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ABO Incompatibility is Associated with Increased Non-Relapse and GVHD Related Mortality in Patients with Malignancies Treated with a Reduced Intensity Regimen: A Single Center Experience of 221 Patients

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ABSTRACT

The effect of ABO-incompatibility on transplantation outcome remains a controversial issue, with many of the reported studies showing conflicting results. In this study, we evaluate: the association between ABO-incompatibility and myeloid engraftment; the incidence and severity of acute and chronic graft-versus-host disease (GVHD); non-relapse mortality (NRM); GVHD-associated mortality, relapse and overall survival (OS). Our study includes 221 patients with malignant diseases treated in the same institution with the same reduced intensity regimen. Other variables known to affect the transplantation outcome such as age, disease, disease risk, and donor characteristics were well-balanced between ABO-matched and ABO-mismatched transplants. Analysis of our data shows increased incidence of NRM during the first months after transplantation in the groups of patients with major and minor ABO-incompatibility. Although neither incidence nor severity of GVHD differed significantly among the different groups, we found increased mortality associated with GVHD in the major ABO-incompatible groups. Long-term OS and relapse rate were not different, although we observed a trend for decreased OS during the first year post transplantation in the group of patients with major ABO-incompatibility. Our study showed that ABO-incompatibility has an adverse impact on the transplantation outcome.

KEY WORDS

Hematopoietic stem cell transplantation • ABO blood groups • GVHD • NST • Survival • Engraftment

INTRODUCTION

The ABO blood group system discovered by Karl Landsteiner in 1900 is one of the most important discoveries in clinical medicine; it resulted in the introduction of safe blood transfusions [1]. Allogeneic bone marrow transplantation (BMT) or peripheral blood stem cell (PBSC) transplantation has a high curative potential in various hematological malignancies, as well as in various inherited non-malignant disorders. Although major ABO-incompatibility is a serious barrier to solid organ transplantation, allogeneic BMT can be performed successfully across ABO-mismatching between the donor and the recipient [2]. ABO-mismatching can be classified as follows: major ABO-incompatibility is characterized by the presence of anti-donor isohemagglutinins (e.g. A to O); minor ABO-incompatibility by the ability of the transplanted marrow to produce anti-host isohemagglutinins (e.g. O to A); and bi-directional incompatibility by a combination of both (e.g. A to B) [2]. BMT across major ABO-incompatibility can be accomplished following elimination of red blood cells (RBC) prior to infusion of stem cells. In the earliest days of allogeneic BMT there was significant concern that ABO-mismatching might lead to increased complications such as graft rejection, graft-versus-host disease (GVHD), and/or

severe hemolytic reactions. Early studies suggested that ABO-mismatched allogeneic BMT fared nearly as well as ABO identical transplants with the exception of occasional delayed RBC engraftment because of prolonged hemolysis or pure red cell aplasia (PRCA). However, these early studies were based on a small number of patients and were performed decades ago using myeloablative conditioning [3,4]. In contrast, recent studies evaluating the significance of ABO-mismatching gave conflicting results, and this issue remains controversial [5,6]. In particular, several lines of evidence suggest that ABO-incompatible stem cell transplantation (SCT) may be associated with an increased risk of GVHD and treatment related mortality (TRM) [7]. The most recent multi-center assessment of 3,103 patients who received conventional myeloablative BMT for leukemia did not show any substantial effect of ABO-incompatibility on the outcome [8]. Moreover, this issue is further complicated by recent developments in the area of allogeneic SCT. PBSC is being increasingly used instead of marrow because it allows more rapid hematologic and immune reconstitution. In contrast to bone marrow, PBSC contains high numbers of T and B lymphocytes, as well as erythrocyte precursors. Indeed, in PBSC transplantation, delayed massive and fatal immune hemolysis from rapid alloantibody production by the high number of donor passenger B lymphocytes has been reported in cases of minor or bi-directional ABO incompatibility [9-11]. Also, in recent years, successful SCT has been established following non-myeloablative (NST) or reduced intensity regimens (RIC) [12-14]. The RIC related toxicity associated with these so-called "mini-transplants" resulted in the extended application of allogeneic SCT to older patients with concurrent co-morbidities, formerly considered ineligible for conventional myeloablative SCT. Recent studies evaluating the significance of ABO-incompatibility in the setting of NST (RIC), resulted in controversial findings [15,16].

