Quality Assessment of Platelet Concentrate Preparations at the Nairobi Regional Blood Transfusion Centre in Kenya

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ABSTRACT

Background: Evaluation of platelet concentrate was based on platelet volume, count, residual WBC and Hemoglobin. The study findings were compared with standards set by National Blood Transfusion Service (NRBTC). Objective: To evaluate platelet concentrates quality prepared at NRBTC. Study Design: This was a descriptive cross-sectional study was adopted. Setting: The present study was conducted at NRBTC. Subject: Nighty one (91) platelet concentrates were selected for the study. Results: Of the samples analyzed, 95.6% (87/91), 79.1% (72/91) met the criteria on platelet count and residual white blood cells respectively. Of note, all the 91 sampled platelet concentrate fell within the NRBTS volume criteria. Conclusion: The findings reveals that the quality parameters assessed in this study from the platelet concentrate were not met. Therefore, there is need to review the quality assurance protocol. Focus should be directed on the platelet concentrates preparation by plasma centrifugation to attain the desirable standards set by NBTS.

Keywords: Blood transfusion, platelets, quality

1. INTRODUCTION

Most blood components are prepared from whole blood (WB) immediately after blood collection. Blood is often stored for a maximum of 8 hours at 20-24°C before platelet preparation(1). In Kenya, Nairobi regional Blood Transfusion Centre (NRBTC) is one of the six regions running transfusion services such as blood donation, banking, screening, platelet preparation, sorting/labeling and dispatch of platelets to different hospitals for transfusion AABB(1). Platelets prepared by NRBTC are used to manage haematological conditions e.g. thrombocytopenia, bone marrow failure and solid malignancies, Biplabendu Talukdar(2). Platelet concentrates are randomly prepared from platelet rich plasma derived from WB of donors Nor Raihan M(3). With continuous agitation, platelets concentrates can be stored for 5 days at 22 ± 2°C(Njoroge(4) HolmeS(5), G. Moroff(6), Welch M(7)). The AABB(1) is tasked with the responsibility of setting standards for transfusion medicine on blood collection, spinning, platelet separation, storage and agitation. Moreover, the AABB(1) prescribed minimal requirements for

platelet concentrates processed by the PRP method for platelet count, volume, RBC content, and residual WBC count. These standards have been adopted by NBTS standards committee. Table 1 shows the specifications for parameters in Kenya, United States and Europe.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>United States</th>
<th>KNBTS</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count ($\times10^{10}$)</td>
<td>&gt;5.5</td>
<td>&gt;5.5</td>
<td>&gt;6.0</td>
</tr>
<tr>
<td>Volume</td>
<td>To Maintain pH&gt;6.2</td>
<td>&gt;50?</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Hb</td>
<td>&gt;12.5g/dl</td>
<td>&gt;12.5</td>
<td>&gt;12</td>
</tr>
<tr>
<td>WBC</td>
<td>&lt;0.83</td>
<td>&lt;0.83</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

2. METHODS

Study Design
The study adopted a descriptive cross-sectional design.

Sampling method
The present study adopted a systematic sampling technique. Every 3rd platelet concentrate prepared was picked. Averagely, 18 platelet concentrates are prepared daily at NRBTC for four days in a week. Consequently, 91 platelet concentrates were selected for the study.

Process Assessment
The principal investigator evaluated weighing, spinning, separation, storage and agitation of the WB units and platelet concentrates. Raw data were recorded and kept in MS Excel.

Laboratory Testing
Assessment of platelet concentrate involved analysis of volume, platelet counts/bag, Residual WBC and Haemoglobin using haematological analyzer.

Data analysis
Data analyses were conducted using STATA version 13. Descriptive statistics were used to present data into pie chart.

Ethical approval
Ethical review and approval was obtained from the KNH/UoN ERC.

3. RESULTS

Blood donor units
This study enrolled 200 platelet concentrates units that were used for quality control by PRP-PC. Every 2nd blood units was picked. Nighty-one blood units were selected for the study. Table 2 shows the concentrate volume, platelet count/bag and residual WBC count/bag expressed in Mean± (standard deviation (SD)).

