Impact of ABO-blood group incompatibility on the outcome of recipients of bone marrow transplants from unrelated donors in the Japan Marrow Donor Program

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ABSTRACT

Background
Although the ABO blood group is one of two major antigen systems of relevance for transplantation in humans, there are still conflicting data concerning the influence of ABO incompatibility on transplant outcome. This study investigated the effect of ABO incompatibility in recipients of bone marrow transplants from unrelated donors.

Design and Methods
We retrospectively analyzed data from 5,549 patients who underwent bone marrow transplantation from unrelated donors in the Japan Marrow Donor Program.

Results
Overall survival rates in the group with major and minor mismatches were significantly lower than the rate in the ABO-identical group (ABO-identical 63.0%; major mismatch, 56.9%; minor mismatch, 57.1% at 1 year). Treatment-related mortality was higher in the major and minor mismatch groups, but there was no significant difference in the rate of relapse. Cox proportional hazards modeling showed that both major and minor ABO incompatibility were significant risk factors for transplant-related mortality, independently of disease, patients' age, and HLA incompatibility. Delayed engraftment of neutrophils, platelets, and erythrocytes was observed in transplants with major incompatibility. There was a high incidence of grade 3 and 4 acute graft-versus-host disease in the groups with major and minor mismatches, which was caused by a high incidence of stage 2 to 4 liver graft-versus-host disease. Interestingly, the risk of grade 2 to 4 graft-versus-host disease in the major mismatch group was higher in patients with early engraftment of erythrocytes. Among the patients receiving reduced-intensity conditioning, the transplant-related mortality was also increased in ABO-incompatible transplants.

Conclusions
Major and minor ABO incompatibility have specific effects on transplant-related mortality and acute graft-versus-host disease in recipients of bone marrow transplants from unrelated donors.

Key words: bone marrow transplantation, unrelated donor, ABO blood type, acute graft-vs-host disease, graft failure.

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Introduction

The AB0 blood group and human leukocyte antigens (HLA) are two major antigen systems of relevance for transplantation in humans. In organ transplantation, AB0 compatibility between the donor and recipient is critical, while the role of the HLA system is minor.[1] High-titer anti-A/B in patients usually induces hyperacute rejection of grafts expressing foreign A or B antigen. In contrast, successful allogeneic hematopoietic transplantation was developed using an HLA-identical stem cell source. AB0 incompatibility is not supposed to be a barrier to stem cell transplantation. However, the recovery of erythrocytes is, in fact, delayed by the persistence of recipient anti-A/B against donor red cells, and there are still conflicting data concerning the influence of AB0 incompatibility on transplant outcome, graft-versus-host disease (GVHD), relapse, and survival.[2-7]

There are two problems in studying the effect of AB0 blood group incompatibility in stem cell transplantation. The first is that there are three types of incompatibility: major incompatibility, which occurs when a patient has anti-A/B against donor red cells; minor incompatibility, which occurs when donor plasma contains anti-A/B against the recipient red cells; and bidirectional incompatibility, which occurs when both donor and recipient have anti-A/B against each other. If the number of analyzed patients is not sufficiently large, the data cannot be analyzed for each mismatched group. Many previous studies failed to identify a significant effect of incompatibility, but it may be that a larger volume of data needs to be analyzed in order to identify an effect. Stem cell sources other than bone marrow are currently available,[8,9] and non-myeloablative or reduced-intensity conditioning regimens are now used for stem cell transplantation.[10-12] AB0 incompatibility may have different effects when different stem cell sources and conditioning regimens are used.

In bone marrow transplantation from an unrelated donor (UR-BMT), some patients can find a number of suitable donors through a donor program. Identifying the effects of AB0-blood type incompatibility can help physicians select better donors. In this study, we analyzed data from 5,549 patients who underwent UR-BMT through the Japan Marrow Donor Program (JMDP) to study the effects of AB0 incompatibility on various outcomes of UR-BMT.