In the present study, we analyzed the effects of ABO-mismatching in a group of 221 patients with malignant disorders treated with the same RIC regimen in a single institution.

PATIENTS AND METHODS

Patients

We analyzed 221 consecutive patients with malignant diseases, referred to the Department of Bone Marrow Transplantation and Cancer Immunotherapy of the Hadassah University Hospital in Jerusalem between 1996 and 2005, and treated with the same RIC regimen. The study protocol was approved by the ethical committee of our hospital. All patients signed informed consent agreements. The patients were divided into the following 3 groups according to the ABO status between donor and recipient: Group 1, patients transplanted from ABO-matched donors (n=127); Group 2, patients transplanted from donors with minor ABO-incompatibility (n=38); Group 3, patients transplanted from donors with major ABO-incompatibility, or from donors with bi-directional incompatibility (n=56). Characteristics of the patients are listed in Table 1.

Transplantation Procedure

Conditioning regimen. For 221 patients with malignant disease, allogeneic SCT was performed using the same RIC protocol. Conditioning consisted of fludarabin 30 mg/m^2/day x 6, oral busulfan 4 mg/kg/day (or i.v. busulfex 3.2 mg/kg/day) x 2, and antithymocyte globulin (ATG) (Fresenius) 10 mg/kg/day x 4. Oral busulfan was given to 163 patients, whereas 58 patients were treated with the intravenous formulation. The day of a first marrow infusion was designated as day (d) 0.

GVHD prophylaxis. All patients received GVHD prophylaxis with cyclosporine A (CSA) with an initial i.v. dose of 3 mg/kg or equivalent oral dose of 6 mg/kg with subsequent control of serum levels and renal function tests, initially from d-1 and subsequently from the d-4.

Donors. The donors were HLA-A, -B, -C and high resolution -DR fully matched siblings in 149 cases, matched unrelated donors in 50 cases, and matched non-sibling family members in 4 of the cases. One-locus mismatched family members were donors in 12 patients, whereas one-locus mismatched unrelated donors were used in 6 cases. PBSC was the source of stem cells in 201 patients; PBSC in combination with BM was the source for 8 patients; BM alone was used in 20 cases. BM was collected from iliac crest under epidural, spinal, or general anesthesia. For PBSC collections, donors were injected subcutaneously with granulocyte-colony stimulating factor (G-CSF; Neupogen ©, 5 μg/kg twice daily for 5 days) and mobilized PBSC were collected on days 5 and 6.

Supportive care. All patients were treated in HEPA-filtered positively pressured rooms. Prior to transplantation patients received acyclovir (1500 mg/m^2 per day) for prophylaxis of herpes simplex virus (HSV) and cytomegalo virus (CMV) infection. Patients transplanted from alternative donors received ganciclovir 10 mg/kg for 5 days before BMT. CMV infections (diagnosed by 2 successive positive PCRs or 1 positive CMV pp65 antigenemia) were treated with ganciclovir 10 mg/kg/day. TMP/SMX (10 mg/kg/d trimethoprim) was given to all patients from the beginning of conditioning until d-2 and after engraftment (absolute neutrophil count (ANC) $>0.75x10^9$/L) twice a week for 6 months as a preventive measure against *Pneumocystis carinii*.

