A Successful and Simplified Filgrastim Primed Single Apheresis Method Without Large Volume Apheresis for Peripheral Blood Stem Cell Collection

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Background: There is a tendency to use only one apheresis collection to reduce the morbidity and the cost of peripheral blood stem cell collection. We studied whether rapid and complete engraftment could be achieved by single apheresis by using only Filgrastim without large volume apheresis in previously treated patients.

Methods: Engraftment of single apheresis in 25 patients was compared with those of multiple apheresis in 26 patients; 52% of patients in the single apheresis group and 62% of patients in the multiple apheresis group were heavily pretreated. All patients received 10–15 µg/kg/day of Filgrastim starting on day 14 after 3–4 cycles of induction chemotherapy. Apheresis was performed using Cobe Spectra on day 4, 5 or 6 in the single apheresis group and every other day in the multiple apheresis group after day 3.

Results: The median collection volume was 250 ml (250–300 ml) in the single apheresis group and 750 ml (200–1500 ml) in the multiple apheresis group. The median CD34(+) cell number was not significantly different in the two groups (11.79 vs 9.38 × 10⁶/kg). The median times to achieve leukocytes ≥1 × 10⁹/l and platelets ≥50 × 10⁹/l counts were 10 days (8–21 days) and 15 days (9–38 days) in the single apheresis group vs 11 days (8–23 days) and 20 days (10–32 days) in the multiple apheresis group, respectively (p < 0.05). Antibiotic use was less in the single apheresis group than the multiple apheresis group (9 vs 12 days, p < 0.05).

Conclusion: Adequate numbers of peripheral stem cells were harvested by G-CSF in a single apheresis without large volume apheresis even in heavily pretreated patients. Rapid and complete engraftment occurred in all patients and it was faster in single than multiple apheresis.

Key words: filgrastim – autologous PBSCT – apheresis

INTRODUCTION

For the last decade, peripheral blood stem cell (PBSC) transplantation has been increasingly used for the treatment of haematological and oncological diseases. However, for autologous PBSC transplantation, an optimal mobilization technique has not yet been determined. However, mobilization techniques in which chemotherapy plus growth factors (GF) and multiple apheresis are used in order to collect an appropriate amount of CD 34(+) stem cells have often been used (1–3). On the other hand, this approach gives rise to an increase in morbidity and cost. Also, the apheresis procedure itself is a time- and cost-consuming attempt for both the patient and technician. Therefore, the clinical trials aiming for an optimization of leukapheresis and mobilization techniques, in which an appropriate amount of CD34(+) stem cell is obtained with a single apheresis have gained importance (4–6). Generally, obtaining an appropriate amount of CD34(+) stem cells requires a large volume leukapheresis (LVL) with a high flow-rate (>90 ml/min) in which at least three times the blood volume (15–35 l) is processed (6–9). Another uncertainty is the time for optimal
apheresis. The time for optimal apheresis has been well determined for allogeneic conditions (10), whereas it has not been clearly defined for an autologous setting (11). For conditions combining chemotherapy with GF, GF is usually given one day after the termination of chemotherapy and apheresis is often started when the leukocyte count is ≥1 × 10^9/l in the first (15), 1–2 days after that of 10 × 10^9/l in the second (16), and immediately after that of 5–10 × 10^9/l in the third (17). These numbers are normally obtained between 11 and 18 days after chemotherapy. In the light of these data, a clinical trial was conducted to decrease the cost and develop a more appropriate technique in our centre. In this study, whether an adequate number of stem cells could be obtained with a single apheresis using G-CSF administration 14 days after the induction chemotherapy was investigated and the findings of post-transplant engraftment and the requirements of supportive therapy were evaluated.