Design and Methods

Patients

We retrospectively analyzed the data of patients who underwent a first UR-BMT through the JMDP between 1993 and 2005 and for whom complete data on AB0-blood group compatibility, age, and HLA compatibility were available. A total of 5,549 patients were transplanted with marrow from AB0-matched (n=2,820, 50.8%), major-incompatible (n=1,384, 24.9%), minor-incompatible (n=1,202, 21.7%), and bidirectional-incompatible donors (n=143, 2.6%) and the survivors were followed up over a median period of 1,831 days (range, 84–5,187 days). Acute leukemia, malignant lymphoma, and multiple myeloma in first or second remission, chronic myeloid leukemia in first or second chronic phase, and myelodysplastic syndromes without leukemic transformation were considered standard-risk diseases, whereas other malignant hematologic diseases were considered high-risk diseases. Diseases that are not malignant were considered benign diseases. Causes of death were categorized as graft failure, disease progression, GVHD, interstitial pneumonia, sepsis, bleeding, hepatic veno-occlusive disease, renal failure, liver failure, and others. All causes of death except disease progression were considered treatment-related mortality. Engraftment was defined as a peripheral granulocyte count more than 0.5×10^9/L for 3 successive days. Secondary graft failure was defined as a peripheral granulocyte count of less than 0.5×10^9/L with a finding of severe hypoplastic marrow in engrafted cases. The occurrence of acute GVHD was evaluated according to grading criteria in patients who survived more than 7 days after transplantation. The occurrence of chronic GVHD was evaluated according to standard criteria in patients who survived more than 100 days after transplantation. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and the study protocol was approved by the JMDP Institutional Review Board.

Statistical analysis

Statistical analysis was performed with JMP (version 5.1, SAS Institute Inc, Cary, NC, USA). For the Cox proportional hazards model and logistic regression model, multiple dichotomous variables were created, corresponding to each blood group compatibility and then incorporated into the models, with the other categories being a reference group. Overall survival and secondary graft failure were analyzed by the Kaplan-Meier method, and the log rank test was used to test the significance of differences. The incidence of blood cell recovery and GVHD were estimated by the cumulative incidence method considering death without recovery and death without GVHD as competing risks, respectively. Factors that significantly affected survival, blood cell recovery, and GVHD were evaluated by the Cox proportional hazards model. The cumulative incidence and grade of acute GVHD were also analyzed using a contingency table with likelihood-ratio χ^2 statistics. Factors involved in the development of GVHD were estimated with logistic regression analysis.

Results

Patients

Among the four groups defined according to AB0-compatibility, excluding age, HLA compatibility, and number of infused nucleated cells, there were no significant differences in the gender distributions of patients and donors, the number of transplantations, performance status before transplantation, conditioning regimen, GVHD prophylaxis, administration of colony-stimulating factors, or underlying diseases on the likelihood-ratio χ^2 test (Table 1).

Table 1. Patients' characteristics.

ABO-compatibility	Total	Match	Mismatch			p
			Major	Minor	Bidirectional	
Patient n.	5549	2820	1384	1202	143	
Age						
Median		31	30	31	36	0.02
Range		0-70	1-69	0-70	1-64	
Gender of patient						
Male	3364	1704	860	724	76	0.19
Female	2185	1116	524	478	67	
Gender of donor						
Male	3392	1718	850	737	87	0.95
Female	2144	1099	527	461	56	
N/A	13	3	7	4	0	
Performance status before BMT						
0	1335	691	333	267	44	0.29
1	1039	517	262	235	25	
2	244	122	66	52	4	
3	83	52	15	14	2	
4	14	6	3	3	2	
N/A	2834	1432	705	631	66	
Conditioning						
TBI regimen	3525	1764	896	779	86	0.31
non-TBI regimen	2024	1056	488	423	57	
Conventional	2009	1014	518	429	48	0.09
Reduced Intensity	666	348	152	136	30	
N/A	2784	1548	0	637	65	
Antithymocyte globulin						
Yes	463	222	128	105	8	0.25
No	5060	2583	1250	1092	135	
N/A	26	15	6	5	0	
CSF						
G-CSF	4923	2476	1254	1062	131	0.05
M-CSF	51	23	13	14	1	
G-CSF + M-CSF	22	12	7	2	1	
No CSF	477	274	87	107	9	
N/A	553	306	110	124	10	
HLA						
Identical	3433	1891	745	713	84	<0.001
Mismatch	2116	929	639	489	59	
Disease						
Benign	445	223	115	101	6	0.12
Malignant						
(standard risk)	2767	1451	672	570	74	
(high risk)	2337	1146	597	531	63	
GVH prophylaxis						
CsA + MTX	2676	1363	658	598	57	0.35
FK + MTX	2619	1321	663	555	80	
Others	254	136	63	49	6	
Number of infused nucleated cells Mean ($\times 10^8$ cells/kg)	2.65	3.07	1.79	2.80	1.74	< 0.001
Number of harvested nucleated cells Mean ($\times 10^8$ cells/kg)	3.19	3.12	3.31	3.22	3.49	0.21

N/A: data not available; BMT, bone marrow transplantation; TBI: total body irradiation; CSF: colony-stimulating factor; G-CSF: granulocyte CSF; M-CSF: macrophage CSF; CsA: cyclosporine A; FK: tacrolimus; MTX: methotrexate.

