LEUKOCYTOsis INTERFERENCE IN CLINICAL CHEMISTRY: SHALL WE STILL INTERPRET TEST RESULTS WITHOUT HEMATOLOGICAL DATA?

INTERFERENCIJA LEUKOCITOZE U KLINIČKOJ HEMIJI: DA LI ĆEMO I DALJE INTERPRETIRATI REZULTATE TESTA BEZ HEMATOLOŠKIH PODATAKA?

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Summary

Background: Extreme leukocytosis is known to induce remarkable variations of some clinical chemistry tests, thus leading to possible clinical misinterpretation. This study aimed to define whether also moderate leukocytosis may influence the stability of glucose and blood gases.

Methods: Blood samples are sent to the local laboratory through a pneumatic tube system. Clinical chemistry testing is routinely performed using lithium-heparin tubes (for glucose) and heparin blood gases syringes (for blood gas analysis). Stability of glucose (in uncentrifuged blood tubes) and blood gases (in syringes) was hence evaluated in samples maintained at room temperature. Results were also analyzed in 2 subgroups of samples with different leukocyte counts, i.e., those with leukocytes <15 × 10^9/L and those with leukocytes >15 × 10^9/L.

Results: An accelerated decrease of pH was observed in blood gases syringes with leukocytosis (i.e., >15 × 10^9/L), while no difference was noted for other blood gases parameters (PCO2, PO2). Spurious and time-dependent hypoglycemia was noted in uncentrifuged blood tubes of patients with leukocytosis.

Conclusions: The results of our study suggest that even modest leukocytosis (i.e., around 15 × 10^9/L), which is

Kratak sadržaj

Uvod: Poznato je da ekstremna leukocitoza izaziva značajne varijacije nekih kliničkih hemijskih testova, što dovodi do moguće pogrešne kliničke interpretacije. Cilj ovog rada bio je da se utvrdi da li i umerena leukocitoza može uticati na stabilnost glukoze i gasova u krvi.

Metode: Uzori krvi su poslani u lokalni laboratorij preko pneumatskog sistema cevi. Klinička hemijska ispitivanja se rutinski izvodive koristeči lijmijum-heparinske epruvete (za glukozu) i heparinske gasove (za analizu gasa u krvi). Stabilnost glukoze (u necentrifugiranim epruvetama krvi) i gasova u krvi (u špricem) je zbog toga procjenjena na uzorcima koji su održavani na sobnoj temperaturi. Rezultati su takođe analizirani u 2 podgrupe uzoraka sa različitim brojem leukocita, to jest, sa leukocitima <15 × 10^9/L i sa leukocitima >15 × 10^9/L.

Rezultati: U špricama sa gasovima u krvi sa leukocitozom (tj. >15 × 10^9/L) primitivno je ubrzano smanjeno pH vrednost, pri čemu nije zabeležena razlika za druge parametre gasova krvi (PCO₂, PO₂). Lažna i vremenski zavisna hipoglikemija zabeležena je u necentrifugiranim epruvetama pacijenata sa leukocitozom.

Zaključak: Rezultati našeg istraživanja ukazuju da čak i skromna leukocitoza (tj. oko 15 × 10^9/L), koja se često
Introduction

It has been convincingly shown that some clinical chemistry parameters may display spurious changes in samples with extreme leukocytosis, thus potentially disturbing both clinical decision making and patient management.

Monitoring blood glucose is essential, since hyperglycemia may be associated with many symptoms such as infections or late-onset nerve damage, while hypoglycemia may also cause serious health consequences. The basic symptoms of hypoglycemia are poorly specific and include fatigue, mood changes, difficulty in concentrating and completing mental tasks, blurred vision, extreme hunger, nervousness, headache, excessive perspiration and tachycardia. Extremely low glucose levels can trigger convulsions and coma (1). An accurate diagnosis of hypoglycemia is also essential for diagnosing metabolic diseases (2).

Glucose is usually measured in clinical laboratories using plasma, serum or whole blood samples. The term »artifactual hypoglycemia« was proposed when results of laboratory measurement do not reflect the actual blood glucose concentration, regardless of the presence of symptoms of both hypo- or hyperglycemia (3). Artifactual hypoglycemia has also been related to in vitro glucose consumption by blood cells after blood sample has been drawn (4, 5).

