A Comparative Assessment of Quality of Platelet Concentrates Prepared By 3 Different Methods.

ABSTRACT

Platelet concentrate, Platelet Rich Plasma -Platelet Concentrate, Buffy Coat-Platelet concentrate, Apheresis-Platelet Concentrate

Introduction
Platelets play a pivotal role in haemostasis through adhesion to the injured vessel wall, aggregation, propagation of coagulation & thrombus formation. Platelets were described by Addison in 1841 as extremely minute granules in clotting blood & were termed platelets by Bizzozero who also observed their tissue adhesive qualities as increased stickiness when a vascular wall is damaged. Subsequently platelets are also involved in fibrinolysis & the repair of the vessel wall, restoring blood flow & vascular integrity.

Platelets concentrates are prepared from three different methods i.e. Platelet Rich Plasma (PRP), Buffy Coat (BC) and Apheresis method. The quality of platelet concentrates is affected by the preparation method and the storage conditions including the duration of storage, type of storage container and storage solution (plasma or additive solution). Different in vivo and in vitro techniques can be used to analyse platelet concentrates. The basic principle behind the preparation of components from whole blood is that each component has its own specific gravity and by applying differential centrifugation, each component is separated and removed, thus allowing the transfusion of the desired component according to the need of the patient.

Material and Methods
The present study was conducted in the Department of Immunohematology and Blood Transfusion Medicine, Govt. Medical College, Jammu over a period of 1 year Nov. 2010 to Oct 2011.

Blood Collection (Phlebotomy)
After appropriate Donor history and physical examination, whole blood was collected in 450 ml blood bags through a single smooth venepuncture. Mechanical blood mixers were used for all donations & blood bags maintained at room temperature (20 - 24 C) until processing. All platelet concentrates were prepared within 6-8 hours of collection. The average time for collection was less than 10 minutes. Cryofuge 6000i, refrigerated centrifuge was used for preparation of components from whole blood is that each component has its own specific gravity and by applying differential centrifugation, each component is separated and removed, thus allowing the transfusion of the desired component according to the need of the patient.

Preparation of Platelet Rich Plasma - platelet concentrate (PRP-PC)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Quality Requirement for PRP-PC</th>
<th>Quality Requirement for BC-PC</th>
<th>Quality Requirement for Apheresis-PC</th>
<th>Frequency of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swirling</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>4 Units/month</td>
</tr>
<tr>
<td>Volume</td>
<td>40 - 70 ml</td>
<td>70 - 90 ml</td>
<td>200 - 300 ml</td>
<td>4 Units/month</td>
</tr>
<tr>
<td>Platelet count/unit</td>
<td>≥5.5 × 1010</td>
<td>≥5.5 × 1010</td>
<td>≥3 × 1011</td>
<td>4 Units/month</td>
</tr>
</tbody>
</table>

In the present study, the following criteria of Quality Control of Platelets as recommended by DGHS (Directorate General of Health Services), India was followed:

Table 1 - DGHS Criteria
The platelet units were randomly selected and tested for the following parameters:

1. **Platelet concentrate volume**
   
   Vol. = Wt. of the full bag (g) - wt. of the empty bag (g)
   
   Specific gravity
   
   Weight is expressed in grams.
   
   Specific gravity of PC is 1.03

2. **Swirling**
   
   Swirling was evaluated by examining the units against light and graded as positive or negative.
   
   Positive swirling was graded as:
   
   - 3: Very clear homogen swirling in all parts of the bag.
   - 2: Clear homogen swirling in all parts of the bag.
   - 1: Homogen swirling only in some part of the bag and is not clear.
   - 0: Homogen turbid and is not changed with pressure.[4]

3. **Platelet count/unit**
   
   The platelet count/unit was calculated by multiplying platelet concentration (count) with platelet concentrate volume. The platelet count was determined with automated cell counter, Celtech.

4. **WBC count/unit**
   
   The WBC count/unit was calculated by multiplying WBC concentration with platelet concentrate volume. WBC count was determined with automated cell counter, Celtech.

5. **pH**
   
   pH was determined with the pH meter (Labtronics) at the end of volume.