Survival

Overall survival differed significantly among the four groups (log rank test, p=0.0003); the estimates of 1-year overall survival for each group were 63.0% (AB0-matched), 56.9% (major incompatibility), 57.1% (minor incompatibility), and 63.5% (bidirectional incompatibility) (Figure 1A). Differences between the AB0-identical group and either the major or the minor mismatched group were statistically significant (log rank test; major incompatibility, p<0.0001; minor incompatibility, 0.0051); however, the difference between the AB0-identical group and the bidirectional mismatched group was not statistically significant (p=0.1884). The proportion of HLA-identical donors was significantly higher in the AB0-matched group than in the AB0-incompatible group (Table 1). Even among cases of HLA-matched transplants (n=3,433), a similar difference in overall survival was observed among the four groups (log rank test, p=0.006), and the overall survival rates of the major and minor mismatched groups were inferior to the rate of the AB0-compatible group (log rank test; major incompatibility, p=0.009; minor incompatibility, p=0.002; Figure 1B).

In AB0-mismatched transplants, the bone marrow must be processed to prevent hemolysis of donor or recipient red blood cells as a result of the infusion of AB0-incompatible red blood cells or plasma. This procedure may reduce the number of hematopoietic stem cells. In fact, the mean number of total infused nucleated cells $\times 10^8$ per patient body weight (kg), in each group was 3.07 (AB0-matched), 1.79 (major incompatibility), 2.80 (minor incompatibility), and 1.74 (bidirectional incompatibility) with a statistically significant difference (Kruskal-Wallis, p<0.001). To examine whether the difference in overall survival depended on the number of transplanted cells, HLA compatibility, or other factors that differed among the four groups, we used time-dependent Cox proportional hazards modeling (Table 2A). While the type of disease (standard and high-risk malignant disease or benign disease; p<0.001), patients' age (p<0.001), HLA mismatch (p<0.001), and major AB0 incompatibility (p=0.016) were significant risk factors, the number of infused cells was not (p=0.093), indicating that the number of infused nucleated cells did not reflect the number of hematopoietic stem cells. The number of harvested nucleated cells $\times 10^8$ per patient body weight (kg), was 3.12 (AB0-matched), 3.30 (major incompatibility), 3.22 (minor incompatibility), and 3.49 (bidirectional incompatibility) with no significant difference (Kruskal-Wallis, p=0.209), and was not a significant risk factor for overall survival in the proportional hazards model (p=0.116).

The cumulative incidences of transplant-related mortality differed significantly among the four groups (Figure 1C, p<0.0001), with the 1-year rates being 27.9% (AB0-matched), 35.8% (major incompatibility), 34.2% (minor incompatibility), and 30.7% (bidirectional incompatibility). In multivariate analysis, major and minor AB0 incompatibility significantly increased the risk of treatment-related mortality (Table 2A). Viewed from a different angle, the rates of disease-related death were significantly lower in the major and minor mismatched groups, although there was no significant difference in relapse rate (likelihood-ratio χ^2 test, p=0.068, *data not shown*).

We also analyzed the outcome of UR-BMT in the subgroup of patients who received reduced-intensity conditioning. Although the overall survival of AB0 incompatible groups was inferior to that of the group receiving AB0-compatible grafts, the difference among the four groups was not statistically significant (*data not shown*).

There was, however, a statistically significant difference in transplant-related mortality among the four groups (p=0.014, Figure 1D). In multivariate analysis, AB0 incompatibility significantly increased the risk of treatment-related mortality: the relative risks (RR) were 1.22 (95% CI, 1.04 - 1.48, p=0.014) for the major incompatibility group, 1.27 (1.09-1.48, p=0.003) for the minor incompatibility group and 1.42 (1.06 - 1.83, p=0.022) for the bidirectional incompatibility group.

Engraftment

Significant differences in the engraftment of red blood cells (1% reticulocytes), neutrophils (0.5×10^9/L), and platelets (50×10^9/L) were found among the four groups (log rank test, p<0.001, p<0.001, and p<0.001, respectively). Comparisons between the AB0-identical group and each mismatched group demonstrated that only the group with major mismatching showed a significantly delayed recovery of these blood cells (log rank test, p<0.001, p<0.001, p<0.001, respectively) (Figure 2A-C). There was no statistically significant difference between the AB0-identical group and the minor mismatched group (log rank test, p=0.962, p=0.431, p=0.068, respectively) or between the AB0-identical group and the bidirectional mismatched group (log rank test, p=0.379, p=0.120, p=0.200, respectively). Time-dependent Cox proportional hazards modeling demonstrated that major AB0 incompatibility, number of infused cells, and HLA identity significantly affected the recovery of these blood cells (Table 2B).

