ABO blood group incompatibility as an adverse risk factor for outcomes in patients with myelodysplastic syndromes and acute myeloid leukemia undergoing HLA-matched peripheral blood hematopoietic cell transplantation after reduced-intensity conditioning

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BACKGROUND: ABO incompatibility is not a contraindication to hematopoietic cell transplantation (HCT), but it has been associated with additional risks including delayed engraftment, pure red cell aplasia (PRCA), and higher transfusion needs. Data on these events and on patient survival after reduced-intensity conditioning (RIC) HCT are limited.

STUDY DESIGN AND METHODS: A total of 127 consecutive patients, 86 with acute myeloid leukemia and 41 with myelodysplastic syndromes, who underwent HLA-matched peripheral blood RIC allogenic HCT between 2005 and 2014 were retrospectively analyzed.

RESULTS: Eighty ABO-compatible, 26 major/bidirectional, and 21 minor-ABO-mismatch HCT were identified. Compared to the ABO-compatible group, major/bidirectional mismatches had increased red blood cell (RBC) transfusion requirement during the first 100 days ($p = 0.009$), delayed RBC and PLT engraftment ($p = 0.0011$ and $p = 0.005$, respectively), and higher incidence of grade II to IV acute graft-versus-host disease (aGVHD; $p = 0.037$). In multivariable analysis, major/bidirectional mismatches had significantly higher non-relapse mortality (NRM) and inferior disease-free survival (DFS) and overall survival (OS) compared with ABO-compatible patients ($p = 0.01$, $p = 0.04$, and $p = 0.035$, respectively). Minor ABO mismatch had no impact on survival ($p = 0.99$). Four (15%) of 26 major/bidirectional mismatches developed PRCA. There was a significant association between fludarabine plus busulfan conditioning and PRCA ($p = 0.0046$).

CONCLUSION: Major/bidirectional ABO mismatch is associated with higher NRM and shortened DFS and OS in the setting of RIC HCT. Increased transfusion need, delayed RBC and platelet engraftment, PRCA, and increased severity of aGVHD are additional complications contributing to the morbidity.

Reduced-intensity conditioning (RIC) for hematopoietic cell transplantation (HCT) is better tolerated in comparison to myeloablative conditioning (MA) and is hence an option for elderly patients and those with associated comorbidities.[1] Although RIC is associated with less toxicity and lower overall transfusion requirements,[2] there are indications that it can lead to more severe immunohematologic complications such as hemolytic reactions, delayed red blood cell (RBC) engraftment, increased RBC transfusion, and pure red cell aplasia (PRCA)

ABBREVIATIONS: aGVHD = acute graft-versus-host disease; AML = acute myeloid leukemia; BM = bone marrow; cGVHD = chronic graft-versus-host disease; CR = complete remission; DFS = disease-free survival; DLI = donor lymphocyte infusion; Flu/Mel = fludarabine plus melphalan; Flu/Bu = fludarabine plus busulfan; HCT = hematopoietic cell transplantation; HR(s) = hazard ratio(s); IHA = isohemagglutinin antibody; MA = myeloablative conditioning; MDS = myelodysplastic syndromes; NRM = non-relapse mortality; OS = overall survival; PB = peripheral blood; PLX = plasma exchange; PRCA = pure red cell aplasia; RIC = reduced-intensity conditioning; RTX = rituximab.

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when used in the setting of ABO-mismatched HCT.[2-4] More importantly, the impact of ABO incompatibility on survival remains highly controversial, with some studies showing a negative impact[5-8] while others show no difference[3,9,10] or even improved outcome after RIC HCT.[11] This conflicting body of literature is partly driven by the heterogeneous nature of study populations in terms of underlying hematologic conditions, type of conditioning regimens, and other transplant characteristics. A meta-analysis of cohort studies by Kanda and colleagues[12] demonstrated no adverse association between ABO mismatching and survival among related HCT recipients. However, marginally lower overall survival (OS) was found in recipients of minor or bidirectional mismatched grafts from unrelated donors, especially in patients with acute leukemia. A recent large retrospective analysis of Center for International Blood and Marrow Transplant Research data on patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) demonstrated a significantly higher non-relapse mortality (NRM) and inferior OS in major ABO-mismatched transplants. There was also a non-significant trend toward decreased OS in minor ABO mismatch grafts.[5] Although this was a large and robust study focusing exclusively on AML and MDS patients, the transplant process was still largely heterogeneous, including both MA and RIC conditioning regimens and both peripheral blood (PB) and bone marrow (BM) grafts. We therefore examined the effect of ABO incompatibility on transplant-related outcomes, including relapse, NRM, and survival, as well as on potential adverse events, such as delayed engraftment, PRCA, and graft-versus-host disease (GVHD) in a large group of AML and MDS patients undergoing a uniform transplant process with allogenic RIC HCT after HLA-matched PB grafts in a single-institution setting.

