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RESEARCH ARTICLE

Risk factors for chronic graft-versus-host disease after anti-thymocyte globulin-based haploidentical hematopoietic stem cell transplantation in acute myeloid leukemia

Meng Lv[1], Xiaohui Zhang[1], Lanping Xu[1], Yu Wang[1], Chenhua Yan[1], Huan Chen[1], Yuhong Chen[1], Wei Han[1], Fengrong Wang[1], Jingzhi Wang[1], Kaiyan Liu[1], Xiaojun Huang[1,2], Xiaodong Mo (✉)[1]

[1]*Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing 100044, China;* [2]*Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100044, China*

Abstract **Chronic graft-versus-host disease (cGVHD) is a major complication following unmanipulated haploidentical hematopoietic stem cell transplantation (haplo-HSCT). We aimed to identify the risk factors for cGVHD in patients who underwent anti-thymocyte globulin-based haplo-HSCT for acute myeloid leukemia (n = 280). The diagnosis of cGVHD was in accordance with the National Institutes of Health consensus criteria. A total of 169 patients suffered from cGVHD. The patients who had 3 loci mismatched had a higher 8-year incidence of cGVHD (total, 66.0% vs. 53.7%, P = 0.031; moderate to severe, 42.4% vs. 30.1%, P = 0.036) than the patients who had 1 to 2 loci mismatched. The patients who had maternal donors had a higher 8-year incidence of moderate to severe cGVHD (49.2% vs. 32.9%, P = 0.024) compared with the patients who had other donors. The patients who had grades III to IV acute GVHD (aGVHD) had higher 8-year incidence of cGVHD (total, 88.0% vs. 50.4%, P < 0.001; moderate to severe, 68.0% vs. 27.0%, P < 0.001) compared with the patients without aGVHD. In multivariate analysis, grades III to IV aGVHD was the only independent risk factor for cGVHD. Thus, further interventions should be considered in patients with severe aGVHD to prevent cGVHD.**

Keywords acute graft-versus-host disease; chronic graft-versus-host disease; National Institutes of Health consensus criteria; acute myeloid leukemia; anti-thymocyte globulin

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective post-consolidation therapy and curative option for patients with acute myeloid leukemia (AML) [1–3]. There is a shortage of human leukocyte antigen (HLA)-identical sibling donors (ISDs) and unrelated donors (URDs) in China [4]. Meanwhile, HLA-haploidentical related donors (haplo-RDs) have become the largest source of allo-HSCT donors in China since 2013 [5,6]. Thus, haplo-RD HSCT (haplo-HSCT) is a valuable option for AML patients who need allo-HSCT in China [7,8].

Although reduced by granulocyte colony-stimulating factor (G-CSF) and anti-thymocyte globulin (ATG) induced immune tolerance, chronic graft-versus-host disease (cGVHD) was inevitable after haplo-HSCT. Nevertheless, we observed that patients with cGVHD had lower risk of relapse and better disease-free survival (DFS) compared with the non-cGVHD group after haplo-HSCT [9]. This may be due to the fact that graft-versus-leukemia (GVL), which might be the most critical mechanisms for the reduction of relapse risk, was associated with cGVHD [10–13]. However, we observed that even mild cGVHD can significantly decrease the risk of relapse [9]; and patients with moderate to severe cGVHD should receive systemic immunosuppressive therapies inevitably for several months [14]. In addition, Chang *et al*. [15] reported that moderate to severe cGVHD significantly increased the risk of multiple late complications in long-term survivors. The relapse risk of patients with AML in complete remission (CR) was relatively low [7,16], therefore it is reasonable to control the severity of

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Correspondence: Xiaodong Mo, mxd453@163.com

cGVHD and prevent the occurrence of moderate to severe cGVHD.