Sodium fluoride is largely used as glycolysis inhibitor in fluoride tubes since it assures longer glucose stability in uncentrifuged or non-separated blood tubes. Nevertheless, lithium-heparin blood tubes without glycolysis inhibitors are very frequently used to measure glucose in clinical and laboratory practice, since it helps to reduce the number of samples drawn and minimizing blood wasting, especially in critically ill patients with severe anemia (6, 7).

A recent study showed that normal blood cell counts and volumes might both have an impact on glucose concentration in uncentrifuged serum and lithium-heparin blood tubes (8), while no data are available on the impact of non-leukemic leukocytosis on glucose stability in blood samples to the best of our knowledge. We suspect that the impact on glucose may be even greater with leukocytosis than with normal leukocyte counts.

Blood gases are conventionally requested as emergency tests. By measurement of dissolved gases in blood and pH, blood gas analysis provides essential clinical information for monitoring alveolar ventilation and acid-base balance (9). The stability of these parameters in blood samples is strongly influenced by storage temperature and by the possible presence of hemolysis (10, 11). Previous studies showed that blood gases are stable for up to 30 minutes after collection (12), while the analysis of blood gases in samples (typically syringes) after this period may yield unreliable results. Since blood gas analysis is an essential test for patients with acute conditions, and recollection of a new blood sample is not always feasible in short-stay units, blood gas analysis is occasionally carried out after the recommended 30-min stability threshold. Nevertheless, blood cells are known to gradually modify the acid-base balance over time, by consuming both oxygen and glucose (13–15), and thus potentially leading to unreliable test results. A case of pseudohypoxemia in a patient with chronic lymphocytic leukemia was also identified, showing that blood gases stability is inversely correlated with the leukocyte count (16).

Therefore, the aim of this study was to define whether moderate leukocytosis may also influence the stability of some clinical chemistry parameters, namely glucose and blood gases.

Materials and Methods

Patient selection and data collection

The study population consisted of patients undergoing routine laboratory testing at Lille University Hospital. Patients received detailed information that their clinical data and/or residual blood samples could be used for research purposes after routine testing had been completed. All data were retrieved from a human biological database authorized by the French Ministry of Research (No. DC-2008-642). Therefore, no written informed consent was collected from this population.

The stability of glucose was assessed in lithium-heparin tubes (4 mL BD Vacutainer™; Becton Dickinson, Franklin Lakes, NJ, United States) and blood gases in heparin syringes (3 mL Smiths Medical; Minneapolis, MN, United States).

To perform our study, we selected samples containing enough blood to allow repeated analyses (full draw). Samples were selected upon arrival from
patients who had had a recent leukocyte count available. Samples were divided into 2 groups according to the leukocyte count: i.e. <15 $\times 10^9$/L or >15 $\times 10^9$/L.

**Laboratory measurements**

Glucose concentrations were measured using Roche Cobas 8000® (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Blood gas parameters were measured on Radiometer ABL800 FLEX (Radiometer, Copenhagen, Denmark). White blood cell count (WBC), hematocrit and platelets count were assayed in BD Vacutainer™ ethylenediaminetetraacetic acid tubes (Becton Dickinson, Franklin Lakes, NJ, United States), using Sysmex XN (Sysmex Corporation, Kobe, Japan).

All blood tubes and syringes were sent to the laboratory by a pneumatic tube system (PTS) (Swiss-log, Switzerland) within 30 min of collection.

For glucose stability experiments, one blood sample (i.e., lithium-heparin blood tube) was collected from each patient and was immediately centrifuged after arrival at 4000×g for 5 min. Lithium-heparin tubes were maintained on a roller mixer at room temperature throughout the study to prevent sedimentation of blood cells in between measurements. Blood cells would this way continue in vitro glucose consumption and better reflect the impact of a delay in sample handling. Before each plasma glucose measurement, samples were centrifuged at 4000×g for 5 min.

Plasma glucose was successively measured at 0, 2, 4, 6 and 8 hours after the reception in the laboratory, and its variation was compared in 2 different groups of patients, displaying WBC count <15 $\times 10^9$/L or >15 $\times 10^9$/L.

For blood gases stability experiments, one whole blood sample (i.e., heparin blood gas syringe) was collected from each patient. Blood gas analysis was then carried out at 0, 60, 120 and 180 min after the reception in the laboratory, on the same syringe maintained at room temperature. Results were compared in 2 groups of patients, i.e., those with leukocyte counts <15 $\times 10^9$/L or >15 $\times 10^9$/L. Before each measurement, the syringe was gently mixed to avoid sedimentation and kept sealed afterwards.