**MATERIALS AND METHODS**

**PATIENTS**

Fifty-one patients with various malignancies underwent autologous PBSC transplantation. Twenty-five patients had a single apheresis (SA) whereas 26 had a multiple apheresis (MA). Of the patients in the SA group, 11 were male (15 female) and the median age was 35 years (range, 19–67), whereas in the MA group 20 were male (six female) and the median age was 24 years (range, 20–53). The patients in the SA group consisted of 12 patients with breast cancer, six with non-Hodgkin’s lymphoma, one with Hodgkin’s disease, two with multiple myeloma, two with osteosarcoma, one with Ewing’s sarcoma and one with small cell lung carcinoma, whereas those in the MA group consisted of five patients with breast cancer, three with non-Hodgkin’s lymphoma, 11 with Hodgkin’s disease, three with testis tumour, one with small cell lung carcinoma, one with brain tumour and one with acute myelocytic leukaemia. Fifty-two percent (n = 13) of the patients in the SA group and 62% (n = 16) of those in the MA group had previously received chemotherapy, with a number of chemotherapy cycles ≥12. In addition to chemotherapy, radiotherapy had been administered to 48% of the patients (n = 12) in the SA group and 50% (n = 13) of those in the MA group. The median time from diagnosis to transplantation was 330 days (90–5110) in the SA group and 575 days (165–3160) in the MA group. Before the transplantation, all patients gave written consent. The distribution and characteristics of cases are shown in Table 1.

**PBSC MOBILIZATION AND TECHNIQUE**

The mobilization was performed after 3–4 cycles of induction chemotherapy in both groups. The drug combinations used as induction therapy included CAF (cyclophosphamide 500 mg/m² day 1, doxorubicin 50 mg/m² day 1, 5-fluorouracil 500 mg/m² days 1 + 8) for patients with breast cancer, DHAP (dexamethasone 40 mg/day 4 days, ara-C 4 g/m² day 2, cisplatin 30 mg/m² 3 days) for those with lymphoma, VAD (vincristine 0.4 mg/day 4 days, doxorubicin 9 mg/m² 4 days, dexamethasone 40 mg/day 12 days) for those with multiple myeloma, VIP (etoposide 75 mg/m² 5 days, ifosfamide 1200 mg/m² 5 days, cisplatin 20 mg/m² 5 days) for those with testis and brain tumours, IPA (ifosfamide 2000 mg/m² 3 days, cisplatin 30 mg/m² 3 days, doxorubicin 50 mg/m² 1 day) for those with osteosarcoma, VACA (vincristine 2 mg/day 1, 8, 15, doxorubicin 30 mg/m² 2 days, cyclophosphamide 1200 mg/m² 1 day, daclomycin 0.8 mg/day 3 days) for Ewing’s sarcoma and EP (etoposide 125 mg/m² 3 days, cisplatin 75 mg/m² 1 day) for those with small cell lung carcinoma. For the patient with

<table>
<thead>
<tr>
<th>Table 1. Patient characteristics</th>
<th>SA group</th>
<th>MA group</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Median age (years)*</td>
<td>35 (19–67)</td>
<td>24 (20–53)</td>
</tr>
<tr>
<td>Male/female†</td>
<td>11/14</td>
<td>20/6</td>
</tr>
<tr>
<td>Diagnoses:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Testis tumour</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Ewing’s sarcoma</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Small-cell lung carcinoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brain tumour</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Acute myelocytic leukemia</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Patients who had intensive therapy (&gt;12 cycles)</td>
<td>13(52%)</td>
<td>16(62%)</td>
</tr>
<tr>
<td>Patients who had previous RT</td>
<td>12 (48%)</td>
<td>13 (50%)</td>
</tr>
<tr>
<td>Time from diagnosis to transplantaion (median) (days)</td>
<td>330 (90–5110)</td>
<td>575 (165–3160)</td>
</tr>
<tr>
<td>High-dose chemotherapy regimens:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICE</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>BEAM</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>BEAM + RT</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>CyEAM</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CyEAM + RT</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>Cy + TBI</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Melphalan</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>BECM</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

