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Evaluation of Hemoglobin and Hematocrit Values by the CO-Oximetry and Cyanide-Free Sodium Lauryl Sulphate Methods: A Retrospective Study

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ABSTRACT

Introduction: Blood gas analyzers (BGA) have recently been widely used as a rapid testing devices for the determination of hemoglobin (Hb) in the intensive care units and emergency services of hospitals. We compared the Hb and calculated-hematocrit (Hct) values by the CO-oximetry and cyanide-free sodium lauryl sulphate (SLS) methods.

Methods: Between January and June 2019, 12,049 patients who applied to the emergency department of İstanbul Training and research Hospital, for whole blood count and venous blood gas analysis were included. Samples were analyzed using SLS- Hb and CO-oximetry methods. Bland-Altman plot and Passing-Bablok regression analysis were performed to evaluate the accordance of the methods.

Results: The correlation coefficients of the methods for Hb and Hct were 0.89 and 0.87, respectively ($p<0.0001$). Passing-Bablok regression analysis showed a significant deviation from linearity ($p<0.01$). Bland-Altman plot showed insufficient agreement between of the two methods for each variable. Bias % calculated as 2.5% for Hb, and 1.1% for Hct. Total error calculated as 4.08% for Hb. Total error of CO-oximetric Hb value was within the limits of allowable total error.

Conclusion: Although each test shows a significant deviation from linearity, BGA's could be used for Hb measurements since the bias and total error were still acceptable.

Keywords: Blood gas analyzers, CO-oxymetry, hemoglobin, sodium lauryl sulphate, method comparison

Introduction

Hemoglobin (Hb) is a complex protein responsible for the transport of oxygen from the lungs through the arteries to the tissues. A lower level of Hb in the erythrocytes red blood cells (RBC) is one of the important factors that shows reduced tissue oxygenation (1). Hematocrit (Hct) is the ratio of the volume of RBC to the whole blood volume. Simultaneous measurements of Hb and Hct concentrations are used to evaluate conditions such as anemia, bleeding or hemorrhage (2).

Hb and Hct can be measured by different methodologies such as hematology analyzers, Hct centrifuge, cyanmethemoglobin method, gravimetric copper sulfate method and color code Hb estimation (3). The cyanmethemoglobin method is accepted as the gold standard for measuring Hb concentration by the International Council for Standardization in Hematology (ICSH). The principle of this method is to measure the absorbance of the final product at 540 nm wavelength by converting Hb to cyanmethemoglobin by adding potassium cyanide and ferricyanide. However, this method is not suitable for automated auto analyzers due to a low Hb conversion rate, and multiple sample processing is required. Furthermore, since cyanide, which has toxic effects on the environment and human health, is wasted in large volumes, laboratories have turned to alternative methods. So non-cyanide measurement methods have become the clinical standard (4,5).

In 1981, Oshiro et al. (6) developed a cyanide-free Hb assay method based on the Lambert-Beer Spectrophotometry principle using a non-toxic compound, sodium lauryl sulfate (SLS). CO-oximeter is another Hb based spectrophotometric method depending on Lambert-Beer laws. CO-oximeters are multi-wavelength photometers that determine the total amount of Hb and Hb derivatives such as oxyhemoglobin, reduced Hb, carboxyhemoglobin, and methemoglobin (7).

Blood gas analyzers (BGA) are used in intensive care units and emergency departments for assessing and monitoring oxygenation status, ventilation, and acid-base status of critically ill patients. Modern BGA have integrated CO-oximeter modules to estimate total Hb and Hb derivatives (8). The use of BGA has increased significantly over the years because of their ease of use and accessibility. The reliability of the results obtained from these devices should be tested using appropriate statistical tools.

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In this study, we assessed the concordance of CO-oximetry and SLS- Hb methods in the terms of measuring Hb and calculating Hct values.

Methods

Samples and Data Collection

This is a retrospective study, conducted in the emergency laboratory of İstanbul Training and Research Hospital. We scanned the records of patients from the hospital information system between January and June 2019. Blood samples were collected in tubes containing lithium heparin (Ayset 2 mL sterile single-use syringe) for blood gas analysis and tubes with K2EDTA (BD Vacutainer™ Plastic Blood Collection Tubes with K2 EDTA) for complete blood count. The study was approved by the institutional ethical committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 1929, date: 09.08.2019).

Exclusion criteria were applied to the patients who underwent an emergency procedure, postoperative cases, and patients with hemoglobinopathy, and severe infection or sepsis. Inclusion criteria were determined as the simultaneous testing for both venous blood gas analysis and complete blood count at the same period. Following the establishment of the exclusion and inclusion criteria, the analytical results of 12,049 subjects were evaluated.

