INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2021.09825
Int J Med Biochem 2021;4(3):161-5

Research Article

Saline replacement as a practical solution to matrix interference effect when a leukocyte differential count cannot be measured in chemotherapy patient samples

Hatice Bozkurt Yavuz[1], Huseyin Yaman[2], Mustafa Tat[3], Osman Akidan[4], Asim Orem[2], Suleyman Caner Karahan[2], Yuksel Aliyazicioglu[2]

[1]Department of Medical Biochemistry, Sebinkarahisar State Hospital, Giresun, Turkey
[2]Department of Medical Biochemistry, Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey
[3]Department of Medical Biochemistry, Kirklareli Training and Research Hospital, Kirklareli, Turkey
[4]Department of Hematology, Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey

Abstract

Objectives: An automated complete blood cell count (hematology) analyzer cannot measure the white blood cell (WBC) differential of some patient sample, notably those of chemotherapy patients. This is often due to the presence of atypical cells in the sample or matrix changes caused by drugs. A technique to use isotonic sodium chloride solution (0.9% saline) as a replacement for plasma has previously been described. The aim of this study was to evaluate to use of saline replacement as a means to resolve the matrix interference effect and achieve a reliable WBC differential count for chemotherapy patients.

Methods: Samples of 29 chemotherapy patients whose WBC differential count could not be calculated using a Beckman Coulter LH-780 hematology autoanalyzer (Beckman Coulter, Inc., Brea, CA, USA) were evaluated. A peripheral blood smear was performed and the saline replacement technique was applied and the samples were then re-analyzed using the same autoanalyzer. The WBC count and differential count of WBC of a peripheral smear and a saline-replacement sample were compared.

Results: There was no statistically significant difference between the peripheral blood smear and saline replacement autoanalyzer measurements of the WBC differential count and percent of WBC. Strong relationships in WBC (r=0.99), neutrophil (r=0.98), lymphocyte (r=0.98), monocyte (r=0.91), and eosinophil (r=0.77) counts were observed in a comparison of the peripheral blood smear and saline replacement measurements.

Conclusion: A saline replacement technique may be a practical solution to resolve the difficulty of the matrix interference effect seen in chemotherapy patient samples and provide a WBC differential count.

Keywords: Chemotherapy, complete blood count, leukocyte differential count, matrix interference, saline replacement

A complete blood cell count (CBC) is one of the most commonly used routine tests performed in clinical laboratories. It helps to diagnose and treat disease and conditions that affect blood cells. The white blood cell (WBC) differential count is a widely used means to assess the status of a patient's immune system and blood-born cancers [1]. A peripheral blood smear is a gold standard diagnostic method [2]. However, it is labor-intensive and time-consuming. Therefore, CBC analysis using automated hematology analyzers is common for an initial evaluation of hematological abnormalities. The automated hematology analyzer in our laboratory could not measure WBC differential parameters in some samples, particularly in patients receiving chemotherapy. A specific count of neutrophils, lymphocytes, eosinophils, monocytes, and basophils could not be determined, though total WBC, erythrocyte, hemoglobin, and thrombocytes measurement was achieved.

The inability to perform a WBC differential measurement for chemotherapy patients may be a result of the presence of

Address for correspondence: Hatice Bozkurt Yavuz, MD. Department of Medical Biochemistry, Sebinkarahisar State Hospital, Giresun, Turkey
Phone: +90 536 646 55 54 **E-mail:** haticebozkurtyavuz@gmail.com **ORCID:** 0000-0003-0468-2486

Submitted Date: February 05, 2021 **Accepted Date:** May 21, 2021 **Available Online Date:** September 11, 2021

atypical cells in the samples or matrix changes caused by the therapy drugs. The matrix comprises all of the components of the sample other than the analyte to be measured, and the contents may interfere with the measurement of many parameters [3, 4]. The neutrophil count is a very important parameter for chemotherapy patients. Neutropenia, defined as an absolute neutrophil count of $<1.5\times10^9$/L, is one of the most frequent causes of chemotherapy dose reduction and dose delay. A count of $<0.5\text{x}10^9$/L represents a severe degree of neutropenia. If a fever develops in these patients, the ability to diagnose neutropenic fever is critical for treatment [5]. Therefore, it is important to be able to accurately measure the WBC differential in chemotherapy patients.

