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LETTER TO THE EDITOR

Long-term survival after HLA-haploidentical SCT from noninherited maternal antigen-mismatched family donors: impact of chronic GVHD

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Allo-SCT from an HLA-haploidentical family donor has been the treatment of choice in patients with high-risk hematologic malignancies, who are expected to have a better prognosis with SCT but lack immediate access to a conventional stem cell source.[1,2] With intent to minimize the risk of severe GVHD, most haploidentical SCT protocols employ *ex vivo* or *in vivo* T-cell depletion, albeit at the expense of an increased risk of infection or relapse as a result of poor post-transplant immune reconstitution. To develop an alternative strategy to perform haploidentical SCT that confers improved immune recovery and acceptable risk of GVHD, we have explored the feasibility of T-cell-replete SCT from family donors mismatched for noninherited maternal HLA antigens (NIMA); NIMA-mismatched donor selection is based on the hypothesis that the detection of long-term maternal or fetal microchimerism in the donor's peripheral circulation is associated with immunological hyporesponsiveness against NIMA (in the case of NIMA mismatch in the graft-vs-host direction) or against inherited paternal HLA antigens (IPA) (in the case of NIMA mismatch in the host-vs-graft direction).[3] According to this scenario, we and other groups showed that T-cell-replete HLA-haploidentical SCT from a NIMA-mismatched family donor is feasible in selected patients with poor-risk hematologic malignancies.[3,4] However, late complications and long-term outcomes in patients undergoing such transplantations have been so far largely unknown. Therefore, we retrospectively studied the severity of chronic GVHD, requirement for immunosupressive treatment, and status of primary disease in long-term survivors who received T-cell-replete NIMA-mismatched haploidentical SCT.

We collected data on 16 consecutive patients who had survived more than 3 years after NIMA-mismatched SCT performed between January 2001 and July 2004 at 11 institutions that participated in our previous nationwide study (Table 1).[3] At the time of SCT, they had a median age of 19 years (range, 2–56) and received BM ($n=5$) or G-CSF-mobilized peripheral blood ($n=11$) as treatment for acute myeloid leukemia ($n=6$), acute lymphoblastic leukemia ($n=3$), chronic myeloid leukemia ($n=4$) and other B-cell neoplasms ($n=3$); 6 patients had a chemosensitive disease and 10 had a refractory disease. Early outcomes of 14 of these transplantations have been described elsewhere.[3–5] All patients received tacrolimus-based GVHD prophylaxis after myeloablative ($n=10$) or reduced-intensity conditioning ($n=6$). The type of donor was NIMA-mismatched sibling in 9 cases, mother in 6 and daughter in 1; all patient-donor pairs had two or three serologic mismatches at HLA-A, HLA-B and HLA-DR antigens in the graft-versus-host direction. The presence of long-term maternal or fetal microchimerism was detected in all donors through NIMA- or IPA-specific nested PCR as described earlier.[3] Karnofsky score was employed to record the performance status for patients who were 16 years or

Table 1 Characteristics of long-term survivors after NIMA-mismatched haploidentical SCT

Median age at transplant, years (range)	19 (2–56)
Sex	
Male	10
Female	6
Diagnosis	
Acute leukemia[a]	9
Chronic myeloid leukemia	4
Diffuse large B-cell lymphoma	1
Plasma cell myeloma	2
Disease status	
CR or chronic phase	6
Chemorefractory or blastic phase	10
Donor type	
NIMA-mismatched sibling	9
Mother	6
Daughter	1
No. of mismatched HLA antigens[b]	
Two antigens	10
Three antigens	6
Stem cell source	
BM	5
Peripheral blood	11
Conditioning	
Myeloablative	10
Reduced-intensity	6
GVHD prophylaxis	
Tacrolimus alone	1
Tacrolimus + MTX	13
Tacrolimus + MTX + corticosteroids	2
Acute GVHD	
None or grade 1	9
Grade 2	6
Grade 3	1

[a]One patient had secondary acute myeloid leukemia developed after treatment for acute lymphoblastic leukemia.
[b]The number of serologic mismatch at HLA-A, HLA-B and HLA-DR antigens in the graft-versus-host vector was shown.

Table 2 Clinical scoring of organ-specific symptoms related to chronic GVHD in long-term survivors after NIMA-mismatched haploidentical transplantation[a]

Involved organ site[b]	*No. of evaluable patients*	*Score 0*	*Score 1*	*Score 2*	*Score 3*
PS	14[c]	6 (43%)	7 (50%)	1 (7%)	0
Skin	16	5 (31%)	7 (44%)	2 (13%)	2 (13%)
Mouth	16	11 (69%)	5 (31%)	0	0
Eyes	16	10 (63%)	3 (19%)	3 (19%)	0
GI tract	16	14 (88%)	2 (13%)	0	0
Liver	16	10 (63%)	3 (19%)	3 (19%)	0
Lungs	16	9 (56%)	2 (13%)	1 (6%)	4 (25%)
Joints and fascia	16	12 (75%)	2 (13%)	2 (13%)	0

Abbreviations: GI = gastrointestinal; PS = performance status.
[a]Scoring was based on the worst symptoms associated with chronic GVHD.
[b]Symptoms involving female genital tract were not reported.
[c]Two patients succumbed to pulmonary complications were excluded.

older, whereas Lansky score was applied for those who were younger than 16 years of age. Organ-specific symptoms related to chronic GVHD and their severity were diagnosed and evaluated by consensus criteria proposed by the National Institutes of Health Chronic GVHD Diagnosis and Staging Working Group.[6]

At a median follow-up of 56 months (range, 38–74), 13 (81%) of 16 patients were alive and free of their primary disease. One patient was alive with relapsed disease, and two died from pulmonary complications at 51 and 52 months after transplantation in continuous remission. Fifteen (94%) patients developed classical chronic GVHD; type of onset was *de novo* in five, quiescent in six and progressive in four. According to the National Institutes of Health Clinical Scoring Systems the affected organs scored two or greater, which included lungs ($n=5$), skin ($n=4$), eyes ($n=3$), liver ($n=3$) and joints/fascia ($n=2$) (Table 2). Overall severity of chronic GVHD among these patients was classified as mild in three (20%) cases, moderate in seven (47%) and severe in five (33%). It is noted that immunosuppressive agents were successfully withdrawn from eight (50%) patients with *de novo* or quiescent onset of disease at a median of 19 months (range, 3–46) after transplantation, although two of four patients who experienced severe chronic GVHD (score 3) of lung eventually succumbed to bronchiolitis obliterans. Karnofsky or Lansky performance score at the time of last follow-up among the 14 surviving patients was 100% in 6 (43%), 80–90% in 5 (36%), 70% in 2 (14%) and less than 70% in 1 (7%).

In the present study, we found that substantial proportion of long-term survivors after NIMA-mismatched haploidentical SCT could discontinue administration of immunosuppressive agents despite the frequent occurrence of moderate-to-severe chronic GVHD. This paradoxical observation contrasts sharply with the conventional assumption that the establishment of robust tolerance across multiple HLA disparities is hardly possible in the setting of marrow or peripheral blood SCTs without employing T-cell depletion. However, clinical significance of NIMA-mismatched donor selection has been controversial because the mechanisms underlying the tolerogenic effect against NIMA or IPA have not been fully elucidated.[7] Using murine BM transplant models, Matsuoka *et al.*[8] showed that transplant from donors exposed to NIMA *in utero*, but not from those exposed to IPA, reduced the morbidity and mortality associated with GVHD in an antigen-specific and a $CD4^+CD25^+$ T cells-dependent manner. In contrast, Opiela *et al.*[9] showed that murine neonates exposed to low levels of NIMA can develop vigorous *in vivo* cytotoxic rather than tolerogenic responses against NIMA, which might explain the inconsistent severity of GVHD after NIMA-mismatched SCTs in humans. Intriguingly, Stern *et al.*[10] recently reported that recipients of T cell-depleted haploidentical SCT using mother as the donor had the better overall survival than those using father as the donor, implying a mechanism by which previous exposure to paternally derived antigens in maternal donors would positively affect transplant outcomes.

In conclusion, our observations in this study suggested that long-term survival without continuous immunosuppressive treatment is possible after T-cell-replete HLA-haploidentical SCT from a microchimeric NIMA-mismatched donor. Although the small number of patients limits the interpretation of our results, further studies are warranted to compare late sequelae after HLA-haploidentical SCTs with various protocols in larger cohorts.

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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

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BACKGROUND: The impact of donor-recipient ABO matching on outcomes after allogeneic stem cell transplantation has been a matter of controversy.
STUDY DESIGN AND METHODS: Individual patient data–based meta-analysis was conducted with a pooled data set provided through six published and one unpublished cohorts. Outcomes in recipients of peripheral blood or bone marrow transplantation for hematologic malignancies were evaluated. A multivariate Cox model was used to adjust differences in outcomes of patients receiving ABO-matched grafts with those receiving major, minor, or bidirectional mismatched grafts. Considering multiple testing, p values of less than 0.05 and 0.001 were considered significant for the primary and secondary endpoints, respectively.
RESULTS: In all, 1208 cases, including 697 ABO-matched and 202 major, 228 minor, and 81 bidirectional mismatched transplants, were analyzed. Overall, adverse impact of ABO matching on overall survival (OS), as a primary endpoint, was not observed (adjusted hazard ratios [95% confidence intervals]: major, 1.03 [0.82-1.30], p = 0.81; minor, 1.19 [0.97-1.47], p = 0.10; bidirectional, 1.25 [0.91-1.72], p = 0.17). Among related stem cell recipients, ABO matching had no significant influence on OS, while the minor and bidirectional mismatched groups among unrelated stem cell recipients exhibited lower OS with marginal significance, especially in patients with acute leukemia, patients who received transplants after 1998, and patients who underwent transplants at Asian centers.
CONCLUSIONS: Our meta-analysis demonstrates no adverse association between any ABO mismatching and survival. However, marginally lower OS found in recipients of minor or bidirectional mismatched grafts from unrelated donors suggested the need for larger studies focusing on unrelated transplants.