Analytical Measurements

The calibration and control of blood gas and complete blood count analyzers were performed according to manufacturers' instructions. The emergency laboratory participates in the RIQAS Blood Gas and the RIQAS Haematology External Quality Assessment programs, monthly.

Blood gas analyses were performed on RAPIDlab 1,265 BGA (Siemens Healthcare Diagnostics, Eschborn, Germany). Hb values were obtained from the CO-oximetry module using multiple-wavelength in the range of 500-680 nm. Hct values were calculated from the obtained Hb values using the formula.

Complete blood count analyses were performed on Sysmex XN1000™ (Sysmex, Norderstedt, Germany). The SLS Hb method was used for measuring Hb. The principle of this method is the binding of SLS to the heme group after the conversion of Hb to methemoglobin by oxidation of heme groups. The SLS-Heme complex is analyzed using a photometer.

Statistical Analysis

We used Excel 2013 (Microsoft, WA, USA), Excel XLSTAT 2019 (Addinsoft, New York, USA), SPSS 18 (IBM, New York, USA) for the statistical analyses. The normality of the distribution of the variables was tested using the Kolmogorov-Smirnov test. Since the data did not pass the normality test, a non-parametric Wilcoxon matched-pairs signed-rank test was performed to evaluate the significance of the difference between each set of matched pairs, and a comparison of each sample against the median values was performed. Passing-Bablok and Bland-Altman analyses were performed to evaluate the concordance of the analytical methods. A p-value less than 0.05 was set as the level of significance.

We calculated Bias % and total error for both tests by using the following formulas, respectively;

$$BIAS = ((C_1 - C_2) / C_2)) \times 100$$

$$TE = BIAS + 2\%CV$$

C_1 represents the mean concentrations obtained from Siemens Rapidlab-1,265, whereas C_2 stands for the mean concentration of the Sysmex XN1000 results. TE represents the total error and CV represents the coefficient of variation (9).

Results

The study included 12,049 patients (7,129 F, 4,920 M) with a mean age of 42.56±24.67 years (Table 1).

The mean and median values and interquartile range for each test using the two different methods are shown in Table 2. The difference between the two methods for each variable was statistically significant ($p<0.0001$). Correlation coefficients between both methods were found to be as $r=0.89$ and $r=0.87$ for Hb and Hct, respectively.

$y=-0.03412+0.9647x$ (intersection confidence interval: -0.08438-0.01569, slope confidence interval: 0.9608-0.9688) equation was obtained from Passing-Bablok regression analysis for Hb. For Hct, $y=3.3000+0.8850x$ (intersection confidence interval: 3.0818-3.5167, slope confidence interval: 0.8792-0.8909) equation was obtained. A deviation from linearity was observed for both variables between the methods ($p<0.01$) (Figure 1).

The Bland-Altman method plot created for the determination of agreeability between the methods, depending on the bias and mean values is shown in Figure 2.

Table 1. The characteristics of the patients for the comparison studies (n=12,049).

Variables	Mean ± SD	Min.-max.
Age (years)	42.56± 24.67	18-65
Gender		
Female	7,129 (59.2%)	
Male	4,920 (40.8%)	
Patient characteristics		
Outpatient clinics	6,921 (57.4%)	
Internalized patients	5,128 (42.6%)	

SD: Standard deviation, min.: Minimum, max.: Maximum

Table 2. Median, p and r-values and interquartile ranges for hemoglobin and hematocrit for two instrument analyses (n=12,049).

	Hemoglobin (g/dL)		Hematocrit (%)	
Instruments	Sysmex XN1000	RAPIDlab 1,265	Sysmex XN1000	RAPIDlab 1,265
Median value	13.1	13.45	40.2	40.9
Interquartile range	31.00-202.00		10.50-60.60	
p-value	<0.0001		<0.0001	
Correlation coefficient (r)	0.89		0.87	