MATERIALS AND METHODS

Patients and study design

Data from 585 consecutive adult patients who underwent allogenic HCT at Mayo Clinic in Rochester, Minnesota between January 2005 and July 2014 were retrospectively reviewed. Of these, 178 patients were included in the study based on the following eligibility criteria: 1) underlying diagnosis of AML and/or MDS, 2) first allogenic HCT after RIC, 3) PB source of grafts, and 4) 10 out of 10 HLA match for unrelated donors or 8 out of 8 HLA match for related donors. Fifty-one patients were excluded for the following reasons: AML arising from an underlying myeloproliferative neoplasm (n = 25), MDS and myeloproliferative neoplasm overlap (n = 2), second allogeneic HCT (n = 14), prior autologous HCT (n = 5), and incomplete records (n = 5). Data from the remaining 127 patients were included in the final analysis. The study was approved by the institutional review board of Mayo Clinic in Rochester, Minnesota.

Transplantation

Donors were HLA-A, HLA-B, HLA-C, and high-resolution HLA-DR fully matched siblings or matched unrelated donors. We used either fludarabine plus busulfan (Flu/Bu; 25 mg/m^2/day fludarabine on Days −6 to −2 and 4 mg/kg/day PO busulfan on Days −5 to −2) or fludarabine plus melphalan (Flu/Mel; 25 mg/m^2/day fludarabine on Days −6 to −2 and 70 mg/m^2 melphalan on Days −3 and −2) as RIC regimens. GVHD prophylaxis consisted of methotrexate plus either cyclosporine or tacrolimus. Grafts were cryopreserved if not infused immediately after collection and were unprocessed except for RBC depletion in the case of major ABO-incompatible transplants to keep RBC content less than 30 mL based on the appropriate isoagglutinin titer. Prophylactic plasma exchange (PLX) to reduce antibody levels before donor cell infusion was not performed. Patients undergoing ABO-incompatible HCT received RBC transfusions of blood group O after the transplant. Plasma transfusions were done with recipient-type plasma in case of minor ABO mismatch and with donor-type plasma in case of major or bidirectional ABO mismatch HCT. Platelet (PLT) transfusions were based on the ABO blood typing of the patient at the time of infusion. Since August 2013, O blood group PLT donors with high anti-A titers were restricted from A blood group recipients. No routine policy was in place to remove incompatible plasma from either PLT or RBC components. All patients received similar supportive care according to Mayo Clinic's institutional protocols.

Definitions

RBC engraftment was defined as the last day of RBC transfusion that was followed by at least 30 days without transfusion. PLT engraftment was defined as the first of 7 consecutive days when PLT count was at least 20×10^9/L without PLT transfusion. Polymorphonuclear leukocyte (PMN) engraftment was defined as the first of 3 consecutive days when absolute neutrophil count was more than 0.5×10^9/L. PRCA was defined as reticulocytopenia of less than 30×10^9/L for more than 60 days after transplantation and lack of BM erythroid precursors. PRCA was diagnosed after excluding RBC alloantibodies, hemolysis, viral and/or bacterial infections, or relapse. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded according to previously published criteria.[13,14] NRM was defined as death unrelated to recurrence or disease progression. Disease-free survival (DFS) was defined as the time from transplantation until relapse or death from any cause. OS was defined as the time from transplantation until death from any cause.

TABLE 1. Patients and transplantation characteristics according to ABO compatibility groups*

Characteristics	Total, n = 127 (100%)	ABO compatible, n = 80 (63%)	Major/bidirectional mismatch, n = 26 (20%)	Minor mismatch, n = 21 (17%)	p value
Age at transplantation (years)	60 (18-71)	60.5 (18-71)	60 (23-69)	62 (44-69)	0.45
Sex					
Male	76 (60)	50 (63)	13 (50)	13 (62)	0.51
Female	51 (40)	30 (37)	13 (50)	8 (38)	
Comorbidity index†					
HCT-CI < 3	64 (50)	40 (50)	11 (42)	13 (62)	0.40
HCT-CI ≥ 3	63 (50)	40 (50)	15 (58)	8 (38)	
Underlying disease					
AML	86 (68)	55 (69)	18 (70)	13 (62)	0.82
MDS	41 (32)	25 (31)	8 (30)	8 (38)	
Disease status at transplantation					
CR	97 (76)	59 (74)	20 (77)	18 (86)	0.51
Untreated or not in CR	30 (24)	21 (26)	6 (23)	3 (14)	
Donor relationship					
Related	75 (59)	52 (65)	13 (50)	10 (48)	0.20
Unrelated	52 (41)	28 (35)	13 (50)	11 (52)	
RIC regimen					
Flu/Bu	38 (30)	26 (33)	8 (31)	4 (19)	0.48
Flu/Mel	89 (70)	54 (67)	18 (69)	17 (81)	
GVHD prophylaxis‡					
TAC + MTX	54 (43)	30 (38)	13 (50)	11 (52)	0.41
CsA + MTX	70 (55)	47 (59)	13 (50)	10 (48)	

* Data are reported as median (range) or number (%).
† HCT-CI was used as previously described by Sorror et al.[34]
‡ Three patients receiving different GVHD prophylaxis not shown.
CsA = cyclosporine; HCT-CI = hematopoietic cell transplantation-specific comorbidity index; MTX = methotrexate; TAC = tacrolimus.

Statistical analysis

Analyses were performed using computer software (JMP, Version 10.0.0, SAS Institute, Inc., Cary, NC). Continuous variables were compared across groups using the Kruskal-Wallis test. Nominal variables were compared across groups using the chi-square or Fisher's exact tests. Relapse, NRM, and survival were estimated using the Kaplan-Meier method and compared across groups using the log-rank test. Multivariable survival analyses were performed using Cox's proportional hazards regression, with the findings summarized using hazard ratios (HRs) and 95% confidence intervals (CIs). Significance was set to 0.05 without adjustment for multiple testing.