A few reports analyzing small numbers of patients have indicated that red blood cell recovery in patients with major mismatching is markedly delayed among those undergoing non-myeloablative hematopoietic stem cell transplantation.[10,11] In our analysis, there were some cases for which data from conventional (n=2009) or reduced-intensity transplantation (n=666) were available: we did not find a statistically significant difference in red blood cell recovery between these two groups with major AB0 incompatibility (log rank test, p=0.244, Figure 2D).

There was a significant difference in secondary graft failure among the four groups (log rank test, p=0.007).

Table 2A. **Multivariate analysis of overall survival and treatment-related mortality.**

Factor	*Overall survival*		*Treatment-related mortality*	
	Relative risk (95% CI)	*p*	*Relative risk (95% CI)*	*p*
Age	1.01 (1.01 - 1.01)	< 0.001	1.01 (1.02 - 1.02)	< 0.001
Cell number (x10^8/kg)	0.98 (0.96 - 1.00)	0.093	0.99 (0.96 - 1.01)	0.379
Disease risk				
Standard	1.00	< 0.001	1.00	< 0.001
High	1.63 (1.52 - 1.75)		1.30 (1.20 - 1.40)	
Benign	0.76 (0.67 - 0.85)		0.98 (0.87 - 1.11)	
HLA compatibility				
Matched	1.00	< 0.001	1.00	< 0.001
Mismatched	1.12 (1.08 - 1.16)		1.17 (1.12 - 1.22)	
ABO compatibility				
Major mismatched	1.06 (1.01 - 1.11)	0.016	1.12 (1.06 - 1.18)	< 0.001
Minor mismatched	1.03 (0.99 - 1.09)	0.163	1.08 (1.06 - 1.18)	0.009
Bidirectional	1.02 (0.90 - 1.14)	0.797	1.07 (0.93 - 1.22)	0.344

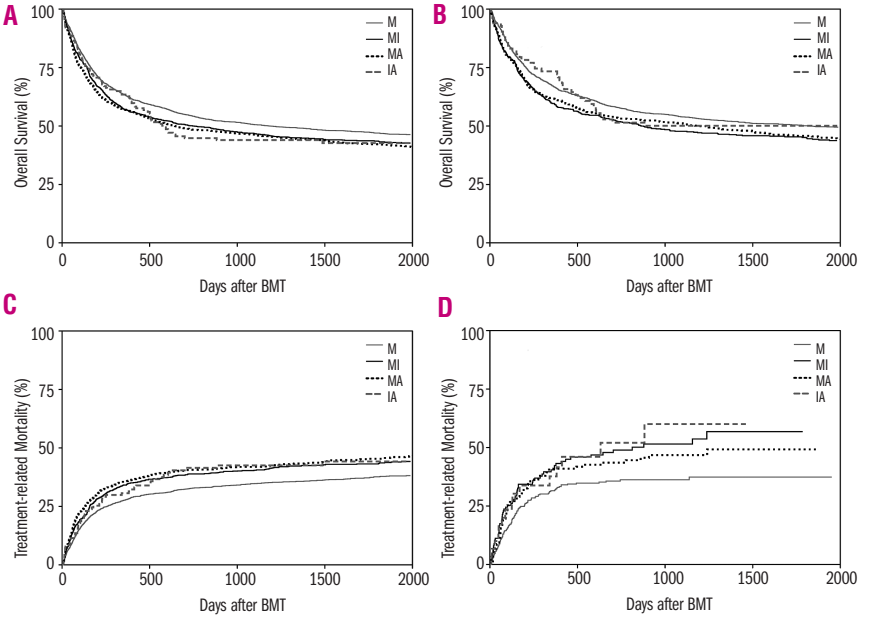

Figure 1. **ABO incompatibility and survival. Kaplan-Meier curves showing overall survival in patients transplanted with ABO-compatible (M) versus ABO-incompatible marrow (MI; minor mismatch, MA; major mismatch, IA; bidirectional mismatch) from all unrelated donors (A) or HLA-matched unrelated donors (B). Cumulative incidence curves showing the probability of treatment-related mortality in patients transplanted with ABO-compatible versus ABO-incompatible marrow with any conditioning (C) or with reduced-intensity conditioning (D).**

The incidence of graft failure in the mismatched groups was higher than that in the AB0 matched group (major incompatibility, p=0.007; minor incompatibility, p=0.031; bidirectional incompatibility, p=0.008; *data not shown*); however, Cox proportional hazards modeling failed to show that any AB0 incompatibility significantly affected secondary graft failure (*data not shown*).