SA, single apheresis; MA, multiple apheresis; CR, complete remission; CT, chemotherapy; RT, radiotherapy; I, ifosfamide; C, carboplatin; E, etoposide; B, BCNU; A, ara-C; M, melphalan; Cy, cyclophosphamide; RT, involved area radiotherapy; TBI, total body irradiation. *p < 0.01. †p < 0.02.
AML the mobilization was performed after the intensification regimen (ara-C, etoposide). Fourteen days after the last cycle of induction therapy, rh G-CSF (Filgrastim, Roche) 10–15 µg/kg/day i.v. was given as a 2 h infusion. Using Cobe Spectra (Cobe Lakewood, USA), the leukapheresis procedure was performed with a three-way catheter 6–8 h after the last dose of G-CSF on the day 4, 5 or 6 in the SA group and on every other day after day 3 in the MA group. Harvested autologous plasma was mixed with dimethyl sulfoxide (DMSO) to a final DMSO concentration of 10%, frozen at −100°C using a computerized freezing device (R 201 Planar) and then stored at −197°C in liquid nitrogen until the day of use.

**IMMUNOFLUORESCENCE ANALYSIS**

1 × 10⁶ MNC of the leukapheresis product were incubated for 30 min at 4°C with 10 ml of fluorescein isothiocyanate-conjugated anti-c antibody (HPCA-2) obtained from Becton Dickinson (Heidelberg, Germany). Isotype-identical antibodies served as controls. Immunofluorescence analysis was performed using FACSscan (Becton Dickinson).

**HIGH-DOSE CHEMOTHERAPY REGIMENS**

The conditioning regimens included ICE (ifosfamide 15 g/m², carboplatin 1.5 g/m² in 6 days in divided doses) for 17 cases in the SA group and 11 cases in the MA group; BEAM (BCNU 300 mg/m²/day, etoposide 200 mg/m²/day × 4 days, ara-C 400 mg/m²/day × 4 days, melphalan 140 mg/m²/day) for two cases in the SA group and three cases in the MA group; CyEAM (cyclophosphamide 1.5 g/m²/day, etoposide 200 mg/m²/day × 4 days, ara-C 400 mg/m²/day × 4 days, melphalan 140 mg/m²/day) for one case in the SA group and three cases in the MA group; BEAM + RT (involved field, starting day after day 3 in the MA group. Harvested autologous plasma was mixed with dimethyl sulfoxide (DMSO) to a final DMSO concentration of 10%, frozen at −100°C using a computerized freezing device (R 201 Planar) and then stored at −197°C in liquid nitrogen until the day of use.

**ENGRAFTMENT FINDINGS**

A complete and persistent engraftment developed in all cases. The median time for leukocyte engraftment (≥1 × 10⁹/l) was 10 days (8–21) in the SA group and 11 days (8–23) in the MA group; platelet engraftment (50 × 10⁹/l) developed earlier in the SA group [15 days (9–38)] vs 20 days (10–32), p = 0.03] (Table 3). No correlation was found between CD34(+) cell number and the number of nucleated cells (r = 0.30, p = 0.036), whereas there was no relationship between CD34(+) cell number and the blood processing rate or processed blood volume.

**SIDE-EFFECTS**

No serious side-effects were observed during the mobilization and harvesting in both groups. Side-effects such as fever (18%) and bone pain (24%) were simply controlled with antipyretic and analgesic drugs.

**POST-TRANSPLANT SUPPORTIVE THERAPY**

The median period with fever was 2 days (0–6) in the SA group and 2 days (0–13) in the MA group; the median duration of hospitalization was 11 days (8–49) in the SA group and 14 days (8–51) in the MA group; the median number of erythrocyte transfusions was 3 units (0–6) in the SA group and 3 units (0–10) in the MA group; the median number of platelets was 2 units (1–8) in the SA group and 2 units (0–10) in the MA group. There was no difference between the SA and MA groups in terms of the number of days with fever, the duration of hospitalization after transplantation and the number of erythrocyte and platelet transfusions, whereas the number of...
days that antibiotics were used was statistically less in the SA group (median 9 vs 12 days, \( p = 0.039 \)) (Table 4). In correlation analysis, there was a negative correlation \( (r = -0.37, p = 0.019) \) only between CD34(+) cell number and the fact that antibiotics were used. The same correlation was more apparent in the SA group \( (r = -0.48, p = 0.015) \).

