Original Article

Correlation of CD34+ Cell Yield in Peripheral Blood Progenitor Cell Product with the Pre-leukapheresis Cell Counts in Peripheral Blood

RB Sawant, SB Rajadhyaksha

Abstract
Introduction: Accurate timing of the leukapheresis procedures is of paramount importance to get the best possible CD34+ cell yield in the minimum number of leukapheresis procedures.

Aim: To find if pre-harvest CD34+ cell concentration in peripheral blood correlates with CD34+ cells in the product.

Material and Methods: Sixty Leukapheresis procedures were performed for 25 patients (8 autologous and 17 allogeneic transplants) with hematological malignancies. Statistical analysis was performed to correlate the pre-harvest CD34+ cell count and the CD34+ cell yield. Volume processed during PBPC harvests was three times the blood volume.

Results: The best correlation was found between the leukapheresis product CD34+ cell count and the pre-harvest PB-CD34+ cell count (PCC=0.674) when compared with the other pre-harvest PB cell counts viz., WBC (PCC=0.229) and MNC (PCC=0.324). This correlation was better in the allogeneic harvest (PCC = 0.645) than the autologous harvest procedures (PCC = 0.348). Correlation analysis based on paired samples from the 60 leukapheresis procedures showed that when the pre-leukapheresis PB-CD34+ cell count was >20x10^3/ul a yield of >1x10^6 CD34+ cells/Kg could be obtained in 95% of the cases and >2x10^6 CD34+ cells/Kg could be harvested in 68% of cases whereas when the pre-leukapheresis PB-CD34+ cell count was <5x10^3/µl the yield was <1x10^6 CD34+ cells/Kg in 81% of the procedures.

Conclusion: The yield of CD34+ cells in PBPC harvests depends on the pre-harvest CD34+ cell concentration and therefore it is more useful than the pre-harvest WBC or MNC counts for predicting the appropriate timing of the harvests and also to achieve the best possible yield of CD34+ cells.

INTRODUCTION
The number of progenitor cells collected can be maximized by appropriate timing of the collection of PBPC for transplantation. Various peripheral blood cell counts have been used to reach a decision on when to initiate leukapheresis procedures. The most commonly used values are the WBC count, MNC count CD34+ cell count and the number of CFU–GM. The last two values stated above have been shown to correlate with the number of progenitor cells finally collected in the harvested product.

Failure to harvest the desired number of CD34+ cells results in emotional disappointment for the patient and a financial burden on the hospital. Therefore a clearly defined cut-off value for circulating CD34+ cells which can be used as a criterion to initiate leukapheresis and also to predict the yield is highly desirable.

Although PBPC collections can be performed on consecutive days for patients who do not reach the target after the first collection, this may be prohibitive in some cases because of poor general condition of the patient, the fast decrease of CD34+ progenitor cells in the PB and the high cost.

Many studies report the maximum levels of circulating progenitor cells after mobilization with G-CSF on day 5-7. In our institution, the PBPC harvest is initiated on day 5 of the mobilization with G-CSF. We have retrospectively analysed data from 60 PBPC harvests to study which of the parameters viz. the pre-leukapheresis PB-WBC count, MNC count or CD34+ cell concentration correlated best with the CD34+ cell content of the PBPC product obtained on the same day. We also evaluated the CD34+ cell concentration in PB as a predictive parameter of the leukapheresis CD34+ cell yield.
**MATERIAL AND METHODS**

Sixty leukapheresis procedures were performed for stem cell transplantation in 25 patients (8 autologous and 17 allogeneic transplants) with hematological malignancies. Immediately prior to these PBPC collection procedures, the WBC, MNC and CD34+ cell counts in PB were determined and correlated with the MNC and CD34+ cell counts in leukapheresis product. The leukapheresis product parameters were expressed as a function of patient body weight.

Informed consent was obtained from all patients and donors for the PBPC harvest procedure.

**Mobilization regimen**

In all the cases (patients and donors), PBPCs were mobilized with growth factors i.e., G-CSF (Filgrastim) as prescribed by the physician.

