INCREASED RISK OF EXTENSIVE CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION USING UNRELATED DONORS*

Short title: Comparing PBSC with BM using unrelated donors.

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Scientific heading: Clinical observations

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ABSTRACT

The long-term follow-up of a study including 214 patients receiving either peripheral blood stem cells (PBSC) or bone marrow from an HLA-A, -B and -DR-compatible unrelated donor is presented. Median follow-up were 4.4 (2.3-7.3) and 5.0 (0.7-8.4) years in the two groups, respectively. Cumulative incidence of overall chronic GVHD were similar in the two groups (78% vs. 71%), while extensive chronic GVHD was significantly more common in the PBSC group compared to the BM group (39% vs. 24%, p=0.03). The five-year transplant-related mortality (TRM) were 37% in the PBSC group and 35% in the BM controls (p=0.7) and overall survival were 42% in both groups. The relapse incidences were 26% and 27% in the two groups, respectively, resulting in a disease-free survival of 41% in both groups.

In conclusion, PBSC from HLA-compatible unrelated donors results in similar outcome compared to BM, but imply an increased risk for extensive chronic GVHD.

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Abstract: 150 words.
INTRODUCTION

Only few patients have been reported who received peripheral blood stem cells (PBSC) from unrelated donors. Reasons for the reluctance to use PBSC from unrelated donors has been the ethics of administering granulocyte colony-stimulating factor (G-CSF) to healthy volunteers and that the 10-to 15-fold higher donor T-cell content of PBSC would increase the risk of graft-versus-host disease (GVHD). Some reports have indicated a higher incidence of chronic GVHD after sibling PBSC. Previously we reported the initial results comparing PBSC with BM from unrelated donors at three centres. Here we report the long-term results.

PATIENTS AND METHODS

Patients. The study group consisted of 107 consecutive patients who received PBSC from unrelated donors between February 1993 and September 1999 (Table 1). Approval for this study was obtained from the local institutional review board and local ethical committee at each centre. Informed consent was provided according to the Declaration of Helsinki.

BM control group. For each unrelated PBSC transplanted patient we selected a control who had received unrelated bone marrow (BM) at almost the same time. Controls were matched for diagnosis, stage of disease, age (<20ys or >20ys) and GVHD prophylaxis. No difference between the two groups existed except that slightly more patients in the BM group received TBI-based conditioning (84% vs. 70%, p=0.03).

Donors. All donors were HLA-A, -B and -DRβ1 compatible with the patients. Before 1997, class I HLA typing was serological. Since then, PCR-SSP low resolution typing for class I was used. For HLA class II, genomic high resolution DNA based typing (PCR-SSP) was used. All patients from Huddinge (n=64) were retyped using PCR-SSP for HLA class I (A, B and C) and II (DR, DP and DQ)

Conditioning. Most patients received cyclophosphamid (Cy) 120 mg/kg, combined with total body irradiation (TBI), dose ranging from 10 to 13.5 Gy (Table 1).
Thirty-eight patients were given busulfan (Bu) 16 mg/kg followed by 120 mg/kg Cy. Detailed description of the therapy and care was given elsewhere 1,8,9.

**GVHD prophylaxis.** The commonest immunosuppression was cyclosporine A (CsA) and 4 doses of methotrexate (MTX)(Table 1). Twelve patients did not receive MTX as part of GVHD prophylaxis.

**Graft-versus-host disease.** Chronic GVHD was defined as limited or extensive according to standard criteria10.

**Statistics.** Analysis was performed on December 10, 2003.

Separate statistical analyses were performed for each endpoint (relapse, TRM, DFS and chronic GVHD). Overall survival and disease-free survival (DFS) were calculated with the Kaplan-Meier method,11 comparing the groups using the log-rank test (Mantel-Haentszel)12. The incidence of TRM, relapse and chronic GVHD were estimated using a non-parametric estimator of cumulative incidence curves. The Cox regression model was used to analyze predictive factors for chronic GVHD, TRM, survival, relapse and DFS 13.

In analysing risk factors for relapse and chronic GVHD, only patients surviving more than 90 days after transplantation were included. Four patients relapsed before day 90 and were included in the risk factor analysis of relapse. Factors significant at the 10% level in the univariate analysis were included in the multivariate analysis. The following factors were analysed: methotrexate, nucleated cell-dose, CD34 dose, patient and donor age and sex, ATG, G-CSF posttransplant, stem cell source, disease, disease stage, pretransplant CMV serology, conditioning and GVHD.

