



## Original article

# Assessing the impact of ABO incompatibility on major allogeneic hematopoietic stem cell transplant outcomes: a prospective, single-center, cohort study



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## ABSTRACT

**Background:** ABO blood group incompatibility between donor and recipient is associated with a number of immunohematological complications, but is not considered a major contraindication to allogeneic hematopoietic stem cell transplantation. However, available evidence from the literature seems to be conflicting as to the impact of incompatibility on overall survival, event-free survival, transplant-related mortality, graft-versus-host disease, and time to neutrophil and platelet engraftment.

**Methods:** This single-center, prospective, cohort study included patients with hematological malignancies who underwent a first allogeneic hematopoietic stem cell transplantation between 2008 and 2014. Patients receiving umbilical cord blood as the stem cell source were excluded from this analysis. The impact of ABO incompatibility was evaluated in respect to overall survival, event-free survival, transplant-related mortality, acute graft-versus-host disease and engraftment.

**Results:** A total of 130 patients were included of whom 78 (60%) were males. The median age at transplant was 36 (range: 2–65) years, 44 (33%) presented ABO incompatibility, 75 (58%) had acute leukemia, 111 (85%) had a related donor, 100 (77%) received peripheral blood hematopoietic stem cells as graft source and 99 (76%) underwent a myeloablative conditioning regimen. There was no statistically significant association between ABO incompatibility and overall survival, event-free survival, transplant-related mortality, grade II–IV acute graft-versus-host disease, neutrophil or platelet engraftment in multivariate analysis.

**Conclusion:** These results show that ABO incompatibility does not seem to influence these parameters in patients undergoing allogeneic hematopoietic stem cell transplantation.

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## Introduction

Allogeneic hematopoietic stem cell transplantation (alloHSCT) constitutes a potentially curative approach to a number of malignant and non-malignant hematological diseases. During the past few decades, results of alloHSCT have improved considerably, particularly with advances in donor selection, hematopoietic stem cell source, supportive measures, management of post-transplant complications, and development of less toxic conditioning regimens, as well as novel post-transplant treatment platforms. This improvement has broadened alloHSCT indications significantly worldwide and has expanded the number of transplant-eligible patients.<sup>1,2</sup>

Donor-recipient ABO blood group incompatibility (ABOi) is not a contraindication to alloHSCT. ABOi is observed in 30–40% of human leukocyte antigen (HLA)-matched transplants, since ABO genetic inheritance is independent to that of HLA haplotypes. Several complications related to ABOi have been described in studies, such as acute and chronic hemolytic transfusion reactions,<sup>3–5</sup> pure red cell aplasia,<sup>6–8</sup> delayed engraftment,<sup>9,10</sup> and increased incidence of acute graft-versus-host disease (GvHD),<sup>11,12</sup> but this has not been confirmed in others.<sup>13–15</sup> Conflicting results seem to be more prominent with regard to the occurrence of GvHD in this setting.<sup>16,17</sup>

Considering the conflicting evidence as to the real impact of ABOi on alloHSCT outcomes, this study aimed to assess the impact of ABOi on major outcomes in patients with hematological malignancies undergoing a first alloHSCT at a transplant referral center in Brazil.

## Methods

This was a prospective, single-center, cohort study, comprising patients with hematological malignancies (acute leukemia, myelodysplastic syndrome, primary myelofibrosis, chronic myeloid leukemia, lymphoma, and multiple myeloma) undergoing a first related or unrelated alloHSCT at Hospital das Clínicas, Universidade Federal de Minas Gerais, Brazil, between the 1st April, 2008 and 31st December, 2014. Patients receiving umbilical cord blood as stem cell source and those undergoing haploidentical transplantation were excluded from this analysis. This study was approved by the institutional Research Ethics Committee, which abides by the Declaration of Helsinki principles for research in human beings, and an informed consent form was obtained from all study participants.

