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Allogeneic peripheral blood stem cell transplantation for standard risk leukemia: experience of İbni Sina Hospital

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Summary:

Fifty-three patients with standard risk leukemia who underwent allogeneic peripheral blood stem cell transplantation (alloPBSCT) from their HLA-identical siblings were analyzed for engraftment, incidence and severity of GVHD, and relapse rate. Standard risk leukemia was defined as AML in first complete remission or CML in first chronic phase within the first year after diagnosis. The median age was 34.5 years (range 13–47). Stem cells were mobilized by using 10 μg/kg G-CSF subcutaneously for 5 days. A median of 5.7 (2.1–21.4) $\times 10^6$/kg CD34$^+$ cells was collected over a median of 2 (range 1–5) apheresis procedures. Cyclosporin A (CsA) plus short-course MTX were used for GVHD prophylaxis. Recovery to granulocytes $>0.5 \times 10^9$/l and platelets $>20 \times 10^9$/l occurred at a median of day +13 (range 8–32) and +13 (range 8–51), respectively. Day +100 transplant-related mortality was 13.2% (7/53). Acute GVHD occurred in 20 of 49 (41%) evaluable patients and only six (12.3%) of them had severe disease (grade III-IV). Chronic GVHD occurred in 30 of 42 (71.4%) evaluable patients. Relapse rate at 2 years was 7.5%. The median overall and leukemia-free survivals were 22 (4–44) and 20 (3–44) months, respectively. Estimated 4 year leukemia-free and overall survival rates were 60% and 62%, respectively. In conclusion, alloPBSCT in standard risk leukemia seems to be associated with a low relapse rate and no increased risk of acute GVHD, but there is a trend for higher incidence of cGVHD. *Bone Marrow Transplantation* (2000) **25,** 1229–1232.

Keywords: allogeneic peripheral blood stem cell transplantation; standard risk leukemia; graft-versus-host disease

The use of G-CSF-mobilized allogeneic PBSC transplantation (alloPBSCT) has increased during the last 5 years.[1–7] This procedure now constitutes up to 30% of all allogeneic hematopoietic stem cell transplants in Europe and USA. No serious side-effects due to G-CSF have been reported so far. While faster neutrophil and platelet engraftment with shorter duration of hospitalization and lower antibiotic use have been seen compared to BMT,[8,9] whether or not GVHD is more common and the relapse rate is different, is not yet clear.[8–17]

In this report, we summarize our experience with alloPBSCT in standard risk leukemia: AML in first complete remission (CR), or CML in early first chronic phase.

Patients and methods

Patients

Fifty-three patients with standard risk leukemia underwent alloPBSCT at the İbni Sina Hospital between 1995 and 1999. Patient characteristics are shown in Table 1. Standard risk leukemia was defined as AML in first CR (CR1) (26 patients), and CML in first chronic phase within the first year after diagnosis (27 patients).[18]

Donors

Stem cell donors were HLA-identical siblings. The median age was 30.5 (range 11–47). Twenty-nine were male and 24 were female. There were 26 donor–recipient sex mismatches. Informed consent was obtained from the donors and patients.

HLA typing

Class I and II HLA antigens were analyzed serologically and by molecular techniques (SSO-SSP), respectively.

Table 1 Patients' and donors' characteristics

n	53
Recipient age (median)	34.5 (13–47)
Donor age (median)	30.5 (11–47)
Recipient sex male/female	36/17
Donor sex male/female	29/24
Sex mismatch	26
ABO blood group mismatch	21
Diagnosis	
AML	26
CML	27
Median time to transplant (months, range)	
CML	6.0 (4–11)
AML	7.5 (5–9)

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PBSC mobilization and collection

Recombinant human granulocyte colony-stimulating factor (rhG-CSF, Neupogen; Amgen, Roche, Istanbul, Turkey) was used for mobilization. Ten μg/kg G-CSF was given daily subcutaneously for 5 days. Apheresis was performed on day 5 using a continuous flow cell separator (COBE Spectra; COBE BCT, Lakewood, CO, USA). A total of 119 leukapheresis cycles (median 2, range 1–5) were performed using peripheral veins. The target was $>4 \times 10^6$/kg $CD34^+$ cells and processing twice the total blood volume of the donor. No manipulation was performed on the collected material and products were transfused immediately on the same day.

Flow cytometry

PBSC were analyzed by flow cytometry for hematopoietic progenitor cells (CD34; HPCA-2, Becton Dickinson, San Jose, CA, USA) and lymphoid sub-populations ($CD3^+$) using standard procedures.