Quality parameters of platelet concentrate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood volume</td>
<td>447.703</td>
<td>28.179</td>
<td>446.000</td>
<td>37.000</td>
<td>374.000</td>
<td>519.000</td>
</tr>
<tr>
<td>Platelets in whole blood</td>
<td>242.495</td>
<td>67.004</td>
<td>237.000</td>
<td>88.000</td>
<td>52.000</td>
<td>451.000</td>
</tr>
<tr>
<td>Platelets Rich plasma</td>
<td>290.506</td>
<td>66.270</td>
<td>297.000</td>
<td>86.000</td>
<td>105.000</td>
<td>540.000</td>
</tr>
<tr>
<td>Platelets concentrate</td>
<td>783.813</td>
<td>232.240</td>
<td>764.000</td>
<td>275.000</td>
<td>362.000</td>
<td>1662.000</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>14.085</td>
<td>1.985</td>
<td>14.000</td>
<td>1.600</td>
<td>10.500</td>
<td>25.400</td>
</tr>
<tr>
<td>WBC in whole blood</td>
<td>5.049</td>
<td>1.319</td>
<td>4.700</td>
<td>1.560</td>
<td>2.910</td>
<td>8.620</td>
</tr>
<tr>
<td>RWBC</td>
<td>0.497</td>
<td>0.467</td>
<td>0.350</td>
<td>0.297</td>
<td>0.037</td>
<td>3.190</td>
</tr>
</tbody>
</table>

All the 91 blood units met the WB volume requirement (350-480ml). Platelet count WB ranged from $52 \times 10^9$ to $451 \times 10^9$ cells/ml. WBC cells ranged from $2.9 \times 10^9$ to 8.62 *10^9 cells/ml.


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Table (3): Residual WBC count in platelet concentrate when whole blood has normal WBC count (4.0-10.5cells/ml)

<table>
<thead>
<tr>
<th>Level/source</th>
<th>Parameters [Normal Reference]</th>
<th>Frequency</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood (n=91)</td>
<td>White blood cell count [4.0-10.5]</td>
<td>76</td>
<td>83.5</td>
</tr>
<tr>
<td>2nd spin-Platelet concentrate (n=76)</td>
<td>Residual white blood cells [&lt;0.83 cells/ml] &amp; Platelet concentrate volume [40-70ml]</td>
<td>63</td>
<td>82.9%</td>
</tr>
</tbody>
</table>

Out of 91 whole blood units, 76 (83.5%) were within the WBC normal range (4.0*10^9-10.5*10^9 cells/ml). The 76 units were considered in the final platelet concentrate quality assessment with respect to residual WBC. After the second spin, 63 (82.9%) fell within the residual WBC quality standard (<0.83cells/ml) and platelet concentrate volume (40-70ml). Implying that the proportion of error was 17.1%

Table 4 Platelet count in concentrate when whole blood has normal platelet count (150-450 cells/ml)

<table>
<thead>
<tr>
<th>Level/source</th>
<th>Parameters [Normal Reference]</th>
<th>Frequency</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood (n=91)</td>
<td>Platelet count [150-450 cells/ml]</td>
<td>87</td>
<td>95.6</td>
</tr>
<tr>
<td>1st spin- Platelet rich plasma (n=87)</td>
<td>Platelet count [≥200cells/ml]</td>
<td>87</td>
<td>100</td>
</tr>
<tr>
<td>2nd spin- Platelet concentrate (n=87)</td>
<td>Platelet count [≥450 cells/ml] &amp; Platelet concentrate volume [40-70ml]</td>
<td>83</td>
<td>95.4</td>
</tr>
</tbody>
</table>

Out of the 91 whole blood units processed, 87 (95.6%) met the normal range of platelet concentrate (150-450 cells/ml) requirement for platelet count. All the 87 (100%) met the requirement for platelet count after the first spin. After the second spin yielding the platelet concentrate, 83/87 (95.4%) met the requirement for platelet count (≥450 cells/ml) and concentrate volume (40-70 ml). The error in this case occurred at the second spin (proportion of error = 4.6%).

The results for whole blood Volume a platelets are shown in table 5

Table (5): Residual white blood cell and platelet count in concentrate when whole blood has normal WBC and platelet count.