**PBPC Collection**

Leukapheresis was performed with a continuous flow blood cell separator i.e., the Fenwal CS 3000 plus (Baxter Healthcare, Deerfield IL, US) using acid citrate dextrose adenine (ACD-A) as anticoagulant in the ratio of 11:1 (WB:ACD-A). Venous access was established by either peripheral vein or central venous catheter of the appropriate size. The maximum blood flow rate was maintained upto 50 ml/minute. The total blood volume processed in each leukapheresis procedure was thrice the blood volume in all the cases.

Leukapheresis was continued daily till a target number of 2x10^6 CD34+ cells per Kg were collected.

**CD34+ cell count determination**

The circulating PB-CD34+ cell counts were determined pre and post-leukapheresis from the donor / patient and the leukapheresis product CD34+ cell counts were analyzed using flow-cytometry following the recommendations of the International Society of Hematology and Graft Engineering.

**Statistical Methods**

Patient and donor characteristics as well as peripheral blood and PBPC product counts were reported as median and range. Associations between various factors were assessed using Pearson’s correlation. Linear regression analysis was used to evaluate the predictable parameters of leukapheresis yield. A ‘p’ value of <0.05 was considered statistically significant. All statistical analysis was performed using SPSS software (version-11.5) for Windows.

**RESULTS**

The patient characteristics, leukapheresis procedure parameters in donors and patients and PBPC product cell count are represented in Table 1, 2 and 3 respectively. To find whether any of the blood cell counts were of predictive value for circulating CD34+ cells, counts were estimated of WBCs and MNCs from PB for potential correlations with pre-leukapheresis PB-CD34+ cell counts. The correlations obtained are shown in Table 4.

The best correlation was found between the leukapheresis product CD34+ cell count and the pre-harvest PB-CD34+ cell count (PCC=0.674) [Fig. 1] when compared with the other pre-harvest PB cell counts viz., WBC (PCC=0.229) and MNC (PCC=0.324).

The correlation between leukapheresis product MNC count and CD34+ cell count was significant (PCC=0.671 and P<0.01).

The correlation between the pre leukapheresis MNC count and product CD34+ cell count was poor (PCC=0.324).

**Linear regression analysis**

Correlation analysis based on paired samples from the 60 leukapheresis procedures showed that when the

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### Table 1: Patient characteristics (n=8)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Myeloma</td>
<td>4</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table 2: Patient and donor characteristics

<table>
<thead>
<tr>
<th></th>
<th>Autologous harvests</th>
<th>Allogeneic harvests</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transplants</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Total No. of leukapheresis procedures</td>
<td>24</td>
<td>36</td>
</tr>
<tr>
<td>No of leukapheresis procedures per patient: Median (Range)</td>
<td>3 (2-5)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>Median age in years (Range)</td>
<td>35 (19-52)</td>
<td>30 (10-56)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/1</td>
<td>10/7</td>
</tr>
<tr>
<td>Wt (Kg) Median (Range)</td>
<td>52 (45-68)</td>
<td>55 (30-78)</td>
</tr>
<tr>
<td>WBC count x10^9 / L on Day 1 of PBPC collection Median (Range)</td>
<td>36.2</td>
<td>39.2</td>
</tr>
<tr>
<td>PB-CD 34+ count x 10^7 / 1 on Day 1 of PBPC collection Median (Range)</td>
<td>6.8</td>
<td>23.2</td>
</tr>
<tr>
<td>CD34+ cells collected (x10^6/Kg) Median (Range)</td>
<td>1 (1-3)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>Volume of leukapheresis product (ml) Median (Range)</td>
<td>52 (50-54)</td>
<td>52 (50-54)</td>
</tr>
</tbody>
</table>

