The Role of ABO Incompatibility in Allogeneic Peripheral Blood Stem Cell Transplantation

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ABSTRACT

ABO incompatibility is not a contraindication for allogeneic bone marrow transplantation, but this procedure requires an extra effort for erythrocyte or plasma depletion in certain well established conditions. Some acute or delayed immunohematological complications such as acute or chronic hemolysis and pure red cell aplasia may be encountered. In this study the outcome and transplant related complications of ABO incompatible and identical cases, who have received allogeneic peripheral blood stem cells from their HLA identical siblings were compared with each other. Ninety-one patients (CML 36, AML 37, other 18) were analyzed retrospectively including 51 (60.4%) ABO identical patients and 36 (39.6%) ABO mismatched (MM) patients, who have a bi-directional MM (n= 5), major MM (n= 16), minor MM (n= 9) and Rh MM (n= 6). Median follow up was 13 (0.5-43.0) months. We did not observed any significant differences between two groups (identical vs non-identical) in terms of acute hemolysis preceding stem cell infusion, peritransplant transfusion demand, acute- and chronic graft versus host disease. There was no change in estimated disease free survival and overall survival durations. We did not observed any influence of ABO/Rh incompatibility on short term outcome in allogeneic peripheral blood stem cell transplantation in our series and did not recommend further manipulation of the infused stem cells.

Key Words: ABO incompatibility, Allo-PBSCT, Hemolysis.

Turk J Haematol 2002;19(3): 401-409

Received: *21.11.2001* ***Accepted:*** *30.06.2002*

INTRODUCTION

Over the last few years there has been an increase in the use of allogeneic peripheral blood stem cell transplantation (alloPBSCT) for the treatment of various hematological malignancies. Advantages of using peripheral blood stem cell (PBSC) include the relative ease of collection and the rapid hematological reconstitution compared to bone marrow (BM). ABO incompatibility is not a

contraindication for allogeneic BM and stem cell transplantation, but this procedure requires an extraeffort for erythrocyte or plasma depletion in certain circumstances[1]. Some acute or late immunohematological complications such as acute or chronic hemolysis and transient pure RBC aplasia (aregeneratoric anemia) might be encountered during posttransplant follow-up[2]. In this retrospective analysis, ABO incompatible cases underwent alloPBSCT in a single center were compared with ABO identical patients in terms of engraftment, transfusion requirements, GVHD and overall survival.

MATERIALS and METHODS

Patients

From 1995 until January 1999, ninety-one patients (CML 36, AML 37, other 18) who received alloPBSC form their HLA identical siblings were included into the analysis. The characteristics of the patients are summarized in Table 1. Fifty-five patients (60.4%) were ABO/Rh compatible and 36 (39.6%) were ABO/Rh incompatible (Table 2). There were no statistically significant difference between the groups.

Conditioning Regimen

All patients received busulfan (Bu) 1 mg/kg PO. four times a day x 4 days and cyclophosphamide (Cy) 60 mg/kg IV daily x 2 days). Median follow up was 13 (0.5-43.0) months.

ABO/Rh Incompatibility

The transplants were designated as ABO identical, if donor and recipient had the same blood type. If the recipient has hemagluttinins against donor's red blood cells this condition was designated as major ABO incompatibility and if vice versa minor. Bi-directional incompatibility was the presence of both major and minor ABO mismatches. Rh incompatibility was defined as patients with the same ABO blood group but different Rh group.

PBSC Collection

After priming the donors with rhG-CSF at the dose of 10 µg/kg s.c. for 5 days, PBSCs were collected using continuous flow cell separators (Cobe Spectra, COBE BCT, Inc, Lakewood, CO. or Fenwall CS 3000, Baxter Heatthcare Systems, Deerfield, IL). PBSC enumeration was performed by Procount® progenitor cell enumeration system kit (Becton Dickinson, Sanhose, USA). Donor's venous access was obtained always through peripheral veins. Median number of leukapheresis cycles was 2 (range 1-4). After each collection, apheresis products were transfused immediately on the same day without manipulation and this day was taken into account as day "0".