Definitions. Day of neutrophil engraftment was defined as the first of 3 consecutive days with

Table 1. *Patient characteristics by ABO blood groups*

	ABO Matched	ABO Minor Mismatched	ABO Major Mismatch	Significance (chi-square)
Number of Patients:	127	38	56	
Age (Median)	40	38	41	NS ($P = .70$)
Sex (M:F)	81:46	28:10	33:23	NS ($P = .33$)
Disease:				
ALL	11	5	3	NS ($P = .35$)
AML	34	12	17	
CML	23	8	7	
NHL	34	4	10	
MDS/MF	10	5	8	
Other	15	4	11	
Disease Status:				
Low risk	37	11	15	NS ($P = .95$)
High risk	90	27	41	
Donor:				
HLA-matched siblings	91	20	38	NS ($P = .29$)
Other HLA-matched relatives	3	0	1	
Matched unrelated donors	25	13	12	
Mismatched donors (related/unrelated)	8 (6/2)	5 (2/3)	5 (4/1)	
Female to male	37	10	18	NS (P=.83)

ANC>$0.5x10^9$/L. Day of platelet engraftment was defined as the first of 3 days with platelets (PLT) >$20x10^9$ /L without any transfusion support. For acute and chronic GVHD (aGVHD, cGVHD) diagnosis and staging, standard previously published criteria were used [17,18]. The overall peak grade of aGVHD and cGVHD was reported in our study for all the statistical evaluations. Evaluable for aGVHD analysis were all patients that had a successful engraftment and survived for at least 4 weeks, whereas evaluable for cGVHD analysis were all patients who survived for 100 days. Non-relapse mortality (NRM) was defined as mortality not directly associated with relapse or progression of the original disease. Deaths from GVHD provoked by immunotherapeutic approaches such as donor leukocyte infusions (DLI) given for disease relapse or progression were not classified as NRM. GVHD-related mortality was defined as mortality associated with GVHD either directly or indirectly. Death from infection in a patient treated with an immunosuppressive regimen for GVHD was considered a GVHD-related death. Patients were stratified to high or low risk categories according to the disease status prior to the transplantation. Namely, patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL) in first complete remission, chronic myelogenous leukemia (CML) in first chronic phase, myelodysplastic syndrome (MDS) without excess of blasts (RA or RARS), and non-Hodgkin lymphoma (NHL), Hodgkin disease (HD) or multiple myeloma (MM) without chemorefractory disease were classified as low-risk patients. All other patients were classified as high-risk patients [19,20]. Survival was calculated from the time of transplantation until the time of death from any cause.

Statistical analysis. For statistical analysis and graphic data representation we used SPSS software, version 12.0 for Windows. Assessments of median (M), average (X), standard deviation, and Student's t-test with two tailed distribution were used as parametric criteria, whereas categorical data were compared by χ^2 or Fisher exact tests. Logistic regression analysis was used to evaluate the relative contribution of various predictors to the differences of categorical parameters, whereas linear regression analysis was used to evaluate the relative contribution of parametric variables. Cox's regression and Kaplan-Meier models were used for survival analysis.

RESULTS

The median follow-up period was 12 months with a range of 2 to 114 months. Factors known to affect the transplantation outcome such as age, disease, disease risk, donor status, sex disparity between donor and recipient, female donor to male recipient, were analyzed and were found to be well-balanced among the 3 groups of patients (Table 1).

Engraftment

Neutrophil and platelet engraftment were not different among the 3 groups. Neutrophil engraftment was achieved in a median of 16 days in all 3 groups. Platelet engraftment was achieved in a median of 12, 13, and 13 days in the ABO-matched, major ABO-mismatched, and minor ABO-mismatched group, respectively. No differences in the platelet and RBC transfusion requirements were found between the 3 groups of patients either (data not shown). In the group of major ABO-mismatch, 1 case of PRCA, and

1 case of prolonged hemolysis were observed; in the minor ABO-mismatch group, 1 case of transient hemolysis was observed. The rate of rejection was very low in our study (2.7%). Six of 221 patients rejected their grafts; 5 in the group of ABO-match and 1 patient in the group of major ABO-mismatch. Three of 6 patients who rejected the graft had been transplanted from matched unrelated donors, whereas 1 patient was transplanted from his one-locus HLA-mismatched mother. The rate of rejection did not vary among the 3 groups ($P > .50$).