The isotonic sodium chloride solution (0.9% saline) replacement technique is defined as the replacement of sample plasma with the same volume of saline. It is recommended for cold agglutinin cases in which erythrocyte indices cannot be measured at 37°C [6]. Saline replacement is also an established method to resolve rouleaux formation and lipemic sample interference [7, 8]. To the best of our knowledge, this is the first study to evaluate saline replacement in non-lipemic samples as a means to measure the WBC differential.

The objective of the present study was to evaluate the possibility of solving the problem of matrix interference and the inability to provide a WBC differential count in chemotherapy patient samples by using the saline replacement technique.

Materials and Methods

This study was performed in the Karadeniz Technical University Faculty of Medicine clinical biochemistry department in November-December 2017. Samples of 29 chemotherapy patients for whom a differential count of WBC could not be measured in the hematology autoanalyzer were evaluated. Venous blood samples were collected into 2-mL K3EDTA Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Insufficient, hemolyzed, lipemic, and icteric samples were excluded from the study.

The samples were analyzed in a Beckman Coulter LH-780 hematology autoanalyzer (Beckman Coulter, Inc., Brea, CA, USA) located in the hospital clinical biochemistry laboratory. This device uses volume, conductivity and scatter (VCS) parameters to measure the WBC differential and the WBC is determined using the Coulter Principle [9, 10].

Once the samples had been analyzed twice in the biochemistry laboratory, they were taken to the hematology laboratory and a peripheral blood smear was prepared and examined according to CLSI document H20-A2 by hematology specialists. Double-blind conditions were observed. Two slides were prepared for each patient and evaluated by different qualified individuals who were unaware of the origin of the slide. Blood films were prepared using the manual wedge-pull technique. One drop (approximately 0.05 mL) of well-mixed blood was placed near one end of a glass microscope slide. The end of a second, narrower spreader slide with polished edges was held against the surface of the first slide at about a 45° angle and drawn back to contact the drop of blood. The blood was allowed to spread almost to the width of the slide and then the spreader slide was pushed forward to the opposite end of the slide, pulling the blood and spreading it into a moderately thin film. The slides were then stained with Wright's stain. Two qualified examiners each performed a 200-cell differential on 1 of the 2 slides; a 400-cell differential count was performed on each patient sample. The samples were returned to the biochemistry laboratory and the saline replacement procedure was performed.

Saline replacement was conducted as follows: The samples were centrifuged at 1600g for 10 minutes (5804R; Ependorf AG, Hamburg, Germany). The plasma was carefully removed without disturbing the cell layer with an automatic pipette (100-1000 µL; Scilogex llc., Rocky Hill, CT, USA). Approximately 0.5 mL of plasma was left on the cell layer. The quantity of plasma removed was recorded. A solution of 0.9% sodium chloride was added to replace the volume of plasma and then homogenized with at least 20 soft and complete inversions. The samples were then re-analyzed with the Beckman Coulter LH-780 hematology autoanalyzer and the WBC differential results were noted.

Statistical analysis

Data were expressed as the mean and SD for variables with parametric distribution and the median and interquartile range (IQR) for those with nonparametric distribution. The distribution was assessed using the Shapiro-Wilks test. Statistical differences between the data from pre- and post-saline replacement were determined according to a paired t-test (parametric) or the Wilcoxon test (nonparametric). $P<0.05$ was considered statistically significant. Pearson or Spearman correlation analysis was used to assess the relationships between the parameters based on the skewness of data distribution. Deming or Passing-Bablock regression analysis was performed using MedCalc Statistical Software version 19.8 (MedCalc Software bv, Ostend, Belgium) to demonstrate the relationship between study groups. IBM SPSS Statistics for Windows, Version 23.0 software (IBM Corp., Armonk, NY, USA) was used to conduct additional statistical analysis.

Results

According to the peripheral blood smear analysis, none of the samples had rouleaux formation or atypical cells.

There was no statistically significant difference in the WBC count between pre-saline replacement (median: $7.7\text{x}10^9$/L, IQR: $5.8\text{-}9.1\text{x}10^9$/L) and post-saline replacement (median: $7.6\text{x}10^9$/L, IQR: $5.8\text{-}9.0\text{x}10^9$/L) ($p=0.06$). There was also a strong correlation between the pre- and post-saline replacement in the WBC count ($r=0.99$ $p<0.01$) (Fig. 1a). The post-saline replacement results and peripheral blood smear results for lymphocyte (%), monocyte (%), neutrophil (%), eosinophil (%), and basophil (%) revealed no statistically significant difference (Table 1). There were also strong correlations between

Table 1. Differential white blood cell count results using saline replacement and peripheral blood smear

