Cytokine generation in stored platelet concentrate: Comparison of two methods of preparation

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Received February 16, 2006

Background & objectives: Contaminating white blood cells (WBCs) in stored platelet concentrates (PC) are the main source of pro-inflammatory cytokines including interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) that are implicated in transfusion reactions. We compared the levels of these cytokines in stored platelet preparations prepared by two methods. Effect of pre-storage leucofiltration on these cytokine levels was also studied.

Methods: Twelve units of pooled PCs were prepared by platelet rich plasma (PRP) method and buffy-coat (BC) method each and stored for 5 days. IL-6, IL-8 and TNF-α levels were measured in platelet supernatants on day 0, 1, 3 and 5 of the storage using commercially available immunoassays. Pre-storage leucofiltration was done in 4-pooled units of PRP-PC and cytokine levels compared with unfiltered PCs.

Results: Median IL-6 levels increased from day 0 to day 5 in both PRP-PC and BC-PC. In PRP-PC, IL-8 increased from <3 pg/ml on day 0 to 817 pg/ml on day 5, while in BC-PC the corresponding levels were 10 and 346.5 pg/ml, respectively. No significant increase in levels of TNF-α was observed in BC-PC during storage period, while levels increased significantly in PRP-PC on day 1 only. There was no significant change in the levels of all three cytokines in leucofiltered PCs over 5 days of storage.

Interpretation & conclusion: Findings of our study showed that method of preparation and WBC content are the critical factors in determining the cytokine levels in stored PCs.

Key words Cytokine - febrile non haemolytic transfusion reaction - platelet concentrate

Platelet transfusions are frequently accompanied with febrile non-haemolytic transfusion reaction (FNHTR). Various proinflammatory cytokines, such as interleukin -1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-αβ), generated during storage of platelet concentrates (PCs) have been implicated in FNHTR. Leucocyte reduction by leukofiltration can prevent FNHTR as white cells are the main source of cytokines in the stored PCs. This approach may not
be feasible in developing nations due to high costs involved.

Preparation of PCs by buffy coat method (BC-PC) can also decrease the risk of FNHTR because of reduction in WBC count by 80 to 90 per cent. British Council on Standardization in Hematology recommends the use of BC-PC for prevention of FNHTR. We at our center prepare PCs by buffy coat and platelet rich plasma (PRP-PC) methods. We therefore undertook this study to measure levels of various cytokines (IL-6, IL-8 and TNF-α) in the supernatants of PCs prepared by these two methods and its comparison with leucofiltered PCs.

Material & Methods

This prospective study was conducted at Blood Center of Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, after taking approval from the Institute Ethical Committee and donor consent. PRP-PCs were prepared from whole blood following standard method, while BC-PCs were prepared using OPTI press I (Baxter, USA) following manufacturer’s instructions. Two units of ABO group matched PCs were pooled using sterile connecting device (Terumo, Japan). A total of 12 units of such pooled BC-PCs and PRP-PCs each were prepared and stored on a linear platelet agitator/incubator (Helmer, USA) at 22°C for 5 days. Another set of 4 pooled PRP-PCs were filtered using sterile connecting device (Terumo, Japan). A total of 12 units of such pooled BC-PCs and PRP-PCs each were prepared and stored on a linear platelet agitator/incubator (Helmer, USA) at 22°C for 5 days. Another set of 4 pooled PRP-PCs were filtered using sterile connecting device (Terumo, Japan) and stored similarly.

Approximately 5 ml of sample was obtained from each bag through sampling site coupler (Baxter, USA) after thorough stripping the segment of the bag on day 0 (day of preparation), 1, 3 and day 5. The samples were then immediately centrifuged at 1300 g for 15 min and platelet poor supernatant was stored frozen at -80°C till further assay. Similarly, another 1 ml sample from each bag was collected in K<sub>2</sub>EDTA to measure platelet and WBC counts (Micros 60, ABX Diagnostics, France). WBC counts on leukofiltered PC were performed using Nageotte counting chamber (Weber Scientific International, UK).