Several factors, such as acute GVHD (aGVHD), were associated with cGVHD in patients who received ISD or URD allo-HSCT [17–22]. However, these factors were not validated in unmanipulated haplo-HSCT recipients. Wang *et al*. [23] reported that disease status and the number of infused CD3 cells were associated with extensive cGVHD after unmanipulated haplo-HSCT. However, National Institutes of Health (NIH) consensus criteria were not used in this study, and heterogeneous group of patients with either AML or acute lymphoblastic leukemia (ALL) were enrolled. Thus, the risk factors for cGVHD in AML patients receiving unmanipulated haplo-HSCT were unclear.

Thus, we aimed to investigate cGVHD risk factors, particularly for the moderate to severe cGVHD, in a consecutive cohort of AML patients who received ATG-based unmanipulated haplo-HSCT.

Materials and methods

Patients

This study included consecutive patients who underwent haplo-HSCT for AML (CR1 or CR2) at the Institute of Hematology, Peking University, Beijing, China between January 2006 and December 2011. The final study cohort comprised of 280 patients (Table 1). The influence of cGVHD on clinical outcomes after haplo-HSCT was previously reported in 2015 [9]. These patients were enrolled and followed up further in this study. The last follow-up visits for endpoint analysis were conducted in 31 December 2018. Patients who received minimal residual disease (MRD)-directed donor lymphocyte infusion (DLI) [24] after transplantation in the same period were analyzed separately (n = 28). Informed consent was obtained from all patients, and the study was conducted in accordance with the *Declaration of Helsinki*. The study protocol was approved by the ethics committee of Peking University People's Hospital.

Transplant regimens

The preconditioning treatment consisted of cytarabine at 4 g/(m^2·day) for 2 days, busulfan at 4 mg/(kg·day) administered orally for 3 days before January 2008 or 3.2 mg/(kg·day) administered intravenously for 3 days after January 2008, cyclophosphamide at 1.8 g/(m^2·day) for 2 days, and simustine at 250 mg/m^2, for 1 day. Rabbit ATG (thymoglobulin; rabbit ATG from Imtix Sangstat, Lyon, France) was also administered at 2.5 mg/(kg·day) for 4 days. All patients received granulocyte colony stimulating factor (G-CSF)-mobilized, fresh, and unmanipulated bone marrow (G-BM) cells plus peripheral blood (G-PB) stem cells. They also received cyclosporine A, mycophenolate mofetil, and short-term methotrexate for GVHD prophylaxis (Supplementary methods) [25,26]. Donor selection, HLA typing, and stem cell harvesting have been described in detail in previous studies [26].

Definitions and assessments

Diagnosis of cGVHD was in accordance with the NIH consensus criteria [27]. cGVHD was diagnosed if the patient presented at least one diagnostic clinical sign of cGVHD or at least one distinctive manifestation confirmed by pertinent biopsy or other relevant tests and the exclusion of any other possible diagnosis. Mild cGVHD was one or two organs (except the lung) with score 1. Moderate cGVHD was three or more organs with score 1 or lung score 1, or one or more organs with score 2. Severe cGVHD was any organ with score 3 or lung score 2. Cytogenetic risks were assessed and classified into three risk groups (good, intermediate, and poor) according to the criteria of the South-west Oncology Group/Eastern Cooperative Oncology Group for patients diagnosed with AML [28]. The incidence of comorbidities in HSCT recipients was assessed based on the hematopoietic cell transplantation-specific comorbidity index (HCT-CI) [29]. Relapse was defined as morphological evidence of disease in samples from the peripheral blood, bone marrow, or extramedullary sites or by the recurrence and sustained presence of pre-transplantation chromosomal abnormalities. Patients who exhibited MRD were not classified as relapse. Non-relapse mortality (NRM) was defined as death without disease progression or relapse. Overall survival (OS) was defined as the time from transplantation to death from any cause. DFS was defined as survival in continuous CR. GVHD-free/relapse-free survival (GRFS) events were defined as grades III–IV aGVHD, as well as cGVHD requiring systemic immunosuppressive treatment, disease relapse, or death from any cause after haplo-HSCT. GRFS was calculated from the transplant day to the date of the first event.