The local reference ranges were 65 to 100 mg/dL (3.58–5.50 mmol/L) for glucose and 4–10 $\times 10^9$/L for WBC count.

**Statistical analyses**

Data analyses were performed with paired Wilcoxon ranked test and Pearson correlation using R software (www.R-project.com) and GraphPad Prism software v6.0. The statistical methods used included t-tests, ANOVA and regression analysis. Statistical significance was set at p < 0.05.

**Results**

**Glucose**

A total number of 64 lithium-heparin samples were included in our analysis, which were sorted according to WBC count, platelet count and hematocrit values, as shown in Table I. The baseline glucose levels, White Blood Cells counts, Platelets counts and Hematocrit levels (Mean +/- SEM) measured in blood. Regression and correlation between glucose and time for each group, p-value obtained by Pearson t-test.

| Glucose t0 (mmol/L) | White Blood Cells (x10^9/L) | Platelets (x10^9/L) | Hematocrit (%) | Glucose-Time Regression |
|---------------------|-----------------------------|---------------------|----------------|-------------------------|
|                     | N | Mean (SE) | Min-Max | Mean (SE) | Min-Max | Mean (SE) | Min-Max | Mean (SE) | Min-Max | Slope | r | p-value |
| Low Leukocytes (<15 $\times 10^9$/L) | 54 | 6.32 (1.39) | 3.77–12.9 | 7.77 (2.75) | 0.3–14.51 | 249 (110) | 24–785 | 31 (4.8) | 19.9–44.2 | -0.34 | -0.42 | <0.001 |
| High Leukocytes (>15 $\times 10^9$/L) | 10 | 6.32 (1.49) | 3.55–9.55 | 109 (88) | 15–346 | 214 (206) | 13–682 | 27 (4.3) | 19.4–36.6 | -0.63 | -0.67 | <0.001 |
| Low Platelets (<150 $\times 10^9$/L) | 18 | 6.93 (1.83) | 3.55–12.9 | 57 (71) | 0.3–346 | 61 (30) | 13–148 | 26.9 (4) | 19.4–42.5 | -0.27 | -0.22 | 0.028 |
| High Platelets (>150 $\times 10^9$/L) | 46 | 6.05 (1.22) | 3.77–10.7 | 10 (4) | 2.7–41.26 | 316 (109) | 157–785 | 32.5 (4.2) | 23.5–44.2 | -0.39 | -0.26 | <0.001 |
| Low Hematocrit (<30%) | 32 | 6.49 (1.49) | 3.55–14.1 | 37 (47) | 0.3–346 | 118 (116) | 13–413 | 24–785 | 36 (3.1) | 30.2–44.2 | -0.41 | -0.56 | <0.001 |
| High Hematocrit (>30%) | 32 | 6.16 (1.27) | 3.77–10.7 | 9.5 (3.5) | 3.8–40.5 | 300 (142) | 24–785 | 36 (3.1) | 30.2–44.2 | -0.41 | -0.56 | <0.001 |
Figure 1 Glucose values (Mean +/- SEM) measured at 2 hours intervals in blood from patients with white blood cell (WBC) values < 15 x 10^9/L (gray lines) or > 15 x 10^9/L (black lines), with platelets < 15 x 10^9/L (gray lines) or > 15 x 10^9/L (black lines), and hematocrit < 30% (grey lines) and > 30% (black lines). B. Correlation between variation of glucose (mmol.L^{-1}.hour^{-1}) and WBC, platelets or hematocrit.

Figure 2 pH, PaO_2 and PCO_2 values (Mean +/- SEM) measured at 60 minutes intervals in whole blood collected from patients with white blood cell (WBC) values < 15 x 10^9/L (black lines) or > 15 x 10^9/L (grey lines). B. Correlation between WBC count and variation of pH, pO_2 and pCO_2 in min^{-1}. 
concentration did not significantly differ in each subgroup. No difference was noted when the analysis was carried out in subgroups of patients with different values of platelets and hematocrit (Figure 1A).

Interestingly, a strong association was found between the value of WBC and decrease of glucose values (r = –0.633, p < 0.0001), but not between glucose and platelet count (r = 0.048; p = 0.704) or hematocrit (r = 0.239; p = 0.057) (Figure 1B). Glucose variations over time (from baseline to 2, 4, 6 and 8 hours after blood draw) was significantly accelerated in samples with WBC count > 15 × 10^9/L (n = 10) as opposed to in those with WBC count < 15 × 10^9/L (n = 54).