Graft-versus-host disease

Log rank testing of cumulative incidence curves showed a statistically significant difference in grade 3 or 4 acute GVHD among the four groups (p<0.0001, Figure 3A). More grade 3 and grade 4 GVHD occurred in the major mismatched and minor mismatched groups than in the AB0-identical group (major incompatibility, p<0.0001; minor incompatibility, p=0.0002; bidirectional incompatibility, p=0.605). Multivariate analysis indicated that both major and minor AB0 incompatibility had a significant effect, similar to that of patients' age, HLA-compatibility, disease risk, and GVHD prophylaxis (Table 2C). There was no significant difference in chronic GVHD (*data not shown*).

The grade of acute GVHD is calculated from each stage of acute GVHD in the skin, gut, and liver. There was no significant difference in the occurrence of GVHD of the skin and gut among the four groups (likelihood-ratio χ^2 test; skin, p=0.67; gut, p=0.34, *data not shown*). However, the incidence of stage 2 to 4 hepatic GVHD was higher in the major and minor mismatched groups than in the AB0 matched group (log rank test, p<0.0001 and p<0.0001, respectively, Figure 3B). Cox proportional hazards modeling indicated that both major and minor incompatibility were significant risk factors for severe hepatic GVHD (Table 2C).

The stage of hepatic GVHD is determined by the total bilirubin level, which raises the possibility of increased indirect bilirubin causing a higher stage. Consequently, we analyzed the overall survival of patients with hepatic GVHD. The overall survival rates of the major and minor mismatched groups were comparable to the rate in the AB0-identical group, suggesting that hepatic GVHD in mismatched patients is not false (log rank test; major incompatibility, p=0.232; minor incompatibility, p=0.551; *data not shown*).

Patients with major mismatching were divided into two groups based on whether their reticulocytes reached 1% within 28 days after transplantation. Interestingly, grade 2 to 4 acute GVHD occurred more frequently among patients who had early recovery of erythrocytes than in those with delayed recovery (likelihood-ratio χ^2

Table 2B. Multivariate analysis of recovery of blood cells.

Factor	*1% reticulocytes*		*0.5x10⁹/L neutrophils*		*50x10⁹/L platelets*	
	Relative risk (95% CI)	*p*	*Relative risk (95% CI)*	*p*	*Relative risk (95% CI)*	*p*
Age	0.99 (0.99-0.99)	< 0.001	1.00 (1.00-1.00)	0.028	0.99 (0.99 - 1.00)	0.408
Cell number (x10⁸/kg)	1.03 (1.02-1.04)	< 0.001	1.03 (1.02-1.04)	< 0.001	1.03 (1.02 - 1.04)	< 0.001
HLA compatibility						
matched	1.00	0.009	1.00	< 0.001	1.00	< 0.001
mismatched	0.96 (0.94 - 0.99)		0.95 (0.93 - 0.98)		0.91 (0.88 - 0.93)	
ABO compatibility						
major mismatched	0.85 (0.82 - 0.88)	< 0.001	0.95 (0.92 - 0.98)	0.004	0.93 (0.90 - 0.96)	< 0.001
minor mismatched	1.01 (0.97 - 1.05)	0.603	0.99 (0.96 - 1.03)	0.711	0.98 (0.94 - 1.01)	0.211
bidirectional	0.99 (0.91 - 1.08)	0.781	0.94 (0.87 - 1.03)	0.220	0.96 (0.88 - 1.05)	0.420

Table 2C. Multivariate analysis of grade 3/4 acute graft-versus-host disease and stage 2 to 4 hepatic graft-versus-host disease.

Factor		*Acute GVHD 3/4*		*Hepatic GVHD 2-4*	
		Relative risk (95% CI)	*p*	*Relative risk (95% CI)*	*p*
Age		1.00 (1.00 - 1.01)	0.002	1.01 (1.01-1.01)	< 0.001
Disease risk	Standard	1.00	< 0.001	1.00	< 0.001
	High	1.40 (1.29 - 1.51)		1.49 (1.36-1.64)	
	Benign	0.88 (0.77 - 1.00)		1.02 (0.88-1.18)	
HLA compatibility	Matched	1.00	< 0.001	1.00	< 0.001
	Mismatched	1.22 (1.17 - 1.28)		1.24 (1.17-1.31)	
GVHD prophylaxis	CsA + MTX	1.00	< 0.001	1.00	< 0.001
	FK + MTX	0.76 (0.70 - 0.82)		0.70 (0.64-0.77)	
ABO compatibility	Major mismatched	1.11 (1.05 - 1.17)	< 0.001	1.17 (1.10-1.25)	< 0.001
	Minor mismatched	1.09 (1.03 - 1.16)	0.004	1.12 (1.04-1.20)	0.002
	Bidirectional	1.02 (0.87 - 1.18)	0.770	0.99 (0.82-1.19)	0.948