ABO matching between donor and recipient in solid organ transplantation is generally thought to be essential for better outcomes.[1] In contrast, blood or marrow stem cell transplantation (SCT) from an ABO-mismatched donor is sufficiently

ABBREVIATIONS: AL = acute biphenotypic or unclassifiable leukemia; ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CLL = chronic lymphocytic leukemia; CML = chronic myelogenous leukemia; HR(s) = hazard ratio(s); IPD = individual patient data; MDS = myelodysplastic syndrome; ML = malignant lymphoma; MM = multiple myeloma; OS = overall survival; SCT = stem cell transplantation; TRM = treatment-related mortality.

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feasible and is performed in routine clinical practice. However, several complications have been reported in ABO-mismatched SCT. Major mismatched transplantation, characterized by the presence of preformed anti-donor hemagglutinin, is sometimes complicated by delayed red blood cell (RBC) engraftment and pure red cell aplasia[2-7] and by hemolytic anemia.[8,9] In minor mismatched transplantation, characterized by the ability of donor B lymphocytes to produce anti-recipient hemagglutinin, acute hemolytic anemia, known as passenger lymphocyte syndrome, can occur shortly after SCT.[9-12] In bidirectional mismatched transplantation, characterized by the combination of major and minor characteristics, both sets of complications can occur. Owing to these reasons, clinicians are very interested in determining whether ABO mismatching affects the final outcome of SCT, especially when several donor candidates with various ABO-matching pairs are available. To resolve these issues, the impact of ABO mismatching on overall survival (OS) in SCT settings has been evaluated in many studies; however, all these studies obtained conflicting results. Some studies reported the association of poorer OS,[13-16] increased nonrelapse mortality,[17] or increased incidence of acute graft-versus-host disease (GVHD) with a single or any type of ABO mismatch compared with ABO-matched SCT.[16,18] In contrast, one report indicated better OS and decreased relapse rate in ABO-mismatched transplantation.[19] In addition to these contradictory reports, many studies reported that ABO mismatching had no impact on OS, incidence of acute GVHD, or relapse rate in SCT.[2,20-26] These contradictory results could have originated due to the following reasons: 1) in many studies, each ABO-mismatched pair is not analyzed independently; 2) the number of bidirectional mismatched transplants is often small; 3) transplant centers may employ differing treatment and supportive care regimes; and 4) the background of the studied populations is heterogeneous. To obtain more robust results, a few large retrospective studies analyzing more than 1000 patients have recently been performed. Seebach and coworkers[18] showed no impact of ABO mismatching on OS in an analysis of 3103 patients who had received bone marrow transplantation from a human leukocyte antigen (HLA)-identical sibling for early-stage acute leukemia and chronic myelogenous leukemia (CML). On the other hand, Michallet and colleagues[27] demonstrated an adverse impact of a minor mismatch on OS by analyzing 1108 patients who received SCT with a reduced-intensity conditioning regimen. Therefore, these results need further evaluation with other methods or populations. To reevaluate and summarize conflicting results from previously published studies and to provide better evidence, we designed a meta-analysis based on individual patient data (IPD) with a pooled data set. IPD-based meta-analysis is a relatively new approach to systemic reviews, aimed to reduce the bias in systemic reviews compared to meta-analysis based on abstracted data without IPD retrieval during central collection and reanalysis of IPD from each study.[28,29] We conducted the IPD-based meta-analysis using data sets, including those obtained from six previously published articles as well as an unpublished data set from one center that did not participate in previous studies.

MATERIALS AND METHODS

Study design

An IPD-based meta-analysis was designed to evaluate the impact of donor-recipient ABO matching on clinical outcomes after peripheral blood and marrow SCT for hematologic malignancies. The primary endpoint was OS, which was compared among patients receiving an ABO-matched graft and those receiving a major, minor, or bidirectional ABO-mismatched graft. The other endpoints analyzed were treatment-related mortality (TRM); GVHD-related mortality; and engraftment of reticulocytes, neutrophils, and platelets (PLTs).

Selection of studies for meta-analysis

Inclusion criteria for the selection of studies were as follows: 1) the studies were original articles published in English after 1995 and 2) the endpoints considered by the studies included the comparison of OS between ABO-matched and any mismatched SCTs. Exclusion criteria were as follows: 1) the studies included 80 or fewer SCT subjects and 2) the median follow-up period of the studies was less than 6 months. An initial literature search of the PubMed database was conducted using the following free-text terms: ABO blood-group system* and ("blood grouping and crossmatching"[Mesh] or blood group incompatibility*[Mesh]) and (bone marrow transplantation*[Mesh] or hematopoietic stem cell transplantation*[Mesh] or peripheral blood stem cell transplantation*[Mesh]). The date of the last search was June 30, 2007. The initial PubMed literature search identified 194 articles published between 1970 and 2007; 11 articles were found to be eligible for the analysis (Fig. 1).[13-16,18-24] Letters were sent to the corresponding authors of these 11 articles asking them to join the IPD-based meta-analysis and 6 of the corresponding authors agreed to participate. The 6 participating studies included 2 multicenter studies,[13,14,20,22-24] and the other 5 nonparticipating studies included 3 multicenter retrospective studies.[15,16,18,19,21] Patients receiving SCT from unrelated donors were present in 4 of the 6 participating studies and in 4 of the 5 nonparticipating studies. Two of the nonparticipating studies were relatively large, analyzing data of more than 1000 patients. In addition, Kyoto University, where this study was designed, participated in the study,

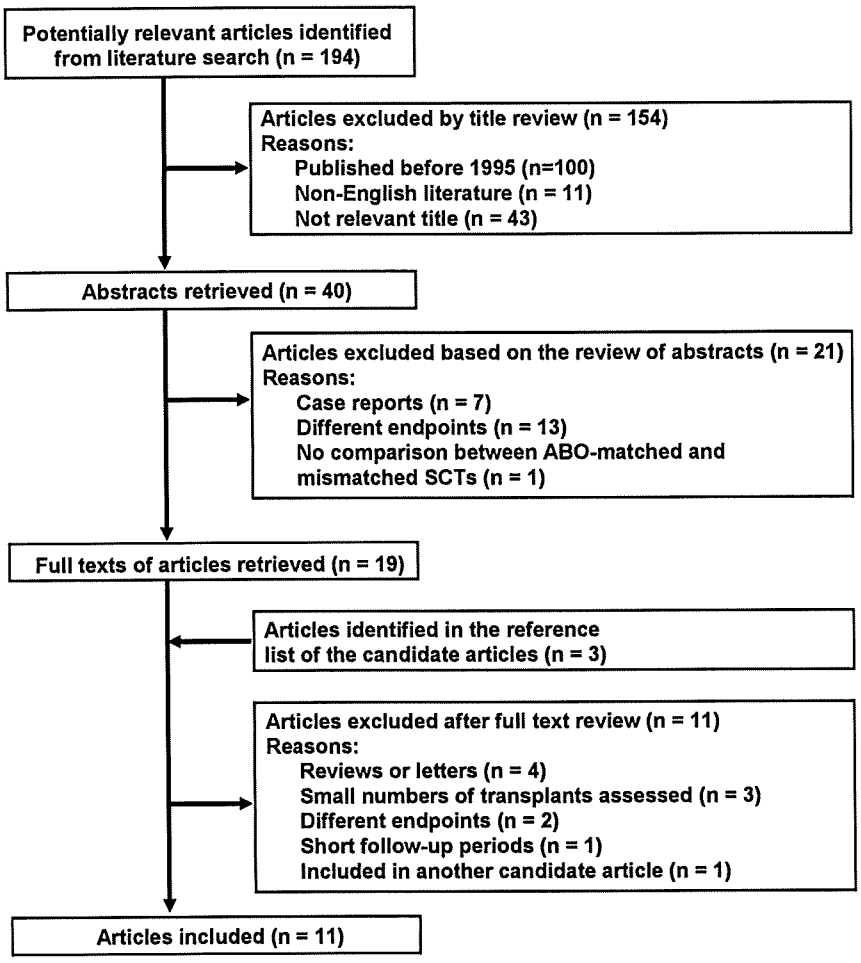

Fig. 1. A flow chart illustrating the process of article selection.

providing its data set on SCT that had not been subjected to survival analysis with reference to ABO matching.

Data collection

We first established the following exclusion criteria for IPD collection: 1) patients who did not meet the minimum data requirements in the following criteria, 2) patients who received SCT for diseases other than hematologic malignancies, 3) patients who received cord blood graft or both peripheral blood and marrow graft, and 4) patients who had experienced prior SCT or had no information regarding their SCT history. Further, we also excluded patients enrolled in the other pooled cohort studies so that the results of our study can be interpreted independently. Second, we defined all the variables required in the present study and made a report form for this data. We then asked the corresponding authors of the participating studies to fill the forms with data. Some authors sent all the raw data sets, which were converted to the report format of our study at the center. Ambiguous definitions were discussed and resolved with the principal investigators, corresponding authors, or data managers. Data from each study were verified against the reported results in some centers, and queries were resolved with the principal investigator, corresponding authors, data managers, or statisticians. The minimum data requirements for participation in this study were data on age and sex of recipients, diagnosis (acute myelogenous leukemia [AML], acute lymphoblastic leukemia [ALL], acute biphenotypic or unclassifiable leukemia [AL], CML, chronic lymphocytic leukemia [CLL], myelodysplastic syndrome [MDS], malignant lymphoma [ML], or multiple myeloma [MM]), type of stem cell source (marrow or peripheral blood stem cell), type of donor (related or unrelated), status of survival (alive, dead, or censored), days of survival after transplantation at the latest follow-up period, and donor-recipient ABO matching (matched or major, minor, or bidirectional mismatched pairs). Additional information requested included donor-recipient compatibility of HLA-A, HLA-B, and HLA-DR antigens by low-resolution typing (matched or mismatched); intensity of conditioning regimen (reduced intensity or myeloablative intensity); GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or other prophylaxes); primary cause of death (disease progression or treatment-related death or detailed information regarding primary cause of death); disease status at SCT; and days to reticulocyte, neutrophil, and PLT engraftment. Data were excluded for patients who met any of the following criteria: patients undergoing SCT for other than hematologic malignancies, those receiving cord blood transplant, those with a history of prior SCT, or those included in a previous large multicenter study published before June 30, 2007. This study was approved by the institutional review board of Kyoto University and other institutions.