**RESULTS AND DISCUSSION**
PBSC grafts vs. BM grafts showed faster engraftment of ANC and platelets, which was presented previously². In this long-term follow-up we found no difference in overall chronic GVHD, 78% and 71% in patients receiving PBSC and BM grafts, respectively. This is in accordance with other studies with unrelated donors¹-³.

Using HLA-identical sibling donors, some studies have reported a higher incidence of overall chronic GVHD when using PBSC⁴-⁶. The reason for absence of a difference may be the higher incidence of chronic GVHD using unrelated donors, compared to HLA-identical siblings¹⁵-¹⁶.

As previously reported, CML and acute GVHD were correlated to chronic GVHD¹⁷. We also found that patients not receiving ATG had more chronic GVHD. Absence of ATG is correlated to more acute GVHD and acute GVHD triggers chronic GVHD ¹⁷.

Extensive chronic GVHD was more common in the PBSC group compared to the BM group (39% vs. 24%, p=0.03). This has previously been reported using sibling donors⁴ but not with unrelated donors. In multivariate analysis GVHD grades II-IV (RH 1.88, CI 1.07-3.29, p=0.03) and PBSC (RH 1.73, CI 1.00-3.00, p<0.05) were correlated to extensive chronic GVHD. As acute GVHD triggers chronic GVHD it is logical to assume that more severe acute GVHD triggers more severe chronic GVHD. This was previously shown¹⁸. Some information in patients with extensive chronic GVHD are displayed in table 2.

TRM was 28% and 29% at one year and 37% and 35% at five years in the PBSC and BM group, respectively. This is in line with previous studies with related and unrelated donors. Acute GVHD is one of the main causes for TRM and most studies comparing PBSC and BM showed similar incidence of acute GVHD.

In the multivariate analysis, acute GVHD grades II-IV (RH 6.05, CI 3.67-9.97, p<0.001), patient age (RH 1.34, CI 1.09-1.63, p=0.007) and absence of MTX as GVHD prophylaxis (RH 3.10, CI 1.28-7.46, p=0.012) were independent risk factors for TRM.
The five-year probability of survival was 42% in both groups. Among patients with early disease (CR1/CP1), the probability of survival was 56% and 54% and for patients in later stages it was 24% and 26% in the PBSC and the BM group, respectively (ns).

Primary causes of death were: relapse in 19 and 25, infection 17 and 13, GVHD 18 and 13 and other causes 6 and 10 in the PBSC and BM group, respectively (ns). In the multivariate analysis, absence of chronic GVHD (RH 3.29 CI 1.99-5.42 p<0.001), late disease (RH 2.32, CI 1.49-3.60, p<0.001) and acute GVHD II-IV (RH 2.34, CI 1.42-3.86, p<0.001) were independent risk factors for death.

The cumulative incidence of relapse was 26% and 27% in the PBSC and BM group, respectively. Among patients with early disease the five-year incidence of relapse was 9% and 15% and for patients in late disease it was 37% in both groups (ns).

In the multivariate analysis, advanced disease (RH 4.05, CI 2.03-8.08, p<0.001), absence of chronic GVHD (RH 3.22, CI 1.77-5.87, p<0.001) and non-CML diagnose (RH 2.25 CI 1.04-4.85, p=0.037) were independent risk factors for relapse. Using unrelated donors, the incidence of chronic GVHD is higher than in HLA-identical siblings, which also carries a graft-versus leukaemia effect 19-20, thus reducing the risk of relapse.

Disease-free survival (DFS) at five years was 41% in both groups. Among patients with CML, DFS was 57% and 52% in the PBSC and the BM group, respectively. Corresponding figures for patients with acute leukaemia was 26% and 31% (ns).

Two studies and a meta-analysis demonstrated a survival advantage with PBSC limited to patients with advanced disease 21-23. In our material, we found no difference in DFS among patients given PBSC or BM with CML or acute leukaemia with early or late disease.

However, among patients not in remission at transplant (n=33), we found a trend for a better OS and DFS (24% vs. 11%, p=0.11) in patients receiving PBSC compared to BM. However, the number of patients is small.
In the multivariate analysis, chronic GVHD (RH 3.42, CI 2.14-5.47, p<0.001), early disease (RH 2.66, CI 1.72-4.14, p<0.001) and acute GVHD 0-I (RH 1.80, CI 1.13-2.89, p=0.016) were independent prognostic factors for a better DFS.

Factors associated to a better DFS in our study agree with other studies 24-25.

With regard to TRM, survival, relapse and disease-free survival the outcome was the same in patients receiving PBSC or BM. This accords with most reports comparing PBSC with BM using related or unrelated donors1,2,4-6,26 while a study from EBMT showed better outcome with rich bone marrow27.

