

Impact of recipient ABH secretor status on outcome in minor ABO-incompatible hematopoietic stem cell transplantation

Andreas Holbro,^{1,2} Martin Stern,¹ Laura Infanti,^{1,2} Alix O'Meara,¹ Beatrice Drexler,¹ Beat M. Frey,³ Jean-Marie Tiercy,⁴ Jakob R. Passweg,¹ Christoph Gassner,³ Andreas Buser,^{1,2} and Joerg-Peter Sigle^{2,5}

BACKGROUND: The impact of ABO incompatibility on hematopoietic stem cell transplantation (HSCT) outcome is controversial. As ABH substances are expressed on tissues and secreted in body fluids, they could drive an immune response in minor ABO-incompatible HSCT. The aim of the study was to investigate the prognostic role of the recipients' ABH secretor status.

STUDY DESIGN AND METHODS: Patients who underwent minor ABO-incompatible HSCT were included. Secretor status was determined either serologically or by molecular genetics.

RESULTS: Between March 1996 and June 2012, a total of 176 patients received minor ABO-incompatible HSCT and 150 (85%) were secretors. Incidence and severity of acute graft-versus-host disease (GVHD) and chronic GVHD did not differ between secretors and nonsecretors (cumulative incidences \pm standard errors: acute GVHD on Day 100, 41 ± 11 and $46 \pm 5\%$, $p = 0.59$; chronic GVHD at 2 years, 52 ± 13 and $56 \pm 5\%$, $p = 0.62$, for secretors and nonsecretors, respectively). Additionally, nonrelapse mortality (NRM) and overall survival (OS) were similar in the two groups (2-year NRM, 27 ± 9 and $23 \pm 3\%$, $p = 0.45$; 4-year OS, 64 ± 10 and $55 \pm 4\%$, $p = 0.28$, for secretors and nonsecretors, respectively).

CONCLUSION: The recipients' ABH secretor status in minor ABO-incompatible HSCT has no prognostic impact on major transplant outcomes.

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment approach for different malignant and nonmalignant diseases.¹ Several given factors such as patient age, comorbidities, donor type, and donor-recipient sex combinations have been shown to affect survival and major outcomes after HSCT.² Scoring systems integrate these pretransplant determinants into a global transplant risk assessment.³

ABO incompatibility is not considered an obstacle for HSCT and occurs in approximately 30% to 50% of transplants.⁴ Different types of donor-recipient ABO incompatibilities exist and are classified as either major, minor, or bidirectional.⁵ In major ABO-incompatible HSCT the patient has preformed antibodies (i.e. isohemagglutinins) against A and/or B antigens expressed on donor red blood cells (RBCs). Minor ABO-incompatible HSCT is characterized by the transfer of donor isohemagglutinins against recipient RBC antigens and of the corresponding immune cells (i.e., lymphocytes). A bidirectional blood group

ABBREVIATIONS: HR = hazard ratio; HSCT = hematopoietic stem cell transplantation; NRM = nonrelapse mortality; OS = overall survival.

From the ¹Division of Hematology, University Hospital, and the ²Blood Transfusion Centre, Swiss Red Cross, Basel, Switzerland; the ³Blood Transfusion Centre, Swiss Red Cross, Zurich, Switzerland; the ⁴National Reference Laboratory for Histocompatibility, Department of Internal Medicine, University Hospitals, Geneva, Switzerland; and the ⁵Blood Transfusion Centre, Swiss Red Cross, Aarau, Switzerland.

Address reprint requests to: Joerg-Peter Sigle, MD, Blood Transfusion Centre, Swiss Red Cross, Kantonsspital Aarau, 5001 Aarau, Switzerland; e-mail: joerg.sigle@blutspende-aarau.ch.