Definition of disease risks, engraftment, and primary cause of death

Complete remission in AML, ALL, AL, CLL, ML, and MM; chronic phase in CML; and untreated or complete remission in MDS were considered indicative of standard-risk diseases. Statuses other than complete remission in AML, ALL, AL, CLL, ML, and MM; accelerated phase and blastic crisis in CML; and statuses other than complete remission

in MDS after treatment were considered indicative of high-risk diseases. As described in previous studies,[2,5] the day of reticulocyte engraftment was defined as the first day when the percentage of reticulocytes in peripheral blood exceeded 1 percent. The day of neutrophil engraftment was defined as the first day of 3 consecutive days when the absolute neutrophil count exceeded 0.5×10^9 per L and that of PLT engraftment, the first day of 3 consecutive days when the PLT count exceeded 20×10^9 per L without PLT transfusions. The primary cause of death was classified into two categories: disease-associated death or treatment-related death. Among patients who experienced treatment-related death, GVHD-related death was defined as death primarily associated with acute or chronic GVHD.

Statistical analysis

Patient and transplant characteristics among ABO matching groups were compared by using Kruskal-Wallis test or chi-square analysis, as appropriate. Survival was estimated according to Kaplan-Meier product limit methods. Cumulative incidences of TRM, GVHD-related mortality, and engraftment were assessed using methods described elsewhere to eliminate the effect of competing risk.[30] The competing event in cumulative incidence analyses was defined as death without an event of interest. Disease-associated death was considered a competing risk in the analysis of cumulative incidence of TRM. Death other than GVHD-related death was considered a competing risk in the analysis of cumulative incidence of GVHD-related death. When appropriate, Gray's test was applied to assess the impact of the factor of interest. Multivariate proportional hazard modeling of subdistribution functions in competing risks was applied to assess the impact of potential prognostic factors.[31] Cox regression analysis was used to determine the impact of ABO matching on the primary endpoint with adjustment for age (continuous), sex (male or female), and center effects in the seven data sets. When appropriate, the following items were added as confounders in addition to age, sex, and center effects: diagnosis (acute leukemia or others), risk (standard-risk, high-risk, or unknown), donor (related or unrelated), stem cell source (bone marrow or peripheral blood), conditioning regimen (reduced intensity, myeloablative intensity, or unknown), GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or unknown), transplant year (1990-1997, 1998-2007, or unknown), and transplant centers (Asian or non-Asian centers). All of the confounders were also considered in the multivariate analysis of TRM, GVHD-related mortality, and engraftment. p Values of less than 0.05 were considered significant for the comparison of baseline characteristics and the primary endpoint. With regard to secondary endpoints, p values of less than 0.001 were considered significant to eliminate false-positive associations possibly induced by multiple testing, and p values of less than 0.05 and equal to 0.001 or more were defined as marginally significant. All analyses were conducted using computer software (STATA, Version 10, STATA Corp., College Station, TX; R, Version 2.6.3, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Collection of data

Seven data sets containing data on a total of 1424 SCT patients were collected from six published data sets and one unpublished data set from one center. A total of 133 patients not meeting the minimum data requirements or those who received SCT for diseases other than hematologic malignancies were excluded. Twenty-eight patients who received cord blood graft or both peripheral blood and marrow graft were also excluded. In addition, 6 patients enrolled in the other pooled studies were excluded. Forty-nine patients who had experienced prior SCT or had no information regarding their SCT history were also excluded. In the end, 1208 transplants, including 697 ABO-matched cases and 202 major, 228 minor, and 81 bidirectional mismatched cases, were included in the study. With regard to the additional data requests, data on disease status at transplant were obtained for five data sets; type of conditioning regimen, GVHD prophylaxis, and transplant year for six data sets; reticulocyte engraftment for two data sets; neutrophil and PLT engraftment for five data sets; and binary data on either disease-associated death or treatment-related death for one data set and for five data sets with detailed information on the primary cause of death.

Characteristics of patients and transplants

Table 1 shows the patient characteristics. The cases included 709 related SCTs and 184 unrelated SCTs from Western centers as well as 214 related SCTs and 101 unrelated SCTs from Asian centers. The median age of the recipients was 39 years (range, 1-69 years). Marrow and peripheral blood stem cell was used for 915 and 293 cases, respectively. There were no significant differences among ABO-matched and mismatched groups for any category except for the type of donors and centers of transplantation. With regard to donor type, bidirectional ABO-mismatched grafts were more frequently used among unrelated SCTs when compared to the ABO-matched group. With regard to transplant centers, SCTs from bidirectional mismatched donors were more frequently performed in Asian centers.

OS

The median follow-up period of survivors was 37 months (range, 3-268 months). The unadjusted probabilities of OS

TABLE 1. Characteristics of patients and transplants

Characteristic	Match (%) (n = 697)	Major mismatch (%) (n = 202)	Minor mismatch (%) (n = 228)	Bidirectional mismatch (%) (n = 81)	p Value
Age					
Median (range)	39 (1-67)	39 (1-66)	39 (2-69)	43 (4-62)	0.074
Sex					
Male	393 (56.4)	129 (63.9)	118 (51.8)	45 (55.6)	0.087
Female	304 (43.6)	73 (36.1)	110 (48.3)	36 (44.4)	
Diagnosis					
AML/MDS	323 (46.3)	70 (34.7)	102 (44.7)	37 (45.7)	0.115
ALL	102 (14.6)	36 (17.8)	45 (19.7)	14 (17.3)	
AL	6 (0.9)	1 (0.5)	0 (0.0)	0 (0.0)	
CML	168 (24.1)	58 (28.4)	50 (21.4)	17 (21.0)	
CLL	5 (0.7)	6 (3.0)	4 (1.8)	0 (0.0)	
ML	67 (9.6)	26 (12.9)	18 (7.9)	10 (12.4)	
MM	26 (3.7)	5 (2.5)	9 (4.0)	3 (3.7)	
Risk					
Standard	341 (48.9)	75 (37.1)	91 (39.9)	39 (48.2)	0.597
High	112 (16.1)	31 (15.4)	50 (21.9)	17 (21.0)	
Unknown	244 (35.0)	96 (47.5)	87 (38.2)	25 (30.9)	
Type of donors					
Related					<0.001
HLA-matched	374 (53.7)	83 (41.1)	103 (45.2)	31 (38.3)	
HLA-mismatched	31 (4.5)	8 (4.0)	9 (4.0)	5 (3.7)	
HLA matching unknown	168 (24.1)	49 (24.3)	51 (22.4)	11 (13.6)	
Unrelated					
HLA-matched	121 (17.4)	62 (30.7)	63 (27.6)	31 (38.3)	
HLA-mismatched	3 (0.4)	0 (0.0)	2 (0.9)	3 (3.7)	
Stem cell source					
BM	519 (74.5)	155 (76.7)	177 (77.6)	64 (79.0)	0.649
PB	178 (25.5)	47 (23.3)	51 (22.4)	17 (21.0)	
Conditioning regimens					
Reduced intensity	101 (14.5)	27 (13.4)	41 (18.0)	8 (9.9)	0.209
Myeloablative intensity	515 (73.9)	144 (71.3)	158 (69.3)	69 (85.2)	
Unknown	81 (11.6)	31 (15.4)	29 (12.7)	4 (4.9)	
GVHD prophylaxis regimen					
CyA based	413 (59.3)	120 (59.4)	122 (53.6)	44 (56.8)	0.052
FK based	153 (22.0)	44 (21.8)	69 (30.3)	29 (35.8)	
Others	3 (0.4)	0 (0.0)	0 (0.0)	1 (1.2)	
Unknown	128 (18.4)	38 (18.9)	37 (16.2)	5 (6.2)	
Transplant year					
1990-1994	123 (17.7)	32 (15.8)	30 (13.2)	8 (9.9)	0.065
1995-1997	189 (27.1)	74 (36.6)	74 (32.5)	25 (30.9)	
1998-2000	147 (21.1)	40 (19.8)	36 (15.8)	18 (22.2)	
2001-2003	102 (14.6)	30 (14.9)	36 (15.8)	15 (18.5)	
2004-2007	58 (8.3)	15 (7.4)	31 (13.6)	12 (14.8)	
Unknown	78 (11.2)	11 (5.5)	21 (9.2)	3 (3.7)	
Transplant centers					
Asian centers	169 (24.3)	46 (22.8)	67 (29.4)	33 (40.7)	0.007
Non-Asian centers	528 (75.8)	156 (77.2)	161 (70.6)	48 (59.3)	

BM = bone marrow; CyA = cyclosporine; FK = tacrolimus; PB = peripheral blood.

(95% confidence interval [CI]) at 5 years among patients receiving ABO-matched grafts and major, minor, and bidirectional mismatched grafts were 48% (44%-52%), 48% (40%-56%), 45% (38%-51%), and 37% (26%-49%), respectively (Fig. 2A). Because different backgrounds and heterogeneity of results in stem cell sources were found, the impact of ABO matching among recipients of either related or unrelated SCT in each stratified category was assessed (Figs. 2B and 2C and 3A and 3B).