RESULTS

Patient and transplantation characteristics

Of the 127 patients analyzed, 80 (63%) underwent ABO-compatible HCT. Of the remainder, 22 (17%) were major ABO mismatched, 21 (17%) were minor ABO mismatched, and four (3%) were bidirectional ABO mismatches. Given the small number, bidirectional mismatches were combined with major mismatches to form the major/bidirectional ABO mismatch group (n = 26). The median age at transplantation was 60 years (range, 18-71 years) for the entire cohort. Seventy-five (59%) patients received allogenic PB HCT from related donors and 52 (41%) from unrelated donors. The underlying diagnosis was AML in 86 (68%) and MDS in 41 (32%) patients. Of patients with MDS, 25 had excess blasts (>5%) and 16 did not. At the time of transplantation, 97 (76%) patients were in complete remission (CR), and 30 (24%) were untreated or not in CR. As shown in Table 1, no significant difference in patients and transplant characteristics was observed across the three ABO compatibility groups.

Engraftment, transfusions, and hemolysis

Transfusion and engraftment data according to the ABO compatibility groups are shown in Table 2. Engraftment data were evaluable in 125 patients who had close follow-up and did survive beyond 30 days after HCT. Time to RBC and PLT engraftment was significantly different across the three groups. RBC engraftment was delayed from a median of 12 days in the ABO-compatible group to 32 days in the major/bidirectional mismatch group ($p = 0.0014$). Time to PLT engraftment was delayed from a median of 11 days in the ABO-compatible group to 13.5 days in the major/bidirectional mismatch group ($p = 0.005$). No significant difference in time to RBC and PLT engraftment was seen between ABO-compatible and minor-ABO-mismatch groups ($p = 0.88$ and $p = 0.52$, respectively). Time to PMN engraftment was not significantly different across the three groups ($p = 0.56$).

TABLE 2. Transfusion and engraftment data according to ABO compatibility groups*

Total (n = 127)	ABO compatible (n = 80)	Major/bidirectional mismatch (n = 26)	Minor mismatch (n = 21)	p value
Engraftment				
Number evaluable (n = 125)	78	26	21	
Days to RBC engraftment	12 (0-185)	32 (0-294)	10 (0-137)	0.003†
Days to PLT engraftment	11 (0-185)	13.5 (0-97)	12 (0-53)	0.014†
Days to PMN engraftment	18 (12–54)	17 (10-23)	16 (12–22)	0.56
0-100 day transfusion				
Number evaluable (n = 118)	76	23	19	
Patients received RBCs	69 (91)	20 (87)	14 (74)	0.15
Patients received PLTs	67 (88)	21 (91)	16 (90)	0.83
RBC units transfused	3 (0-36)	8 (0-41)	2 (0-21)	0.009†
PLT units transfused	3.5 (0-64)	6 (0-43)	3 (0-23)	0.27
101–365 day transfusion				
Number evaluable (n = 82)	56	13	13	
Patients received RBCs	15 (27)	6 (46)	2 (15)	0.22
Patients received PLTs	13 (23)	6 (46)	1 (8)	0.08
RBC units transfused	0 (0-44)	0 (0-43)	0 (0-16)	0.19
PLT units transfused	0 (0-89)	0 (0-72)	0 (0-16)	0.07

* Data are reported as median (range) or number (%).
† Significant

TABLE 3. Incidence and severity of aGVHD and cGVHD according to ABO compatibility groups*

Patients (n = 127)	ABO compatible (n = 80)	Major/bidirectional mismatch (n = 26)	Minor mismatch (n = 21)	p value
aGVHD				
Number evaluable (n = 125)	78	26	21	
Grade 0-I	53 (55)	9 (35)	7 (33)	0.015†
Grade II-IV	35 (45)	17 (65)	14 (67)	
cGVHD				
Number evaluable (n = 118)	76	23	19	
Absent-mild	31 (41)	16 (70)	9 (47)	0.053
Moderate-severe	45 (59)	7 (30)	10 (53)	

* Data are reported as number (%).
† Significant.

Transfusion requirement at 100 days was evaluable in 118 patients who had close follow-up and did survive beyond 100 days after HCT. The majority of patients required RBC and PLT transfusion during the first 100 days (74% to 91% for RBCs and 88% to 91% for PLTs), with no significant difference in incidence across the three ABO compatibility groups. The major/bidirectional mismatch group required a significantly higher number of RBC units compared to the ABO-compatible group (median, 8 units vs. 3 units; $p = 0.009$). PLT transfusion requirement was not significantly different across the three groups ($p = 0.27$).

Transfusion requirement after 100 days and until 1 year after HCT was evaluable in 82 patients who were alive more than 1 year after HCT. During this period, a greater proportion of major/bidirectional mismatches required RBC and PLT transfusion compared to the ABO-compatible group (46% vs. 27% for RBC transfusion and 46 vs. 23% for PLT transfusion), although the difference was not significant ($p = 0.15$ for RBC and $p = 0.09$ for PLT). RBC and PLT units transfused after 100 days and until 1 year after HCT were not significantly different across the three ABO compatibility groups (median, 0 for both RBC and PLT units; $p = 0.19$ and $p = 0.07$ for RBCs and PLTs, respectively).