Transfusion Support

Patients were transfused with packed red cells to maintain a hematocrit greater than 24% and with platelets to keep the platelet count above 20 x 10^9/L. The ABO type of blood components transfused after transplantation was defined by the blood groups of donors and recipients. Our center's transfusion policy in such conditions was reviewed very recently[3].

Prophylaxis and Treatment of GVHD

Diagnosis and grading of acute GVHD was based on the Glucksberg clinical criteria. Patients were evaluated for acute GVHD if they survived 21 days and had evidence of engraftment. All patients received GVHD prophylaxis consisting of cyclosporine (CsA) 1.5 mg/kg/ IV twice a day started on day-1 and continued to day + 180 followed by 15 mg/m^2 methotrexate IV on day-1 and 10 mg/m^2 on days 3 and 6, as previously described. When clinical grade II-IV acute GVHD was established, methylprednisolone was initiated. ATG or ALG was added to this treatment if no improvement or progression were observed. Chronic GVHD was treated with CsA plus methylprednisolone and supportive measures.

Engraftment

The day of granulocyte engraftment was defined as the first of 3 consecutive days with a granulocyte count of > 0.5 x 10^9/L. Platelet engraftment was defined as an unsupported platelet count of > 20 x 10^9/L for 3 consecutive days. The identification of sex or blood group mismatches and VNTR polymorphism were used for detection and monitorization of patients' chimeric status.

Table 1. Patients' characteristics

	ABO/Rh identical n= 55	ABO/Rh mismatched n= 36
Median recipient age, years (range)	35 (14-58)	33 (16-47)
Median donor age, years (range)	30.5 (15-49)	31.5 (11-46)
Recipient sex (female/male)	19/36	14/22
Donor sex (female/male)	25/30	14/22
Sex mismatch	25	14
Median time from diagnosis to Transplantation, months (range)	7 (6-15)	8 (3-17)
Diagnosis		
AA	2	-
ALL	2	3
AML	25	12
HD	1	-
CML	18	18
MDS	3	2
MM	3	0
NHL	1	1

AA: Aplastic anemia, ALL: Acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, HD: Hodgkin's disease, CML: Chronic myeloid leukemia, MDS: Myelodysplastic syndrome, MM: Multiple myeloma, NHL: non-Hodgkin's lymphoma.

Table 2. ABO compatibility between donor and recipient

Type	n	%
ABO identical	55/91	60.4
ABO mismatched	36/91	39.6
Major	16	44.4
Minor	9	25.0
Bi-directional (major&minor)	5	13.8
Rh	6	16.6

Statistics

Differences between two groups in terms of recipient and donor age, time to transplantation, mononuclear and $CD34^+$ cells count, RBC and platelets transfusion were compared by Wilcoxon rank-sum (Mann-Whitney) test. Chi-square test was used to compare differences among the two groups for; recipient and donor sex mismatch, ABO mismatch, diagnosis, aGVHD grade I-II and grade III-IV, cGVHD, relapse, transplant related mortality, and causes of death. Disease free survival and overall survival were compared by using the method of Kaplan Meier with long-rank analysis.

RESULTS

The median value of collected MNC and $CD34^+$ cells in ABO/Rh identical and ABO/Rh mismatched group were 6.6 vs. 6.2 x 10^8/kg and 5.8 vs 5.4 x 10^6/kg ($p > 0.05$), respectively (Table 3). Median time to neutrophil and platelet recovery was similar in both groups (15 vs 14 days) ($p > 0.05$) and (14.5 vs 15 days) ($p > 0.05$), respectively (Table 3).

Acute hemolysis due to transfusion was observed only in one patient with major ABO incompatibility. The patient had a stable course under hyperhydration and there was no need for further therapeutic approach. In each group there was one case of transient pure RBC aplasia.

Transfusion requirements of both groups were summarized in Table 3. There was no significant difference between two groups for RBC and PLT transfusion demand (p > 0.05).