Among recipients of related SCT, no significant difference in OS was observed between the ABO-matched group and any other mismatched group. These results were consistent across each stratified group. In contrast, minor and bidirectional mismatched groups among unrelated SCT recipients tended to be associated with poorer OS when adjusted for age and sex (adjusted hazard ratio [HR]: minor, 1.71 [95% CI, 1.15-2.53], p = 0.008; bidirectional, 1.73 [95% CI, 1.05-2.86], p = 0.031). The adverse impact of minor and bidirectional mismatched grafts on OS in unrelated SCT was strongly observed in the following stratified categories: patients with acute leukemia, patients who received SCT after 1998, and patients who underwent transplants at Asian centers.

In multivariate regression analysis of OS adjusted for potential confounders listed in Table 2, no adverse impact of ABO matching on OS was observed among all or the

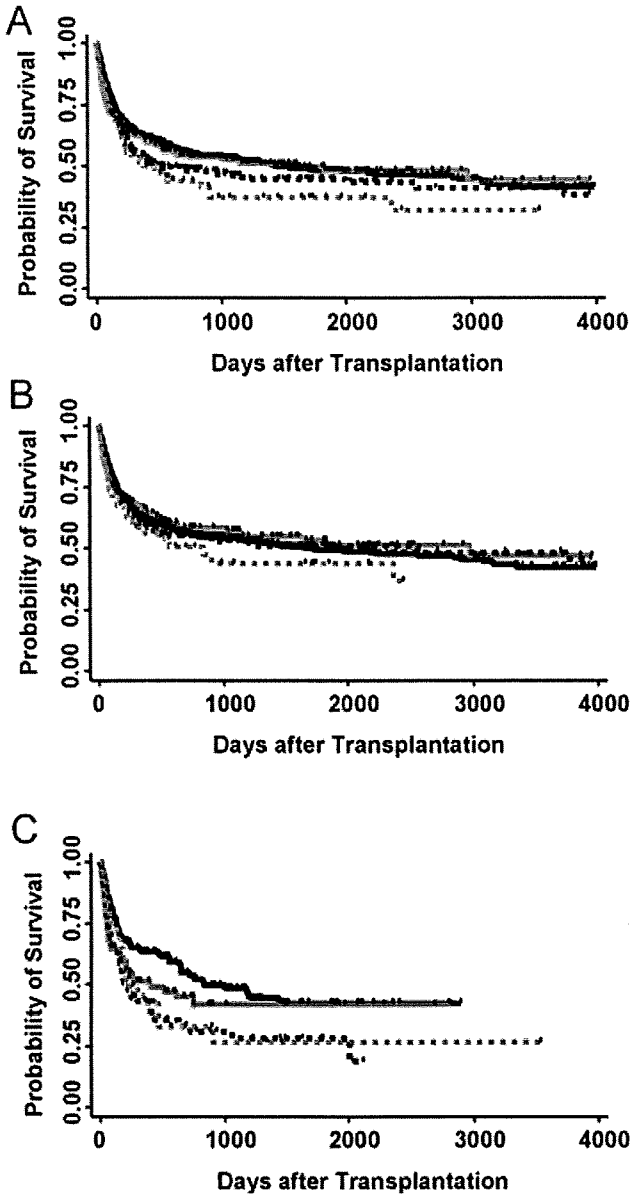

Fig. 2. Kaplan-Meier survival estimates of OS in all patients (A), those who received a related graft (B), and those who received an unrelated graft (C). (—) ABO-matched transplantation; (—) major mismatched; (· · ·) minor mismatched; (-···) bidirectional mismatched.

subset of related SCTs, while minor and bidirectional mismatched groups showed tendency of poorer OS among the subset of unrelated SCT (adjusted HR: major, 1.38 [95% CI, 0.87-2.17], p = 0.17, minor, 1.68 [95% CI, 1.12-2.51], p = 0.012; bidirectional, 1.81 [95% CI, 1.08-3.00], p = 0.023) (Table 2).

TABLE 2. Impact of ABO mismatching on OS

	OS (n = 1208)	
Category	HRs (95% CI)*	p Value
Overall		
Match	1.00	
Major	1.03 (0.82-1.30)	0.81
Minor	1.19 (0.97-1.47)	0.10
Bidirectional	1.25 (0.91-1.72)	0.17
Related SCT		
Match	1.00	
Major	0.93 (0.70-1.23)	0.62
Minor	1.02 (0.79-1.32)	0.88
Bidirectional	1.09 (0.71-1.68)	0.70
Unrelated SCT		
Match	1.00	
Major	1.38 (0.87-2.17)	0.17
Minor	1.68 (1.12-2.51)	0.012
Bidirectional	1.81 (1.08-3.00)	0.023

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

TRM

Data on the primary cause of death were available for 1026 patients (85%). To evaluate the effect of ABO mismatch on treatment-related complications, we analyzed overall TRM at 5 years and early TRM within 100 days of transplantation. Although the cumulative incidences of overall TRM among the ABO-matched group and any mismatched groups did not show any significant difference in multivariate regression analysis, an increased risk of early TRM was observed among the bidirectional mismatched group (adjusted HR: 2.08 [95% CI, 1.14-3.79], p = 0.017; Table 3). This impact remained marginally significant among recipients of related SCTs (adjusted HR: 2.08 [95% CI, 1.04-4.15], p = 0.038). To evaluate whether early TRM was associated with acute GVHD, GVHD-related mortality within 100 days was analyzed using the available data sets (964 patients, 80%). Based on multivariate regression analysis adjusted for the confounding factors, the risk of acute GVHD-related mortality was significantly higher for the bidirectional mismatched group (adjusted HR, 9.35 [95% CI, 3.24-26.93], p < 0.001); however, further stratification by donor type could not be performed due to insufficient number of the data sets.

Engraftment

The data on days to reticulocyte, neutrophil, and PLT engraftment were available for 269 (24%), 667 (55%), and 662 (55%) patients, respectively. As shown in Table 4, multivariate regression analysis adjusted for confounders revealed no impact of ABO mismatching on reticulocyte, neutrophil, or PLT engraftment among patients who received related SCTs. In contrast, there was a marginally significant impact of ABO matching among recipients of unrelated SCTs. This analysis demonstrated a marginally significant impact of minor and bidirectional mismatched grafts on delay in reticulocyte engraftment compared to matched grafts among unrelated SCT recipients (major,

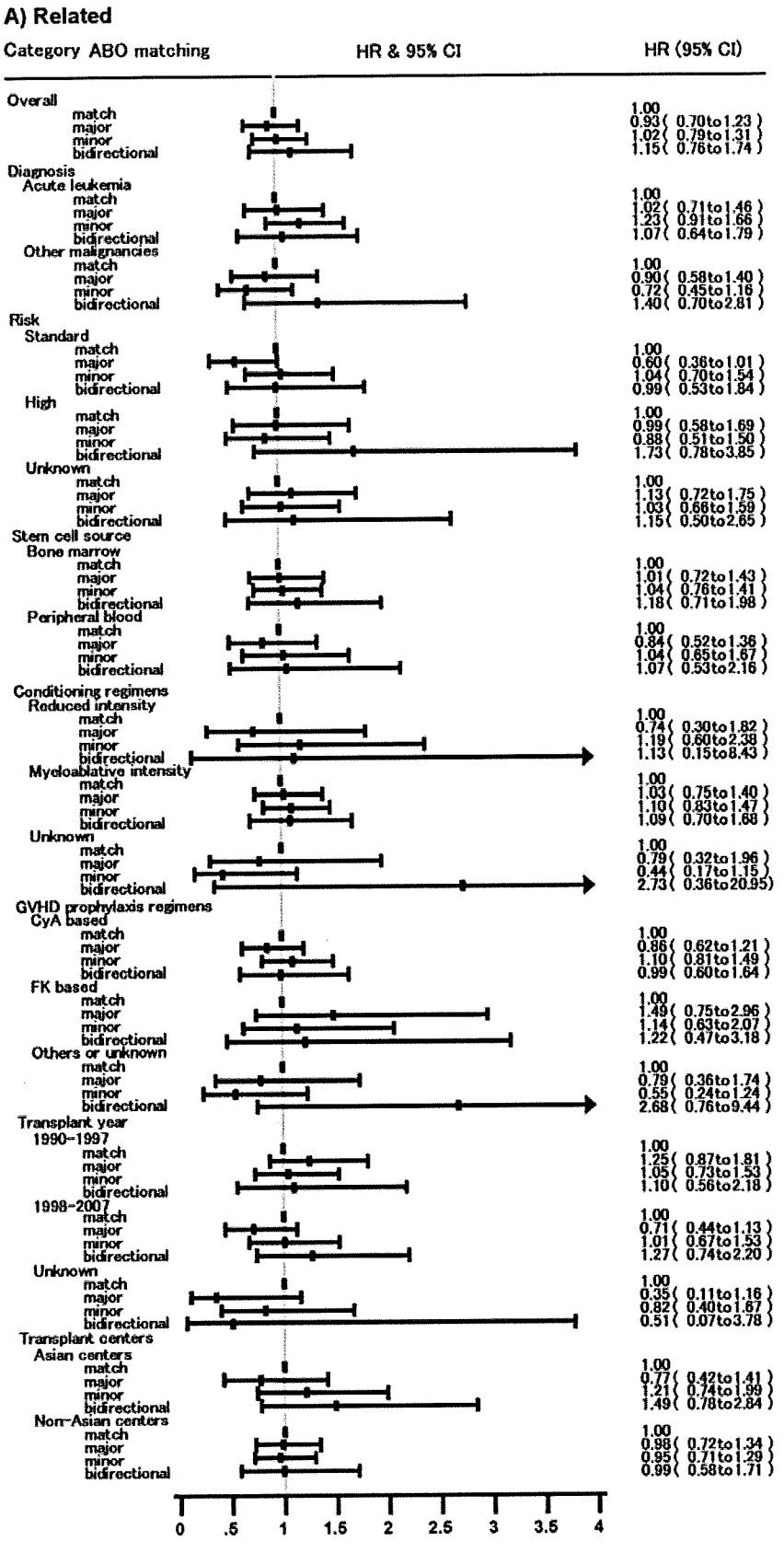

Fig. 3. Impact of ABO mismatching on OS in each stratified category among related (n = 923) (A) and unrelated stem cell transplantation (n = 285) (B). HRs were adjusted for age and sex. Square boxes on lines indicate HRs, and horizontal lines represent 95% CI.

p = 0.010; bidirectional, p = 0.012). Among recipients of unrelated SCTs, neutrophil engraftment tended to be delayed in the bidirectional mismatched group compared to the matched group (p = 0.019), and PLT engraftment tended to be delayed in the minor and bidirectional mismatched groups when compared to the matched group (minor mismatch, p = 0.023; bidirectional, p = 0.024).