Acute hemolysis was not observed in any of the patients. One patient developed warm antibody-mediated autoimmune hemolytic anemia 3 months after a minor mismatched allogenic HCT for AML (O+ to A+). This was managed with a combination of high-dose steroids, intravenous gammaglobulin and rituximab (RTX) and resolved after 3 months of therapy.

aGVHD and cGVHD

The incidence and severity of aGVHD and cGVHD according to ABO compatibily groups are shown in Table 3. aGVHD was evaluable in 125 pateints who had been

TABLE 4. Patient outcomes according to ABO compatibility groups

Patients (n = 127)	ABO compatible (n = 80)	Major/ bidirectional mismatch (n = 26)	Minor mismatch (n = 21)
Relapse			
Number of events (%)	19 (24)	7 (27)	2 (9)
HR (95% CI)	1	1.23 (0.48-2.80)	0.46 (0.07-1.61)
3-year probability	29%	27%	12%
p value		0.63	0.25
NRM			
Number of events (%)	18 (23)	13 (50)	6 (29)
HR (95% CI)	1	2.70 (1.28-5.56)	1.54 (0.55-3.71)
3-year probability	23%	49%	33%
p value		0.009*	0.37
DFS			
Number of events (%)	37 (47)	20 (77)	8 (38)
HR (95% CI)	1	1.78 (1.00–3.08)	0.86 (0.37-1.77)
3-year probability	52%	37%	59%
Months, median	18.5	10.5	26
p value		0.048*	0.71
OS			
Number of events (%)	36 (45)	18 (69)	8 (38)
HR (95% CI)	1	1.82 (1.01–3.18)	1.0 (0.43-2.05)
3-year probability	56%	39%	59%
Months, median	23	13.5	26
p value		0.046*	0.99

* Significant.

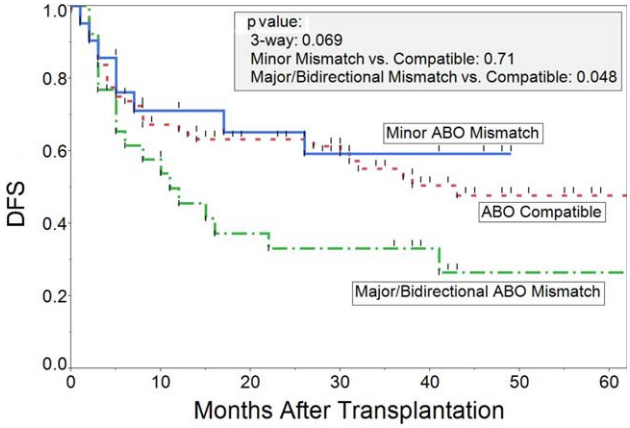

Fig. 1. Probabilities of DFS according to ABO compatibility groups.

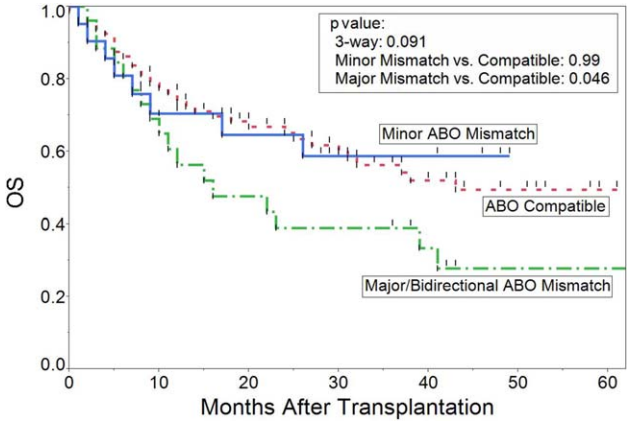

Fig. 2. Probabilities of OS according to ABO compatibility groups.

successfully engrafted and had survived at least 30 days after HCT. The incidence of grade II to IV aGVHD was significantly higher in the major/bidirectional (65%) and in the minor-ABO-mismatch groups (67%) compared to ABO-compatible patients (45%; $p = 0.037$ and $p = 0.047$, respectively). cGVHD was evaluable in 118 patients who lived at least 100 days after HCT. The incidence of moderate to severe cGVHD was lower in the major/bidirectional ABO-mismatched group compared to ABO-compatible patients (30% vs. 59%, $p = 0.028$), but not significantly different from the minor-ABO-mismatch group (53% vs. 59%, $p = 0.79$).

Outcomes

With a median follow-up of 19 months (range, 3 days-86 months), relapse occurred in 28 (22%), NRM in 37 (29%), and death in 62 (49%) patients (Table 4). Thirty-six (45%) of 80 ABO-compatible patients died, with the main causes being relapse in 15 (42%), infection in seven (20%), GVHD in three (8%), and "other" in 11 (30%) patients. Death occurred in 18 (69%) of 26 major/bidirectional mismatches, with the main causes being infection in six (33%), relapse in five (28%), GVHD in three (17%), and "other" in four (22%) patients. Eight (38%) of 21 patients with minor ABO mismatches died, with the main causes being infection in four (50%), GVHD in two (25%), and "other" in two (25%) patients. As shown in Table 4, the major/bidirectional mismatch group had a significantly higher NRM (estimated 3-year probability of 49% vs. 23%, $p = 0.009$) compared with the ABO-compatible group. NRM within the first 100 days comprised 17, 24, and 33% of the cumulative NRM in ABO-compatible, major/bidirectional ABO-mismatched, and minor ABO-mismatched transplants, respectively (data not shown in the table). No significant difference was observed in the cumulative incidence of relapse across the three groups ($p = 0.46$).