### Table 3: PBPC product cell counts

<table>
<thead>
<tr>
<th></th>
<th>Autologous Median</th>
<th>Transplantation Range</th>
<th>Allogeneic Median</th>
<th>Transplantation Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNC (x10^6)</td>
<td>9.6</td>
<td>1.1 – 24.9</td>
<td>12.5</td>
<td>3.19 – 32.05</td>
</tr>
<tr>
<td>CD34+ cells (x10^7)</td>
<td>4.7</td>
<td>0.7 – 23.8</td>
<td>12</td>
<td>1.15 – 53.4</td>
</tr>
<tr>
<td>CD34+/Kg (x10^6)</td>
<td>1</td>
<td>1 – 3</td>
<td>2</td>
<td>1 – 3</td>
</tr>
</tbody>
</table>
pre-leukapheresis PB-CD34+ cell count was >20x10³/µl a yield of >1x10⁶ CD34+ cells/Kg could be obtained in 95% of the cases and >2x10⁶ CD34+ cells/Kg could be harvested in 68% of cases whereas when the pre-leukapheresis PB-CD34+ cell count was <5x10³/µl the yield was <1x10⁶ CD34+ cells/Kg in 81% of the procedures. This has been summarized in Table 5.

Analysis of outliers

False negative outcome i.e., a good collection (PBPC CD34+ cells >1.0x10⁶/Kg) when PB-CD34+ cell count predicts a poor collection (PB-CD34+ cells <5.0x10³/µl) occurred in 3 (5%) procedures in our study. All these cases were normal healthy donors mobilized with G-CSF. False positive outcome i.e., a poor collection (PBPC CD34+ cells <2x10⁶/Kg) when PB-CD34+ cells predict a good collection was not observed in any procedure.

The PB-CD34+ cell count was lesser on Day 1 compared to Day 2 on six occasions and it was <5x10³/µl in 5 of these cases. Sixteen leukapheresis harvests were initiated at PB-CD34+ cell count <5x10³/µl and a yield of 1x10⁶/Kg CD34+ cells was obtained in only 3 of these cases.

**DISCUSSION**

The optimal timing of PBPC collection is critical to maximize the efficiency of the leukapheresis procedures.

For operational and economical efficiency, it would be beneficial if sufficient PBPCs could be obtained from the fewest collections and the optimal timing of the leukapheresis could be reliably predicted. A further benefit of the higher CD34+ cell yield per single apheresis is reduction in the total volume of the progenitor cell component. Besides the decrease in the contaminating RBCs within the graft, a reduction in the total volume of the PBPC component may reduce the amount of dimethyl sulfoxide required for cryopreservation. Both factors diminish the risk of adverse side effects related to the graft transplant. It is essential that a cut-off value of the circulating CD34+ cells can be defined that discriminates patients with a significant probability of collecting an adequate number of CD34+ cells by a single leukapheresis from those who need more than one procedure.

Various criteria like the number of days post mobilization, the PB-WBC, MNC counts have been used to determine the exact time to initiate leukapheresis. A PBPC graft CD34+ cell content of at least 2x10⁶/kg CD34+ cells is considered as adequate and to reliably predict engraftment post-transplantation. It is important that the leukapheresis procedures be scheduled to achieve the desired target dose of CD34+ cells in a cost-effective and efficient manner i.e., in minimum number of leukapheresis procedures. Our study aimed at determining whether any of the pre-leukapheresis peripheral blood cell counts could reliably predict the PBPC CD34+ cell yield.

We have therefore examined the correlation between the PB-WBC, MNC and CD34+ cell count and CD34+ cell content in PBPC product. In our institution PBPC collection is initiated on Day 5 of G-CSF mobilization. We found that, 73.4% of our pre-leukapheresis CD34+ cell counts were above 5x10³/µl whereas only 36.6% were above 20x10³/µl. Leukapheresis could have been avoided in these 16 sessions (26.6%) with PB-CD34+ cell count <5x10³/µl. We found a poor correlation between WBC or MNC count in PB and the yield of CD34+ cells in PBPC product as also reported by other investigators. Most studies have reported a close correlation between the number of CD34+ cells in the PB and the number of CD34+ cells in the PBPC product. In our study also the number of CD34+ cells in the PB have correlated favourably and found to be a good predictor for the
timing of leukapheresis and also for the number of CD34+ cells in the PBPC product.