DISCUSSION

To integrate the previous contradictory results, and to provide new data regarding the impact of ABO matching on survival after allogeneic blood and marrow SCTs, we performed an IPD-based meta-analysis using seven independent data sets including more than 1200 ABO-matched and mismatched transplants. Consistent with the results of the previous large retrospective analyses, our study confirmed and externally validated a lack of association between the use of ABO-mismatched grafts and OS among patients who underwent related SCTs. In contrast, we found marginally significant impact of minor and bidirectional mismatch among those who received unrelated SCTs. This observation suggested the need for larger studies focusing on unrelated SCTs that include various ethnic backgrounds as the next step in assessing the clinical significance of ABO mismatching in SCTs.

In this study, the adverse impact of minor and bidirectional mismatch on OS after unrelated SCTs was observed in the following stratified categories: patients with acute leukemia, patients who received SCT after 1998, and patients who underwent transplants at Asian centers. These associations might

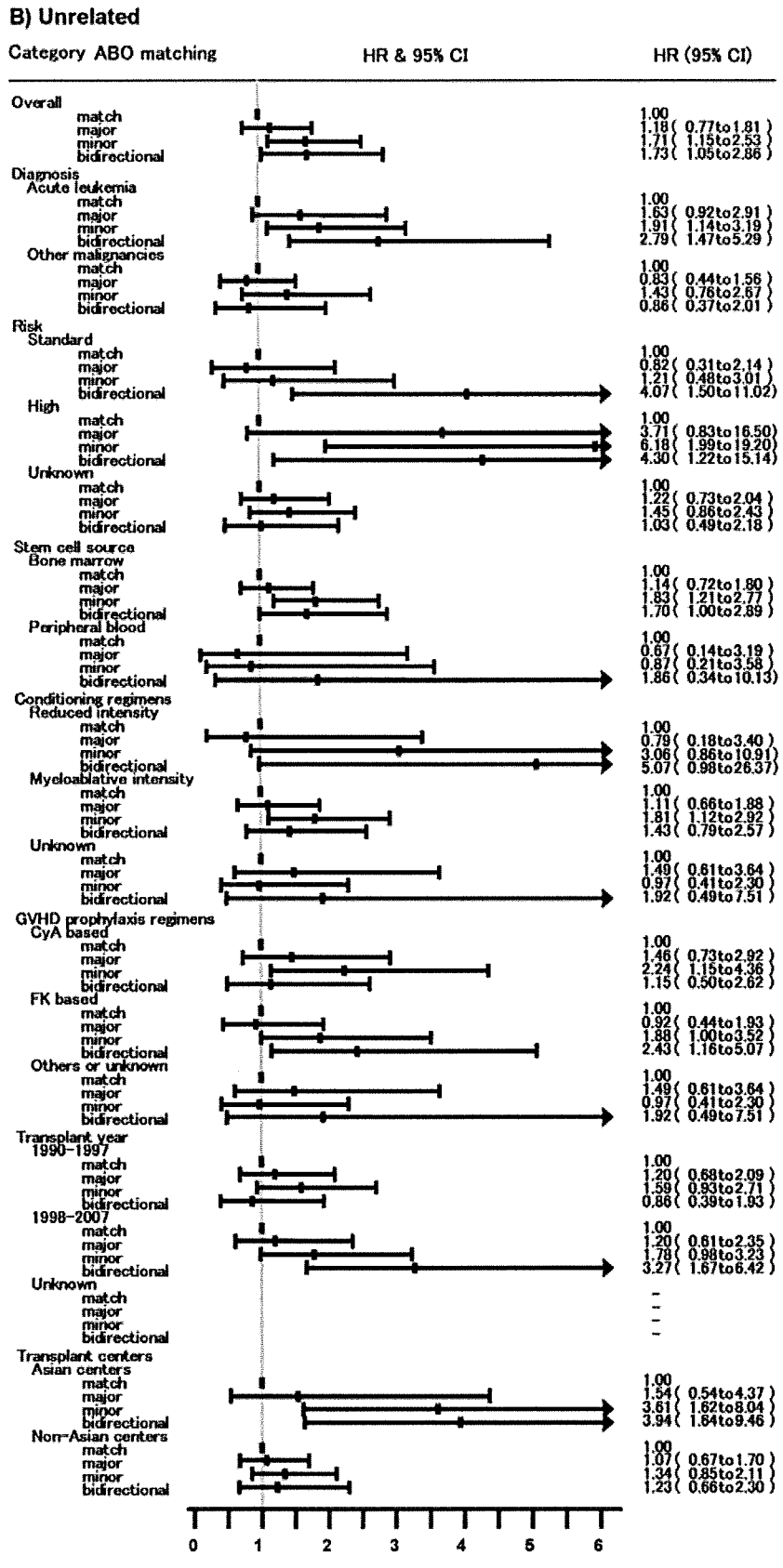

Fig. 3. Continued.

be biased by the relatively small size of unrelated transplant recipients in our analysis, because the previous study on the effect of ABO compatibility in unrelated SCTs among non-Asian populations reported that OS was not influenced by ABO mismatching.[21] However, more recently, a retrospective analysis of more than 5000 HLA-matched or mismatched unrelated SCTs facilitated by the Japan Marrow Donor Program revealed that the major ABO-mismatched group as well as minor mismatched group had inferior OS when compared to the ABO-matched group.[32] These varying results may partly be attributable to differences in the genetic backgrounds between Asian and non-Asian populations, such as cytokine gene polymorphisms and minor histocompatibility antigens;[33] it might be possible that the impact of minor and bidirectional mismatch is amplified by the increased immune dysregulation more likely to be seen in unrelated transplants compared with related transplants. Otherwise, ABO mismatching may exacerbate any underlying tendency toward complications seen in allogeneic transplantation, and these effects might be more prominent in unrelated SCTs. Recently, Michallet and coworkers[27] reported the results of a large retrospective study using the transplant data registered at the Société Française de Greffe de Moëlle et de Thérapie Cellulaire registry. The study analyzed 1108 patients who received related or unrelated SCTs after reduced-intensity conditioning for hematologic malignancies and it showed that minor ABO-mismatched grafts were associated with poorer OS. Although the background of patient characteristics in their study was different from that in this study, these results partly support our observation that minor and bidirectional mismatched grafts could have an adverse impact on OS.

However, the mechanism that underlies inferior survival after minor and bidirectional mismatched SCTs is presently unknown. In minor or bidirectional mismatched SCTs with marrow or peripheral blood grafts, passenger

TABLE 3. Impact of ABO mismatching on early TRM within 100 days and overall TRM

	Treatment-related death within 100 days (n = 1026)		Treatment-related death (n = 1026)	
Category	HRs (95% CI)*	p Value	HRs (95% CI)*	p Value
Overall				
Match	1.00		1.00	
Major	1.40 (0.84-2.32)	0.19	0.85 (0.57-1.28)	0.45
Minor	0.91 (0.52-1.59)	0.71	0.94 (0.65-1.34)	0.73
Bidirectional	2.08 (1.14-3.79)	0.017	1.45 (0.91-2.29)	0.11
Related SCT				
Match	1.00		1.00	
Major	1.10 (0.59-2.06)	0.75	0.81 (0.51-1.27)	0.36
Minor	0.81 (0.41-1.62)	0.56	0.85 (0.54-1.31)	0.45
Bidirectional	2.08 (1.04-4.15)	0.038	1.58 (0.95-2.64)	0.08
Unrelated SCT				
Match	1.00		1.00	
Major	2.10 (0.70-6.29)	0.19	0.84 (0.33-2.18)	0.72
Minor	1.17 (0.36-3.84)	0.79	1.15 (0.53-2.50)	0.72
Bidirectional	3.35 (0.95-11.80)	0.059	1.57 (0.63-3.92)	0.33

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

donor B lymphocytes are known to often produce anti-recipient hemagglutinin 1 or 2 weeks after SCT.[10-12,34] For certain periods of time, such hemagglutinin could be continuously absorbed on widely expressed A/B antigens in tissues and residual RBCs of the recipient. Therefore, in addition to complication of delayed hemolysis, production of immune complexes on the surfaces of recipient tissues shortly after SCT could be a target for alloreaction or could dysregulate immunity. In addition, different transfusion policies may affect survival in minor and bidirectional mismatched transplants, because Benjamin and Antin[35] suggested that the transfusion of plasma containing anti-A,B antibodies in group O PLTs and RBC may exacerbate the cytokine storm that follows allogeneic transplant. Assessing the number of components transfused and the presence and/or development of anti-A/B antibodies would be a worthwhile consideration in future studies.