### Results

#### Quality Parameter No.1: Swirling

**Table 2: Intergroup comparison of Swirling**

<table>
<thead>
<tr>
<th>Swirling Grade</th>
<th>PRP-PC</th>
<th>BC-PC</th>
<th>Apheresis-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>2(4.17%)</td>
<td>5(10.42%)</td>
<td>...</td>
</tr>
<tr>
<td>Grade 2</td>
<td>30(62.5%)</td>
<td>28(58.33%)</td>
<td>4(8.33%)</td>
</tr>
<tr>
<td>Grade 3</td>
<td>16(33.33%)</td>
<td>15(31.25%)</td>
<td>44(91.67%)</td>
</tr>
</tbody>
</table>

#### Quality Parameter No.2: Volume

**Table 3: Intergroup comparison of Volume**

<table>
<thead>
<tr>
<th>Volume per unit (ml)</th>
<th>PRP-PC</th>
<th>BC-PC</th>
<th>Apheresis-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>58.45 ± 13.05</td>
<td>60.68 ± 16.09</td>
<td>209.65± 10.86</td>
</tr>
<tr>
<td>Range</td>
<td>33.98 - 97.08</td>
<td>24.13 - 87.38</td>
<td>189.32-233.01</td>
</tr>
</tbody>
</table>

#### Quality Parameter No.3: Platelet count/unit

**Table 4: Intergroup comparison of Platelet count/unit**

<table>
<thead>
<tr>
<th>Platelet count per unit</th>
<th>PRP-PC (1010)</th>
<th>BC-PC (1010)</th>
<th>Apheresis-PC (1011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>5.68 ± 1.83</td>
<td>5.13 ± 1.75</td>
<td>2.72 ± 0.48</td>
</tr>
<tr>
<td>Range</td>
<td>1.9 - 9.38</td>
<td>0.61 - 9.23</td>
<td>1.36 - 3.66</td>
</tr>
</tbody>
</table>

#### Quality Parameter No.4: WBC count/unit

**Table 5: Intergroup comparison of WBC count/unit**

<table>
<thead>
<tr>
<th>WBC count per unit</th>
<th>PRP-PC (107)</th>
<th>BC-PC (107)</th>
<th>Apheresis-PC (106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>7.6 ± 4.87</td>
<td>3.72 ± 1.63</td>
<td>4.16 ± 0.71</td>
</tr>
<tr>
<td>Range</td>
<td>3.7 - 34.95</td>
<td>2.26 - 7.48</td>
<td>2.04 - 5.68</td>
</tr>
</tbody>
</table>

#### Quality Parameter No.5: pH

**Table 6: Intergroup comparison of pH**

<table>
<thead>
<tr>
<th>pH</th>
<th>PRP-PC</th>
<th>BC-PC</th>
<th>Apheresis -PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>6.9 ± 0.32</td>
<td>6.9 ± 0.33</td>
<td>7.02± 0.26</td>
</tr>
<tr>
<td>Range</td>
<td>6.4 - 7.5</td>
<td>6.4 - 7.4</td>
<td>6.5 - 7.6</td>
</tr>
</tbody>
</table>

### Discussion

**Quality Parameter No.1: Swirling**

Swirling is a simple & non-invasive procedure performed visually. Its presence indicates pH within the required range adequate for in vivo survival.

In the present study, Score 3 swirling was seen in 33.33%, 31.25% and 91.67%; score 2 swirling was seen in 62.5%, 58.33% and 8.33% of PRP-PC, BC-PC and Apheresis-PC units respectively while score 1 swirling respectively was seen in 41.7% & 10.42% of PRP-PC and BC units.

Statistically no significant difference was observed between PRP-PC and BC-PC units for swirling. Apheresis-PC units showed better swirling than PRP-PC and BC-PC units; this difference was statistically significant (p<0.0001).

In a similar study by Singh RP et al.,4 score 3 swirling was observed in 79.7%, 83.9% and 90% while score 2 swirling was noticed in 20.3%, 16.1% and 10% of PRP-PC, BC-PC and Apheresis-PC units respectively. No unit had score 1 swirling.

**Quality Parameter No.2: Volume**

The mean volume of PRP-PC, BC-PC and Apheresis-PC units was 58.45 ± 13.05 ml, 60.68 ± 16.09 ml and 209.65 ± 10.86 ml and ranged from 33.98 - 97.08 ml, 24.13 - 87.38 ml and 189.32 - 233.01 ml respectively. PRP-PC and BC-PC units were comparable in terms of volume (p=1.00); whereas a statistically significant difference was found between Apheresis-PC units and PRP-PC & BC-PC units (p=0.0001). 81.25% (39/48), 27.08% (13/48) and 85.42% (41/48) of PRP-PC, BC-PC and Apheresis-PC units respectively fulfilled the quality control criteria of volume. On comparing the maximum number of units meeting the desired quality control criteria of volume for PRP-PC units, BC-PC units & Apheresis-PC units (91/2875) (10% vs 30% vs 100%) respectively.

The mean platelet count of PRP-PC, BC-PC and Apheresis-PC was 5.6 ± 1.83 x 10 /unit and 2.8 ± 0.73 x10 /unit. In the study on 94 plateletpheresis procedures on CS 3000 (Baxter) & found a mean volume of 200.6 ± 14.6 ml. Das SS et al.41 on analysing the quality of 267 platelet concentrate units found a mean volume per unit of 54 ml.