GVHD

In the ABO-matched group, 116 patients were evaluable for aGVHD. Among them aGVHD grade II-IV, and III-IV were observed in 40 (34%), and 28 (24%) patients, respectively. Of 52 evaluable patients in the major ABO-mismatched group, 23 (44%), and 19 (36%) patients developed aGVHD grade II-IV, and III-IV, respectively. In the minor ABO-mismatched group among 31 evaluable patients, 12 (39%), and 9 (29%) developed aGVHD grade II-IV and III-IV, respectively. We observed a trend ($P < .10$) for increased incidence of severe aGVHD grade III-IV in the ABO-mismatched group (36%) in comparison with the ABO-matched group (24%) (Figure 1).

In the ABO-matched group, 100 patients were evaluable for cGVHD. Among them, 42 (42%) developed cGVHD; the cGVHD was extensive in 27 (27%). Among 39 evaluable patients in the major ABO-mismatched group, 15 (38%) developed cGVHD, and the cGVHD was extensive in 10 (26%). In the minor ABO-mismatched group, the cumulative incidence of cGVHD was 54% (13 of 24 evaluable patients), whereas extensive cGVHD was observed in 7 (29%) patients. The differences in the incidence and severity of cGVHD between the 3 groups were not significant ($P = .68$).

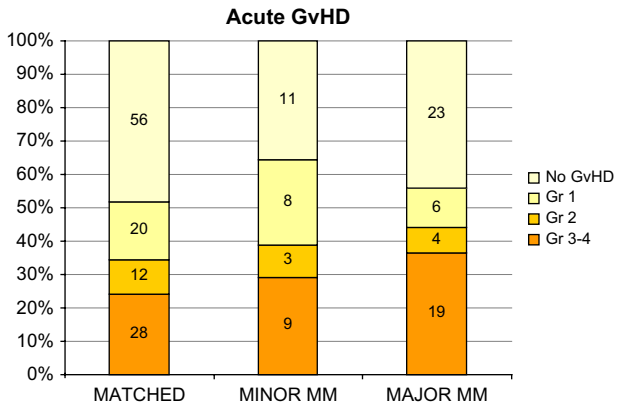

Figure 1. Cumulative incidence of acute GVHD (aGVHD): there is a trend for more severe aGVHD grade III-IV in ABO major mismatched (MM) group compared to ABO matched group ($P = .098$). (Here and in subsequent figures, absolute numbers of patients are shown in the appropriate fields.)

Non-relapse Mortality

In the ABO-matched group, the 1-year NRM was 22% (29 deaths among 127 patients). In the major ABO-mismatched group, the 1-year NRM was 39% (22 deaths among 56 patients). In the minor ABO-mismatched group the 1-year NRM was 31.6% (12 deaths among 38 patients). The 1-year NRM was significantly increased in the major-ABO ($P = .023$) and minor-ABO ($P = .045$) mismatched groups in comparison with ABO-matched group (Figure 2). NRM at 3 months was also significantly increased in the ABO-mismatched groups in comparison with the ABO-matched group ($P < .01$).

aGVHD and cGVHD associated Deaths

We tried to evaluate the cause of increased NRM in ABO-mismatched groups. Therefore, we analyzed the incidence of GVHD-associated deaths in the 3 groups of patients. In ABO-matched group, 10 of 127 patients died during the first year due to aGVHD and cGVHD-related causes (7.9%). In the major ABO-mismatched group, 11 of 56 (19.6%) patients died from GVHD, whereas in the minor ABO-mismatched group 5 of 38 (13.5%) patients died from the same causes. Distribution analysis showed that in comparison with the ABO-matched group the incidence of GVHD associated deaths was significantly higher ($P = .021$) in the major ABO-mismatched group (Figure 3).