Subgroup analyses regarding TRM and engraftment were performed with available data sets to evaluate other effects of ABO mismatching. Those analyses showed that the use of bidirectional mismatched grafts was associated with an increased risk of early TRM when compared with matched grafts ($p = 0.017$), while the overall TRM was similar. The higher TRM observed in the early period after bidirectional ABO-mismatched SCTs may be due to the combination of major and minor ABO mismatching with additive or synergistic enhancement of single adverse effects. Theoretically, major ABO mismatching leads to antidonor cell damage and release of cytokines soon after transplantation. That may enhance the subsequent activation of antihost donor-derived lymphocytes in the minor mismatch direction. Therefore, fatal transplant complications such as severe acute GVHD may occur more often among the bidirectional mismatched group.[18] This hypothesis was supported by our observation that the incidence of GVHD-related death within 100 days was significantly higher among recipients of bidirectional mismatched SCTs ($p < 0.001$). Furthermore, delayed engraftment of neutrophils and PLTs could potentially affect early transplant complications, such as infection and bleeding, although we could not clearly identify an increased risk of such complications among a subgroup of patients who received bidirectional mismatched grafts from an unrelated donor. To assess the effect of immunologic reactions between ABO-mismatched pairs, the genotype of genes regulating the secretor status of ABO substances and glycosyltransferases are worth exploring in future studies. First, it is well known that only "secretors," that is, individuals who possess the appropriate secretor genotype, can secrete the soluble H and ABO substances into the body fluids and plasma. In secretor patients, hemagglutinin may form immune complexes with secreted ABO substances in circulation. In contrast, in nonsecretor patients, it may react with the endothelial compartment as well as blood cells. These different immune reactions can modify treatment-related complications. Second, Eiz-Vesper and coworkers[36] have recently demonstrated that a genotype mismatch with regard to glycosyltransferases among phenotypically ABO-matched donor-recipient pairs can induce an alloreaction in vitro. Therefore, the genotypic difference may be a source of minor histocompatibility antigens and affect the risk of GVHD in addition to ABO mismatching.

Reticulocyte engraftment tended to be delayed for the major and bidirectional mismatched groups among recipients of unrelated SCTs ($p = 0.010$ and 0.012, respectively), consistent with previous reports.[2-6] The delay in reticulocyte engraftment may become more evident through the enhanced host-versus-graft reactions in some unrelated SCTs than in related SCTs. In addition, neutrophil and PLT recovery tended to be delayed among patients receiving bidirectional mismatched unrelated grafts ($p = 0.019$). Late recovery of neutrophils after ABO-mismatched transplantation was also observed in the major mismatched group of both related and unrelated SCTs,[18,24,37] although these findings were not confirmed in the present study. Rozman and colleagues[24] hypothesized that immune complexes formed after ABO-mismatched transplantation can cause a pseudo-delay in neutrophil engraftment because immune complexes can be constantly recognized by the Fc receptors on immune cells,

TABLE 4. Impact of ABO mismatching on reticulocyte, neutrophil, and PLT engraftment

	Reticulocytes (>1%) (n = 269)			Neutrophils (0.5×10^9/L) (n = 667)			PLTs ($>20 \times 10^9$/L) (n = 662)		
	Median (day)	HRs (95% CI)*	p Value	Median (day)	HRs (95% CI)*	p Value	Median (day)	HRs (95% CI)*	p Value
Overall									
Match	10	1.00		16	1.00		18	1.00	
Major	26	0.67 (0.47-0.96)	0.029	16	1.01 (0.83-1.23)	0.92	21	0.91 (0.75-1.11)	0.37
Minor	20	0.91 (0.64-1.30)	0.61	16	0.93 (0.73-1.17)	0.51	19	0.85 (0.69-1.06)	0.15
Bidirectional	21	0.84 (0.58-1.21)	0.35	17	0.76 (0.56-1.03)	0.079	20	0.66 (0.45-0.96)	0.031
Related SCT									
Match	18	1.00		16	1.00		17	1.00	
Major	23	0.89 (0.57-1.39)	0.61	17	1.05 (0.83-1.31)	0.70	21	0.92 (0.74-1.14)	0.44
Minor	19	0.81 (0.51-1.29)	0.37	16	0.90 (0.68-1.19)	0.47	19	0.90 (0.70-1.16)	0.43
Bidirectional	18	1.17 (0.75-1.84)	0.49	16.5	1.02 (0.70-1.47)	0.93	17.5	0.78 (0.48-1.29)	0.34
Unrelated SCT									
Match	22	1.00		16	1.00		21.5	1.00	
Major	30	0.42 (0.21-0.81)	0.010	16	0.85 (0.54-1.33)	0.47	22	0.98 (0.65-1.48)	0.92
Minor	20	0.85 (0.47-1.53)	0.58	15.5	0.93 (0.62-1.40)	0.74	24.5	0.61 (0.40-0.93)	0.023
Bidirectional	26	0.43 (0.22-0.83)	0.012	18	0.52 (0.30-0.90)	0.019	25	0.47 (0.24-0.91)	0.024

* HRs were adjusted for age, sex, diagnosis, risk, stem cell source, conditioning regimen, GVHD prophylaxis, transplant year, transplant centers, and donor, if appropriate.

including neutrophils, which are subsequently removed from circulation. Finally, it should be mentioned that the presence of HLA antibodies, HLA allelic mismatching, or infused stem cell doses in unrelated donor SCTs could affect engraftment. It is desirable to include these factors in future studies of unrelated SCTs.

Limitations of this study should be noted. First, our data sets included heterogeneous diseases and various transplant methods, which made it difficult to elucidate the factors potentially associated with OS among minor and bidirectional mismatched transplantations. Second, the existence of missing data may have biased the results. In addition, data regarding the secondary endpoints were not available in some data sets. Therefore, these endpoints should be cautiously interpreted. Third, since we collected IPD from 6 of 11 candidate studies, there might be a potential selection bias. The findings of the meta-analysis should be interpreted in reference to the other large studies. Fourth, we performed the meta-analysis of nonrandomized cohort studies, which might limit our interpretation due to the potential selection bias. However, truly randomized control trials for SCT have rarely been conducted. Fifth, generally speaking, the effect of multiple testing should be taken into account when we interpret secondary endpoints. Finally, missing data on HLA matching between related donors and recipients might reduce the statistical power in the analysis of related SCTs. However, with regard to unrelated SCTs (n = 285), exclusion of patients receiving SCT from HLA-mismatched unrelated donors (n = 8) did not alter the main result (data not shown).

In conclusion, our IPD-based meta-analysis demonstrates no adverse association between any type of ABO mismatching and survival in allogeneic SCTs for hematologic malignancies, although the possible association of minor or bidirectional ABO mismatching with lower OS was observed among recipients of unrelated SCTs. Larger studies focusing on the effects of ABO matching in unrelated SCTs from various ethnic backgrounds with complete HLA allele information are warranted.

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Clinical Significance of Serum Hepcidin Levels on Early Infectious Complications in Allogeneic Hematopoietic Stem Cell Transplantation

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The association of iron overload with complications of allogeneic hematopoietic stem cell transplantation (HSCT) has been suggested in previous studies. Because hepcidin plays a central role in the regulation of iron homeostasis, we analyzed the association between pretransplant serum hepcidin-25 levels and early infectious complications after allogeneic HSCT. We studied 55 consecutive adult patients with a median age of 47 years (range: 20–64 years) who underwent allogeneic HSCT for hematologic malignancies at our institution. Thirty-two patients had myelogenous malignancies; the remaining 23 had lymphogenous malignancies. The median pretransplant serum hepcidin level of patients in the study was 21.6 ng/mL (range: 1.4–371 ng/mL), which was comparable to that of healthy volunteers (median: 19.1 ng/mL [range: 2.3–37 ng/mL]; n = 17). When cumulative incidences of documented bacterial and cytomegalovirus (CMV) infections at day 100 were compared according to pretransplant hepcidin-25 levels, the incidence of bacterial, but not CMV, infection, was significantly higher in the high-hepcidin group (≥50 ng/mL; n = 17) than in the low-hepcidin group (<50 ng/mL; n = 38) (65% [95% confidence interval, 38%–82%] versus 11% [3%–23%]; $P < .001$). This finding was confirmed by multivariate Cox analysis adjusted for confounders, including pretransplant ferritin and C-reactive protein (CRP) levels. No fungal infection was documented in either group. These results suggest that the pretransplant serum hepcidin-25 level may be a useful marker for predicting the risk of early bacterial complications after allogeneic HSCT. Larger prospective studies are, however, warranted to confirm our findings.

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KEY WORDS: Hepcidin, Bacterial infection, Allogeneic stem cell transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has been widely performed as a potentially curative treatment for intractable hematologic malignancies with conventional chemotherapy. However, despite recent advances in the treatment of infectious diseases and conditioning regimens for transplantation, treatment-related complications remain a major problem. Therefore, it is particularly important to identify a good biomarker that can predict treatment-related complications before transplantation. A recently accumulated body of evidence suggests that iron overload is associated with adverse clinical outcomes in HSCT [1-10]. Armand et al. [2] showed that a high pretransplant serum ferritin level was strongly associated with lower overall and disease-free survival (OS, DFS) in patients with allogeneic HSCT that was performed as a treatment for acute leukemia and myelodysplastic syndrome (MDS). Other studies have shown that pretransplant iron overload in autologous or allogeneic HSCT was a risk factor associated with posttransplant complications, such as mucositis, bacterial, and fungal infection, and hepatic veno-occlusive disease (VOD) [3-6,8-11].

Hepcidin, first identified in human blood and urine as an antimicrobial small peptide [12,13], is now considered to be a central molecule that regulates iron metabolism. Hepcidin decreases iron absorption from the intestine and blocks its release from iron stores by

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downregulating the expression of the cellular iron exporter, ferroportin [14,15]. Hepatic expression of hepcidin can be upregulated by iron loading [16,17] as well as by inflammatory stimuli such as interleukin-6 (IL-6) [18]. Therefore, we hypothesized that serum hepcidin level could be a useful predictor of iron overload and inflammatory condition prior to HSCT. Here, we performed a single-center retrospective study at our institution to evaluate the significance of serum hepcidin levels as a predictor of early treatment-related complications after allogeneic HSCT with special reference to infectious complications.