### Conditioning regimen

The conditioning regimens administered in the included patients were classified according to the National Marrow Donor Program (NMDP) and the Center for International Blood and Marrow Transplant Research (CIBMTR), as follows<sup>18,19</sup>:

a. *Reduced intensity conditioning (RIC)*: use of an oral busulfan formulation at a dose equal to or less than 9 mg/kg/body

weight and intravenous melphalan at a dose equal to or less than 140 mg/m<sup>2</sup>/body surface area.

b. *Myeloablative conditioning (MAC)*: use of oral busulfan and intravenous melphalan doses greater than 9 mg/kg/body weight and 140 mg/m<sup>2</sup>/body surface area, respectively.

### ABO compatibility

Transplants were classified according to ABO compatibility between donor and recipient, as follows:

- (a) ABO iso-group: donor and recipient had the same ABO blood group;
- (b) Minor ABO incompatibility: when the donor had iso-hemagglutinins against recipient red blood cell antigens;
- (c) Major ABO incompatibility: when the recipient had iso-hemagglutinins directed against donor red blood cell antigens; this group also included bidirectional ABO incompatibility (i.e., when there were iso-hemagglutinins against both donor and recipient red blood cell antigens).

### Clinical outcome definitions

**Neutrophil engraftment**: the first of three consecutive days with a neutrophil count equal to or greater than  $0.5 \times 10^9$  cells/L. For this analysis, the incidence of neutrophil engraftment was considered within the first 30 days post-transplant.

**Platelet engraftment**: the first of seven consecutive days with a platelet count equal to or greater than  $20 \times 10^9$ /L without transfusion support. The incidence of platelet engraftment was considered within the first 100 days post-transplant.

**Transplant-related mortality**: death associated with alloHSCT complications and not related to relapse. The cumulative incidence of transplant-related mortality was assessed at one year post-transplant.

**Event-free survival**: probability of being alive after transplant without having any events during the first two years post-transplant. For this analysis, 'event' was defined as death or relapse.

**Overall survival**: probability of being alive at two years post-transplant.

Acute GvHD was classified and graded according to the Glucksberg-Seattle criteria.<sup>20</sup> The cumulative incidences of grade II–IV and grade III–IV acute GvHD were assessed within the first 100 days post-transplant.

### Statistical analysis

Frequency (*n*) and proportion measures were used for categorical variables and median, minimum and maximum values were considered for continuous variables. Transplant-related mortality was defined as death from all causes not related to relapse. Event-free survival was defined as the interval between transplant and death or relapse, whereas overall survival comprised the interval between transplant and death from all causes. Event-free and overall survival were estimated using the Kaplan–Meier method and the log-rank test was used to compare these survival curves in the univariate analysis. Data was censored at the time of death or of

last follow-up. The Gray method was used for the analyses of competing events: in the analyses of neutrophil and platelet engraftment and of acute GvHD cumulative incidences, death was considered a competing event, whereas, in the analysis of the cumulative incidence of transplant-related mortality, relapse was considered as a competing event. A significance level of 5% ( $p$ -value = 0.05) with a 95% confidence interval (95% CI) was used for the hazard ratio (HR) estimates. Cox's proportional hazards model was used for the multivariate analyses of overall and event-free survival, and the Fine and Gray multivariate method for competing events was applied for the analyses of cumulative incidence of transplant-related mortality, neutrophil and platelet engraftment, and acute GvHD. The following variables were included in the univariate analysis: source of hematopoietic stem cells, type of conditioning regimen, type of donor, and type of ABOi. Variables with a  $p$ -value  $\leq 0.30$  in the univariate analysis, as well as the type of ABOi were included in the multivariate analysis. All statistical analyses were performed using the Easy R software package.

## Results

### Patients

A total of 130 patients were included, of whom 86 (66%) were ABO iso-group with their donors, 20 (15.3%) had minor ABOi, 20 (15.3%) had major ABOi, and four (3%) had bidirectional ABOi. There was a predominance of males (60.0% of cases). The median age at transplant was 36 (range: 2–65) years. Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) were the main primary hematological diseases, comprising 46 (35.3%) and 29 (22.3%) cases, respectively. Patients' main characteristics are shown in [Table 1](#).