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barrier is a combination of major and minor ABO incompatibility. Various specific complications—for example, pure RBC aplasia in major ABO-incompatible HSCT or delayed hemolysis through passenger lymphocyte syndrome in minor ABO-incompatible HSCT—can occur. Several approaches to prevent complications after ABO-incompatible HSCT have been proposed, including preventive measures in the recipient and different graft processing steps.⁵

Several studies have addressed the impact of ABO incompatibility on HSCT outcome. A large study found no difference in overall survival (OS), transplant-related mortality, and Grade II to IV acute graft-versus-host disease (GVHD) after ABO-identical, major, minor, or bidirectional ABO-incompatible HSCT from HLA-identical siblings.⁶ On the other hand, Kanda and coworkers⁷ found a lower OS in a subgroup of patients after minor ABO-incompatible, unrelated HSCT with bone marrow as stem cell source. As A and B antigens and their precursor, the H glycoprotein, are expressed not only by RBC, but also many other tissues including vascular endothelium (“histo-blood group”), one may speculate that in the presence of an ABO barrier the tissue expression of ABH antigens can trigger or sustain an inflammatory reaction similar to that occurring in GVHD.⁸ In particular, in minor ABO-incompatible HSCT a humoral immune response mediated by antibodies produced by donor lymphocytes against recipient ABH antigens may trigger GVHD by binding to and thus damaging the recipient’s endothelium. Data regarding the effect of minor ABO-incompatible HSCT on rate and severity of GVHD are conflicting. Some studies have shown an increased risk of GVHD in minor ABO-incompatible HSCT.^{5,9} The study by Stussi and coworkers⁹ showed similar OS after minor ABO-incompatible HSCT compared to ABO-compatible HSCT, but a higher incidence of acute GVHD (Grade I-IV). Other studies failed to demonstrate a significant effect of minor ABO-incompatible HSCT on either rate or severity of GVHD.⁵

However, the above-mentioned studies did not include the recipients’ secretor status, which could explain the conflicting results. Eighty percent of all individuals, who are defined as secretors, do not only express their ABH antigens on tissues, but are also capable of secreting soluble ABH substance in their body fluids, including plasma.¹⁰

Soluble A/B antigens in the recipient’s plasma potentially neutralize in vivo circulating anti-A and/or anti-B derived from donor lymphocytes in minor ABO-incompatible HSCT and thus mitigate possible immunologic and/or inflammatory responses. This could affect incidence and severity of GVHD, disease relapse, and OS. The aim of this retrospective study was to investigate the prognostic role of the recipients’ ABH secretor status after minor ABO-incompatible HSCT.

MATERIALS AND METHODS

All adult patients that underwent minor or bidirectional ABO-incompatible allogeneic HSCT at our institution between March 1996 and June 2012 were included in this retrospective study. Patients with blood group A1, who received a HSCT from an A2 donor, were also included in the analysis. We excluded patients who received more than one HSCT, cord blood as stem cell source, or highly T-cell-depleted haploidentical HSCT. Patient, disease, and transplant characteristics were collected by chart review and through the electronic database of our institution. All patients provided written informed consent to have their data on disease, treatment, and outcome reported.

ABH secretor status

According to the Lewis (LE) phenotype and the secretor gene (α 1,2-L-fucosyltransferase; *FUT2*), individuals can be classified as secretors and nonsecretors.¹¹ Thus *Se* and *se* (the two alleles of *FUT2*, *Se* being dominant over *se*) determine the presence or absence of the ABH substance in body fluids.

The ABH secretor status was assessed through determination of LE phenotype or by *Se* genotyping. At our institution serologic typing for all clinically relevant blood groups, including LE, is routinely performed before HSCT in all patients by gel test (Gel Test ID-system, Bio-Rad Laboratories DiaMed GmbH, Cressier, Switzerland) or by conventional agglutination test in tubes (antisera from Immucor, Inc., Norcross, GA). *Se* genotyping was performed in patients where serologic LE phenotyping was missing, equivocal (mixed field after recent transfusions), or negative for both *Le^a* and *Le^b*.

Molecular determination of secretor status

Genomic DNA was isolated from peripheral blood mononuclear cells with the use of a DNA isolation kit (MagnaPure LC, Roche Diagnostics, Mannheim, Germany). The classic human secretor locus (*Se*) *FUT2* encodes α 1,2-L-fucosyltransferase and is located on Chromosome 19. A nonsense mutation involving Codon 143 (numbered from the putative initiator methionine of the short *FUT2* protein) is responsible for the nonsecretor phenotype.¹² The nonsense mutation is due to a G-to-A transition at Nucleotide 428. Wild-type (*Se*, 428G) and mutant (*se*, 428A) alleles of *FUT2* gene were detected by polymerase chain reaction using sequence specific priming technology in two independent reactions. Heterozygous individuals would give positive amplification in both reactions, and homozygous individuals in one reaction only. Primers for the wild-type allele (428G) were *FUT2*-all+523R (CCGGCTCCCGTTCACCTG-3′) and *FUT2*-*Se*+428G-F (CCGGTACCCCTGCTCGTG-3′), and