Acute GVHD-associated Deaths

We tried to evaluate the response to therapy and the mortality from aGVHD between the 3 groups as well. In the ABO-matched, major ABO-mismatched, and minor-ABO-mismatched groups, 7.5%, 30%, and 25% of patients with aGVHD grade II-IV died from aGVHD, respectively ($P = .05$). Significantly

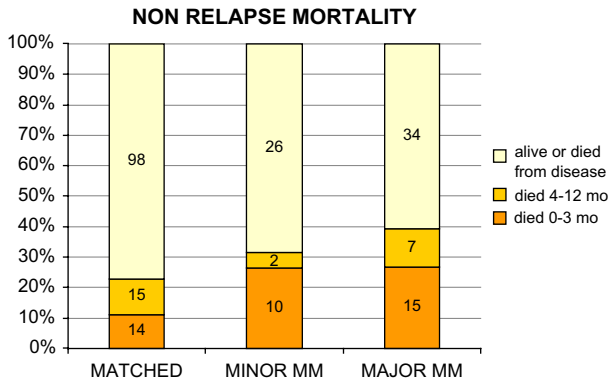

Figure 2. Non-relapse mortality is higher in ABO mismatched groups: $P = .045$ for minor and $P = .023$ for major mismatched groups, respectively.

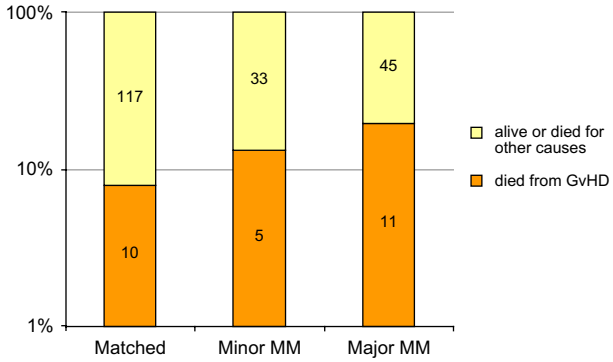

Figure 3. GVHD-associated mortality is higher in the ABO major mismatched group compared with the ABO matched group ($P = .021$).

more patients in the major ABO-mismatched group had aGVHD that was resistant to treatment and died from this complication in comparison with patients in the ABO-matched group ($P = .014$). Also, a trend for increased aGVHD-related mortality was observed among the minor ABO- mismatched group.

Relapse, Other Causes of Death, and Overall Survival

Within a median follow up of 12 months, 35 patients in the ABO-matched group died from relapse or disease progression, whereas 10 and 7 patients died from the same cause in the major ABO-mismatched and minor ABO-mismatched group, respectively. The relapse rate was not significantly different between the 3 groups. In addition, the minor mismatched group demonstrated a slightly higher incidence of deaths associated with infectious complications, such as sepsis including adult respiratory distress syndrome (ARDS) and development of multiorgan failure (MOF), as well as opportunistic infections, for example, invasive aspergillosis (37% vs. 22% and 28% in matched and major mismatched groups, respectively). These differences in distribution are significant according to chi-square test ($P = .037$, Figure 4). Except for a trend towards decreased overall survival (OS) in the major ABO-mismatched group during the first year after BMT, OS was not significantly different between the 3 groups (Figure 5).

Results of Multifactorial Analysis

OS, NRM in 3 and 12 months, and development of aGVHD and cGVHD were analyzed by a multifactorial model. Independent variables such as patient's age, female-to-male transplantation, disease risk group, aGVHD and cGVHD presence, grade (severity) and timing were included in the model. Using logistic regression and Cox's analysis, we confirmed that ABO-matching characteristics remained insignificant for OS ($P = .098$), whereas major poor prognostic factors were disease risk category ($P = .0001$), aGVHD, especially grade IV ($P < .0001$) as well as older age ($P = .049$, all according to Cox's analysis). At the same time, ABO-mismatching remains a significant predictor factor for NRM and GVHD- associated deaths at 3 or 12 months after BMT, as well as for development of cGVHD ($P = .031$).