### Neutrophil and platelet engraftment

The cumulative incidence of neutrophil engraftment at 30 days was 83.8% (median: 19 days; range: 3–30 days). In univariate analysis ([Table 2](#)), type of ABOi was associated with an increase in the time to neutrophil engraftment (88.4% of neutrophil engraftment in the ABO iso-group versus 62.5% in the major/bidirectional ABOi transplants;  $p$ -value = 0.02). Neither the type of ABOi nor the other studied variables showed any statistically significant predictive association with neutrophil engraftment in the multivariate analysis. Regarding the cumulative incidence of platelet engraftment, it occurred in 76.2% of cases during the first 100 days post-transplant (median: 21 days; range: 3–100 days). None of the studied variables showed any predictive association with platelet engraftment ([Table 2](#) and [Table 3](#)).

### Graft-versus-host disease

The cumulative incidences of grade II–IV and grade III–IV acute GvHD during the first 100 days post-transplant were 33.1% and 7.7%, respectively. Neither the type of ABOi nor the other studied variables showed any statistically significant association with the cumulative incidence of grade II–IV and grade III–IV acute GvHD in the univariate analysis ([Table 2](#)). In multivariate

**Table 1 – Characteristics of the population studied (n = 130).**

Characteristic		
Gender: male – n (%)	78	(60.0)
Age at transplant (years) – median (range)	36	(2–65)
ABO incompatibility (ABOi) classification – n (%)		
ABO iso-group	86	(66.1)
Minor ABOi	20	(15.3)
Major ABOi	20	(15.3)
Bidirectional ABOi	4	(3.3)
Primary disease – n (%)		
AML	46	(35.4)
ALL	29	(22.3)
CML	20	(15.4)
HL	4	(3.1)
NHL	11	(8.5)
MM	1	(0.8)
MDS	12	(9.2)
PMF	7	(5.1)
Graft source – n (%)		
BMHSC	30	(23.1)
PBHSC	100	(76.9)
Conditioning regimen – n (%)		
MAC	98	(75.4)
RIC	32	(24.6)
Type of donor – n (%)		
Related	111	(85.4)
Unrelated	19	(14.6)

ABOi: ABO blood group incompatibility; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BMHSC: bone marrow hematopoietic stem cells; CML: chronic myeloid leukemia; HL: Hodgkin lymphoma; MAC: myeloablative conditioning; MDS: myelodysplastic syndrome; MM: multiple myeloma; NHL: Non-Hodgkin's lymphoma; PBHSC: peripheral blood hematopoietic stem cells; PMF: primary myelofibrosis; RIC: reduced intensity conditioning.

analysis, neither the type of ABOi nor the other studied variables were associated with the cumulative incidence of grade III–IV acute GvHD ([Table 3](#)).

### Transplant-related mortality

The cumulative incidence of transplant-related mortality during the first year of transplant was 38.5%. Neither the type of ABOi nor the other studied variables showed any statistically significant predictive association with transplant-related mortality in either the univariate or the multivariate analyses ([Tables 2 and 3](#), respectively).

### Event-free and overall survival

The estimated event-free and overall survival at two years post-transplant were 32.0% (95% CI: 21.3–36.9%) and 35.4% (95% CI: 26.3–42.6%), respectively. Univariate ([Table 2](#)) and multivariate ([Table 3](#)) analyses did not show any statistically significant association between ABOi ( $p$ -value = 0.46) or the other studied variables with event-free or overall survival.

**Table 2 – Univariate analysis of the main post-transplant outcomes of 130 patients submitted to hematopoietic stem cell transplant at a single center in Brazil.**