<table>
<thead>
<tr>
<th>Level/source</th>
<th>Parameters [Normal Reference]</th>
<th>Frequency</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood (n=91)</td>
<td>WBC count [4.0-10.5cells/ml] &amp; Platelets [150-450cells/ml]</td>
<td>72</td>
<td>79.1</td>
</tr>
<tr>
<td>2nd spin- Platelet concentrate (n=72)</td>
<td>WBC count [4.0-10.5cells/ml] &amp; Platelets [150-450cells/ml] &amp; Concentrate volume [40-70ml]</td>
<td>59</td>
<td>81.9</td>
</tr>
</tbody>
</table>

Out of 91 whole blood units that were processed, 72 (79.1%) fell within the baseline standard reference range for WBC count (4.0-10.5 cells/ml) and platelet count (150-450 cells/ml). After the second spin yielding the platelet concentrate, 59/72 (81.9%) met the standard platelet quality requirement for residual white blood cell count (<0.83), platelet count (≥450 cells/ml) and platelet concentrate volume (40-70ml). The proportion of error is 18.1%.

Assuming an acceptable level of error in platelet concentrate preparation of less than 5%, the proportion of error of 18.1% was statistically high (z-statistic = 5.100; p-value<0.001) therefore the null hypothesis was rejected and concluded that not all platelet concentrates prepared at NBTS meet the standard quality requirement.

Comparison of parameters
The graphs (Figure 1 and Figure 2) showed no linear relationship between platelet count and hemoglobin level. The graphs Fig. 3 and Fig. 4 showed no linear relationship between platelet count and volume of whole blood.

**Figure 1**: Platelet count in whole blood vs. hemoglobin level

**Figure 2**: Platelet concentrate count vs. hemoglobin level
4. DISCUSSION

Functions of circulatory transfused platelets depend upon ex-vivo storage lesion and the status of the in-vivo environment of transfused individuals by Njoroge(1) Platelet concentrate. These concentrates are transfused within 24 to 48 hours of donation without significant storage interval, they have uniformly high recovery, good survival and preserved function. This concurs with other studies done by Nor Raihan M(9). This study assessed the quality processes and procedures used in the preparation of platelet concentrates at the NRBTC, the process of centrifugation, separation, storage and agitation, with standard procedures set by NBTS as adopted from AABB(1), N. Tynngard(9). The study also determined quality parameters of platelet concentrates that included platelet counts, Hb level, Residual WBC count, platelet volume. The results were compared with the Standards Committee of NBTS guidelines. All the 91 blood samples, haemoglobin level were measured in the Initial whole blood using the haematological analyzer. In this study, the mean±SD
of Hb level was 14.085±1.985 with a volume ranged from 10.5g/dl to 25.0g/dl. However, the KNBTS standard operating procedure range for haemoglobin level for donors is 12.5g/dl-16.5g/dl for female and male 12.5-17.5g/dl. However, some samples did not meet the criteria of AABB because of low count or high count above the reference range referring to set standards for blood transfusion.

In this study all the 91 blood units met the whole blood volume requirement (350-480ml) with the mean and SD of 447.703 ± 28.179. Initial whole blood platelet count and haemoglobin level was measured using haematological analyzer. The cell volume, resulting in a cell count, platelet count measured between 52 * 10⁹ cells/mL to 451 * 10⁹ cells/L. Moreover, white blood cells ranged between 2.9 * 10⁹ cells/L to 8.62 *10⁹ cells/mL. The mean±SD platelet concentrates count in whole blood was 242.495 ± 67.004 while WBC count was 5.049 ± 1.319 respectively. 87(95.6%) of concentrates recorded a platelet count of >5.5 * 10⁹. The mean±SD for platelet count was 783.8±232.2* 10⁹ with a median of 764 * 10⁹ and with range of ≥450. Fourpercent of the concentrates returned platelet concentrate of less than 5.5* 10⁹ per bag. Their parameters were compared with those that returned higher platelet counts Biplabendu(10,11). In the concentrates with lower platelet count and Residual WBC counts were lower while Haemoglobin was higher. The converse was true in those concentrates with platelet count above 5.5* 10⁹. This may be due to variability in centrifugation and separation process. Inadequate centrifugation or delay in separation after spinning may result in low platelet count. The error in this case occurred at the second spin (proportion of error = 4.4%).