DISCUSSION

The discrepancy of the results between different studies devoted to effects of ABO-incompatibility on transplant outcome can be explained in several ways. Reported studies are different in many aspects that may augment or reduce the significance of ABO-incompatibility. Some of the factors can be summarized as follows:

1) Variance in the intensity of the preparative regimen. RIC, in contrast with myeloablative preparative regimens, usually establishes a transient state of mixed chimerism that gradually converts to complete donor chimerism, either spontaneously or after manipulations such as withdrawal of cyclosporine or DLI. From a theoretical point of view, RIC regimens are anticipated to be associated with increased incidence of complications related to major ABO-incompatibility, because of the delayed elimination of host anti-donor B-lymphocytes. Indeed, delayed donor cell chimerism and increase incidence of PRCA were found by Bolan et al [21] in a group of patients with major ABO-mismatched grafts, who were treated with a non-myeloablative conditioning.
2) Difference in the source of stem cells. PBSC grafts, in contrast to bone marrow, contain at least 1 log more B-lymphocytes. This observation may be of importance in cases of minor ABO-mismatches because passively transferred donor B-lymphocytes can lead to production of significant amounts of anti-host isohemagglutinins [9,11].
3) Differences in the GVHD prophylaxis regimens. CSA, in contrast with methotrexate (MTX), does not have any cytolytic effect on lymphocytes and is less efficient in preventing the activation of B than of T-lymphocytes. It has been suggested that CyA used alone, without the addition of MTX, can be associated with increased incidence of prolonged hemolysis or PRCA in cases of ABO-incompatibility [22,23]. Also the effect, if any, of ABO-mismatch on the transplantation outcome can be altered by the use of antibodies given *in vivo* or *ex vivo* for prevention of GVHD. Alemtuzumab, a humanized monoclonal antibody (mAb) with reactivity against the pan-lymphocyte antigen CD52, is effective in promoting apoptosis of T and B lymphocytes through complement-dependent and antibody-dependent cell-mediated cytotoxicity

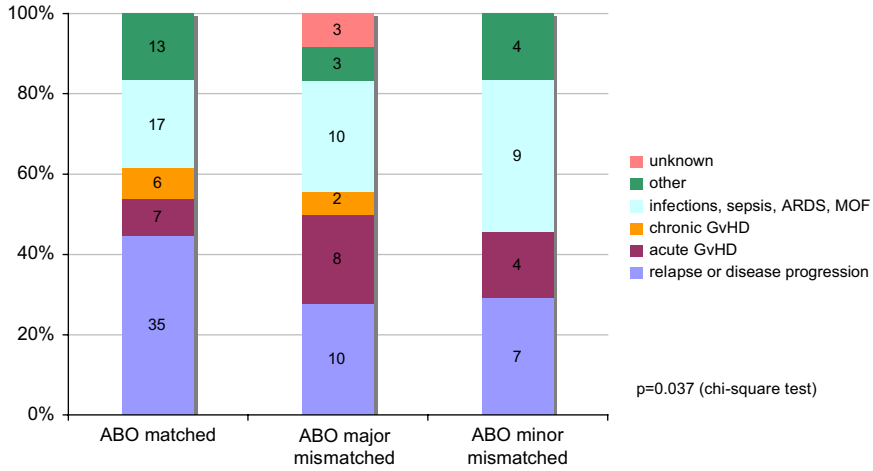

Figure 4. Distribution of major causes of death is different in ABO matched and mismatched groups, $P = .037$ according to chi-square test (ARDS indicates adult respiratory distress syndrome; MOF, multi-organ failure).

mechanisms. Peggs et al [24] did not observe any cases of PRCA or hemolysis in a group of 19 patients transplanted from major ABO-mismatched donors. In their study, alemtuzumab was used as part of the conditioning regimen.