FUT2-all+523R and FUT-se+428A-F (ACCGGCTACCCC TGCTCGTA-3') for the mutant allele (428A), respectively. Concentrations of the primers in the final reaction volume were 200 nmol/L, and those of the control primers 90 nmol/L. Sequences of the control primers, reaction, and cycling conditions have been described previously.¹³

Statistical analysis

Patient, disease, and transplant characteristics were compared between secretors and nonsecretors using Pearson's chi-square tests for categorical variables. For acute and chronic GVHD and nonrelapse mortality (NRM), competing risks analysis was used. For univariate analysis of OS, the Kaplan-Meier method was used. Multivariable Cox analysis was used to adjust for donor type and disease stage. All comparisons were two-sided, and p values of less than 0.05 were considered significant. All analyses were carried out with computer software (Stata, Version 12, StataCorp, College Station, TX).

RESULTS

Patients

Between March 1996 and June 2012, a total of 788 adult patients underwent allogeneic HSCT at our institution. A total of 201 patients received a minor ABO-incompatible HSCT. Patients who received more than one HSCT (n = 19) or a highly T-cell-depleted haploidentical HSCT (n = 5) were excluded from the analysis. One additional patient had to be excluded because of missing LE phenotyping and no available DNA for molecular testing. Overall, 176 patients were included in the analysis. ABO donor-recipient combinations were as follows: O/A_{1/2} 91; O/AB 3; O/B 22; A/B 11; B/A 17; A₂/A₁ 19; A₂/A₁B 3; B/AB 3; and A/AB 7. A total of 112 patients (64%) were male, and 64 (36%) were female. Mean age at transplantation was 40 years and was not different between secretors and nonsecretors.

Secretors and nonsecretors

A total of 150 patients (85%) were secretors and 26 (15%) were nonsecretors. Of the nonsecretors, 20 patients (11%) had their secretor status determined by molecular analysis because of missing LE phenotype (n = 6) or because the phenotype was Le(a-b-) (n = 14). Seven of these patients were homozygous (Se/Se) and 10 heterozygous (Se/se) in the secretor gene locus and three were nonsecretors. Overall, 22 nonsecretors (85%) were male.

Disease and transplant characteristics

The main indication for HSCT was acute leukemia (n = 93; 53%), with acute myeloid leukemia being the most fre-

TABLE 1. Patient, disease, and transplant characteristics according to the secretor status

Characteristic	Nonsecretor	Secretor	p value
Patients	26	150	
Male	22	90	0.029
Female	4	60	0.029
Mean age at HSCT (years)	39.6	41.5	0.322
Disease			0.94
ALL	4	25	
AML	10	54	
LPD/PCD	5	30	
MDS	4	19	
MPN	3	16	
Others	0	6	
Disease stage			0.472
Early	14	67	
Intermediate	6	30	
Advanced	6	53	
Conditioning regimen			0.867
Myeloablative	21	119	
RIC	5	31	
Donors			0.384
Matched sibling	13	54	
Mismatched related	2	12	
Unrelated	11	84	
CMV (D/R)			0.024
--	10	49	
-/+	4	34	
+/-	5	10	
+/+	2	44	
Unknown	5	13	
GVHD prophylaxis			0.93
CYA	1	10	
CYA + MMF	7	35	
CYA + MTX	16	95	
T-cell depletion	2	10	

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CMV = cytomegalovirus; CYA = cyclosporine; D = donor; LPD/PCD = lymphoproliferative disorders/plasma cell diseases; MDS = myelodysplastic syndrome; MMF = mycophenolate; MPN = myeloproliferative disorder; MTX = methotrexate; R = recipient; RIC = reduced-intensity conditioning.

quent (n = 64; 36%). Diagnosis distribution and disease stage at HSCT were not different between secretors and nonsecretors. Most patients (n = 140; 80%) received a myeloablative conditioning regimen. Ninety-five patients (54%) received a HSCT from an unrelated donor. GVHD prophylaxis consisted primarily of cyclosporine and methotrexate. Table 1 summarizes patient, disease, and transplant characteristics, according to the secretor status.