CsA: cyclosporine A, FK: tacrolimus, MTX: methotrexate.

test, p=0.007, Figure 3C). This difference was not obvious in the other groups. Logistic regression analysis indicated that the day of reaching 1% reticulocytes, disease risk, and HLA compatibility had significant effects on the occurrence of severe acute GVHD (Wald test, p<0.01).

Among the patients who received reduced-intensity conditioning, grade 3 to 4 acute GVHD was observed more frequently in the major mismatched group than in the group transplanted with ABO-identical grafts (p=0.0372). The incidence of stage 2 to 4 hepatic GVHD in the major mismatched group was higher than that in the ABO-identical group, although this difference was barely significant (log rank test, p=0.0430; Wilcoxon's test, 0.0592). Instead, the incidence of gut GVHD was significantly higher in patients with major incompatibility than in those receving ABO-identical grafts (log rank test, p=0.0017, Figure 3D), and a multivariate analysis showed that major incompatibility was a significant risk factor (RR, 1.27; 95% CI, 1.09 - 1.48; p=0.0024).

Discussion

We examined the effects of ABO-blood group incompatibility on the outcome of UR-BMT in a large-scale analysis. Our results showed poor overall survival in the group of patients with major incompatibility and a higher risk of grade 3 to 4 acute GVHD in both the major and minor mismatched groups. These effects on transplantation outcome are particularly important in the UR-BMT setting, because a given patient can find several suitable donors through our growing marrow donor program.

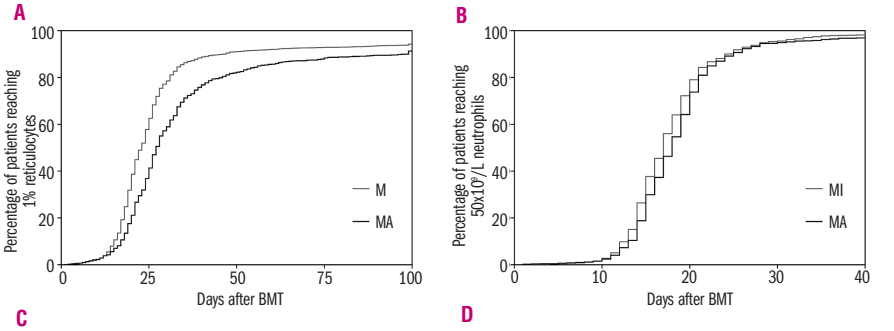

Figure 2. Engraftment of blood cells. Cumulative incidence curves showing the probability of reaching different blood cell endpoints after transplantation in recipients of marrow from ABO-identical (M) or major mismatched donors (MA). Recovery to 1% reticulocytes (A), 0.5x10⁹/L neutrophils (B), and 50x10⁹/L platelets (C) was significantly delayed in the major mismatched group (p<0.0001, p<0.0001, and p<0.0001, respectively). (D) Cumulative incidence curves showing the probability of reaching 1% reticulocytes in recipients of marrow from major mismatched donors with conventional (CST) or reduced intensity conditioning (RIST) regimens.