In both groups, the incidence of aGVHD (grade 2) was similar (Table 4). The incidence of cGVHD was 64.3% in the ABO/Rh identical group compared to 61.4% in incompatible group (Table 4).

The relapse rate was 22.7% in the ABO identical group and 17.9% in the ABO incompatible group (p > 0.05) (Table 4).

In the ABO identical group, 18 patients died; GVHD (n= 6), infection (n=1), aplastic death (n= 3), relapse (n= 6), hepatic failure (n= 1) and cerebral hemorrhage (n= 1) (Table 5). The causes of death in 16 patients of the ABO incompatible group were GVHD (n= 7), relapse (n= 2), aplastic death (n= 2), interstitial pneumonia (n= 1), infection (n= 2), suicide (n= 1) and VOD (n= 1) (p > 0.05).

Overall mortality rate was similar both in the ABO/Rh identical and incompatible group, 32.7% (18/55) and 44.4% (16/36), respectively (p> 0.05).

In the ABO identical group median disease-

Table 3. Infused cells and engraftment kinetics

Variables	ABO/Rh identical n= 55	ABO/Rh inmatched n= 36
Number of apheresis	2 (1-4)	2 (1-4)
$CD34^+$ cells count infused (x 10^6/kg)	5.8 (1.8-18.9)	5.4 (0.8-19.4)
MNC count infused (x 10^8/kg)	6.6 (3.2-9.3)	6.2 (2.5-38.2)
Median time to ANC > 0.5 x 10^9/L, days (range)	13 (8-32)	13 (8-28)
Median time ANC > 1.0 x 10^9/L, days (range)	15 (9-44)	14 (9-30)
Median time to platelet count > 20 x 10^9/L, days (range)	13 (6.35)	13 (9-51)
Median time to platelet count > 50 x 10^9/L, days (range)	14.5 (10-54)	15 (10-85)
No. of red cell concentrates transfused	1 (0-12)	2 (0-14)
No. of random or single donor platelets transfused*	4 (1-25)	4 (1-21)

* Either.
MNC: Mononuclear cells, ANC: Absolute neutrophil count.

Table 4. Transplant related complications and disease outcome

	ABO/Rh identical n= 55	ABO/Rh mismatched n= 36
Acute GVHD	19/48 (35.6%)	13/32 (40.6%)
Grade I-II	14/48 (29.2%)	8/32 (25.0%)
Grade III-IV	5/48 (10.4%)	5/32 (15.6%)
Chronic GVHD	27/44 (61.4%)	18/28 (64.3%)
Relapse	10/44 (22.7%)	5/28 (17.9%)
Pure red cell aplasia	1/44 (2.3%)	1/28 (3.6%)
Median disease free survival, months (range)	11 (0-43)	8 (0-38)
Median overall survival, months (range)	11 (0.5-43)	8 (0.5-38)

* p > 0.05.

Table 5. Survival characteristics

	ABO/Rh identical n= 55	ABO/Rh mismatched n= 36
Alive	37/55 (67.3%)	20/36 (55.5%)
Death*	18/55 (32.7%)	16/36 (44.4%)
Transplant related mortality** (the first 100 days)	10/55 (18.1%)	9/36 (25.0%)
Causes of death		
GVHD (total)	6	7
Acute	3	4
Chronic	3	3
Infection	1	2
Relapse	6/55 (10.9%)	2/55 (3.6%)
< 28 day	3	2
Interstitial pneumonia	0	1
Hepatic failure	1	0
Suicide	0	1
Cerebral hemorrhage	1	0
Veno occlusive disease	0	1

* p = 0.25, ** p = 0.43.

free survival (DFS) and overall survival (OS) were 11 months (range 0-43) and 11 months (range 0.5-43), respectively. In the ABO/Rh mismatched group median DFS and OS were 8 months (range 0-38) and 8 months (range 0.5-38), respectively (Table 4). The estimated DFS on the 36th month was 45.1% for ABO/Rh identical group and 51.7% for ABO/Rh incompatible group (p= 0.52), and the estimated OS was the same 53.4%, both for ABO/Rh identical and incompatible group (p = 0.26).