Conditioning regimen and GVHD prophylaxis

All patients were conditioned with BU 16 mg/kg p.o. and CY 120 mg/kg iv. Cyclosporin-A (CsA) (3 mg/kg/day i.v.) plus short-course MTX (15 mg/m^2 i.v. on day +1 and 10 mg/m^2 i.v. on subsequent days +3, 6 and 11) were used for GVHD prophylaxis in all patients. After day +180 CsA was tapered (25 mg every 3 weeks) if the patient had no sign of GVHD. Diagnosis and grading of acute GVHD (aGVHD) were based on standard criteria.[19]

Engraftment

None of the patients received post-transplant hematopoietic colony-stimulating factors. Engraftment was defined as the first day of a granulocyte count $>0.5 \times 10^9$/l and an unsupported platelet count $>20 \times 10^9$/l for 3 consecutive days.

Chimerism study

In order to detect chimerism, we used HLA polymorphism, sex mismatches, and ABO blood group mismatches. We used VNTR for HLA polymorphism, FISH for sex mismatches and ABO group typing for ABO mismatches. All the above analyses except ABO typing were performed on bone marrow samples on the day of discharge and on day +100.

Statistical analysis

Disease-free survival and overall survival were estimated by using the method of Kaplan and Meier.[20]

Table 2 Engraftment characteristics

Variable	*Median (range)*
Neutrophil $>0.5 \times 10^9$/l (days)	13 (8–32)
Neutrophil $>1.0 \times 10^9$/l (days)	15 (9–44)
Platelet $>20 \times 10^9$/l (days)	13 (8–51)
Platelet $>50 \times 10^9$/l (days)	14 (10–74)
Febrile neutropenic days	4 (0–23)
Duration of antibiotics	10 (0–30)
RBC transfusions	1 (0–12)
PLT transfusions (filtered single donor)	24 (6–156)

Results

Collection and engraftment

The median number of aphereses was 2 (1–5). No severe adverse effects due to apheresis were seen. None of the donors needed central venous catheter insertion. All the collections were performed on an outpatient basis. Administration of G-CSF was well tolerated among donors except for mild to moderate bone pain relieved by oral acetaminophen. Patients received a median of 5.5 (2.6–14) $\times 10^8$/kg MNC or 5.7 (2.1–21.4) $\times 10^6$/kg $CD34^+$ cells. All evaluable patients engrafted. The median time to granulocyte engraftment was day +13 (8–32) and to platelet engraftment day +13 (8–51) (Table 2). Granulocytes $>1.0 \times 10^9$/l and platelets $>50 \times 10^9$/l were achieved on days +15 and +14, respectively. There was no correlation between the $CD34^+$ cell dose and hematological recovery ($r = -0.162$ and $P = 0.29$).

Chimerism study

Complete chimerism (CC) was documented in the majority of our patients. Sex chromosome-based FISH (10 of 10) and conventional cytogenetics revealed CC in five patients (no metaphases could be gained in 11 patients) of sex mismatched patients. ABO blood grouping revealed conversion of blood groups to donor type in 85.7% (18 of 21) of relevant patients. By using variable non-tandem repeats CC was shown in 81.3% (13 of 16) of the patients studied.

GVHD

GVHD profile of the patients are given in Table 3. GVHD occurred in 20 of 49 (41.1%) evaluable patients. Grade III–IV was in six of 49 (12%) patients. Chronic GVHD was observed in 30 out of 42 (71.4%) evaluable patients. Nine

Table 3 GVHD profile of the alloPBSCT patients

AGVHD	
Grade I	2/49
Grade II–IV	18/49
Total	20/49 (41%)
CGVHD	
Limited	25/42
Extensive	5/42
Total	30/42 (71.4%)

Table 4 Organ involvement in chronic GVHD

	n = 28
Liver	25
Skin	14
Eyes	5
Gastrointestinal tract	4
Hematopoietic	3
Pulmonary	5

Table 5 Causes of death (*n* = 18)

Causes of death	*No.*
GVHD (total)	9
Acute	4
Chronic	5
Relapse	1
Aplastic death	4
Interstitial pneumonia	2
Hepatic failure	1
Veno-occlusive disease	1

patients died due to GVHD (four acute, five chronic). Organ involvement in cGVHD is shown in Table 4. Liver was the most commonly involved organ. Deterioration of liver function was detected in almost all the patients while tapering CsA after day +100. Median time to cGVHD was 4 (4–19) months. CsA administration could not be discontinued as planned in these patients because of cGVHD. Median time to CsA cessation was 16 (7–25) months.

Outcome

Median follow-up was 22 (11–44) months. Major causes of death are shown in Table 5. Day +100 transplant-related mortality was 13.2% (seven of 53). Thirty-five patients are still alive, 34 disease-free. Relapse occurred in five patients (three AML, two CML). One patient with CML relapsed with blast crisis 16 months post transplant while suffering from extensive cGVHD. Another patient in second chronic phase is receiving DLIs. One AML patient relapsing 4 months after transplantation achieved CR2 with standard induction therapy. Later on, donor leukocytes were infused from her original donor and complete chimerism was documented after DLI. This patient is still alive in CR2 17 months after DLI. The second AML patient refused further treatment and the third one died during induction therapy. Kaplan–Meier curves of overall and leukemia-free survival of the patients are shown in Figures 1 and 2. The estimated 5 year leukemia free- and overall survival rates are 60% and 62%, respectively.