In conclusion, this study has shown that the use of PBSC from unrelated donors is a safe and well-tolerated procedure, but the risk for extensive chronic GVHD is increased.

ACKNOWLEDGEMENT

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REFERENCES


3. Garderet L, Labopin M, Gorin NC, et al. Patients with acute lymphoblastic leukaemia allografted with a matched unrelated donor may have a lower survival with a peripheral blood stem cell graft compared to bone marrow. Bone Marrow Transplant. 2003;31:23-29.


Table 1. Patients’ and donors’ characteristics, conditioning and GVHD prophylaxis. Absolute numbers or median and range are given.

<table>
<thead>
<tr>
<th></th>
<th>PBSC Group</th>
<th>BM Group</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
<td>Recipient age</td>
<td>35 (5-56)</td>
<td>37 (1-55)</td>
</tr>
<tr>
<td>Donor age</td>
<td>33 (20-53)</td>
<td>36 (18-55)</td>
</tr>
<tr>
<td>Recipient sex (M/F)</td>
<td>56/51</td>
<td>60/47</td>
</tr>
<tr>
<td>Donor sex (M/F)</td>
<td>72/35</td>
<td>62/44</td>
</tr>
<tr>
<td>Diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early / Late disease</td>
<td>12/24</td>
<td>11/24</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early / Late disease</td>
<td>5/11</td>
<td>7/10</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP / AP / BC</td>
<td>43/6/2</td>
<td>43/6/3</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Aspartylglycosaminurea</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Recipient CMV serology (-/+</td>
<td>43/64</td>
<td>47/59</td>
</tr>
<tr>
<td>Donor CMV serology (-/+</td>
<td>61/46</td>
<td>58/48</td>
</tr>
<tr>
<td>Conditioning</td>
<td></td>
<td></td>
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<tr>
<td>TBI containing</td>
<td>75 (70%)</td>
<td>90 (84%)</td>
</tr>
<tr>
<td>Busulfan containing</td>
<td>31</td>
<td>17</td>
</tr>
<tr>
<td>ATG / OKT-3</td>
<td>25 / 11</td>
<td>21 / 16</td>
</tr>
<tr>
<td>GVHD prophylaxis</td>
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<td></td>
</tr>
<tr>
<td>CsA or MTX</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>CsA + Prednisolone</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CsA + MMF ± Pred</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>CsA + MTX ± Pred</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>CsA + MTX + Pred + MMF</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Follow-up, years</td>
<td>4.4 (2.3-7.3)</td>
<td>5.0 (0.7-8.4)</td>
</tr>
</tbody>
</table>

Early; CR1, Late; >CR1, CP; chronic phase, AP; accelerated phase, BC; blast crisis, TBI=Total body irradiation, CsA=Cyclosporine, MTX=Methotrexate, MMF=Mycomofetilphenolate, Pred=Prednisolone
**Table 2.** Bilirubin, platelet levels and immunosuppression in patients with extensive chronic GVHD after PBSCT and BMT.

<table>
<thead>
<tr>
<th></th>
<th><strong>PBSC (n=31)</strong></th>
<th><strong>BM (n=19)</strong></th>
<th><strong>p-value</strong></th>
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<tbody>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>15 (5-255)</td>
<td>16 (4-134)</td>
<td>Ns</td>
</tr>
<tr>
<td>Platelet (x10^9/L)</td>
<td>137 (9-300)</td>
<td>166 (25-300)</td>
<td>Ns</td>
</tr>
<tr>
<td>Response to prednisolone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No/partial/Yes)</td>
<td>12/8/11</td>
<td>4/5/10</td>
<td>Ns</td>
</tr>
<tr>
<td>IS stopped? (Yes/No)</td>
<td>8/23</td>
<td>5/14</td>
<td>Ns</td>
</tr>
<tr>
<td>Time to cessation of IS</td>
<td>963 (405-2182)</td>
<td>1265 (526-2096)</td>
<td>Ns</td>
</tr>
</tbody>
</table>

PBSCT; peripheral stem-cell transplantation, BMT; bone marrow transplantation, IS; immunosuppression.
LEGEND TO FIGURES

Figure 1. Cumulative incidence of extensive chronic GVHD. Time to and cumulative incidence of extensive chronic GVHD after unrelated donor stem cell transplantation with peripheral blood stem cells (PBSC) or bone marrow (BM).
Figure 1.

Extensive chronic GVHD

Cumulative Incidence

PBSC, 39%

BM, 24%

Days after HSCT

0 365 730 1095 1460 1825 2190 2555 2920 3285
Increased risk of extensive chronic graft-versus-host disease after allogeneic peripheral blood stem cell transplantation using unrelated donors

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