**Table 2. Leukapheresis results**

<table>
<thead>
<tr>
<th></th>
<th>SA group ± SD</th>
<th>MA group ± SD</th>
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</thead>
<tbody>
<tr>
<td><strong>Rate (ml/min)</strong></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>41.40 ± 4.13</td>
<td>47.04 ± 6.10</td>
</tr>
<tr>
<td>Median</td>
<td>40.20</td>
<td>47.40</td>
</tr>
<tr>
<td>Range</td>
<td>34.80–49.70</td>
<td>36.20–63.10</td>
</tr>
</tbody>
</table>

| **Processed blood volume (l)** | | |
| Mean                         | 11.32 ± 0.99 | 20.64 ± 6.72 |
| Median                       | 11.26        | 19.13         |
| Range                        | 8.97–12.84   | 11.77–37.83   |

| **Collected volume (ml)** | | |
| Mean                      | 254 ± 13.84 | 657 ± 283    |
| Median                    | 250         | 700          |
| Range                     | 250–300     | 200–1500     |

| **Number of aphereses:** | | |
| Mean                     | 1           | 3 ± 1.13     |
| Median                   | 1           | 2.5          |
| Range                    | 1           | 2–7          |

| **Nucleated cell number (×10⁸/kg)** | | |
| Mean                           | 12.20 ± 6.37 | 7.69 ± 1.91 |
| Median                         | 9.06         | 7.59         |
| Range                          | 5.30–31.42   | 4.07–11.50   |

| **CD34(+) cell number (×10⁶/kg):** | | |
| Mean                        | 11.79 ± 19.12 | 9.38 ± 6.29 |
| Median                      | 6.03         | 8.78         |
| Range                       | 1.82–92      | 1.72–33.89   |

* \( p < 0.0001 \). † \( p < 0.00001 \). ‡ \( p < 0.001 \).

**Table 3. Post-transplant haematological improvement**

<table>
<thead>
<tr>
<th></th>
<th>SA group (days ± SD)</th>
<th>MA group (days ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leukocytes (1 × 10⁹/l):</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.28 ± 2.97</td>
<td>13.19 ± 4.79</td>
</tr>
<tr>
<td>Median</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Range</td>
<td>8–21</td>
<td>8–23</td>
</tr>
</tbody>
</table>

| **Platelets (50 × 10⁹/l):** | | |
| Mean                     | 17.36 ± 6.96         | 21.44 ± 5.91         |
| Median                   | 15                   | 20                   |
| Range                    | 9–38                 | 10–32                |

* \( p = 0.012 \). † \( p = 0.03 \).

**DISCUSSION**

For autologous PBSC transplantation, an optimal mobilization time and technique have not been clearly defined. The mobilization techniques that could be used with autologous procedures include chemotherapy alone, CSF alone, chemotherapy + CSF, CSF combinations and monoclonal antibodies against the integrine receptors of stem cells. Owing to the potential to decrease the patient morbidity and the cost, there is a tendency towards collection procedures with single apheresis. Such procedures require LVL and a mobilization technique in which chemotherapy and CSFs are often combined. With the conditions in which chemotherapy and CSFs are combined, CSF is usually administered 1 day after the termination of chemotherapy and the leukapheresis procedure is often started when the leukocyte count is ≥1 × 10⁹/l (12,13).

Accepting a leukocyte count ≥1 × 10⁹/l to start to leukapheresis is possibly wrong as it subsequently will result in an inadequate number of aphereses. The most ideal method to determine the optimal time for apheresis is daily measurement of CD34(+) cell count in circulating blood (2,18). Hence apheresis must be started when the CD34(+) cell count reaches 0.1% of the number of nucleated cells (13,19). Recent studies have found a highest concentration of CD34(+) cells either 2 days after the leukocyte count reached 2 × 10⁹/l (15) or 1–2
days after 10 × 10^9/l (16) or the day after reaching 5–10 × 10^9/
l (17). Therefore, with the mobilization regimens in which
CSFs and chemotherapy are combined, the best period for
collecting is days 11–18 if the blood concentration of CD34(+)cells is not measured (14). Another study reported days 13–19
for the best collection period (15). On the other hand, the best
collection time for the allogeneic mobilization technique, in
which CSFs alone is used, has been well established as day +4,
+5 or + 6 (10).