Levels of IL-6, IL-8 and TNF-α were measured in the stored platelet supernatants using commercially available assays (Genzyme Corporation, USA) following manufacturer’s instructions. The minimum detection limits were 1 pg/ml for IL-6, 3 pg/ml for IL-8 and 10 pg/ml for TNF-α assay.

Statistical analysis: As the data were not normally distributed (sample size < 30), non-parametric Mann Whitney U test was used for inter-group and Wilcoxon Signed-Rank test for intra-group comparison. *P* < 0.05 was considered significant.

Results & Discussion

The mean platelet and WBC counts in PRP-PC were 705 ± 203 x 10<sup>9</sup>/l and 6.3 ± 3.1 x 10<sup>9</sup>/l respectively on the day of preparation, while the mean platelet and WBC counts in BC-PC were 803 ± 165 x 10<sup>9</sup>/l and 428 ± 108 x 10<sup>9</sup>/l respectively.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Median (range) Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6* (pg/ml)</td>
<td>2.2 (2.1-3.2)</td>
<td>2 (1-5)</td>
<td>34 (2.2-155)</td>
<td>161** (6-400)</td>
</tr>
<tr>
<td>IL-8* (pg/ml)</td>
<td>10 (10-10)</td>
<td>&lt; 3</td>
<td>162 (10-410)</td>
<td>265* (190-880)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>10 (10-10)</td>
<td>&lt; 3</td>
<td>10 (10-35)</td>
<td>47** (10-90)</td>
</tr>
</tbody>
</table>

*Statistically significant increase in IL-6 and IL-8 from baseline (day 0) over the 5 days of storage in both, BC-PC and PRP-PC (P<0.005); **Significant increase in IL-6 in PRP-PC compared to BC-PC on day 1 only (P<0.005); *Significant increase in IL-8 in PRP-PC compared to BC-PC on day 1, 3 and 5 (P<0.005); **No significant increase in TNF-α neither in PRP-PC nor BC-PC over 5 days of storage except on day 1 wherein significant (P<0.005) increase was seen in PRP-PC compared to BC-PC.

BC-PC, buffy coat platelet concentrate; PRP-PC, platelet rich plasma-platelet concentrate
0.5 ± 0.8 x 10^9/l, respectively. A significant (P<0.005) increase was seen in the levels of IL-6 and IL-8 during storage period of 5 days in both types of PCs. However, in TNF-α the increase was significant only in PRP-PC on day 1, while no increase was observed in BC-PC throughout 5 days of storage (Table I).

On comparing both the platelet preparations a significant increase in IL-6 levels was observed (P<0.005) only on day 1 in PRP-PC compared to BC-PC. However, unlike IL-6, the median IL-8 levels were significantly higher in PRP-PC throughout storage period as compared to BC-PC. In leucofiltered-PRP-PCs no increase in cytokine levels was observed from day 0 to day 5 (Table II).

We observed that irrespective of method of preparation PCs contain very high levels of cytokines, which progressively increase during 5 days of storage, particularly IL-6 and IL-8. Muylle et al first demonstrated increased levels of proinflammatory cytokines such as TNF-α, IL-1 and IL-6 in stored PCs and concluded that transfusion reactions might be due to administration of plasma with high cytokine levels.

Generation of cytokines was much lower in BC-PC compared to PRP-PC (Table I). This may be due to low levels of contaminating WBCs in BC-PC, which are the main source of cytokines in stored PC as previously demonstrated. A number of factors can activate WBCs to generate cytokines during PC storage including activated complement components, thrombin or by cytokines released from damaged white cells, or by non-biological surfaces of plastic containers. Once the WBCs are activated, the released cytokines are probably responsible for further WBC activation.