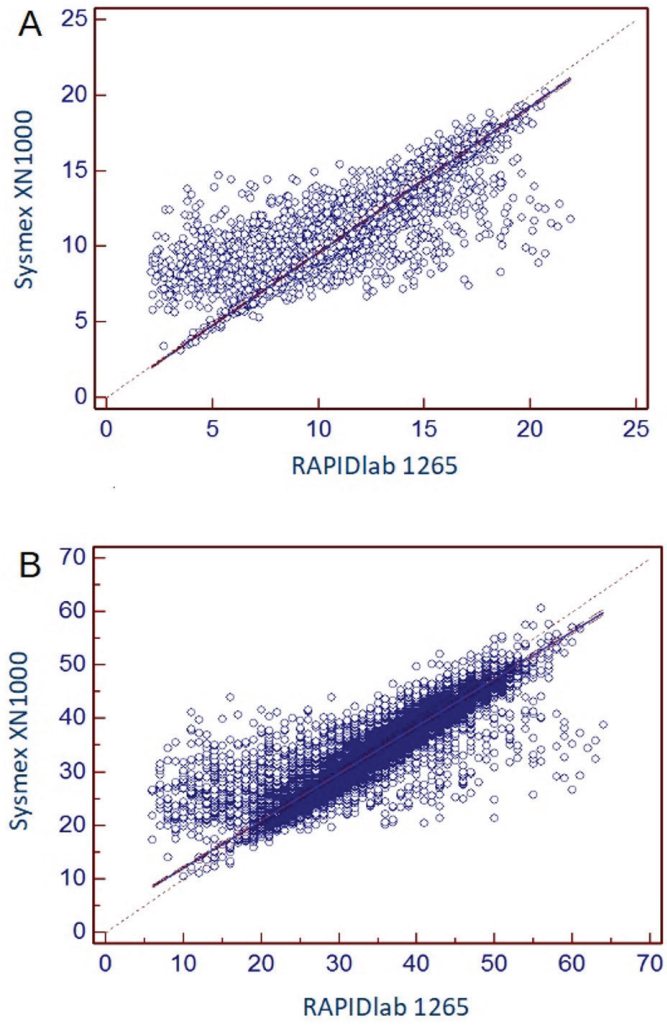

Figure 1. Passing Bablok regression analysis between the two measurement methods for hemoglobin A) and hematocrit B) (R^2=0.79 for hemoglobin and R^2=0.76 for hematocrit)

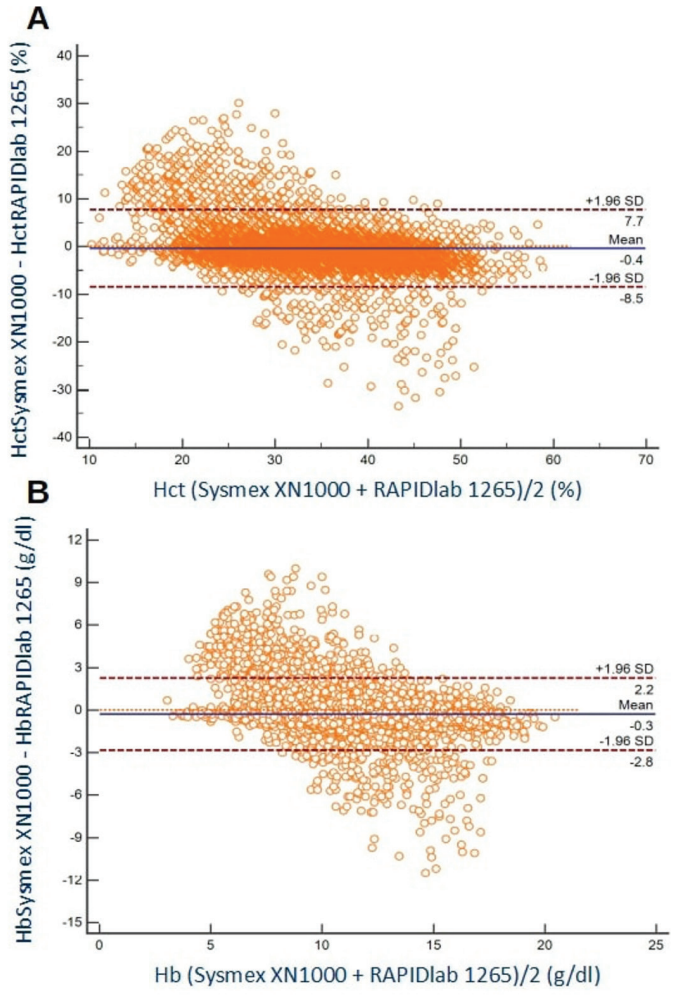

Figure 2. Bland-Altman analysis plots for the agreement of hematocrit A) and hemoglobin B) concentration with CO-oximetry (blood gas analyzer) and SLS-hemoglobin in 12,049 patients. The dashed lines represent the limits of agreement within which 95% of differences between measurements. Y-axes present the bias; X-axes present the average of the measurement results
SLS: Sodium lauryl sulphate

The formula calculation for the agreement of Hb results yielded a 2.5% bias and 4.08% TE. Bias for Hct measurement was calculated as 1.1% and the total error was calculated as 3.3%

The interassay coefficient of variations (CVs) for Hb was 0.9% and 1.6% for Sysmex XN1000 and RAPIDlab 1265 devices, respectively. The interassay CVs for Hct were 0.7% and 1.1% for the Sysmex XN1000 and RAPIDlab 1265 devices, respectively.