GVHD

Cumulative incidence of acute (≥Grade II) GVHD on Day 100 was 41 ± 11% for secretors and 46 ± 5% for nonsecretors (p = 0.59). Incidence of chronic GVHD at 2 years was 52 ± 13% for secretors and 56% ± 5% for nonsecretors (p = 0.62; Fig. 1).

Adjusted multivariable Cox analysis confirmed that secretor status was not predictive of GVHD development:

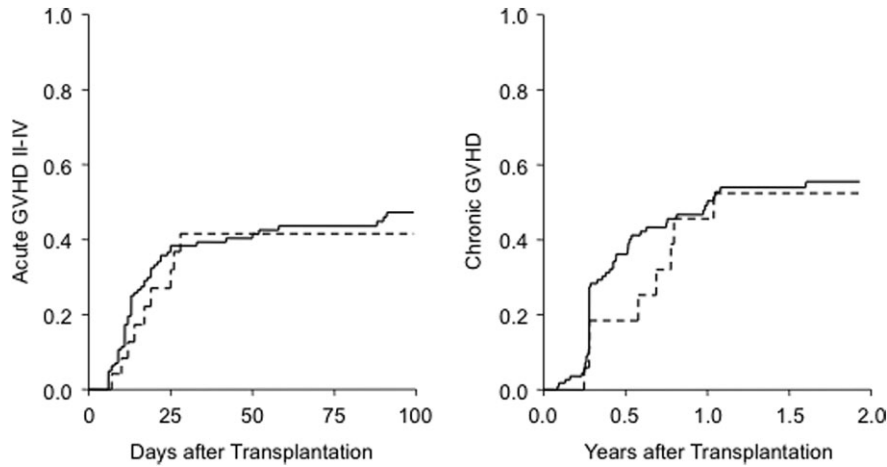


Fig. 1. Acute (\geq Grade II) and chronic GVHD according to secretor status. (—) Nonsecretor; (- - -) secretor.

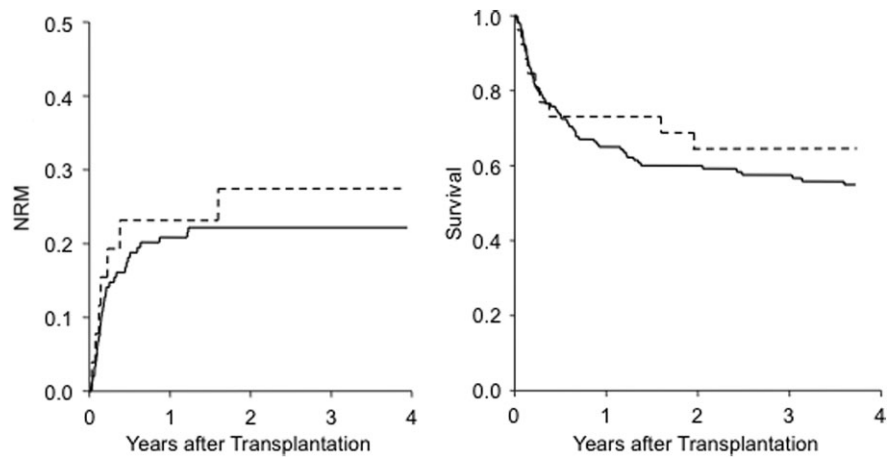


Fig. 2. NRM and OS according to secretor status. (—) Nonsecretor; (- - -) secretor.

hazard ratio (HR) secretor versus nonsecretor for acute GVHD 0.85, 95% confidence interval (CI) 0.42-1.72, $p = 0.64$; and HR for chronic GVHD 1.08, 95% CI 0.58-2.01, $p = 0.82$.

NRM and OS

Two-year NRM was similar between secretors and nonsecretors ($27 \pm 9\%$ and $23 \pm 3\%$ for secretors and nonsecretors, respectively; $p = 0.45$).

The same was seen in the 4-year OS, which was $64 \pm 10\%$ for secretors and $55 \pm 4\%$ for nonsecretors ($p = 0.28$; Fig. 2). Again, adjusted multivariable Cox analysis confirmed that secretor status was not predictive of NRM and OS: HR secretor versus nonsecretor for NRM 1.17, 95% CI 0.54-2.55, $p = 0.70$; and HR for OS 0.79, 95% CI 0.41-1.55, $p = 0.50$.

