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Evaluation of Two Methods for Counting Residual Leukocytes in Leuko-reduced Platelets: Nageotte's Method and Flow cytometry

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Abstract

Introduction:

Leukoreduced (LR) blood components are used for the prevention of several transfusion adverse effects. Advancement in technology has led to newer methods to count residual leukocytes (rWBC) which miss detection on most standard automated hematology analyzers.

Materials and Methods: Samples from thirty eight platelet concentrates (prepared by Buffycoat method) were randomly taken on the day of preparation for rWBC count using Nageotte's chamber and flowcytometer.

Results: The rWBC count on Nageotte's ranged from 2.5 WBC/μL to a maximum of 600 WBC/μL where as the flowcytometric count had a lowest of 1.97 WBC/μL to a highest of 740 WBC/μL. We found that the WBC counts using the Nageotte's method and flowcytometeric method are highly correlated. The

concordance correlation coefficient or intraclass correlation coefficient which is a measure of reliability was 0.78

Conclusion: In view of the high concordance in correlation coefficient between the two methods, Nageotte's method could be skillfully performed for assessing leukoreduction in LR platelet concentrates of resource constrained blood banks of developing nations.

Key Words: Flow cytometry, leukoreduced, Nageotte's

Introduction

Leuko-reduced (LR) blood components are widely used for the prevention of several transfusion adverse effects. Advancement in technology has led to newer methods to count residual white blood cells (rWBC) which miss detection on the most standard automated hematology analyzers.

To ensure good quality of LR products, it is imperative to routinely monitor the efficacy of leukoreduction in blood products. New enumeration methods such as flow cytometry[1-6] and microvolume fluorometry[1-3,6] have replaced manual methods such as Nageotte hemocytometer and microscopy.[1,3,4,6]

We undertook this study at our center to weigh the pros and cons of a newer method over the contemporary ones in measuring rWBC in LR platelet products.

Materials and Methods

Segments of 38 platelet concentrates (prepared by Buffycoat method) were randomly taken on the day of preparation, from October 1, 2015 to October 15, 2015. After stripping the segments of platelet concentrates, the segments were sealed into two parts for measurement using the Nageotte's chamber and flow cytometer which were performed by different persons.

Automated method for counting rWBCs in buffy-coat platelet concentrates: BD Leucocount reagent, USA, was used for Leucocount assay. A volume of 100 μL of platelet sample was mixed with 400 μL of BD Leucocount reagent in each Tru-count test tube. The tubes were gently vortexed (maximum up to 15 s) and then, incubated for 5 minutes in dark at room temperature. Finally, the samples were acquired using Flow cytometer (BD FACSCanto

II, USA) in low flow rate, and minimum of 10,000 events were acquired.

After acquisition, analysis was performed by a predefined template. The template was prepared by processing samples of ten healthy individuals. WBC events (R2) and Beads events (R1) were used for final calculation from the acquired data.

The absolute number of rWBC was then calculated by using the formula given below:

$$\frac{\text{WBC events (R2)}}{\text{Bead events (R1)}} \times \frac{\text{Bead events (R1)}}{\text{Sample volume (}\mu\text{L)}} = \text{Absolute rWBC/ (}\mu\text{L)}$$

counting chamber. Then, the hemocytometer was focused under a microscope using a 20× objective. WBCs appeared as gray-blue refractile cells bearing a nucleus. All the WBC present in 50 μL volume of the Nageotte's counting chamber were counted and the following formula was applied to know the final count.

WBC/μL = Number of WBC/ 50 μL × 5 (dilution factor).

Total WBC count in the LR product = WBC/μL × 1000 × product volume (ml).

Manual method of counting residual white blood cell (Nageotte's)

One hundred microliter of the platelet sample was mixed well with 400 μL of Turk's fluid in a clean test tube by pipetting several times. The mixed sample was loaded on the hemocytometer with a cover slip by using a pipette. The charged hemocytometer was placed in a Petri dish with a amp filter paper for 10–15 minutes so as to allow the WBCs to settle over the

Results

A total of 38 platelet concentrates were analyzed. The rWBC count on Nageotte's ranged from 2.5 WBC/μL to a maximum of 600 WBC/μL whereas the flow cytometry count had a lowest of 1.97 WBC/μl to a highest of 740 WBC/μl. The mean rWBC count was higher in flow cytometry than Nageotte's method [Table 1].

Statistics

The WBC counts using the Nageotte's and flow cytometric methods are

highly correlated (Pearson's correlation coefficient: r = 0.9122). Using the Bland–Altman method, mean difference between the two methods was 15.3 units.

Ninety-five percent limits of agreement is -10 to +41. The concordance correlation coefficient or intraclass correlation coefficient which is a measure of reliability was 0.78.

Discussion

In several studies, Nageotte's method results were lower than those obtained by flow cytometry.[1,2,7-10] However, in some studies, WBC counts in LR red blood cells (RBCs) were higher when measured by Nageotte's method than by flow cytometry.[4,11,12]

In our study, many of our platelet products had a high count on Nageotte's method than that on flow cytometry. This could be because of artifacts as the same Nageotte's chamber, and slide cover was repeatedly used and also ecause the flow cytometry gating was setup to encompass only intact WBCs. It is believed that products having an increased prestorage leukoreduction time increases the number of WBC fragments and cell-free DNA in the LR products,[5,13-18] which may interfere with rWBC counts. Accurate values of rWBC could be achieved by minimizing the time between collection, component preparation, leukoreduction, sample recovery, and addition of Pallfix preservative. Hence, we ensured that all our products were analyzed on the same day of preparation to avoid such inadequacies. Other studies have found no significant difference in the values of rWBC counts for platelet products prepared from citrated or ethylenediaminetetraacetic blood collections, irrespective of the enumeration method.[19]

In fact, Van der Meer *et al.*[18] did not use any WBC preservative to stabilize samples in spite of doing

Table 1: Comparison of measurements by Nageotte's and flow cytometry methods

WBC measurement Range	Range (WBC/ μL)	Mean (WBC/ μL)	Median (WBC/ μL)
Nageotte's method	2.5 - 600	97.75	29.6
Flowcytometry	1.97 - 740	113.05	32.62

leukoreduction after 24 hours. Many studies have reported that rWBC counts by Nageotte's hemocytometer and microscopy in LR RBC and platelet products were not as precise as the results obtained from the automated methods such as microfluorometry or flow cytometry.[1,9,10]

Lutz and Dzik[8] noted a correlation coefficient of r = 0.9995 comparing Nageotte's method and flow cytometry results in the range of 0.3–18.4 WBCs per mL for LR apheresis platelet concentrates. rWBC counts on stabilized samples measured by EPICS XL-MCL flow cytometer gave results which were precise, specific, reliable, accurate, and sensitive. Nageotte's method provided similar sensitivity (0.1 WBC/mL), precision, and reliability, when done by experienced technologists, yet was less acceptable in terms of specificity and accuracy.[20] In our study, the two methods were equally good, and in resourceconstrained settings of developing countries, Nageotte's chamber could be best used to reduce the cost. Even though the Nageotte's method is time-consuming, only 1% of the LR products prepared are to be analyzed in a month for quality control purpose. Hence, it could be practiced conveniently.

Conclusion

Our study demonstrated a good correlation between the Nageotte's and flow cytometry methods for counting rWBC in LR platelet concentrates. Given the high concordance in correlation coefficient between the two methods, Nageotte's method could be skillfully performed for assessing leukoreduction in LR platelet concentrates of resourceconstrained blood banks of developing nations.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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