	Post-saline replacement	Peripheral blood smear	p
LY %[a]	28.5±17.3	29.9±18.9	0.4
MO %[a]	7.9±5.3	9.9±5.9	0.06
NE %[a]	58.5±22.8	56±21.4	0.06
EO %[b]	2.5 (1.05-3.9)	3 (0.25-4.75)	0.15
BA %[b]	0.3 (0.15-0.95)	1 (0.5-3)	0.08

Mean±SD for a parametric distribution and median (interquartile range) values for b non-parametric distribution. BA: Basophil, EO: Eosinophil, LY: Lymphocyte, MO: Monocyte, NE: Neutrophil

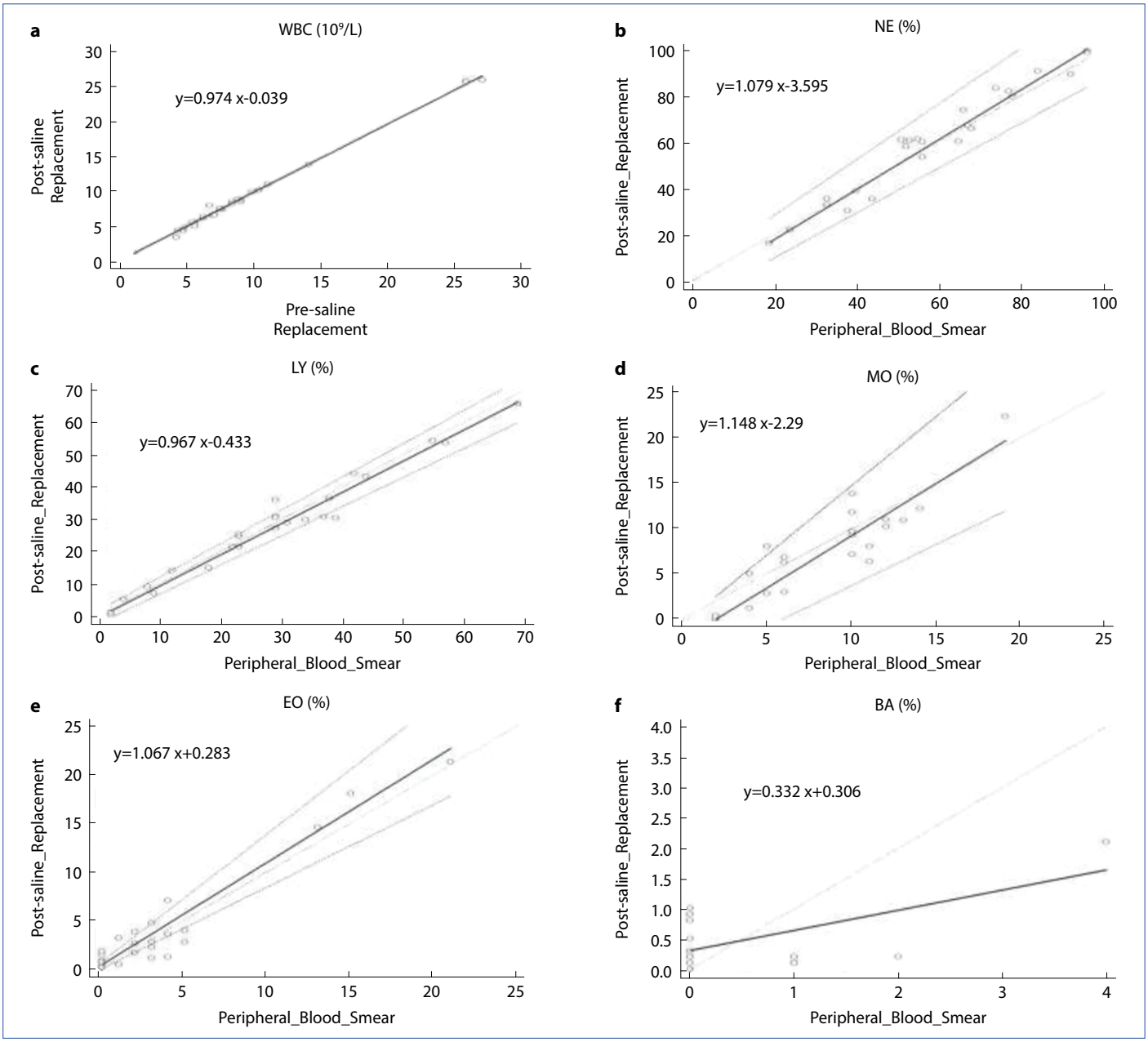

Figure 1. WBC count and differential percent of WBC in post-saline replacement and peripheral blood smear samples. (a) WBC count, (b) neutrophil (%), (c) lymphocyte (%), (d) monocyte(%), (e) Eosinophil (%), and (f) basophil (%).