The Kaplan-Meier curves for DFS and OS according to ABO compatibility groups are shown in Figs. 1 and 2, respectively. The differences in DFS and OS across the three ABO compatibility groups demonstrated a trend, but were not significant ($p = 0.069$ and $p = 0.091$ for DFS and OS, respectively). However, when the major/bidirectional

group was directly compared with the ABO-compatible group, the difference was significant, with major/bidirectional mismatches having an inferior DFS (median, 10 months vs. 18.5 months; p = 0.048) and OS (median, 13.5 months vs. 21.5 months; p = 0.046) compared with the ABO-compatible group.

Multivariable analysis

Multivariable analysis was performed to determine the impact of ABO incompatibility on clinical outcomes (relapse, NRM, DFS, and OS) while adjusting for potential confounders. The following factors were included in a Cox regression model: age (continuous variable), HCT comorbidity index (0-2 vs. ≥3), underlying disease (AML vs. MDS), disease status at transplantation (CR vs. not CR/untreated), donor source (related vs. unrelated), and conditioning regimens (Flu/Bu vs. Flu/Mel). As shown in Table 5, major/bidirectional ABO incompatibility was independently associated with higher NRM (HR, 2.73; p = 0.01), lower DFS (HR, 1.84; p = 0.04), and lower OS (HR, 1.91; p = 0.035). Relapse was not affected by ABO compatibility status in multivariable analysis.

TABLE 5. Multivariable* analysis of ABO incompatibility effect on clinical outcomes

Patients (n = 127)	ABO compatible (n = 80)	Major/ bidirectional mismatch (n = 26)	Minor mismatch (n = 21)
Relapse			
HR (CI)	1	1.36 (0.52-3.12)	0.77 (0.11-2.85)
p value		0.50	0.72
NRM			
HR (CI)	1	2.73 (1.27-5.74)	1.34 (0.47-3.33)
p value		0.010†	0.55
DFS			
HR (CI)	1	1.84 (1.02–3.21)	0.95 (0.40-2.0)
p value		0.041†	0.89
OS			
HR (CI)	1	1.91 (1.04-3.36)	1.04 (0.43-2.20)
p value		0.035†	0.91

* Variables included in the model were age, comorbidity index, underlying condition, disease status at transplantation, donor relationship, and conditioning regimens.
† Significant.

PRCA

PRCA developed in four (15% of major/bidirectional mismatch) patients, who had received an A-to-O HCT. Transplant characteristics and clinical outcomes of these patients are shown in Table 6. The underlying diagnoses were MDS in two patients, and AML in two. PRCA developed in four (50%) of the eight major/bidirectional mismatches who were conditioned with Flu/Bu, compared to none (0%) of the 18 major/bidirectional mismatches who were conditioned with Flu/Mel. Using Fisher's exact test, there was a significant association between Flu/Bu conditioning and PRCA (p = 0.0046). Time to PMN engraftment in patients with PRCA ranged between 16 and 22 days, which was similar to that in patients with major/bidirectional mismatches who did not have PRCA (median, 16.5 days). All patients had achieved a BM chimerism of at least 95% donor at the time of PRCA diagnosis. In all patients, isohemagglutinin antibody (IHA) titers were

TABLE 6. Characteristics and clinical course of four patients with PRCA

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	63	55	60	41
Underlying diagnosis	MDS	MDS	AML	AML
Blood group (donor → recipient)	A+ → O+	A+ → O−	A+ → O+	A+ → O+
Donor relationship	Related	Related	Unrelated	Unrelated
Conditioning regimen	Flu/Bu	Flu/Bu	Flu/Bu	Flu/Bu
GVHD prophylaxis	CsA + MTX	CsA + MTX	Tac + MTX	Tac + MTX
CD34+ cell dose ($\times 10^6$/kg)	5.13	6.32	7.34	4.99
Pretransplantation IHA titer	>2048	>2048	1024	256
IHA titer at PRCA diagnosis	>2048	512	512	512
IHA titer at PRCA resolution	NR	8	32	256
100-day BM chimerism (%)	100	95	100	100
RBC transfusion				
0-100 days	11	11	17	21
101-365 days	NR	28	5	5
Time to PMN engraftment (days)	16	22	21	22
Acute GVHD grade (I-IV)	IV	II	II	II
cGVHD grade (mild-severe)	Absent	Absent	Absent	Severe
Treatment for PRCA, in order	EPO, RTX, CsA taper	RTX, CsA taper, PLX	RTX, TAC taper	EPO, RTX, TAC taper, PLX, DLI
Time to PRCA resolution (months)	NR	11	6	4
Follow-up (months)	5	13	16	43
Relapse (yes/no)	No	No	No	No
Survival status	Died	Alive	Died	Alive