The pooled data from these two settings are the rationale of
our study. We showed that an adequate number of stem cells
could be obtained and a complete and permanent engraftment
occurs with a single apheresis, which was performed on day 4,
5 or 6 following a 2 h G-CSF i.v. infusion of 10–15 µg/kg, 14
days after an induction therapy of 3–4 cycles. The measure-
ment of the CD34(+) cell count on the day of leukapheresis
showed that the number of CD34(+) cells is ≥0.1% of the
number of nucleated cells in 90% of cases in the SA group
(data not shown). It is well known that >2.5 × 10^6 CD34(+)cells/kg of can be collected by a single apheresis when the
peripheral blood CD34(+) cell count is ≥2.5 × 10^9/ml (20). In a
study in which Kanold et al. examined haematopoietic progen-
itor cell kinetics in children with solid tumours and leukaemia,
who were treated with CSF alone (10 µg/kg/day), it was shown
that peak peripheral blood levels of CD34(+) cells were
achieved after the fourth and fifth doses of G-CSF and that,
with a standard volume single apheresis in which one volume
of blood is processed, 2 × 10^6 CD34(+) cells/kg could be
collected (21).

Many studies have shown that there is a poor correlation
between the collected number of CD34(+) cells and the base-
line and pre-apheresis leukocyte count or MNC count in the
product (4,11). Another study has suggested that the CD34*/
CD71 cell concentration (>30/ml) of a steady-state bone
marrow could be a good indicator of the CD34(+) cell content
of a product which was mobilized by G-CSF (22). Other
studies have reported that there is a positive correlation
between pre-apheresis leukocyte count and the number of
CD34(+) cells in the product and that the efficacy of CD34(+)cell collection could increase if the leukocyte count on the day
of apheresis ≥0.030/mm³ (23,24). Among our cases, over
90% in the SA group had a leukocyte count ≥0.030/mm³ on
the day of apheresis (data not shown). In our study, while there
was a positive correlation between the CD34(+) cell content of the
collected product and the number of nucleated cells (r =
0.30, p = 0.036), we observed no relationship between the
blood flow-rate and the processed blood volume. So far, no
study has reported an optimal blood flow-rate for Cobe Spectra
to collect CD34(+) cells. By increasing the total volume of
processed blood (15–35 l), single LVLs have been attempted to
obtain an adequate number of stem cells (6–8). However, this
requires an increased flow-rate (290 ml/min).

The fact that we observed no relationship between CD34(+)cell count and haematologic improvement may be explained
by our approach to give all patients stem cells at much higher
levels than the limit CD34(+) levels. A study by Pettengell et
al. came to the same conclusion (4). This study in which
chemotherapy and G-CSF were combined for mobilization has
shown that an adequate number of CD34(+) cells could be
collected with a single apheresis between days 7 and 9, with a
processed blood volume of 10–15 l. When the haematological
engraftment findings of our study were compared with those in
Pettengell et al.’s study, our results suggested a similarity in
terms of leukocyte and platelet engraftments (10 vs 10 days
and 15 vs 16 days). However, the median unit number of post-
transplant platelet transfusions was two in our study and 24 in
the other study. Jones et al. reported that the platelet require-
ment in the post-transplant period was 15 units (5). This can be
explained by the fact that they had a higher number of
intensely treated patients or that the mobilization procedure
allowed the combination of G-CSF with chemotherapy regi-
mens such as cyclophosphamide.

Our study has some important advantages since it offers a
cost-effective use of an expensive drug such as G-CSF,
requiring less supportive therapy. Further, an adequate number
of CD34(+) progenitor cells can be mobilized by G-CSF only,
which is given 14 days after an induction chemotherapy and
collected with a single apheresis without LVL. Even a complete
and permanent engraftment can occur among patients who were treated with an intense therapy. Using this
effective and inexpensive method, randomized trials in which
the effects of blood flow-rate, processed blood volume and
other factors on the number of CD34(+) cells and engraftment
process are examined may determine the optimal method.

Acknowledgement

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European Group for Blood and Marrow Transplantation, Cour-
mayeur, Italy, March 22–26, 1998, and was published in Bone

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