	OS		EFS		TRM		aGVHD II-IV		NE		PE	
	%	p	%	p	%	p	%	p	%	p	%	p
<b>Classification</b>												
ABO iso-group	37.2	0.42	33.7	0.46	37.2	0.29	38.4	0.17	88.4	0.02	79.1	0.27
Minor ABOi	40.0		36.8		30.0		25.0		85.0		75.0	
Major/bidirectional ABOi	25.0		21.7		50.0		20.8		62.0		58.3	
<b>Graft source</b>												
BM HSC	40.0	0.51	34.5	0.92	30.0	0.29	36.7	0.62	90.0	0.41	76.7	0.96
PBHSC	34.0		31.3		41.0		32.0		82.0		75.0	
<b>Gender</b>												
Male	33.8	0.83	31.5	0.79	41.6	0.40	35.1	0.48	81.8	0.08	76.6	0.67
Female	37.7		32.7		34.0		30.2		86.8		75.5	
<b>Conditioning regimen</b>												
MAC	35.4	0.61	31.6	0.87	41.9	0.69	33.3	0.93	83.8	0.12	75.8	0.10
RIC	35.5		33.3		37.4		32.3		83.9		77.4	
<b>Type of donor</b>												
Related	38.7	0.13	34.9	0.27	36.0	0.23	31.5	0.47	82.9	0.78	77.5	0.36
Unrelated	15.8		15.8		52.6		42.1		84.2		63.2	

ABOi: ABO blood group incompatibility; BM HSC: bone marrow hematopoietic stem cells; PBHSC: peripheral blood hematopoietic stem cells; MAC: myeloablative conditioning; RIC: reduced intensity conditioning; OS: overall survival; EFS: event-free survival; TRM: transplant-related mortality; aGVHD: acute graft-versus-host disease; NE: neutrophil engraftment; PE: platelet engraftment. Significance set for a *p*-value <0.05.

**Table 3 – Multivariate analysis of the main post-transplant outcomes in 130 patients undergoing hematopoietic stem cell transplant at a single center in Brazil.**

Outcome	Variable	Hazard ratio (95% CI)	p-Value	
OS	ABOi	Minor	1.25 (0.72–2.17)	0.41
		Major/bidirectional	0.87 (0.48–1.58)	0.66
	Type of donor	Unrelated	1.43 (0.81–2.52)	0.21
EFS	ABOi	Minor	1.28 (0.73–2.23)	0.37
		Major/bidirectional	1.00 (0.55–1.80)	0.99
TRM	Type of donor	Unrelated	1.24 (0.70–2.19)	0.45
		ABOi	Minor	1.47 (0.73–2.97)
		Major/bidirectional	1.29 (0.64–2.59)	0.42
aGVHD II-IV	Type of donor	Unrelated	0.71 (0.31–1.61)	0.19
		ABOi	Major/bidirectional	1.19 (0.25–5.63)
	Type of regimen	MAC	0.33 (0.04–2.64)	0.30
NE	ABOi	Minor	0.50 (0.30–0.90)	0.20
		Major/bidirectional	1.11 (0.65–1.89)	0.69
	Recipient sex	Female	1.31 (0.89–1.92)	0.17
PE	Type of regimen	MAC	1.30 (0.78–2.15)	0.31
		ABOi	Minor	0.67 (0.39–1.16)
	Type of donor	Major/bidirectional	1.12 (0.62–2.01)	0.70
	Unrelated	1.37 (0.80–2.33)	0.25	

ABOi: ABO blood group incompatibility; OS: overall survival; EFS: event-free survival; TRM: transplant-related mortality; aGVHD II-IV: acute grade II-IV graft-versus-host disease; MAC: myeloablative conditioning; NE: neutrophil engraftment; PE: platelet engraftment; 95% CI: 95% confidence interval. Significance set for a *p*-value <0.05.

## Discussion

The present study evaluated both the presence and the clinical relevance of ABOi in alloHSCT. No significant impact of ABOi was found on overall and event-free survival, nor on the cumulative incidence of transplant-related mortality, neutrophil and platelet engraftment, and grade II-IV and grade III-IV acute GvHD.

The proportion of patients stratified as having ABOi in this study is in accordance with that of the medical literature, where 30-50% of allogeneic transplants are ABO incompatible.<sup>21</sup>

Goldman et al.,<sup>22</sup> in a retrospective study including 153 alloHSCT recipients with hematological malignancies, showed, as in the current study, that the presence of ABOi does not influence overall survival. Their cohort study differs from this study due to its retrospective design and the fact that patients with both Hodgkin's and non-Hodgkin lymphoma and multiple myeloma were excluded from the analysis. Moreover, these authors showed a greater proportion of donor-recipient pairs with ABOi (45.1% versus 33.9%), with a different distribution of ABOi subtypes: minor ABOi accounted for 18.9% of cases compared to 15.3% in this study; major ABOi represented 22.9% (versus 15.3%) and bidirectional ABOi comprised 3.3% of cases (which is equal to the 3.3% observed in this study).