### Blood gases parameters

Overall, 77 blood gases samples were included in our analysis. The results, sorted according to WBC counts, are shown in Table II. No significant difference was found for baseline values of pH, pO_2, and pCO_2 between the two groups.

A larger decrease of pH values over time was observed in samples with WBC count > 15 × 10^9/L (n = 45) compared to those with WBC count < 15 × 10^9/L (n = 32) (Table II and Figure 2A). However, no difference in pO_2 and pCO_2 values were noted between these two groups (Figure 2A).

The variations of blood gases values (pH, pO_2 and pCO_2) over time (i.e., at baseline and 60, 120 and 180 min afterwards) were analyzed with Pearson’s correlation, in which a significant association was found between time and pH in the two groups (r = –0.204; p = 0.0284 and r = –0.430; p < 0.0001), and pCO2 in the low leukocyte count cohort (r = 0.243; p = 0.018). On the contrary no difference was observed for pO2 in both groups (r = –0.130; p = 0.125 and r = –0.110; p = 0.243), and pCO2 in the high leukocyte cohort (r = 0.172; p = 0.116). Notably, a highly significant association was noted between WBC count and pH changes over time (r = –0.707; p < 0.0001), but not between WBC count and variation of both pO2 (r = –0.167; p = 0.121) or pCO2 (r = 0.134; p = 0.348) values over time (Figure 2B).

### Discussion

The stability of laboratory parameters in blood samples is an important matter of concern, since spurious variations occurring before testing may have a profound impact on clinical decision making and care management, thus ultimately jeopardizing patient safety. An important aspect that emerged from our study is that plasma glucose undergoes a substantial, time-dependent consumption in plasma samples displayed in even modest leukocytosis (i.e., ∼15 × 10^9/L). The maximum allowable time before sample centrifugation shall hence be reduced in these patients since glucose values would become otherwise unreliable. The use of glycolysis inhibitors might also be mandatory in all patients with leukocytosis. However, the complete effect of fluoride on gly-

|                     | Low Leukocytes (<15×10^9/L) n = 32 | High Leukocytes (>15×10^9/L) n = 45 |
|---------------------|-----------------------------------|----------------------------------|
|                     | Mean (SE)                         | Mean (SE)                        |
| pH t0               | 7.39 (0.12)                       | 7.36 (0.08)                      |
| pO_2 t0 (mmHg)      | 101 (32)                          | 96 (40)                          |
| pCO_2 t0 (mmHg)     | 36 (6)                            | 46 (14)                          |
| White Blood Cells (×10^9/L) | 8.4 (2.1) | 24.3 (5)                        |

| Regression (pH)     |                     |                     |
| Spectrum            | -0.0004718          | -0.0006996          |
| r                   | -0.204              | -0.430              |
| p-value             | 0.028               | <0.0001             |

| Regression (PaO_2)  |                     |                     |
| Spectrum            | -0.0907             | -0.0658             |
| r                   | -0.130              | -0.110              |
| p-value             | 0.125               | 0.243               |

| Regression (PCO_2)  |                     |                     |
| Spectrum            | 0.035               | 0.032               |
| r                   | 0.243               | 0.172               |
| p-value             | 0.018               | 0.115               |
colysis inhibition could take up to 4h according to leukocytes values, and concentration of glucose in the blood tube may meanwhile considerably decrease (17). Alternatively, a comparison with glucose results obtained with a glucometer may be advisable in case of suspicious results.

Unlike previous studies (18, 19), we failed to observe an association between WBC count and variation of pO2 and pCO2, while we noted a significant association between WBC values and pH, and this may be attributable to the fact that glucose metabolism by leukocytes may contribute to increased lactic acid production, thus generating acidosis.

In conclusion, the results of our study suggest that even modest leukocytosis (i.e., around 15 × 10^9/L), which is frequently encountered in clinical and laboratory practice, may impair the stability of glucose and pH in blood samples. This would lead us to conclude that the results of these parameters should be perhaps accompanied by those of hematologic testing, namely the leukocyte count, to prevent clinical misinterpretation. In case of suspicious biochemistry results associated with high leucocyte levels, verification could be performed short-circuiting pre-analytical transport using glucometer or point of care device.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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