In the study by Ali SF40, the mean volume of BC-PCs and Apheresis-PCs was 70.6 ± 20.3 ml and 225 ± 15.6 ml respectively.

**Quality Parameter No.3: Platelet count per unit**

The mean platelet count of PRP-PC, BC-PC and Apheresis-PC was 5.6 ± 1.83 x 10 /unit, 5.13 ± 1.75 x10 /unit and 2.72 ± 0.48 x10 /unit and ranged from 1.5 - 9.38 x10 /unit, 0.61 - 9.23 x10 /unit and 1.86 - 3.66 x10 /unit respectively. The mean platelet count of PRP-PC and BC-PC was comparable and statistically no significant difference was observed; whereas a statistically highly significant difference was found between Apheresis-PC units and PRP-PC & BC-PC units (p<0.0001), 66.7% (32/48), 52.08% (25/48) and 35.42% (17/48) of PRP-PC, BC-PC and Apheresis-PC units respectively fulfilled the quality control criteria of platelet count/unit.

**Quality Parameter No.4: WBC count per unit**

The mean WBC count of PRP-PC, BC-PC and Apheresis-PC units was 6.5 ± 1.8 x 10 /unit, 2.8 ± 0.73 x10 /unit and 2.8 ± 0.8 x10 /unit respectively. On comparison, BC-PC units had less WBC contamination than PRP-PC units and the difference was statistically highly significant (p<0.0001).

**Quality Parameter No.5: pH**

All PRP-PC and BC-PC units met the desired quality control criteria of WBC count per unit whereas the recommended quality control criteria of WBC count per unit was met in 91.67% (44/48) of Apheresis-PC units.

The mean pH of PRP-PC, BC-PC and Apheresis-PC units was found to be 7.6 ± 1.0, 7.6 ± 0.7 and 7.6 ± 0.8 respectively.
Choudhary R et al. in his study on 94 plateletpheresis procedures on CS 3000, Baxter found a mean WBC count of 5.3 ± 0.6 × 10^6/unit. In the study by Ali SF, the mean WBC contamination in BC-PC and APC units was 41 ± 0.4 × 10^6/unit and 3.1 ± 0.7 × 10^6/unit respectively.

**Quality Parameter No.5: pH**

The mean pH of PRP-PC, BC-PC and Apheresis-PC was 6.9 ± 0.32, 6.9 ± 0.33 and 7.02 ± 0.26 and ranged from 6.4 - 7.5, 6.4 - 7.4 and 6.5 - 7.6. All PRP-PC, BC-PC and Apheresis-PC units met the recommended quality control criteria of pH. Chopra G et al. found an adequate pH within range of 7.4 - 6.6 in their study. Ali SF observed that the mean pH was 6.8 ± 0.1 and ranged from 6.6 - 7.01.

The quality of platelet concentrates is affected by various factors such as donor variables, technique used and personal skills. Blood collection, processing and storage conditions significantly influence the quality of platelet concentrates. Over past few decades, the developed countries are making a gradual transition from WBD-PC (PRP-PC & BC-PC) units to Apheresis-PC units. This transition is largely due to the better quality and reduced donor exposure of Apheresis-PC units.

Our study has also shown that Apheresis-PC units had a better quality with respect to swirling, volume, platelet count per unit & had least WBC contamination as compared to PRP-PC & BC-PC units. The number of Apheresis-PC units meeting the quality control criteria for platelet count per unit was 35.42%. This may be explained on the basis that Apheresis is a technical procedure requiring expertise affected by many variables like baseline platelet count of the donors, the collection time & the volume of blood processed during the procedure. A significant proportion of Indian donors have borderline haemoglobin and platelet counts. Therefore, to meet the desired platelet yield, we need to select the donors with higher base line platelet counts, increase the processing time & also increase the blood volume processed.

On comparing the cost effectiveness, quality & reduced rate of complications of BC-PC units with PRP-PC units, it is recommended that BC-PC units should be used rather than PRP-PC units for the wide range of patients being treated at our centre since it is a Regional Tertiary Care Centre catering to a large subset of population belonging to variable socio-economic strata.

**Conclusion**

Our study suggests that Apheresis-PC units have a better quality with respect to swirling, volume, platelet count per unit & least WBC contamination as compared to PRP-PC & BC-PC units. Therefore, we should make a gradual transition from WBD-PC units to Apheresis-PC units at our centre.

Lack of awareness regarding the benefits of Apheresis-PC units poses a major hindrance in the usage of Apheresis-PC units. Moreover, donor needs to spend 60 - 90 minutes of his or her time for the procedure. And last but not the least the cost of the closed system Apheresis kits is high. Therefore, to increase the recruitment of plateletpheresis donors, we should counsel the donor regarding the better quality & cost effectiveness of Apheresis-PC units over WBD-PC units.

**References**