CsA = cyclosporine; EPO = erythropoietin; MTX = methotrexate; NR = not reached; TAC = tacrolimus.

elevated, with titers being highly variable, both before the transplant (range, 256->2048) and at the time of PRCA diagnosis (range, 512->2048). In patients whose PRCA had resolved (three of four patients), IHA titers decreased but still remained highly variable (range, 8-256) at the time of PRCA resolution. aGVHD was present in all patients (Grade II in three and Grade IV in one patient), but cGVHD was present only in one patient. PRCA treatments included RTX and tapering of immunosuppression for all four patients, followed by PLX in two, and donor lymphocyte infusion (DLI) in one patient. Two patients (one with persistent PRCA and one after PRCA resolution) died after 5 and 16 months, respectively, and two patients were still alive after 16 and 43 months of follow-up. None of the PRCA patients had evidence for residual or recurrent disease.

DISCUSSION

We evaluated the impact of ABO incompatibility on transplant outcomes in 127 patients with AML or MDS who exclusively received HLA-matched PB allogenic HCT after RIC in a single-institution setting. Results demonstrated a significantly higher NRM, lower DFS, and lower OS in the ABO major/bidirectional group compared to ABO-compatible patients. Minor ABO mismatches were not associated with adverse outcomes.

Our results are in keeping with those of Logan and colleagues,[5] who retrospectively analyzed three different transplant populations; they found that ABO minor and major mismatch were associated with worse transplant outcomes, although with inconsistent patterns. Their analysis of two of three cohorts demonstrated that ABO minor mismatch was associated with inferior OS and increased NRM. One of these cohorts included 1,737 patients with lymphoid and myeloid neoplasms receiving PB and BM HCT after RIC and MA conditioning, and the other one included 435 patients with lymphoma undergoing PB HCT after RIC and MA conditioning. Analyzing a third cohort of 5,179 AML and MDS patients undergoing PB and BM HCT after RIC and MA conditioning, however, they did not find an association between ABO minor mismatch and transplant outcomes. Instead, they found a significant association between ABO major mismatch and increased NRM and inferior OS. As suggested by the authors, contradictory results when assessing the impact of ABO incompatibilities on transplant outcomes could be related to the heterogeneity of study populations and transplant characteristics.

Our data add to the evidence that ABO incompatibility is associated with adverse transplant outcomes in the setting of RIC HCT.[8,15] Furthermore, we were able to demonstrate this in a smaller but more homogeneous group of patients. In contrast, the majority of previous studies that failed to demonstrate an adverse survival effect in the context of ABO incompatibility either were limited to MA HCT[16,17] or included a heterogeneous mixture of MA and RIC regimens without subgroup analysis.[10,18] To our knowledge, the only large-scale study limited to RIC HCT that failed to identify a hazard from ABO incompatibility was that of Wang and colleagues,[3] who analyzed a group of 503 patients receiving non-myeloablative HCT. Their study population, however, included a very heterogeneous group of underlying diagnoses, including acute and chronic leukemias of both lymphoid and myeloid lineage, as well as lymphoma and multiple myeloma.

In our study, ABO major/bidirectional mismatch was associated with a significantly delayed RBC and PLT engraftment, with no difference in time to PMN engraftment. Additionally, the number of RBC units transfused during the first 100 days post-HCT was significantly increased in the ABO major/bidirectional mismatch group. PLT transfusion was not significantly affected, possibly due to the smaller increase in time to PLT engraftment (from 11 days to 13.5 days) compared to the increase in time to RBC engraftment (from 12 days to 32 days). Several previous studies have reported delayed RBC engraftment and increased RBC transfusion in the setting of ABO major mismatch with RIC HCT.[9,19] There are also reports that RIC HCT, when used in the context of ABO major mismatch, is associated with higher transfusion requirements than in MA HCT.[3,20] A few studies, however, contradict these results, showing no difference in RBC and PLT transfusion or engraftment time in the setting of RIC HCT and ABO incompatibility.[7,18,21] The majority of these studies, however, included small and heterogeneous populations, with the total number of major/bidirectional ABO mismatches in each study ranging between 10 and 15 (compared to 26 in our study). The only relatively large-scale study on an RIC allogenic HCT population that failed to show a difference in transfusion and engraftment is that of Resnick and colleagues,[8] who analyzed a total of 221 patients, including 56 major-ABO-mismatch patients. They used relatively similar conditioning and immunosuppression regimens to ours, but their study population was heterogeneous in several other aspects, including in HLA matching, graft type, and underlying diagnoses. With regard to PMN engraftment, our results are in keeping with several previous studies that showed no effect from ABO incompatibility.[9,16,19]