All the 91 whole blood required 2 spin to prepare platelets concentrates and these was done using soft spin (2100 rpm for 6 minutes at 220c) which separated RBCs and WBCs from plasma and platelets. Heavy spin (3800 rpm for 9 minutes at 220c) in case platelet in platelet rich plasma (PRP) was forced to the bottom of a satellite bag and 40-60ml of plasma was expelled into another satellite bag, while the bag contains platelet concentrate (40-70ml). Even though the centrifugation speeds were consistent for all concentrates, calibration of the centrifuge for the highest percent of platelet yields was not performed to maintain the quality.

In another study Hirose(10), Murphy S(11) reported higher platelet count in PRP-PC units than BC-PC. Murphy et al.(10,11) also found that platelet recovery was higher in patients receiving PRP-PC (60-70%) than those with BC-PC (40-60%) transfusion. However, the AABB in their technical manual has recommended that all centrifuges be calibrated for the lowest speed and take shortest time that gives the highest platelet percent yield and the lowest residual contaminating of red cells in the preparation of platelets concentrates using the Platelet concentrate method from whole blood. The speed used for centrifugation was 2100 rpm and 3800 rpm for the soft and hard spin respectively, there was need for calibration to ensure that these speeds resulted in the highest percent platelet yield for that particular centrifuge used.

The quality of platelet concentrates was assessed using whole blood units with parameters within the normal standard reference ranges. Quality assessment of platelet concentrates is an important step to evaluate in-vitro functional viability, post transfusion survival and recovery in the recipients. Various parameters were used for assessment of ex-vivo function, volume, platelet count, RWBCs contamination. Although other studies included parameters such as, measurements of ATP, membrane glycoprotein levels (P- selectin, GP Ib, GP IIb-IIIa) swirling, pH which was not performed in this study.

Storage for all 91 platelet concentrates was at room temperature 20-240c in the platelet agitator. Generally, 5 days storage of platelet concentrates are practiced in developed counties AABB1. In NRBTC, platelets are usually used within 24 to 72 hours of preparation. However, the bag can store up to 5 days N. Tynngard(9).

In this study, low residual WBC counts are desired to minimize side effects of residual leucocytes and to minimize the platelet storage lesion that can be enhanced by high leucocyte count. Most of the concentrates in this study had low residual WBC contamination though the White blood cell count ranged between 2.91*10⁹ cells/ml and 8.62*10⁹ cells/ml with the mean ± SD of 5.049 ± 0.467*10⁹. Therefore this study shows only 72 platelet concentrates (81.9%) met the minimum platelet count and 59(81.9%) of platelet concentrates attained the residual WBC quality standard (<0.83cells/ml) for residual WBC count comparing NBTS requirement of >95%.

Similar study, Singh(12) reported mean WBC count of 40.5±4.8*10⁹ for the PRP method that was far lower than the levels established in this study. Assuming the error in platelet concentrate preparation was < 5%,
then 18.1% proportion of error was statistically high ($z$-statistic = 5.1; $p<0.001$).

Similar study at KNH found that only 51% of all concentrates showed platelet count of $>5.5 \times 10^9$ against NBTS requirement of $>95\%$ which fulfilled the minimum requirement for platelet count. The mean and standard deviation for platelet count was $6.63\pm4.73 \times 10^9$ which was well above the minimum threshold. The range of platelet counts was $0.89-21.50 \times 10^9$, showing a wide variation in the counts Njoroge(4).

These results for WBC count, like those for platelet counts suggest that the marked differences in counts is due to variability in process due to lack of standardization in the preparation procedures, particularly in centrifugation and separation in this study.

In another study at a tertiary hospital in Nigeria reveals that platelet concentrates were prepared by PRP method, Fasola(4) found that only 35% of the concentrates met the minimum $5.5 \times 10^9$ platelet count threshold. The mean± SD for that study was $4.17\pm3.95$ showing a range as wide as in this study. A study by Singh(12) reported a mean value of $7.6\pm2.97$ which is well above the $5.5 \times 10^9$ threshold in a study in which he assessed PRP, BC and apheresis platelets. Furthermore, Singh demonstrated a wide variation in platelet concentrate preparation techniques in different countries.