DISCUSSION

ABO/Rh incompatibility is not an absolute contraindication for AHSCT but some important complications such as early or delayed hemolysis, slower RBC recovery and transient pure RBC aplasia are the preceding complications. According to clinical results published in the 1970s, it is believed that the clinical outcome of ABO identical and incompatible AHSCTs are equivalent. Bone marrow and peripheral blood progenitors are the two commonly used stem cell sources. Bone marrow has been still in use as stem cell source since early 1970s. Peripheral blood progenitors are more extensively used after 1990s. Although peripheral blood stem cell transplantation had the advantage of accelerated neutrophil and platelet engraftment kinetics, the marked increase in graft versus host disease shown in our series and by many centers, is an important emerging disadvantage of alloPBSCT[4]. In spite of several reports about the role of ABO compatibility in allo BMT, there are a few reports about the outcome of alloPBSCT in ABO incompatible patients[5-7].

Forty percent of all AHSCTs were ABO/Rh incompatible (15-20% major incompatible and 20% minor incompatible)[8]. Benjamin et al reported the rate of ABO/Rh incompatible patients as 28.9%[9]. In our analysis, total, major, minor, bi-directional, and Rh mismatched transplant rates were 39.6%, 17.6%, 9.9%, 5.5% and 6.6% respectively and was concordant with the literature.