BA: Basophil; EO: Eosinophil; LY: Lymphocyte; MO: Monocyte; NE: Neutrophil; WBC: White blood cell-leukocytes.

the post-saline replacement autoanalyzer results and the peripheral blood smear results in WBC differential parameters, with the exception of basophils. The WBC (r=0.99, p<0.01), neutrophil (r=0.98, p<0.01), lymphocyte (r=0.97, p<0.01), monocyte (r=0.91, p<0.01), eosinophil (r=0.77, p<0.01), and basophil (r=0.02, p=0.96) findings observed in the post-saline replacement and peripheral blood smear results are shown in Figure 1a, 1b,1c, 1d, 1e, 1f, respectively.

Discussion

The results of the total WBC count before and after saline replacement were consistent. The percent difference results of the WBC count in the autoanalyzer after saline replacement were also consistent with the results obtained from the peripheral blood smear. There were statistically significant relationships between post-saline replacement and peripheral blood smear WBC count and subtype counts, other than for basophiles (Fig. 1). The differential parameters of WBC were compatible not only in percentages but also numerically. This enables a clinician to safely diagnose febrile neutropenia, which is evaluated using numerical results. Accurate measurement of differential parameters is also important for other reasons. For example, the neutrophil-to-lymphocyte ratio is accepted as an independent risk factor for mortality in patients with coronavirus 2019 (COVID-19) [11]. Therefore, the results of this study may be of great use to laboratory workers during the current COVID-19 pandemic.

The results of this study suggest that the use of saline replacement may offer a practical solution when a WBC differential cannot be measured using the VCS method. Saline replacement is easier than preparing a peripheral blood smear, it provides results sooner, and does not require dye or special experience. A saline replacement technique for a CBC could be standardized and then each laboratory could prepare a protocol according to their own studies.

The present study also has some limitations. There was no statistically significant difference in the basophil values between the peripheral blood smear and the post-saline replacement results, but the coefficient of determination was dramatically lower than we expected. The reference range for basophils (%) is 0.2-1% [12]. This makes it difficult to find basophils in a peripheral blood smear and thus, with a small number of cells, it is hard to find strong correlation when an autoanalyzer gives decimal results while a hematologist reports results rounded up to an integer. This may be statistically tolerable at high concentration levels, but with low numbers it became a statistical difference (Fig.1f).

We suggest that while reporting the results obtained using this saline replacement method to obtain a WBC differential, it is appropriate state that the results were obtained using this technique and it should be described in a lab protocol.

There are studies in the literature that have investigated the matrix effect in a CBC, but these studies are generally associated with lipemia interference. Lipoproteins are known to interfere with the accurate determination of hemoglobin using photometric measurement. It may be plausible to replace plasma with saline or other diluents for lipemic samples [13, 14]. Er et al. [14] reported significant changes in lymphocyte and monocyte counts in plasma of a lipemic sample replaced with a diluent performed according to the recommendations of a manufacturer. However, that study was confined to an examination of lipemia and did not include other matrix effects. In our study, lipemia was one of the exclusion criteria, and we used saline, rather than a manufacturer's diluent, which may be relevant to the results.

Automated hematology analyzers use various methods to measure parameters. The inability to measure a WBC differential in samples drawn from chemotherapy patients may be related to the VCS method. Further studies are needed to investigate the matrix effect in samples that present this obstacle.

Conclusion

In conclusion, a saline replacement technique may be a practical solution to the matrix interference effect that has precluded a differential WBC count in chemotherapy patient blood samples.

Conflict of Interest: The authors have no competing interests.

Ethics Committee Approval: Our study does not include any human or animal subject. Therefore ethics committee approval was not obtained.

Financial Disclosure: None declared.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept – H.B.Y., M.T.; Design – H.B.Y., M.T.; Supervision – H.Y., A.O., Y.A., S.C.K.; Funding – H.Y., A.O., Y.A., S.C.K.; Materials – H.B.Y., M.T.; Data collection &/or processing – H.B.Y., M.T., O.A.; Analysis and/or interpretation – H.B.Y., H.Y.; Literature search – H.B.Y., H.Y.; Writing – H.B.Y., H.Y.; Critical review – H.Y., A.O., Y.A., S.C.K.

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