PATIENTS AND METHODS

Study Population

The study population comprised 66 consecutive adult patients who underwent allogeneic HSCT for the treatment of hematologic malignancies at Kyoto University Hospital from July 2006 to September 2008. A total of 55 patients, excluding those who had received prior transplantations within 1 year or who had any active infections before the current transplantation, were included in the analysis. This study was approved by the Ethics Committee of Kyoto University Graduate School and the Faculty of Medicine. Written informed consent was obtained from all patients.

Serum Analysis

Before the administration of conditioning regimens, serum samples were obtained at around 8:00 am, allocated in tubes, and stored at –80°C until analysis. The levels of serum hepcidin-25 (the main form of active hepcidin peptide) were quantified using a liquid chromatography-tandem mass spectrometry-based assay system following the method described by Murao et al. [19]. Other serum parameters were measured using standard laboratory techniques.

Prophylaxis, Monitoring, and Diagnosis of Infection

The patients were isolated in a single room equipped with a high-efficiency particulate air filter (HEPA) system from 1 day before transplantation until at least 4 weeks after transplantation. No bacterial prophylaxis was prescribed for the patients according to our institutional protocols [20]. Trimethoprim-sulfamethoxazole (160 mg/day [trimethoprim], 3 times a week) was administered as prophylactic therapy for *Pneumocystis jirovecii* pneumonia from the day of admission until the day of transplantation and restarted after the day of neutrophil engraftment. All patients received fluconazole (200 or 400 mg/day) and acyclovir (1000 mg/day) prophylaxis from the period of conditioning until 30 days after transplantation. After the first 30 days, the patients received fluconazole at a dose of 100 mg/day until at least 100 days after transplantation. The administration of acyclovir (400 mg/day) was continued when patients received steroid therapy for acute graft-versus-host disease (aGVHD). For each febrile episode, 1 or 2 sets of blood samples were cultured, and the cultures of specimens other than blood and imaging examinations were performed according to clinical judgment. The occurrence of cytomegalovirus (CMV) infection was closely monitored by CMV pp65 antigenemia testing with C10/C11 monoclonal antibodies (mAbs) from the day after neutrophil engraftment until at least 100 days after transplantation. Documented bacterial infection included any incidence of bloodstream infection or any other bacterial infection. Bloodstream infection was diagnosed if at least 1 of the following criteria was met: (1) blood culture obtained during a febrile episode was positive, at least once, for bacterial organisms not considered to be common skin contaminants; (2) blood culture obtained during a febrile episode was positive for the same common skin contaminant on separate occasions within 72 hours; (3) blood culture was positive, at least once, for a common skin contaminant, and the patient was diagnosed with septicemia, including hypotension (systolic blood pressure, <90 mmHg) and abnormal coagulopathy. Infections other than bloodstream infection were diagnosed if the following criteria were met: (1) bacterial organisms were observed from specimens such as sputum, urine, and stool at least on 2 occasions, and (2) the patient showed symptoms of infection corresponding to those specimens. *Clositridium difficile* enterocolitis was excluded from the analysis, because this disease is toxin-mediated, and cannot be prevented by administration of common bacterial prophylactic agents such as fluoroquinolones, even if patients with a high risk of bacterial infection can be identified by using a putative biomarker. CMV infection was defined as positive if either C10 or C11 antigenemia assay showed at least 2 positive cells per 150,000 leukocytes. Invasive fungal infection was diagnosed according to the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [21].

Statistical Analysis

Endpoints included cumulative incidences of documented bacterial infection, fungal infection, CMV infection, and infection-related mortality, and OS within 100 days post transplantation. Patient and transplant characteristics between 2 groups were compared using the Mann-Whitney *U*-test or χ^2 analysis, as appropriate. The day of neutrophil

engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count (ANC) exceeded 500/μL. The day of neutrophil engraftment between 2 groups was compared by using the Mann-Whitney *U*-test. To eliminate the effect of competing risk, the cumulative incidences were assessed using methods described elsewhere [22]. The competing event in the cumulative incidence analyses was defined as death without an event of interest within 100 days post transplantation. OS was estimated using Kaplan-Meier methods. Infection-related death was defined as death associated with any infection within 100 days after transplantation. Standard risk disease was defined as complete remission (CR) in cases of acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), adult T cell leukemia/lymphoma (ATL), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), and untreated or CR in MDS and myeloproliferative disorder (MPD). High-risk disease was defined as statuses other than CR in AML, ALL, ATL, HL, and NHL and in MDS and MPD after treatment. The Cox proportional-hazard model was applied to assess the effect of factors that potentially affected the study endpoints. The following items were added as confounders: recipient's sex (male or female), recipient's age (<50 or ≥50 years), diagnosis (myelogenous or lymphogenous malignancies), risk of disease (standard or high risk), conditioning regimen (reduced or myeloablative intensity [RIC, MA]), type of donor (related or unrelated donor), reticulocyte count ($<60 \times 10^9$ or $\geq 60 \times 10^9$/L), ferritin level (<1000 or ≥1000 mg/dL), and C-reactive protein (CRP) level (<0.3 or ≥0.3 μg/dL). The cutoff points for reticulocyte count and the ferritin and CRP levels were chosen such that we could make optimal use of the information with a proviso that the smaller group contained at least 30% of patients. *P* values of < .05 were considered statistically significant. All analyses were conducted using STATA software version 10 (STATA Corp., College Station, TX).

Table 1. Characteristics of Patients and Transplants

Variables	Hepcidin, Low (<50 ng/mL) n = 38	Hepcidin, High (≥50 ng/mL) n = 17	*P* Value
Age at transplant			
Median age (range)	47.5 (23–64)	47 (20–63)	.750
Sex			.171
Male	21 (55%)	6 (35%)	
Female	17 (45%)	11 (65%)	
Disease			.612
Myeloid malignancies	23 (61%)	9 (53%)	
AML	15	8	
MDS/MPD	8	1	
Lymphoid malignancies	15 (39%)	8 (47%)	
ALL	4	4	
ATL	4	1	
HL	1	0	
NHL	6	3	
Risk of disease			.051
Standard	22 (58%)	5 (29%)	
High	16 (42%)	12 (71%)	
Conditioning regimen			.545
Myeloablative intensity	19 (50%)	10 (59%)	
Reduced intensity	19 (50%)	7 (41%)	
Prophylaxis against GVHD			.663
Cyclosporine-based	5 (13%)	3 (18%)	
Tacrolimus-based	33 (87%)	14 (82%)	
Type of donor			.181
Related donor			
HLA*-matched	10 (26%)	3 (18%)	
HLA-mismatched	3 (8%)	1 (6%)	
Unrelated donor			
HLA-matched	18 (47%)	5 (29%)	
HLA-mismatched	7 (18%)	8 (47%)	
Source of stem cells			.259
Bone marrow	29 (76%)	10 (59%)	
Peripheral blood	1 (3%)	0 (0%)	
Cord blood	8 (21%)	7 (41%)	
Serum ferritin (μg/dL)			
mean (±SD)	664 (±796)	1551 (±993)	<.001
CRP (mg/dL)			
mean (±SD)	0.36 (±0.68)	0.70 (±1.63)	.176
Reticulocyte ($\times 10^9$/L)			
mean (±SD)	63.7 (±40.2)	64.0 (±42.2)	.979

AML indicates acute myelogenous leukemia; MDS/MPD, myelodysplastic syndrome and myeloproliferative disorders; ALL, acute lymphoblastic leukemia; ATL, acute T cell leukemia/lymphoma; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; GVHD, graft-versus-host disease; Cyclosporine-based, cyclosporine with or without other agents; Tacrolimus-based, tacrolimus with or without other agents; HLA, human leukocyte antigen; CRP, C-reactive protein.
Data are counts of individuals unless specified otherwise.
*HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, -B, and -DR antigens.

RESULTS

Characteristics of Patients and Transplants

Characteristics of patients and transplants are shown in Table 1. The median age of patients was 47 years (range: 20–64 years). The primary disease in these patients was as follows: AML in 23, MDS/MPD in 9, ALL in 8, NHL in 9, HL in 1, and ATL in 5. The risk of diseases was standard in 27 and high in 28 patients. Nearly half of the patients (n = 26) received a RIC regimen. The stem cell sources used were bone marrow (BM) in 39, peripheral blood (PB) in 1, and cord blood (CB) in 15 patients. The median pretransplant serum hepcidin level was 21.6 ng/mL (range: 1.4–371 ng/mL), which was comparable to that of healthy volunteers (median: 19.1 ng/mL [range: 2.3–37 ng/mL]; n = 17) [23]. Because the lower hepcidin level of the third tertile among the patients in this study was 49.1 ng/mL, we set a cutoff hepcidin level of 50 ng/mL for practical use to divide the patients into low- and high-hepcidin groups (n = 17 and 38, respectively). There was no difference in patient and transplant characteristics between the low- and high-hepcidin groups, except for serum ferritin levels ($P < .001$).