On the other hand, the results of a Japanese marrow donor program that retrospectively analyzed data from 5549 allogeneic transplants with unrelated donors for the treatment of malignant and non-malignant hematological diseases showed that transplants with minor or major ABOi had worse overall survival as compared to ABO iso-group transplants. In that study, no significant difference was noted in overall survival for bidirectional ABOi transplants when compared to iso-group ones. However, worse transplant-related mortality, delayed neutrophil and platelet engraftment, and greater incidence of grade III-IV acute GvHD were observed in both major and minor ABOi groups. That study also observed a greater proportion of HLA-mismatched transplants in the major, minor and bidirectional ABOi groups (43.9% HLA-mismatched transplants in the major ABOi group, 23.1% in the minor ABOi group, and 2.8% in the bidirectional group) in comparison to the ABO iso-group (30.2%) pairs ( $p$ -value <0.001). Such discrepancy between the proportion of HLA mismatch in the ABO iso-group cases and that of the ABOi groups may have influenced the overall survival, transplant-related mortality and acute GvHD estimates found by those authors.<sup>23</sup> Moreover, when compared to this study, important differences emerge with regard to the characteristics of the population studied, such as those related to the type of primary disease (malignant and benign disease versus only malignant hematological disease in this study), the type of donor (100% unrelated donors versus 14.6% in this study), and the proportion of patients with ABOi (49.2% in the Kimura et al.,<sup>22</sup> study versus 33.9% in this study).

Kim et al.,<sup>24</sup> in a retrospective study including 89 patients submitted to alloHSCT using peripheral blood hematopoietic stem cells for the treatment of malignant and non-malignant hematological diseases, showed a proportion of 44.9% of ABOi

transplants, among which 22.5% had major ABOi, 16.8% had minor ABOi, and 5.6% had bidirectional ABOi within donor-recipient pairs. These authors did not observe any statistically significant differences in the incidence of transplant-related mortality, acute GvHD, neutrophil engraftment and platelet engraftment between the ABOi and the ABO iso-group transplants, which is in accordance with the findings of the present study.

In another retrospective study which included a larger number of patients (562) with hematological malignancies (mainly acute leukemia), 35.8% of cases had ABOi (27.0% had minor ABOi, 23.7% had major ABOi, and 4.7% had bidirectional ABOi). In contrast to the findings herein, this study noted a significant association between minor ABOi transplants and the incidence of low-grade acute GvHD, but no differences were observed in respect to the incidence of moderate or severe (II-IV) acute GvHD.<sup>25</sup>

In keeping with most of the aforementioned studies, no differences were observed with regard to overall survival and to transplant-related mortality, nor to the incidence of acute GvHD, between the ABO groups in the current study.

The major drawbacks of this study were that it was limited to a single-center cohort, patients were enrolled based on convenience sampling during a pre-specified time period and included a relatively small number of subjects with ABOi (20 minor ABOi transplants, 20 major ABOi cases, and only four bidirectional ABOi donor-recipient pairs). This may have masked potentially significant differences in outcomes between ABOi subgroups. Likewise, due to the relatively small number of patients who had ABOi and survived after Day 100 post-transplant (i.e., only 13 patients), it was not possible to analyze the potential association of ABOi with the occurrence of chronic GvHD in this population. Moreover, since most of the patients received transfusions at different centers during the late post-transplant period, we were unable to analyze the probable implications of ABOi regarding transfusion dependence and burden (due to a lack of reliable data from the centers).

## Conclusions

This prospective, single-center, cohort study found that ABOi does not seem to influence overall and event-free survival, nor the incidence of transplant-related mortality, acute GvHD, or neutrophil and platelet engraftment in HLA-identical, related and unrelated, alloHSCT patients. Multicenter studies, with a greater number of patients with ABOi, may help to shed light on the effects of ABOi on these and other clinically relevant post-transplant outcomes in this population.

## Conflicts of interest

The authors declare no conflicts of interest.

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