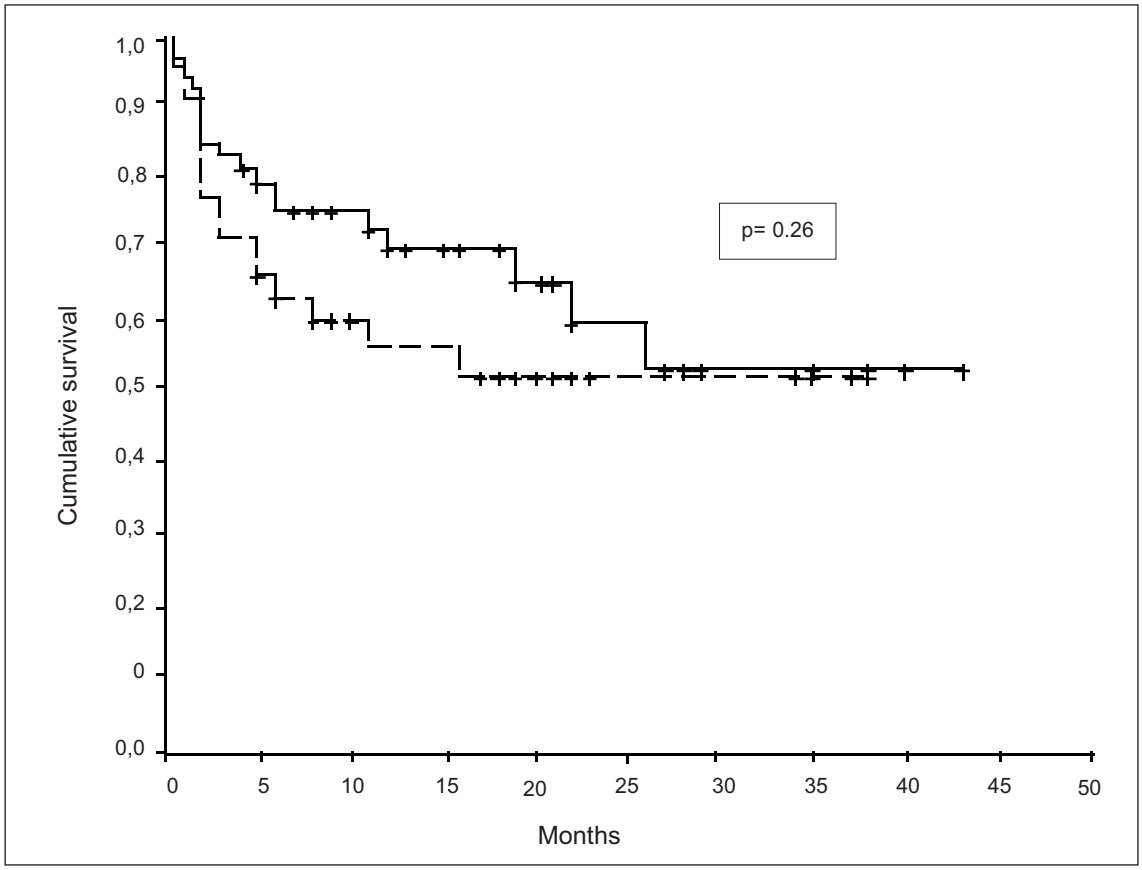

Figure 1. Kaplan-Meier curve of overall survival (— ABO/Rh identical patients, --- ABO/Rh inmismatched patients).

ABO/Rh blood group incompatibility is not an absolute contraindication for AHSCT but some important complications such as early or delayed hemolysis, transient pure RBC aplasia are the preceding complications. According to clinical results published in the 1970s, it is believed that the clinical outcome of ABO identical and incompatible AHSCTs were equivalent.

If hematopoietic stem cell source is bone marrow with major ABO incompatibility, erythrocytes must be removed for the prevention of acute intravascular hemolysis. First complication detected after stem cell infusion is acute intravascular hemolysis, which is seldom detected in minor mismatched patients[10]. Routinely within allo BMT harvest 150-300 mL RBCs are infused, 98% of RBC volume should be removed from stem cell product for successful prevention of acute intravascular hemolysis in case of major ABO MM[11]. Different techniques may be used for red cell depletion such as manual technique or using cell separator[12]. In peripheral blood stem cell products there was no need for erythrocyte depletion in cases of with ABO incompatibility and as a rule there should be less than 15 mL.s RBC within stem cell product[13]. Acute hemolysis is detected in only one patient with major ABO/Rh incompatibility and the patient had a stable clinical transplant course.

Delayed hemolysis is the second common complication seen in ABO incompatible patients. Although most of the patients are major incompatible, delayed hemolysis is detected also in patients with minor ABO incompatibility[13-15]. Delayed hemolysis was treated with erythropoietin and corticosteroids in some cases plasmapheresis may be useful[16,17].