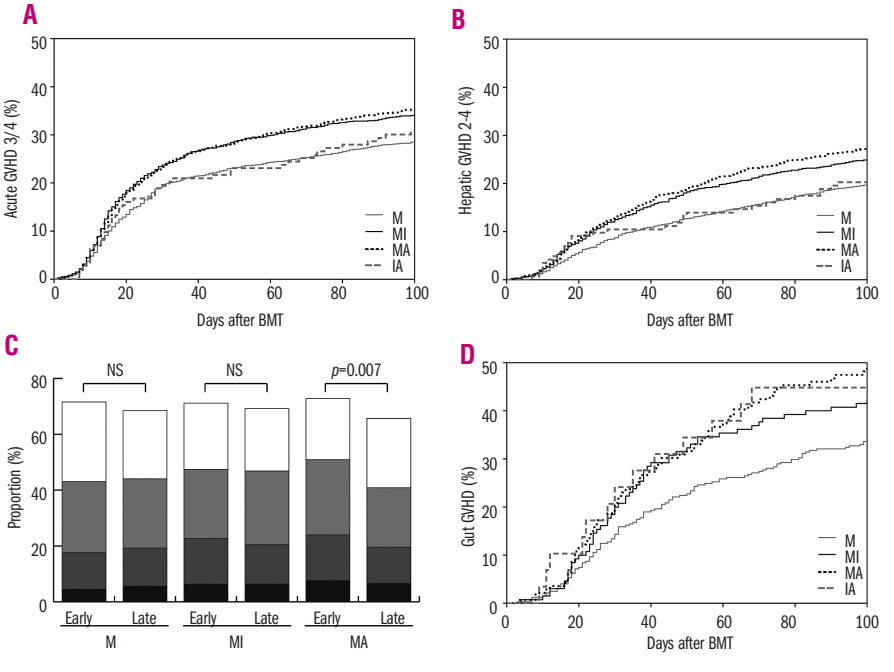

Figure 3. Incidence of acute GVHD after ABO-incompatible bone marrow transplantation. Cumulative incidence curves showing the probability of grade 3/4 acute GVHD (A) or stage 2 to 4 hepatic GVHD (B) in patients transplanted with ABO-compatible (M) versus ABO-incompatible marrow (MI, MA, IA). (C) Engraftment of erythrocytes and acute GVHD. Patients receiving ABO-identical (M), major incompatible (MA), and minor incompatible (MI) grafts were subdivided into two groups according to whether erythrocyte recovery occurred earlier or later than the median (M, 24 days; MI, 24 days; MA, 28 days). There was a higher incidence of grade 2 to 4 GVHD among major mismatched patients with early recovery than in those with late recovery (50.9% *vs.* 40.8%, p=0.007). Grade 1, while column; grade 2, light gray column; grade 3, dark gray column; grade 4, black column. (D) Cumulative incidence curves showing the probabilities of gut GVHD in patients transplanted with reduced-intensity conditioning.

The higher mortality rate in the major mismatched group was statistically significant but not greatly so. Many previous reports failed to find a difference in survival according to the degree of AB0-incompatibility.[7] One of the reasons for this failure may be that too few cases were analyzed. Even in our analysis, only 143 patients underwent a bidirectional incompatible transplantation, which appears insufficient to obtain a definitive conclusion in terms of the Kaplan-Meier curve for overall survival. Only two previous reports considered more than 1000 patients, but neither found any difference in overall survival or incidence of acute GVHD between the subgroups with different degrees of AB0-incompatibility. Mielcarek *et al*. analyzed 1676 patients to demonstrate the relationship between acute GVHD and the disappearance of donor-directed anti-A/B isohemagglutinin.[2] Their study included transplants from both HLA-matched related and unrelated donors, and the comparison was performed among a relatively small number of AB0-incompatible subgroups in 748 allografts from unrelated donors. Seebach *et al.* reported a retrospective analysis of 3102 patients, but they confined the background disease and stem cell source to patients with early-stage leukemia who received a bone marrow transplant from an HLA-identical sibling.[6] Thus, our report is the first large-scale analysis of UR-BMT with AB0-incompatible allografts. Another explanation of why our results differ from those of previous reports may be the use of bone marrow from unrelated donors as a source of stem cells. In addition, we could analyze the effects of AB0 incompatibility in transplantation among patients who received reduced-intensity conditioning, and observed increased transplant-related mortality in the mismatched groups, as recently reported from a single center.[13]

Delayed recovery of red blood cells in AB0-major mismatched transplantation is a well-known problem and is caused by persistent anti-A/B against non-self-AB0 antigens.[14,15] We found delayed recovery not only of red blood cells but also of neutrophils and platelets. A previous report documented a longer period of thrombocytopenia in cases of major AB0 incompatibility.[8] The limitation of this phenomenon to transplants with major AB0-mismatching suggests that ABH antigens are expressed in blood cells other than erythrocytes. Cooling *et al*. reported that platelets express ABH in a relatively stable manner influenced by group A subtype.[16] It would, therefore, be interesting to examine the subtype of ABH antigen that caused more severe delays in platelet engraftment. In the JMDP database, details on AB0 blood group subtype of the donors and recipients were not available. In contrast, ABH antigens could be detected on lymphocytes but not on neutrophils or monocytes.[17] Delayed engraftment of neutrophils was also reported previously by other groups.[6,7] They speculated that anti-donor isohemagglutinin may bind to A/B antigens adsorbed on the surface of neutrophils or their precursors. An increased risk of graft failure after major mismatched transplants was recently found in an analysis of 224 patients who underwent transplantation from unrelated donors.[18] We also found a higher incidence of secondary graft failure after transplants with major incompatibility than after AB0-identical transplants, but we were unable to identify major incompatibility as an independent risk factor for graft failure.