This correlation was better in the allogeneic healthy PBPC donors (PCC=0.656) than in autologous (PCC=0.393) leukapheresis procedures. Z. Gasova et al.12 have reported a weak correlation between the number of CD34+ cells/Kg in the product and pre-collection concentration of CD34+ cells in PB in weakly mobilized patients (PB-CD34+ cell count <20x10^3) as compared to those with a better mobilization. A poor correlation between the number of CD34+ cells in the PB and those in PBPC product has been reported in another study13 and this has been attributed to the variable collection efficiency (CE) of the cell separators. In our study all the leukapheresis procedures were performed on the CS 3000 Plus cell separator and its CE was constant (between 55% to 60%).

In our study the coefficient of correlation between PB-CD34+ cells and CD34+ cell content of leukapheresis product was 0.674. Various other studies14,15 report variable correlation coefficient ranging from 0.57 to 0.95. This can be caused by technical difficulties in the harvesting procedure or inaccuracies in the CD34+ cell measurements, especially when the PB-CD34+ percentage is low.16

We have retrospectively analysed the predictive ability of the CD34+ cell count in PB in a very heterogenous population i.e., patients and healthy allogeneic – PBPC donors. We could use the CD34+ cell count in the PB to time leukapheresis procedures and predict CD34+ cell yield. When the pre-leukapheresis CD34+ cell count in PB was >20x10^3/µl, a PBPC yield of >1x10^6/Kg CD34+ cells could be obtained in 95% of the procedures, whereas to obtain a yield of >2x10^6 CD34+ cells/Kg in PBPC product at least 50 x10^3/µl PB-CD34+ cell concentration was required. On the other hand, when the pre-leukapheresis PB-CD34+ cell count was <5x10^3/µl a yield of <1x10^6/Kg CD34+ cells was obtained in 81% of the procedures. Therefore leukapheresis should be avoided when the PB-CD34+ cell count is below 5x10^3/µl.17

Several investigators have proposed guidelines for the timing of apheresis after mobilization.1,2,9-11 Same report that using 10-L apheresis, greater than 10 CD34+ cells/ml on the day of apheresis predicts collection of greater than 0.5x10^6 CD34+ cells/kg or less than 20 CD34+ cells /µl predicted less than 4x10^6 CD34+ cells/kg; >20 CD34+ cells/mm3 predicts collection of greater than 2-2.5 x10^6 CD34+ cells/kg after one or two procedures, greater than 50 CD34+ cells /µl predicted collection of either greater than 2.5x10^6 CD34+ cells/kg or greater than 4x10^6 CD34+ cells /kg.16

Measurement of circulating CD34+ cells on the day of apheresis require a rapid laboratory turnaround and necessitates the availability of a flow cytometer. As an alternative to this, measurement of PB-CD34+ cells on the day prior to leukapheresis has been reported to correlate with the yield of CD34+ cells and mitigates the need for rapid turnaround in the CD34+ cell measurement.1

The techniques to enumerate CD34+ cells are complex, expensive, require technical skills and time consuming thus resulting in significant delay to start leukapheresis procedure on the same day. Therefore the measurement of circulating immature myeloid cells on the day of leukapheresis to predict the yield of CD34+ cells has also been reported.16

In conclusion our analysis confirms a good correlation between the number of pre-leukapheresis PB-CD34+ cells and the PBPC yield of CD34+ cells and therefore can serve as a more accurate guide than PB-WBC or MNC counts for the initiation of leukapheresis procedures.

A threshold of at least 5x10^3 CD34+ cells/µl in PB for the initiation of leukapheresis procedures is essential to obtain a satisfactory yield of CD34+ cells in the PBPC product. For obtaining a yield of >1x10^6/Kg CD34+ cells in a single apheresis procedure our study recommends a PB-CD34+ cell count >20x10^3/µl.

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REFERENCES


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