Increased incidence and severity of aGVHD were observed in our major/bidirectional and minor-ABO-mismatch patients. This was consistent with the findings of several earlier studies[10,22] and could be related to the fact that A/B antigens are ubiquitously expressed on most tissues.[23] Whether increased incidence and severity of aGVHD translates into increased cGVHD and transplant-related mortality, however, remains controversial. Analyzing 221 RIC HCT patients, Resnick and coworkers[8] showed a significantly higher incidence of aGVHD-related

death in the major-ABO-mismatch group and a trend for increased aGVHD-related mortality among minor-ABO-mismatch patients. Ludajic and colleagues,[10] on the other hand, performed a competing risk analysis on 154 patients (including both RIC and MA transplants) and did not find an association between increased aGVHD and cGVHD or transplant-related mortality. In our study, the incidence of moderate and severe cGVHD was lower in the major-ABO-mismatch group. However, this could be simply related to the inferior OS of major/bidirectional mismatches and hence lower chance of being observed to have cGVHD. As far as cause of death, major/bidirectional ABO mismatches had a higher rate of death due to infection (33% vs. 20%) and GVHD (17% vs. 8%), but lower rate of death caused by relapse (28% vs. 42%) in comparison with the ABO-compatible group. Statistical comparisons were, however, not possible due to small numbers in each category. Other limitations of our study were lack of subgroup analysis based on donor relationship (related vs. unrelated) and diagnosis of AML versus MDS. We did not divide our study population into these smaller subgroups as it would have reduced the power of the study and increased multiple testing error. We did, however, demonstrate that these variables were evenly distributed across the three ABO compatibility groups and did include them in the multivariable analysis of outcomes.

The incidence of PRCA is estimated to be between 0 and 16% after MA HCT[20,24] and between 8 and 38% after RIC HCT.[25,26] Although there is no consensus on its mechanism and risk factors, increased frequencies of PRCA are reported in the setting of myeloid malignancies,[27] RIC versus MA conditioning,[20] related versus unrelated HCT, donor A to recipient O transplant,[20,28] and the absence of aGVHD.[27,29] The incidence of PRCA in our cohort of AML or MDS patients who underwent HLA-matched RIC allogenic HCT from related or unrelated PB grafts was 15%. This is comparable to previous reports for RIC HCT. All four PRCA cases in our study had received an A-to-O transplant, which is consistent with previous reports showing PRCA primarily occurs when the donor is group A and the recipient is group O. This is thought to be due to increased clearance time of anti-A (median, 160 days) versus anti-B (median, 51 days) isohemagglutinins.[30]

There was a significant association between Flu/Bu conditioning and PRCA in our study. Aung and colleagues[27] reported similar results. In a retrospective analysis of 161 major-ABO-mismatched HCTs, they found 12 cases of PRCA, of which nine were conditioned with Flu/Bu and three with other regimens. They raised a possibility that Flu/Bu conditioning might actually be a confounding factor associated with lower incidence of aGVHD, and therefore lower graft-versus-plasma cell activity, which in turn results in a slower decline in donor-specific IHA titers and increases the risk of PRCA. In our study, all patients with PRCA had at least Grade II or higher aGVHD. Therefore, the higher risk of PRCA with Flu/Bu seems to be independent of its impact on the incidence of aGVHD. At PRCA diagnosis, IHA titers were elevated in the presence of nearly full donor myeloid chimerism both in our study and in previous reports.[24,31]

Most studies have reported that PRCA resolves spontaneously or after reduction of immunosuppression.[20,27-29] Other treatments tried by other studies include RTX,[29] PLX,[32] and DLI,[33] although with inconsistent results. All our patients were treated with tapering of immunosuppression and RTX, followed by PLX in two patients and DLI in one patient.

Our study adds to the evidence that ABO incompatibility is associated with increased morbidity and mortality in the setting of RIC allogenic HCT. Delayed RBC and PLT engraftment, increased transfusion needs, PRCA, and severe grades of aGVHD are among the immunohematologic complications of major/bidirectional ABO mismatch HCT, while increased NRM and lower DFS and OS are major adverse outcomes. The risks associated with ABO incompatibility may vary according to the study population and transplant process. Therefore, further studies on homogenous groups of patients are warranted to identify groups at greatest risk.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 1998;91:756-63.
2. Baron F, Vanstraelen G, Beguin Y. Transfusions after non-myeloablative or reduced-intensity conditioning regimens. Leukemia 2006;20:2081-6.
3. Wang Z, Sorror ML, Leisenring W, et al. The impact of donor type and ABO incompatibility on transfusion requirements after nonmyeloablative haematopoietic cell transplantation. Br J Haematol 2010;149:101-10.
4. Worel N, Greinix H, Keil F, et al. Severe immune hemolysis after minor ABO-mismatched allogeneic peripheral blood progenitor cell transplantation occurs more frequently after nonmyeloablative than myeloablative conditioning. Transfusion 2002;42:1293-301.
5. Logan AC, Wang Z, Alimoghaddam K, et al. ABO mismatch is associated with increased non-relapse mortality after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2015;21:746-54.