Two reports have shown that donor red cell chimerism occurs later in major AB0-incompatible non-myeloablative stem cell transplants than in myeloablative ones.[10,11] In our analysis, the time to achieve 0.5% reticulocytes was longer in some patients undergoing non-myeloablative transplantation than those undergoing myeloablation, but the difference was not statistically significant. Peggs *et al.* indicated that the incidence of delayed donor red cell chimerism may differ according to the type of non-myeloablative conditioning regimen used.[11] Differences in the regimen may have reduced the influence of non-myeloablative conditioning on erythrocyte engraftment of major AB0-mismatched marrow in our analysis.

We observed a high incidence of severe acute GVHD in the groups of patients with major and minor incompatibility but not in the bidirectional mismatched group. Some previous reports indicated a higher incidence of severe acute GVHD in groups with major and minor mismatching than in the AB0-identical group, but other reports did not.[4,6,7] We were able to analyze a sufficient number of patients to detect an influence of AB0 mismatching on acute GVHD. In addition, Japanese patients are at a significantly lower risk of severe acute GVHD than American patients, which is suggested to be due to the lower genetic diversity of HLA and cytokine gene polymorphisms.[19] This lesser influence of other factors might be responsible for the influence of AB0 mismatching on acute GVHD.

The incidence of liver GVHD was higher among cases of minor AB0-incompatible transplants. ABH antigens are widely distributed in tissues other than erythrocytes and are also expressed on epithelial cells of large bile ducts.[20,21] Recipient- directed anti-A/B may injure the liver epithelium contributing to an increase in the incidence and severity of liver GVHD. In fact, a high incidence of biliary and hepatic artery complications was observed in AB0-incompatible liver transplants.[22]

In contrast, there is donor-directed anti-A/B instead of recipient-directed anti-A/B in major AB0-incompatible transplants. Mielcarek *et al*. showed that a more rapid clearance of donor-directed anti-A/B was positively correlated with the development of acute GVHD in AB0-incompatible transplants from HLA-matched related donors.[2] They suggested that host-directed donor T cells lead to more rapid elimination of residual mature antibody-producing host lymphocytes and plasma cells, which they termed the *graft-versus-plasma cell effect*. In their analysis, however, there was a less striking correlation between acute GVHD and anti-A/B disappearance in HLA-matched UR-BMT, and the small sample size of UR-BMT patients was speculated to be a reason. In our analysis, patients undergoing major mismatched transplantation were divided into two groups depending on whether the time to achieve 1% reticulocytes was earlier than the median 28 days after major AB0-incompatible transplantation, and patients with earlier recovery of erythrocytes showed a higher incidence of severe acute GVHD. We did not measure the titer of donor-directed

anti-A/B, but the disappearance of anti-A/B is well known to precede the engraftment of erythrocytes. Therefore, our observation suggests that the graft-versus-plasma cell effect also played an important role in the engraftment of erythrocytes in the UR-BMT setting. In AB0 major mismatched transplants, although we could not speculate which specific antigen on host cells was a target of donor-derived lymphocytes, elimination of residual plasma cells producing donor-directed anti-A/B might be necessary but might cause a more severe graft-versus-host reaction leading to engraftment.

The number of bidirectional-mismatched patients in our analysis was relatively small, which may have caused a deviation in GVHD incidence. Another possibility is that the mechanism promoting severe hepatic GVHD differs between cases of major and minor incompatibility. In bidirectional mismatched transplants, these effects might counteract each other. In contrast, however, a recent large analysis by Seebach *et al.* showed that only the group with bidirectional mismatching had a higher incidence of acute GVHD than that in the AB0-identical group.[6] However, only 114 of 3103 patients undergoing transplantation from an HLA-identical sibling had bidirectional-mismatching. The conflict between our observations and those of Seebach *et al.* might be related to the source of stem cells used for the transplants. In either case, the number of patients with bidirectional-mismatching in our study was insufficient, and a larger group of patients will be necessary to draw a definitive conclusion on GVHD as well as survival in patients with bidirectional incompatibility.

Authorship and Disclosures

F.K, KS, SK, TI, HO, MH and KM participated in the conception of this study; FK, KS, TI, HS, SO, KM, SM, and HA performed the transplants; FK and HA analyzed the statistical data; FK wrote the paper; all authors checked the final version of the paper.

The authors reported no potential conflicts of interest.

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