The results this study confirms that lower volumes result in undesirable lower pH than higher final volumes. In another study Fasola(13) reported the mean volume was considerably low 18.52ml compared to(12) reported a mean of 62.30±22.68 ml.

This study also demonstrates that Haemoglobin (Hb) and platelet counts can form an integral part of a rapid, simple and practical method for validation of collection, processing and storage procedures that is useful for routine quality monitoring and pre-release testing of platelet concentrates. Furthermore, in transfusion units involving few platelet concentrates processing, all units can be sampled and then subjected to pre-release quality checks Njoroge(4).

Implementation of these measures will help in continuous quality improvement as well as entrench a standardization and harmonization program for platelet quality monitoring at NRBTC.

In this study, all the 91 (100%) platelet concentrates met the criteria for volume 40-70 ml, separation was achieved using a plasma extractor. Other studies have suggested the PCs might be stored for 5 days with a volume as low as 30 ml without significant changes of in-vitro platelet characteristics that were believed to reflect platelet viability and hemostatic functions by(14).

The volume was determined by subtracting the weight of the empty bag from that of full bag. NBTS has set a minimum volume of 60mls whereas AABB has not specified the minimum volume, simply stating that the final volume should be adequate to buffer the pH to $>6.2$. Various other studies [(Rahaman(15), Njoroge(4)] have shown that a volume $>40$ ml maintained the pH $>6.2$. Despite the fact that pH was not performed in this study. There has been no consensus as to the final volume. Studies have shown that as little as 35-40 mls of plasma is adequate to maintain pH above 6.0 below which the platelet storage lesion is irreversible.

The majority of platelet concentrates sampled were issued on day 1 and the remaining on day 2. Most of platelet concentrate at NRBTC were issued within 24 hours of processing, however the bag used allowed storage of platelet concentrate for up to 5 days. Studies demonstrate that platelets transfused within 24-48 h have few platelet storage lesion compared to platelets stored for long(Rahaman(15), Njoroge(4)).

Out of the 91 whole blood units processed, 87 (95.6%) met the normal range of platelet concentrate (150-450 cells/ml) requirement for platelet count. After first spin at 2100 rpm for 6 minutes, 87 (100%) attained recommended platelet count. After the second spin 3800 rpm for 9 minutes yielding the platelet concentrate, 83/87 (95.4%) had the required platelet count, $(\geq450$ cells/ml) mean±SD was $783.813\pm232.24$ and concentrate volume (40-70 ml).

In a study by Fasola(13), the mean volume was considerably low (18.52mls) whereas Singh reported a mean of 62.30±22.68 ml. NBTS set minimum volume at 60ml whereas AABB do not have a specified minimum volume.

A similar study at Nigeria hospital, platelet concentrates were prepared by PRP method, Fasola(13), reported that only 35% of the platelet concentrates met the minimum $5.5\times10^9$/l platelet count threshold.

5. CONCLUSION

The parameters assessed for quality in this study from prepared platelet concentrates, not all met the criteria for platelet count, residual white cell count, though platelet concentrate volume of all met the criteria set by NBTS. Based on these findings, there is need to review the quality assurance protocol and focus mainly on the processes used to prepare platelet concentrates on centrifugation that is light and heard
spin speed and separation of plasma to attain the quality of platelet concentrates required.

6. RECOMMENDATIONS

Therefore, all the platelet concentrates processed at NRBTC and issued, samples should be subjected to pre-release quality checks. Implementation of these measures will help in continuous quality improvement as well as strengthen a standardization and harmonization program for platelet quality monitoring. This study also demonstrates that Haemoglobin and platelet counts can form an integral part of a rapid, simple and practical method for validation of collection, processing and storage procedures that is useful for routine quality monitoring and pre-release testing of platelet concentrates.

7. STUDY LIMITATION

In this study cell, counts were performed in EDTA tubes after transfer from citrate bag. Of which EDTA facilitates dispersal of the reversible aggregates that may form in citrate. Cell counts were performed in citrate. Therefore, it was not possible to compare the differences in cell counts in citrate from those in EDTA. This study therefore assumed that EDTA dispersed all the reversible aggregates, if any present in the original citrated sample.

REFERENCES


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