Autologous peripheral stem cell transplantation after two-day fludarabine conditioning in patients with acute leukaemia

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Abstract

Background: Autologous stem cell transplantation from peripheral blood stem cells (PBSC) is being increasingly used in place of autologous bone marrow transplantation. PBSC following myeloblastic conditioning regimens reduced the period of marrow aplasia thus reducing morbidity, mortality and resource utilization. We report three cases of patients with acute leukaemia who received PBSC transplantation.

Materials and Methods: Patient-1 is a 37-year old male with acute lymphoblastic leukaemia (ALL), patient-2, a 42-year old male and patient-3 a 57-year old female, both with acute myeloblastic leukaemia (AML) underwent 2-day fludarabine conditioning and peripheral blood stem cell infusion. The routine white blood cell count, haemoglobin and platelets were monitored periodically for four months. Absolute neutrophil count (ANC), haemoglobin and platelets were determined.

Results: At 30 months from procedure the AML patients were reportedly still in good health and showed no evidence of relapse except for the ALL patient who had a relapse after 20 months and passed away one month later. Stem/Progenitor/blast cells showed an average loss of 33.5% after 2-day of storage at 4°C before the infusion. There was a sudden drop in ANC and platelets following post-infusion but returns to stable normal levels after 7- days. The haemoglobin levels showed irregular patterns but remains in normal stable state by four months.

Discussion and Conclusion: The 2-day fludarabine conditioning in PBSC transplantation for acute leukaemia showed promise and sets new challenges for further study.

Keywords: autologous stem cell, fludarabine, acute leukaemia
He was given remission reduction (UK-AML-12) for adults under 60 years of age. He did not undergo maintenance protocol but went directly for mobilization procedure for PBSC.

**Patient-3.** A 57-year old female who came with de novo AML/FAB-M2 underwent first remission induction and achieved complete remission (CR). She refused to continue with further chemotherapy. One month after remission induction she agreed on autologous PBSC and started to undergo mobilization procedure.

The patients underwent 2-day fludarabine conditioning while PBSC was collected and stored at 4°C for two days before infusion.

Mobilization and harvesting procedures. All patients received subcutaneous injection of filgrastim 330 mcg 12 hourly on days 1 to 6 (12 doses). On morning of day 7 apheresis was started using Haemonetics MCS+ apheresis machine and stopped when the cycles have reached the cycle volume equivalent to 1x blood volume. Calculation of progenitor cells; Total progenitor cells: % of progenitor cells x TNCC (cell/mm³) x PBSC volume (mL) x 1,000. This calculation is also used for stem cells and blast cells. The PBSC collected was stored at 4°C for two days before being infused to the patients.

Conditioning. Upon completion of apheresis, patients were given fludarabine (Schering Indonesia) 50 mg/m² iv. push in 30 minutes on days 7 and 8. All patients received oral ciprofloxacin (Bayer Indonesia) 500 mg 8-hourly for infection prophylaxis.

Infusion of PBSC. On morning of day 9, patients were re-infused PBSC through the peripheral line lasting 1 hour. Haemoglobin, absolute neutrophil count and platelets were monitored on post-mobilization, post-conditioning, pre-transplant, day +2, +9, +28 and every month for 4 months.

Laboratory investigation. White blood cell (WBC) count, haemoglobin and platelets were determined in the haematology analyser Sysmex XN2000 (Kobe, Japan). The absolute neutrophil count (ANC) was calculated from percentage neutrophil x WBC and expressed as per mm³.

**Results**

Total number of collected stem, progenitor and blast- cells and loss of potential cells during storage at 4°C

The assessment of the number of potential stem cell/progenitor cells/blast cells were calculated manually based on morphology of the cells with relative percentage of each type of cell were counted into absolute numbers by multiplying the total nucleated cells (TNCC) (cell/mm³) and the volume of the PBSC in the bag (mL) and factor of 1,000. The number of cell collected per bag at Day-1 and Day-2 showed an average storage loss of 33.5% among the three patients (Table1)

**Fig 1:** ANC, haemoglobin and platelets of each patient monitored at post-mobilisation and post-transplantation for 4 months.

**Survival outcome following PBSC transplant infusion**

Patient-1 (ALL) had a second relapse after 20 months post-PBSC transplant infusion and passed away one month later. To date both the patients with AML were reportedly still in good health with no evidence of relapse after more than thirty months following PBSC transplant infusion.
Table 1: Data of potential stem/progenitor/blast cells collected from the three patients with acute leukaemia and the losses during storage (4°C) at Day-2.

<table>
<thead>
<tr>
<th>Apheresis Volume (mL/bag)</th>
<th>Number of cells collected (cells/bag)</th>
<th>Cell losses at Day-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 1</td>
<td>DAY 2</td>
</tr>
<tr>
<td>Patient-1</td>
<td>117</td>
<td>8,281,587</td>
</tr>
<tr>
<td>Patient-2</td>
<td>124</td>
<td>17,745,888</td>
</tr>
<tr>
<td>Patient-3</td>
<td>138</td>
<td>23,843,778</td>
</tr>
<tr>
<td>Average</td>
<td>126.3</td>
<td>16,623,751</td>
</tr>
</tbody>
</table>

Table 2: Absolute neutrophil count, haemoglobin and platelets of the three patients with acute leukaemia at post-mobilisation and after 2-day fludarabine conditioning in peripheral blood stem transplantation monitored for four months.