4) Lastly, the significance of ABO-incompatibility can be masked by other factors known to affect the transplantation outcome such as age, diagnosis, disease status pre-transplantation, donor characteristics, sex disparity between donor and recipient, and minor differences in supportive treatment at different centers.

All the patients included in this study were treated in the same institution with the same preparative regimen, were given the same GVHD prophylaxis regimen, and received the same infection prophylaxis protocols. Other variables known to affect transplantation outcome were analyzed and were found to be well-balanced among the different ABO groups.

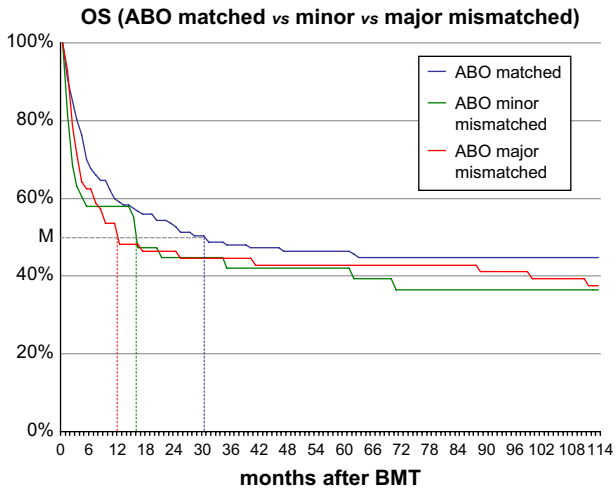

Figure 5. Kaplan-Meier probability of overall survival according to the degree of ABO incompatibility. (M and dotted line indicate median survival.)

Our results show that in the setting of RIC, ABO-incompatibility does not have any effect on the rate of neutrophil and megakaryocyte engraftment. Because rejections were extremely rare in our study population, the effect, if any, of ABO-mismatch on the rate of rejection could not be evaluated. Although exceptional cases of delayed engraftment, higher transfusion requirement, or even rejections have been reported in association with major ABO-mismatch, results from previous studies are in accordance with our findings [8,20,25-27]. These observations are in agreement with laboratory data showing that ABO blood group antigens are highly expressed on the surface of erythroid progenitors, whereas they are expressed on only 5% of more primitive progenitors such as GEMM-CFU [28]. In our study, the incidence of prolonged hemolysis or PRCA in the group of patients with major ABO-mismatch was very low (3.5%). Similar to our study, Kanda et al [29] showed that the use of a reduced intensity regimen consisting of a purine analog and busulfan was not associated with increased incidence of immunohematologic complications in major ABO-mismatched transplantations [27]. This observation may be explained by the hypothesis that the combination of busulfan with a purine analog has high activity against plasma cells resulting in the early elimination of anti-donor isohemagglutinins.

In this study, we observed increased incidence of severe aGVHD in the group of patients with major ABO-incompatibility, although this increased rate did not reach statistical significance. The results of other studies evaluating this issue are conflicting. Goldman et al [30] did not observe any significant effect of ABO-incompatibility on the incidence of aGVHD. However, a study performed by Bacigalupo et al [31] showed more aGVHD in the group of

Table 2. *Summary of influence of ABO incompatibility on major characteristics of myeloablative vs reduced intensity conditioning transplantation*

Type of transplantation	Conventional BMT (Seebach et al., 2005 [8])	RIC (our data)
Number of patients	3,103 (232 centers)	221 (single center)
Cells' source	BMT from matched donors	PBMC in 201 and BMT in 20 from matched donors
Median follow-up	54 mos	77 mos
Engraftment	Slower for neutrophyls and RBC in major ABO incompatibility group	No difference for WBC, platelets and RBC
Rejection	No data	No difference
Acute GVHD	Trend to higher severity in bi-directional mismatched	Trend to higher severity in major mismatched
Chronic GVHD	Similar	Higher
OS	Similar	Similar
NRM	Similar	Higher in 3 mos in mismatched, in 1 yr in major mismatched, and a higher number of GVHD associated deaths

patients with minor ABO-mismatch. Also, in more recent studies, Stussi et al [32], observed more GVHD, and Seebach et al [8] greater severity of aGvHD after bidirectional ABO-incompatible transplantations.