6. Michallet M, Le QH, Mohty M, et al. Predictive factors for outcomes after reduced intensity conditioning hematopoietic stem cell transplantation for hematological malignancies: a 10-year retrospective analysis from the Société Française de Greffe de Moelle et de Thérapie Cellulaire. Exp Hematol 2008;36:535-44.
7. Erker CG, Steins MB, Fischer RJ, et al. The influence of blood group differences in allogeneic hematopoietic peripheral blood progenitor cell transplantation. Transfusion 2005;45: 1382-90.
8. Resnick IB, Tsirigotis PD, Shapira MY, et al. 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. Biol Blood Marrow Transplant 2008; 14:409-17.
9. Canals C, Muñiz-Díaz E, Martínez C. Impact of ABO incompatibility on allogeneic peripheral blood progenitor cell transplantation after reduced intensity conditioning. Transfusion 2004;44:1603-11.
10. Ludajic K, Balavarca Y, Bickeböller H, et al. Minor ABO-mismatches are risk factors for acute graft-versus-host disease in hematopoietic stem cell transplant patients. Biol Blood Marrow Transplant 2009;15:1400-6.
11. Mehta J, Powles R, Sirohi B, et al. Does donor-recipient ABO incompatibility protect against relapse after allogeneic bone marrow transplantation in first remission acute myeloid leukemia? Bone Marrow Transplant 2002;29:853-9.
12. Kanda J, Ichinohe T, Matsuo K, et al. Impact of ABO mismatching on the outcomes of allogeneic related and unrelated blood and marrow stem cell transplantations for hematologic malignancies: IPD-based meta-analysis of cohort studies. Transfusion 2009;49:624-35.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant 1995;15:825-8.
14. Sullivan KM, Shulman HM, Storb R, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. Blood 1981;57:267-76.
15. Kimura F, Sato K, Kobayashi S, et al. Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan marrow donor program. Haematologica 2008;93: 1686-93.
16. Mielcarek M, Leisenring W, Torok-Storb B, et al. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence for a graft-versus-plasma cell effect. Blood 2000;96:1150-6.
17. Seebach JD, Stussi G, Passweg JR, et al. ABO blood group barrier in allogeneic bone marrow transplantation revisited. Biol Blood Marrow Transplant 2005;11:1006-13.
18. Kim JG, Sohn SK, Kim DH, et al. Impact of ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplant 2005;35: 489-95.
19. Badros A, Tricot G, Toor A, et al. ABO mismatch may effect engraftment in multiple myeloma patients receiving nonmyeloablative conditioning. Transfusion 2002;42: 205-9.
20. Bolan CD, Leitman SF, Griffith LM, et al. Delayed donor red cell chimerism and pure red cell aplasia following major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation. Blood 2001;98:1687-94.
21. Kanda Y, Tanosaki R, Nakai K, et al. Impact of stem cell source and conditioning regimen on erythrocyte recovery kinetics after allogeneic haematopoietic stem cell transplantation from an ABO-incompatible donor. Br J Haematol 2002;118:128-31.
22. Stussi G, Seebach L, Muntwyler J, et al. Graft-versus-host disease and survival after ABO-incompatible allogeneic bone marrow transplantation: a single-centre experience. Br J Haematol 2001;113:251-3.
23. Ernst C, Thurin J, Atkinson B, et al. Monoclonal antibody localization of A and B isoantigens in normal and malignant fixed human tissues. Am J Pathol 1984;117: 451-61.
24. Worel N, Greinix HT, Schneider B, et al. Regeneration of erythropoiesis after related- and unrelated-donor BMT or peripheral blood HPC transplantation: a major ABO mismatch means problems. Transfusion 2000;40:543-50.
25. Worel N, Kalhs P, Keil F, et al. ABO mismatch increases transplant-related morbidity and mortality in patients given nonmyeloablative allogeneic HPC transplantation. Transfusion 2003;43:1153-61.
26. Malfuson JV, Amor RB, Bonin P, et al. Impact of nonmyeloablative conditioning regimens on the occurrence of pure red cell aplasia after ABO-incompatible allogeneic haematopoietic stem cell transplantation. Vox Sang 2007; 92:85-9.
27. Aung FM, Lichtiger B, Bassett R, et al. Incidence and natural history of pure red cell aplasia in major ABO-mismatched haematopoietic cell transplantation. Br J Haematol 2013;160: 798-805.
28. Zhu K, Li J, Zhang T, et al. Clinical features and risk factors of pure red cell aplasia following major ABO-incompatible allogeneic hematopoietic stem cell transplantation. Hematology 2007;12:117-21.
29. Helbig G, Stella-Holowiecka B, Wojnar J, et al. Pure red-cell aplasia following major and bi-directional ABO-incompatible allogeneic stem-cell transplantation: recovery of donor-derived erythropoiesis after long-term treatment using different therapeutic strategies. Ann Hematol 2007; 86:677-83.
30. Lee JH, Lee KH, Kim S, et al. Anti-a isoagglutinin as a risk factor for the development of pure red cell aplasia after major ABO-incompatible allogeneic bone marrow transplantation. Bone Marrow Transplant 2000;25:179-84.

31. Griffith LM, McCoy JP, Bolan CD, et al. Persistence of recipient plasma cells and anti-donor isohaemagglutinins in patients with delayed donor erythropoiesis after major ABO incompatible non-myeloablative haematopoietic cell transplantation. Br J Haematol 2005;128:668-75.
32. Damodar S, George B, Mammen J, et al. Pre-transplant reduction of isohaemagglutinin titres by donor group plasma infusion does not reduce the incidence of pure red cell aplasia in major ABO-mismatched transplants. Bone Marrow Transplant 2005;36:233-5.
33. Musso M, Porretto F, Crescimanno A, et al. Donor lymphocyte infusions for refractory pure red cell aplasia relapsing after both autologous and nonmyeloablative allogeneic peripheral stem cell transplantation. Bone Marrow Transplant 2004;33: 769-71.
34. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005; 106:2912-9.