucose solution on erythrocytes and platelets as may occur during a normal venipuncture contaminated by exogenous glucose, using two hematological analyzers with different technology for enumeration and sizing of blood cells.

Materials and methods

The study population consisted in 12 subjects selected from ostensibly healthy undergoing routine laboratory testing. Three venous blood specimens were collected at fasting from each subject in tubes containing K_2EDTA (13 mm × 75 mm, 3.0 mL BD Vacutainer® Plus plastic whole blood tube containing 5.4 mg spray dried K_2EDTA; Becton Dickinson Italia S.p.A., Milan, Italy). The homologous blood of each subject was pooled and then divided into four aliquots of 2.0 mL each. The first aliquot was left untreated (i.e., no contamination), whereas a scalar amount of 100, 200 and 400 μL of a standard 5% glucose-containing solution (25 g of glucose monohydrate in 500 mL of water, 278 mOsm/L; Baxter SpA, Rome, Italy) was added to the other three aliquots, to generate a final glucose contamination of 5%, 10% and 20%, which is representative of the majority of glucose-contaminated specimens received in our laboratory. The blood aliquots were left capped and in upright position for 1 h at room temperature. The complete blood cell count (CBC) was then performed with both Siemens Advia 2120 (Siemens Diagnostic Solutions, Milan, Italy) and Sysmex XE-2100 (Dasit SpA, Cornaredo, Italy). In Advia 2120, the erythrocyte population is analyzed using flow cytometry and 2-angle laser light scatter. The mean corpuscular volume (MCV) is directly measured by hydrodynamic focusing, laser low-angle and high-angle optical scatter, whereas the hematocrit (Ht) is indirectly obtained from the values of RBC count and MCV. The red blood cell distribution width (RDW) is then estimated from the RBC histogram in a window between 60 and 120 fL. In XE-2100, a specific detector counts both erythrocytes and platelets using the principle of hydrodynamic focusing (i.e., impedance technology), whereas the Ht is measured using the RBC pulse height detection method. Therefore, the erythrocyte size (i.e., the MCV) is not directly measured, but is obtained by dividing the Ht for the RBC count. The RDW is then calculated at a relative height of 20% above the baseline of RBC histogram. The mean corpuscular hemoglobin (MCH) is obtained in both analyzers from the equations hemoglobin (Hb)/RBC × 10, whereas the mean corpuscular hemoglobin concentration (MCHC) is calculated from the formula Hb/[RBC × MCV] × 1000 in Advia 2120 and Hb/Ht × 100, respectively. Platelets are counted by optical method in Advia 2120 and primarily with impedentiometric technology in XE-2100. In both instruments, the mean platelet volume (MPV) is then calculated from the platelet histogram, by using instrument-specific calculations. After the CBC was completed, the blood aliquots were centrifuged at 1300 × *g* for 15 min; glucose (hesokinase method) and cell free-hemoglobin (hemolysis index) were measured in plasma using a Beckman D × C (Beckman Coulter Inc., Brea, CA, USA). In D × C the hemolysis index is measured by direct spectrophotometry, and the cell-free hemoglobin values are finally calculated on a linear scale from 0 (0 g/L of hemoglobin) to 10 (hemoglobin concentration from 4.5 to 5.0 g/L).