<table>
<thead>
<tr>
<th>Post-Mobilisation</th>
<th>Pre-Transplantation</th>
<th>Day +2</th>
<th>Day +9</th>
<th>Day +14</th>
<th>Day +21</th>
<th>1month</th>
<th>2month</th>
<th>3month</th>
<th>4month</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANC/mm³</td>
<td>Hb g/dL</td>
<td>Platelets x10⁹/L</td>
<td>ANC/mm³</td>
<td>Hb g/dL</td>
<td>Platelets x10⁹/L</td>
<td>ANC/mm³</td>
<td>Hb g/dL</td>
<td>Platelets x10⁹/L</td>
<td>ANC/mm³</td>
</tr>
<tr>
<td>9,000</td>
<td>14.0</td>
<td>190</td>
<td>30,000</td>
<td>11.5</td>
<td>150</td>
<td>45,000</td>
<td>11.0</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>13.5</td>
<td>140</td>
<td>12,000</td>
<td>10.5</td>
<td>75</td>
<td>6,000</td>
<td>10.5</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>8.6</td>
<td>80</td>
<td>5,000</td>
<td>10.0</td>
<td>80</td>
<td>5,000</td>
<td>11.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>8.6</td>
<td>100</td>
<td>5,000</td>
<td>9.5</td>
<td>70</td>
<td>5,000</td>
<td>12.5</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>5,000</td>
<td>11.8</td>
<td>170</td>
<td>9,000</td>
<td>9.5</td>
<td>100</td>
<td>4,000</td>
<td>14.2</td>
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<td></td>
</tr>
<tr>
<td>10,000</td>
<td>11.8</td>
<td>210</td>
<td>7,000</td>
<td>10.0</td>
<td>220</td>
<td>4,000</td>
<td>12.7</td>
<td>220</td>
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<tr>
<td>5,000</td>
<td>11.0</td>
<td>240</td>
<td>4,000</td>
<td>12.5</td>
<td>220</td>
<td>5,000</td>
<td>12.5</td>
<td>370</td>
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</tr>
<tr>
<td>5,000</td>
<td>11.2</td>
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<td>220</td>
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<td>13.0</td>
<td>300</td>
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<td>12.8</td>
<td>130</td>
<td>4,000</td>
<td>9.5</td>
<td>290</td>
<td>5,000</td>
<td>12.9</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>13.7</td>
<td>260</td>
<td>5,000</td>
<td>12.0</td>
<td>200</td>
<td>5,000</td>
<td>14.4</td>
<td>340</td>
<td></td>
</tr>
</tbody>
</table>

ANC= absolute neutrophil count; Hb = haemoglobin

Discussion

Autologous stem cell transplantation from PBSC following myeloablative conditioning regimens has been increasingly performed reducing the period of marrow aplasia and reducing morbidity, mortality [1, 2]. This small study of three patients especially AML patients had better outcome than the study by the Dutch-Belgian and Swiss- Collaborative Group that showed 50% cumulative percentage of around 14 months [3]. The Eastern Cooperative Oncology Group (ECOG) study in AML patients after first relapse and treated with chemotherapy alone without stem cell transplantation on the 14-month period showed only 12% overall survival [9] suggesting that high risk AML patients treated by chemotherapy alone do not have good survival outcome. To our knowledge there is no reported study investigating the survival of ALL patients who stopped treatment on the first cycle of remission and there is scarce report of autologous haematopoietic stem cell transplantation in ALL.

Our study on autologous PBSCT with 2-day fludarabine conditioning is similar with other groups that use standard myeloablative conditioning and patients in first remission [3]. The average loss in stem/progenitor/blast cell of 33.5% in our study after 2 days storage at 4°C was similar with standard cryopreservation of PBSCT after thawing loss of around 34.8% [10]. The very low fludarabine used in our study for PBSCT is promising as it has less toxic effect on conditioning and short period of recovery time of 3 to 4 days for absolute neutrophil count (ANC) and platelets. The procedure is suitable and may benefit the elderly and physically unfit patients going on haematopoietic stem cell transplantation. The logic on the use of the 2-day fludarabine conditioning followed by PBSCT is better than chemotherapy alone especially in AML or to some degree in ALL is based on the data that 2-day fludarabine is a kind of NMA which refer to the non-myeloablative conditioning regimen which is less toxic compared to the full myeloablation and RIC. On the other hand, transplant itself of abundant number of own potent healthy stem cells engraftment will eliminate or supress some of the remaining malignant hematopoietic cells allowing for the establishment of better hematopoiesis. The true answer of why 2-day fludarabine followed by PBSCT showed benefit in this report needs further investigation. The 2-day fludarabine conditioning in autologous PBSCT especially for acute myeloblastic leukaemia showed promise and sets new challenges for further study.

Conclusion

The 2-day fludarabine conditioning in autologous peripheral blood stem cell transplantation for acute leukaemia especially AML showed promise and sets new challenges for further study.

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Conflict of Interest

The authors declared that they have no conflict of interest.

References