DISCUSSION

In contrast to solid organ transplantation ABO incompatibility is of minor importance for HSCT. HLA matching, on

the other hand, strongly affects NRM, incidence and severity of GVHD, and OS.¹⁴ For several decades, HSCT has been performed—if unavoidable—across the ABO blood group barrier. While in major ABO-incompatible HSCT, acute hemolysis and pure RBC aplasia are the major short-term complications, passenger lymphocyte syndrome is a possible complication in minor ABO-incompatible HSCT, with varying clinical course from asymptomatic laboratory finding to severe and even life-threatening condition.^{15,16} Besides the above-mentioned immunehematologic complications, the data on the impact of minor ABO blood group incompatibility on OS as well as incidence and severity of GVHD is controversial.⁵ As ABO blood group antigens are expressed on different tissues, the presence of antibodies against these antigens could have an impact on the underlying disease, GVHD, and OS. In particular, antibodies produced by donor lymphocytes in minor ABO-incompatible HSCT are directed against recipient ABO antigens and could induce a humoral immune response. Endothelial cells which express A and/or B substances could be a

possible target of this immune response, as there is a persistence of recipient type endothelium after HSCT.¹⁷ Recipients, who are ABH secretors could—on the other hand—neutralize these donor antibodies (“in vivo” adsorption), thus mitigating a possible immunologic reaction in both directions (graft-versus-leukemia and graft-versus-host reaction). To the best of our knowledge, the impact of ABH secretor status in recipients of minor ABO-incompatible HSCT has not been investigated so far.

In this study, 85% of the 176 patients with minor ABO-incompatible HSCT were ABH secretors, which is consistent with the known prevalence in a Caucasian population. We did not find any differences in major transplant outcomes, including acute (\geq Grade II) and chronic GVHD, NRM, and OS between secretors and nonsecretors. Our data therefore do not suggest a clinically significant effect of secretor status on a donor-derived humoral immune response against recipients’ ABO antigens. One possible explanation for this finding could be the lack of a general increase of donor derived anti-A/B after minor ABO-incompatible HSCT.¹⁸ Additionally one may further speculate that transplant-associated microangiopathy as a manifestation of GVHD also represents an antibody-mediated endothelial cell activation and damage. However, in a previous study we did not find ABO incompatibility to be a risk factor for the development of transplant-associated microangiopathy.¹⁹

From a pathophysiologic viewpoint, the transfusion of ABO-compatible nonidentical plasma can mimic a scenario similar to the one described above. Soluble ABH substance in plasma obtained from secretors can interact with recipients’ isohemagglutinins, if transfused in an ABO-compatible but nonidentical manner. A large retrospective study has described a potentially negative impact of ABO-compatible nonidentical plasma transfusions on survival.²⁰ The authors hypothesized that immune complex formation could be a possible cause. However, our finding on NRM and OS suggests that immune complex formation, which could occur in secretors in minor ABO-incompatible HSCT has a negligible impact on outcome.

Our study has various limitations. These include the relatively small overall number of nonsecretors, the lack of data regarding recipient isohemagglutinin titers before and during the course of HSCT and the lack of additional data on immunohematologic complications after minor ABO-incompatible HSCT, including passenger lymphocyte syndrome in secretors and nonsecretors. An additional limitation is the power of our retrospective analysis, which would detect differences in the defined outcome variables with a HR of 2 to 2.5. ABH secretor status was determined either through determination of LE phenotype or by genotyping. Therefore, we could not further analyze the influence of zygosity of the secretor gene.

Even though we found no impact of secretor status in minor ABO-incompatible HSCT, the role of secretor status in ABO-incompatible solid organ transplants should be investigated. It is widely recognized that anti-A and anti-B isohemagglutinins can cause hyperacute rejection of incompatible transplants. However, the current organ shortage has driven new incentives and strategies, including ABO-incompatible solid organ transplantation. Together with immunosuppression and different preparative protocols including rituximab and immunoadsorption and/or plasma exchange in ABO-incompatible solid organ transplantation, ABH secretion could have an impact on short- and long-term transplant outcomes, acting as an “in vivo” adsorption mechanism.

In conclusion, the role of the recipients’ ABH secretor status in minor ABO-incompatible HSCT appears to be not relevant for clinical outcome and is not an explanation for the discrepant results of the published literature. This may not be the case for ABO-incompatible solid organ transplantation, an expanding field that deserves further evaluation.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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