Discussion

In our study, we compared the Hb and Hct values simultaneously obtained from the BGA and the complete blood count autoanalyzer.

Hb levels might be measured by various methods such as the Sahli method, cyanmethemoglobin method, oxyhemoglobin method, and SLS- Hb method. The method recommended by Davis et al. (10) for measuring hemoglobin concentration is cyanmethemoglobin. SLS-Hb method is the method that we accept as the reference method and compared the BGA to evaluate the analytical performance. Although the SLS-Hb method is not considered a reference method, studies are indicating that the results correlate with the cyanmethemoglobin method (11,12). The main advantage of this method is its non-toxic nature and lower interference by lipemia and hemolysis (11).

According to the results of a study comparing the same BGA in our study, the mean Hb-value measured was found to be higher than the SLS- Hb method (135.10 g/L vs 130.60 g/L). Similarly, in our study, Hb values measured by the BGA were found to be higher than those the SLS- Hb method (119.60 g/L vs 116.60 g/L). However, the estimated correlation coefficient in this study was higher than that of our estimations (r=0.96, p<0.001). Contrary to our study, more than 95% of their samples remained within the limits according to the Bland-Altman analysis and were compatible with each other (13). This might be a result of several differences between the two study set-ups. Firstly, their data were normally distributed compared with our findings, with up to 5-fold more samples analyzed (2,548 vs 12,049). Furthermore, the variations between the populations and hospital management systems might result in minor analytical differences despite the use of similar devices. However, their study design was retrospective and based on an emergency laboratory setting.

Studies comparing the analysis results of BGA using venous blood for Hb measurement found higher mean Hb concentrations than the automated hematology analyzers (14). However, reports suggest that noninvasive measurement methods of Hb were more acceptable with the reference method than the measurement of Hb using a blood gas analyzer. However, both methods had significant variations from the reference method that can affect the clinical decision-making processes in the pediatric population (15).

In a multicenter cohort study on adult trauma patients, the initial Hb measurement on the trauma scene by point of care BGA predicted the presence of a significant hemorrhage with an area under the curve value of 0.72. Since early identification of excessive bleeding is crucial for adequate treatment, usage of point of care testing analyzers for measuring these variables in "outside the hospital" setting has the utmost importance to increase survival and decrease the possible morbidity (16).

Study Limitations

A limitation of our study was the lack of strict control of pre-analytical phase due to its retrospective design. Aruga et al. (17) reported that icterus, lipemia, cell free Hb and turbidity could interrupt the measurement of Hb. Although pre-analytical factors were controlled in routine laboratory applications, we believe that well-designed prospective studies with different patient groups such as pediatrics, suprageriatric (>85 years old) patients and individuals with hemoglobinopathies and anemia would be beneficial. Another limitation of our study was that broad clinical exclusion criteria were not applied. Among the 12,049 patient samples we compared, some patients applied to the emergency department and who were hospitalized and had chronic diseases. Due to these limitations, we consider our study not as a method comparison but as an evaluation of the method agreement study. The strong sides of our study are the higher subject number and the distribution of measured Hb values in a broad range. We also participated in an external proficiency testing program that revealed satisfactory performance for the analytes of interest.

Conclusion

Although each variable did not provide a satisfactory result in the Bland-Altman analysis when comparing the two methods, the calculated total errors for each analytes was lower than that reported by Westgard (4.20 % for Hb, 3.97 % for Hct) (9). Thus, the point of care BGA could be used for Hb measurements since the calculated bias remains acceptable. However, it is necessary to emphasize that the user and cost-related factors should also be evaluated within the scope of the laboratory pre-preanalytic phase related to the physician who will request the test. Since the blood count is performed using automatic systems, we must provide efficiency in terms of costs and qualified laboratory staff and manage faster access to test results, especially in healthcare institutions with many patients, in terms of laboratory practice.

Ethics Committee Approval: The study was approved by the Institutional Ethical Committee of University of Health Sciences Turkey, İstanbul Training and Research Hospital (approval number: 1929, date: 09.08.2019).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: O.O., F.S.H., H.S.; Design : O.O., F.S.H., H.S.; Data Collection or Processing: O.O., F.S.H., H.S.; Analysis or Interpretation: O.O., F.S.H., H.S.; Literature Search: O.O., F.S.H., H.S.; Writing: O.O., F.S.H., H.S.

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