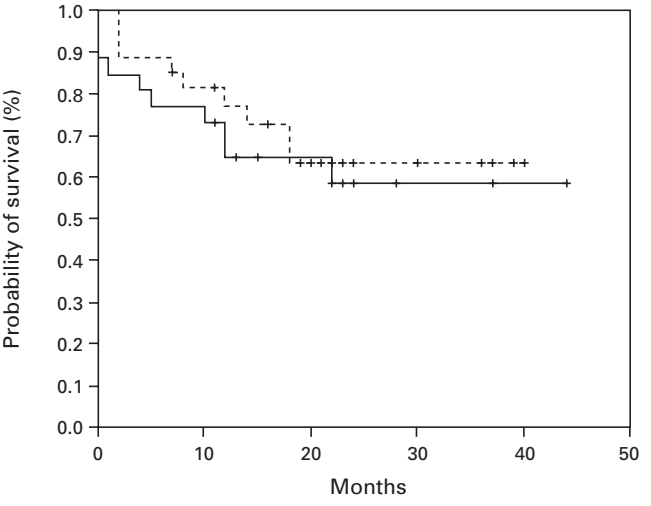


Figure 1 Kaplan–Meier curve for leukemia-free survival (—— AML, – – – CML).

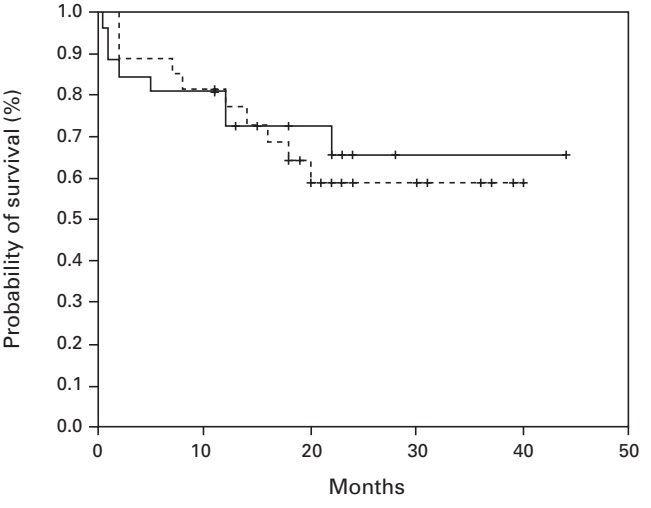


Figure 2 Kaplan–Meier curve for overall survival (—— AML, – – – CML).

Discussion

We have evaluated the kinetics of engraftment, the incidence of acute and chronic GVHD and relapse rate in a considerable number of standard risk leukemia patients, who were treated homogenously. In the current study, median granulocyte and platelet engraftments were on day +13 and +13 respectively. In our historical 40 alloBMT patients, some of whom were high risk patients, engraftment of granulocytes and platelets occurred on days +19 (12–50) and +27 (13–70), respectively.[11] Clearly, faster hematological recovery is seen in standard risk leukemia patients after alloPBSCT. The overall incidence of aGVHD (41%) and of grade III–IV aGVHD (12%) were comparable to those reported in the literature. Whether the incidence of cGVHD increased in patients undergoing alloPBSCT is still controversial.[8–17] There appears to be a trend towards a higher incidence of cGVHD.[18] Our previous retrospective comparative study (containing standard as well as high risk patients) showed an increased cGVHD compared to bone marrow (24.1% *vs* 78.1%).[11] The current study, comprising only standard risk patients, revealed that 30 out of 42 patients (71.4%) developed cGVHD. In AML patients treated with alloPBSCT, we have seen cGVHD in 70% of

the patients.[21] Although randomized trials will resolve this issue, it is our opinion that cGVHD is more common in alloPBSCT compared to bone marrow transplantation.

The relapse rate in this study was low (five of 42). Two reasonable explanations may account for this result. The first is that this study included standard risk leukemia patients. They were under 35 years old and were transplanted during the early phases of their diseases. However, even in these circumstances, according to EBMT and IBMTR data, the 2-year relapse rates for AML and CML patients are 15–20% after allogeneic bone marrow transplantation. The second explanation is that a graft-versus-leukemia effect and/or cGVHD due to increased T cell doses may be responsible. We do not know whether other transplant-related variables specific for alloPBSCT, such as high dendritic cell content of the harvest material, may have contributed to this low relapse rate.

In conclusion, in standard risk leukemia patients, alloPBSCT is an alternative to allogeneic bone marrow transplantation because of the ease of collection and rapid hematological recovery. In our study, alloPBSCT was not associated with increased risk of acute GVHD, but there was a suggestion of increased cGVHD. In addition, it was also associated with an unusually low relapse rate.

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