Our results indicated that there was an active synthesis of cytokines by activated WBCs during storage at 22°C as levels increased significantly from day 0 to day 5 (Table I). Platelet storage temperature is important for active synthesis of cytokines by WBCs as it has been demonstrated that there is negligible increase in IL-8 and IL-1β in red cells stored at 4°C. The fall in median levels of TNF-α from day 1 onwards in PRP-PC in the present study may be attributed to its short serum half-life (approximately 6 min).

Based on the assumption that normal adult dose of random donor platelets (RDP) in a 70 kg individual is 6 pooled RDPs (approximately 300 ml), the total

### Table II. Effect of pre-storage leucofiltration on median cytokine levels in PRP-PC

<table>
<thead>
<tr>
<th>Type of PC</th>
<th>WBC (x10^9/l)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-8 (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfiltered PC (N=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>5.5</td>
<td>105</td>
<td>290</td>
<td>53</td>
</tr>
<tr>
<td>Day 5</td>
<td>4</td>
<td>186</td>
<td>810</td>
<td>20</td>
</tr>
<tr>
<td>Filtered PC (N=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>0.02</td>
<td>4</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Day 5</td>
<td>0.017</td>
<td>4</td>
<td>10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

Unfiltered vs filtered PC: P<0.05 for all cytokines on day 1 and day 5

### Table III. Amounts of cytokines infused per dose** of PRP-PC and BC-PC on day 5 of storage

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>PRP-PC</th>
<th>BC-PC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose/</td>
<td>Dose/</td>
</tr>
<tr>
<td></td>
<td>transfusion</td>
<td>kg*</td>
</tr>
<tr>
<td>IL-6</td>
<td>74.4</td>
<td>1.06</td>
</tr>
<tr>
<td>IL-8</td>
<td>238.2</td>
<td>3.4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>146.4</td>
<td>2.09</td>
</tr>
</tbody>
</table>

*Dose/kg has been calculated considering 70 kg as average adult weight; **One dose is defined as pool of 6 random donor PC either PRP or BC with approximate volume of 300 ml;

BC-PC, buffy coat platelet concentrate; PRP-PC, platelet rich plasma-platelet concentrate
dose of cytokine per kg body weight as well as per transfusion episode for PRP-PC and BC-PC is shown in Table III. For example, a patient transfused with one dose of PRP-PC will receive $1.06 \times 10^3$ pg/kg body weight of IL-6 or $0.57 \times 10^3$ pg/kg if BC-PC is transfused. It is now well established that IL-6 and TNF-α are major pro-inflammatory cytokines that induce acute phase response. Therefore, transfusion of these cytokines in stored PCs can lead to transfusion reactions as it has been demonstrated previously that increased serum IL-6 levels in the recipients correlates with occurrence of fever, chills and rigors.

As these high levels of cytokines in stored PC are related to WBC content, accumulation of cytokines can be prevented by pre-storage removal of WBC from the PC using filters. We demonstrated complete suppression of cytokine synthesis in PRP-PCs that are filtered before storage compared to unfiltered PRP-PCs (Table II). This was in accordance with the findings of others. In addition to filtration, increased cytokine generation can also be prevented by the inactivation of WBCs using UV-B irradiation. However, in view of the technical difficulties associated with UV-B irradiation and the high cost of leucocyte filters, physical removal of buffy coat from PCs seem to be a good alternative to prevent cytokine induced transfusion reactions (FNHTR) in developing nations like India.

The method of preparation and degree of leucocyte contamination may be the critical factors that influence cytokine levels in stored PCs. In view of their role in the pathogenesis of transfusion reactions, every effort should be made to minimize the levels of cytokines in the stored PCs. Though, pre-storage leucocyte filtration is the method of choice, buffy coat removal can also be a good alternative in developing countries.

Acknowledgment

This work is a part of intramural research project funded by Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow. The authors are thankful to Dr Asim Tewari, Senior Resident, Transfusion Medicine at this Institute for collection of samples.

References


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