Documented Bacterial Infections

There was no significant difference between the days of neutrophil engraftment of the low- and high-hepcidin groups (median day: 21 [range: 14–99] and median day: 22.5 [range: 12–53], respectively, $P = .54$). A total of 16 episodes of bacterial infections were documented; these included 15 episodes of bloodstream infections and 1 episode of pneumonia. No patient experienced more than 1 episode of bacterial infection within 100 days after transplantation. The documented bacterial organisms are listed in Table 2. The main organisms were Gram-negative bacilli in both the low- and high-hepcidin groups. In the antimicrobial-susceptibility tests, 12 of the 13 Gram-negative isolates were sensitive to fluoroquinolone. We documented 2 bacterial infections in the late period of transplantation; 1 patient showed infection at day 89 after transplantation, which was attributed to delayed neutrophil engraftment, and another patient showed infection at day 68, when the neutrophil counts had temporarily decreased. The cumulative incidences of the documented bacterial infection in the low- and high-hepcidin groups were 11% (95% confidence interval [CI], 3%–23%) and 65% (95% CI, 38%–82%), respectively (Figure 1A). In the low-hepcidin group, the cumulative incidence of bacterial infection was lower in patients with a hepcidin level of <25 ng/mL than in those with a hepcidin level ranging from ≥25 to <50 ng/mL (10% [95% CI, 2%–23%] versus 17%, [95% CI, 1%–52%]). Univariate analysis of various potential confounders showed that high hepcidin level was the only factor that affected the cumulative incidence of documented bacterial infection (hazard ratio [HR], 8.98; 95% CI, 2.82–28.57; $P < .001$) (Table 3). To exclude the effect of other confounders, the significance of high hepcidin level was assessed in the stratified category of each confounder (eg, in either the high- or low-ferritin group); we noted consistently high HRs in the high-hepcidin group in each stratified category (data not shown). We also found that hepcidin had a significant impact on the patients, excluding the patients in other specific categories, such as those who received a CB transplant or those who underwent a transplant from an unrelated HLA-mismatched donor (data not shown). Furthermore, the significant effect of hepcidin persisted even after the adjustment for confounders in multivariate analysis (HR, 28.46; 95% CI, 2.51–323.34; $P = .007$) (Table 3). Even when the variables were treated as continuous instead of categoric, the significant effect of hepcidin persisted (HR, 1.01; 95% CI, 1.00-1.01; $P = .001$).

Table 2. Documented Bacterial Organisms within 100 Days after Stem Cell Transplantations

Category	Hepcidin, Low (<50 ng/mL) n = 38	Hepcidin, High (≥50 ng/mL) n = 17
Gram-positive cocci (n)	*Staphylococcus epidermidis* (1)	*Enterococcus faecium* (2)
Gram-negative bacilli (n)	*Klebsiella pneumoniae* (2)	*Klebsiella pneumoniae* (3)
	Enterobacter cloacae (1)	*Escherichia coli* (3)
	Prevotella intermedia (1)	*Pseudomonas aeruginosa* (2)
		Klebsiella oxytoca (1)

P. intermedia was detected in the sputum of 1 patient with pneumonia. Other organisms were detected in blood culture bottles.

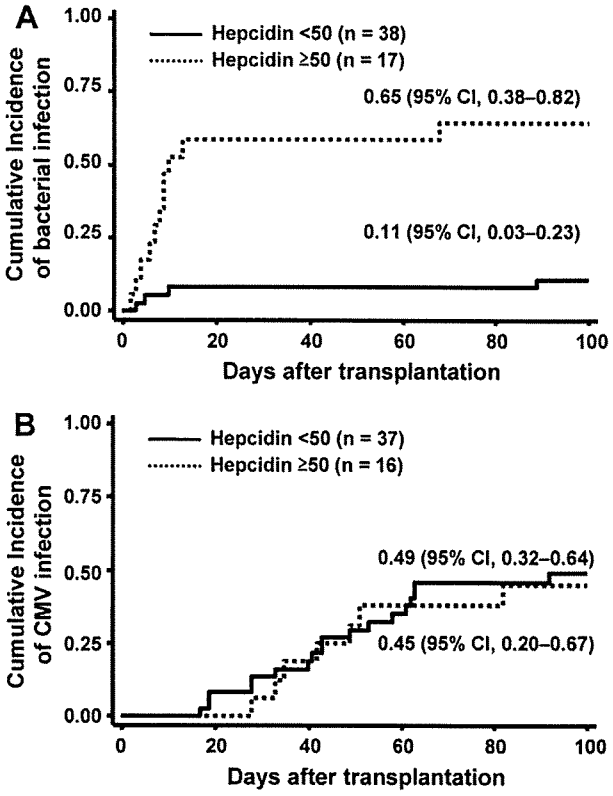

Figure 1. The cumulative incidences of documented bacterial infection (A) and cytomegalovirus (CMV) infection (B) at 100 days after stem cell transplantation. Solid black line, the low-hepcidin group (<50 ng/mL); solid gray line, the high-hepcidin group (≥50 ng/mL); CI, confidence interval. CMV infection was not assessable in 2 patients because of early death before neutrophil engraftment.

Other Transplant-Related Complications and Mortality

The cumulative incidences of CMV infection in the low- and high-hepcidin group were 49% (95% CI, 32%–64%) and 45% (95% CI, 20%–67%), respectively (Figure 1B); univariate and multivariate analyses showed no significant difference between the 2 groups (Table 3). All CMV infections were well treated by the administration of ganciclovir or foscarnet. No fungal infection was documented. Therefore, all infection-related deaths were attributed to bacterial infection. The cumulative incidence of infection-related mortality in the low-hepcidin group was 3% (95% CI, 0.2%–12%), whereas that in the high-hepcidin group was 6% (95% CI, 0.4%–24%),

Table 3. Univariate and Multivariate Analyses of Documented Bacterial Infection, CMV Infection, and Overall Survival at 100 Days after Stem Cell Transplantations

		Univariate Analysis		Multivariate Analysis	
	Number	HR (95% CI)	P Value	HR (95% CI)	P Value
1) Documented bacterial infection					
Hepcidin, low (<50 ng/mL)	5/38	1	–	1	–
Hepcidin, high (≥50 ng/mL)	11/17	8.98 (2.82–28.57)	<.001	28.46 (2.51–323.34)	.007
2) CMV antigenemia (C10 or C11 ≥2)					
Hepcidin, low (<50 ng/mL)	18/37	1	–	1	–
Hepcidin, high (≥50 ng/mL)	7/16	0.97 (0.40–2.32)	.939	0.63 (0.16–2.49)	.511
3) Overall survival					
Hepcidin, low (<50 ng/mL)	36/38	1	–	–	–
Hepcidin, high (≥50 ng/mL)	14/17	3.60 (0.60–21.56)	.161	–	–

CMV indicates cytomegalovirus; CI, confidence interval.
Hazard ratios (HRs) in multivariate analysis were adjusted for recipient's sex (male or female), recipient's age (<50 or ≥50 years), diagnosis (myelogenous or lymphoid malignancies), risk of disease (standard or high risk), conditioning regimen (reduced or myeloablative intensity), type of donor (related or unrelated donor), reticulocyte count (<60 × 109 or ≥60 × 10^9/L), ferritin level (<1000 or ≥1000 mg/dL), and C-reactive protein (CRP) level (<0.3 or ≥0.3 μg/dL). Overall survival was not analyzed in the multivariate model because of the low incidence of death.

with no statistical difference between the 2 groups. OS at 100 days after transplantation in the low- and high-hepcidin groups was 95% (95% CI, 81%–99%) and 82% (95% CI, 55%–94%), respectively (Figure 2). No significant difference in OS was observed (Table 3).

DISCUSSION

In our cohort of patients who underwent allogeneic HSCT for hematologic malignancies, we found a significant association between the pretransplant serum hepcidin levels and the cumulative incidence of documented bacterial infection. To our knowledge, this is the first study that has evaluated the clinical significance of serum hepcidin levels in predicting transplant-related complications; the findings suggest that the pretransplant serum hepcidin level can be used as a good pretransplant biomarker to predict bacterial infection in a patient scheduled for HSCT.

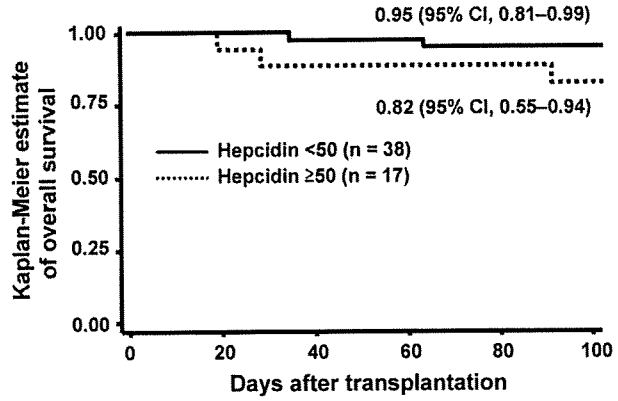

Figure 2. Kaplan-Meier estimate of OS at 100 days after stem cell transplantation. Solid black line, the low-hepcidin group (<50 ng/mL); solid gray line, the high-hepcidin group (≥50 ng/mL); CI, confidence interval.

Hepcidin production is regulated by at least 3 factors: iron load [16,17], inflammation [18], and unknown erythropoietic signals [23-25]. Therefore, the good predictive value of hepcidin with respect to the incidence of documented bacterial infection can be partly explained by the cumulative effect of at least these 3 factors on bacterial infection. Iron overload increases the level of circulating non-transferrin-bound iron, which is known to amplify free-radical reactions in inflammatory or ischemia-related conditions [7,26]. Such reactions could enhance tissue damage such as mucositis during the conditioning regimen, thereby allowing bacterial translocation through the damaged mucosa [27]. In addition, iron is a necessary nutrient for bacteria and fungus [28]. The association between hemochromatosis, 1 of the iron overload disorders, and infection with certain organisms has already been described [29]. Therefore, the high hepcidin levels might reflect iron overload status, which has an adverse effect on bacterial infections. Second, a high hepcidin level may indicate inflammation because of a latent bacterial infection that was undetectable before HSCT, but may surface in posttransplant neutropenic status. Last, a high hepcidin level could reflect suppressed erythropoiesis, probably because of the short duration from the last chemotherapy to the start of the conditioning regimen for transplantation. Repeated cytotoxic chemotherapy in a short period may exacerbate tissue damage and increase the risk of bacterial infection.

Although serum ferritin levels do not necessarily correlate with the amount of iron load in patients with inflammation or specific diseases [1,30,31], it is frequently used and regarded as a indicator of iron overloading, and several studies have demonstrated the association between high ferritin levels and treatment-related mortality (TRM) [3,11]. In this cohort, an elevation of serum ferritin level was not found to be a significant risk factor for bacterial infection,