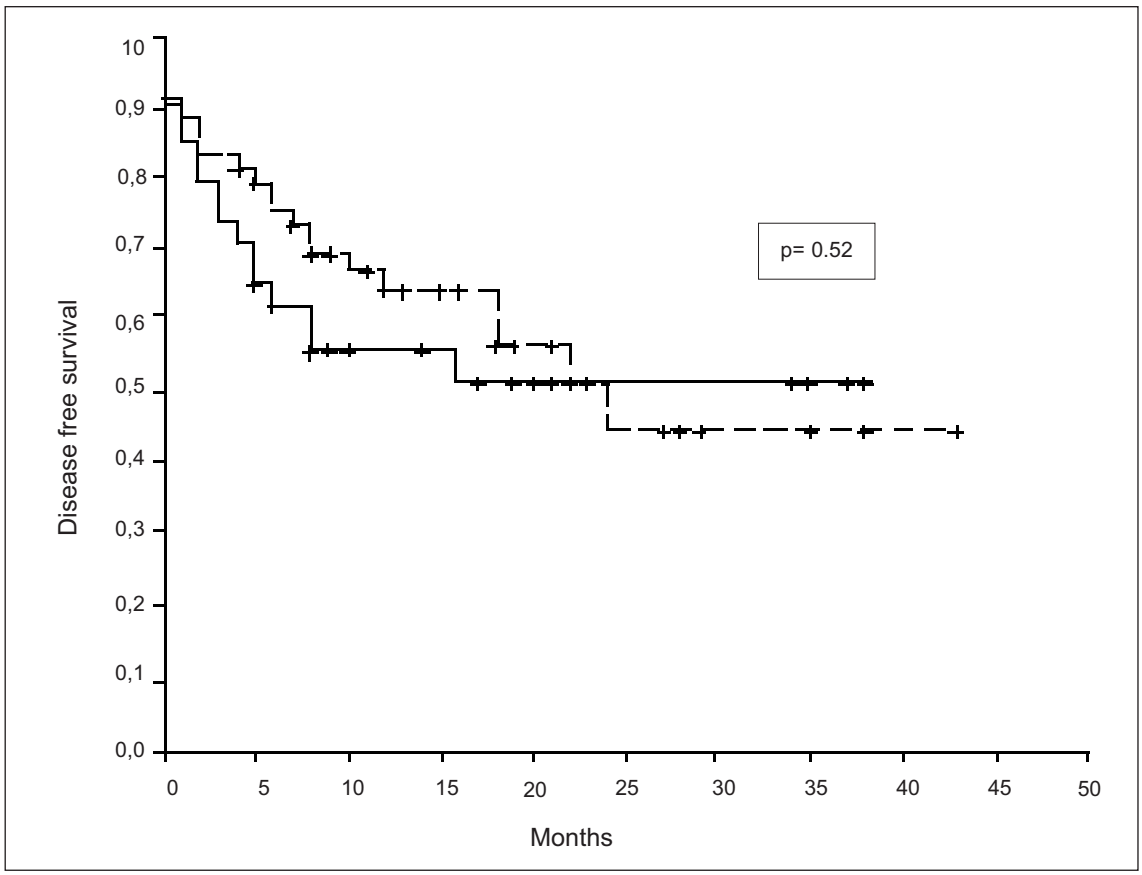

Figure 2. Kaplan-Meier curve of disease free survival (— ABO/Rh identical patients, --- ABO/Rh mismatched patients).

Most of the studies showed that neutro- phil and platelet recovery times were not affected in ABO/Rh incompatible patients[1,18,19]. These results were in concordance with our engraftment kinetics. Erythrocyte engraftment is effected in these patients in contrast to platelet and neutrophil engraftment. Time of erythrocyte engraftment is longer in major ABO incompatible patients than minor ABO/Rh minor incompatible and compatible patients[9]. Though some authors showed increased erythrocyte requirements and transfusion dependence, several studies like ours did not confirm these results[1,8,18-20].

Delayed erythrocyte engraftment is a common entity after ABO incompatible AHSCT. Sometimes this complication lead to transient pure RBC aplasia[18,19,21-23]. Corticosteroids, high dose erythropoietin, intravenous immunoglobulin and seldom anti-thymocyte globulin and in refractory cases therapeutic plasma exchange are recommended therapeutic measures[9,24-26].

Bacigalupo et al and Benjamin et al reported increased aGVHD in patients with ABO incompatibility[16,27,28]. But some authors did not confirm such an unfavourable effect of ABO incompatibility[1,8,18-20]. Overall survival and disease free survival are not different between two groups[16,17]. We did not observed any difference between two groups in terms of either GVHD or survival. Overall survival curve showed that early mortality was higher in ABO/Rh incompatible patients but this difference was not statistically significant.

Risk of 100 days mortality has been found increased to 1.86 fold in ABO/Rh incompatible 292 patients who underwent allogeneic bone marrow

transplantation[14]. This increment was shown both in major and minor ABO incompatible patients and was not associated with GVHD and engraftment failure. The major causes of death were multiple system dysfunction and sepsis. This effect was obvious in patients with AML and MDS but not in CML.

We did not observe such a major drawback in our series of patients and could not demonstrate any detrimental effect of ABO incompatibility in allogeneic PBSCT. Further follow-up and addition of bone marrow stem cell recipients to our database are necessary for drawing firm conclusions about the effect of ABO incompatibility on nonrelapse morbidity and mortality after hematopoietic cell transplantation.

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