Comparison with the latest study published for the GVHD Working Committee of the Center for International Blood and Marrow Transplant (IBMT) Research is presented in Table 2. This table shows that there are some differences between cohorts of patients who underwent myeloablative (IBMT study [8]) or non-myeloablative (our study) transplantation. The most interesting finding of our study was the observation of significantly increased NRM in 3 and 12 months in the groups of patients with major and minor ABO-incompatibility. More importantly, in the ABO major mismatched group, the aGVHD and cGVHD-associated mortality was also significantly increased, suggesting that ABO major mismatched patients suffered from more resistant GVHD. The increased NRM resulted in a trend for decreased 1-year OS in the group of patients with major ABO-mismatch. Taken together, these data raise the possibility of an association between ABO-mismatch and severe GVHD resistant to treatment.

Our analytical findings and the observations from previous studies that showed an adverse impact of ABO-incompatibility on transplantation outcome raised significant questions about the pathogenetic mechanisms of this association. Several explanations have been proposed. Hemolysis observed in the major ABO-mismatch setting can lead to secretion of cytokines resulting in activation of donor anti-host alloreactive T-cell clones. However, in our study, we did not observe an increased incidence of hemolytic reactions. In this regard, it is of interest that ABO-antigens have a broad range of tissue distribution, including endothelial cells and von-Willebrand factor [33]. Donor anti-host isohemagglutinins produced after minor or bi-directional ABO-mismatched transplantations can attack host endothelial cells expressing blood group antigens triggering GVHD. Insight into the system of ABO-glycosyltransferases may offer another explanation of the association between ABO-blood group system and transplantation outcome. The A and B antigenic structures of the ABO-blood group system are carbohydrates that are synthesized by transfer of different sugars (GalNAc and Gal) to the H-substrate. The transfer of different sugars is accomplished by proteins with glycosyltransferase activity. ABO-genes are highly polymorphic and different alleles encode for glycosyltransferases with different enzymatic activity. Blood group A phenotype is associated with allelic variants that encode for proteins with A-transferase activity, while B-phenotype is associated with alleles that encode for proteins with B-transferase activity. Non-functional alleles code for proteins without any enzymatic activity and therefore are associated with O-phenotype [34]. Until now, approximately 100 different allelic sequences of ABO-glycosyltransferases have been identified. However, only few of them are detected with increased frequency in different individuals [35]. From the above data one can conclude that ABO-phenotypically mismatched individuals are also mismatched at the genotypic level, while ABO-phenotypic identity does not necessarily mean genotypical identity. Recent studies showed that ABO-glycosyltransferases might act as minor histocompatibility antigens (mHAgs). Eiz-Veisper and colleagues [36] in an elegant study proved that peptides derived from ABO-transferases are capable of stimulating peptide-specific alloreactive T-cells from individuals lacking the peptide, while the opposite was true for individuals expressing the specific peptide sequence. ABO-phenotypically mismatched donor-recipient pairs express, by definition, different ABO-mHAgs, while matched pairs have a high likelihood of being identical at the ABO-mHAgs level. These data are in agreement with the observed association of increased TRM, GVHD-related deaths in ABO-mismatched transplants.

In conclusion, in our study, we found that ABO-mismatch is associated with increased NRM in the first months post transplantation. In the major ABO-mismatched patients, the increased NRM resulted

from more GVHD-related deaths. These results are in accordance with many of the previous studies. Analysis of large cohorts of transplantation patients will be required to fully assess all the consequences of ABO-mismatching in comparison with other factors. Based on the above, practically speaking, the role of ABO-matching in choosing an optimal donor, when more than one is available, should not be underestimated. Consideration of ABO-identity at the allelic level should be included in future trials.

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