Statistical analysis

Data were censored at time of relapse, NRM, or the last available follow-up. Demographic and clinical characteristics were compared between the cGVHD and non-cGVHD groups. The χ^2 and Fisher's exact tests were used for dichotomous variables, and the Mann–Whitney U test was used for continuous variables. Survival probabilities were estimated using the Kaplan–Meier method. Competing risk analysis was performed to calculate the cumulative incidence of cGVHD, relapse, and NRM. Gray's test was used to examine the differences between the groups [30]. For GVHD, relapse and death without GVHD were competing events. For NRM, relapse was a competing

Table 1 Characteristics of patients

Characteristics	Non-cGVHD (n = 111)	cGVHD (n = 169)	P*
Median age at HSCT, year (range)	25 (3–55)	26 (2–54)	0.298
Time in months from diagnosis to HSCT, median (range)	6 (2–94)	6 (2–60)	0.303
Gender, *n* (%)			
Male	69 (62.2)	96 (56.8)	0.373
Female	42 (37.8)	73 (43.2)	
Disease status at transplantation, *n* (%)			
CR1	99 (89.2)	153 (90.5)	0.714
CR2	12 (10.8)	16 (9.5)	
Cytogenetic risk, *n* (%)			
Good	17 (15.3)	21 (12.4)	0.634
Intermediate	91 (82.0)	140 (82.8)	
Poor	3 (2.7)	8 (4.7)	
Donor–recipient gender match, *n* (%)			
Female–male	23 (20.7)	38 (22.5)	0.726
Others	88 (79.3)	131 (77.5)	
Donor–recipient relation, *n* (%)			
Father–child	35 (31.5)	46 (27.2)	0.558
Mother–child	19 (17.1)	42 (24.9)	
Sibling–sibling	40 (36.0)	62 (36.7)	
Child–parent	11 (9.9)	14 (8.3)	
Other	6 (5.5)	5 (2.9)	
Number of HLA-A, -B, -DR mismatches, *n* (%)			
1	19 (17.2)	13 (7.7)	0.019
2	44 (39.6)	60 (35.5)	
3	48 (43.2)	96 (56.8)	
ABO match, *n* (%)			
Matched	63 (56.8)	94 (55.6)	0.851
Mismatched	48 (43.2)	75 (44.4)	
HCT-CI scores			
0	56 (50.5)	84 (49.7)	0.100
1–2	35 (31.5)	68 (40.2)	
⩾3	20 (18.0)	17 (10.1)	
aGVHD, *n* (%)			
None	57 (51.4)	58 (34.3)	<0.001
Grades I–II	52 (46.8)	88 (52.1)	
Grades III–IV	2 (1.8)	23 (13.6)	
Viral infection			
Cytomegaloviremia	55 (49.5)	96 (56.8)	0.234
Cytomegalovirus disease	2 (1.8)	8 (4.7)	0.324
Epstein–Barr viremia	5 (4.5)	7 (4.1)	1.000
PTLD	3 (2.7)	3 (1.8)	0.684
Median mononuclear cells, $\times 10^8$/kg (range)	8.2 (4.0–16.3)	7.8 (2.7–18.8)	0.141
Median $CD3^+$ counts, $\times 10^8$/kg (range)	1.5 (0.2–6.3)	1.5 (0.2–8.3)	0.249
Median $CD34^+$ counts, $\times 10^6$/kg (range)	2.2 (0.4–55.3)	2.2 (0.3–12.1)	0.424

aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CR, complete remission; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; PTLD, posttransplant lymphoproliferative disorders.
*The criterion for statistical significance was $P < 0.05$.

event. For relapse, NRM was a competing event. Multivariate hazard ratios (HRs) for cGVHD and survival were estimated from Cox regression with a forward-stepwise model selection approach. For time-dependent variables (e.g., aGVHD), the proportional hazard (PH) assumption was examined. Then, a stratified Cox model was used to examine the effects of variables on the observation endpoints and to test the interaction terms with covariates. Factors included in the regression model for cGVHD were age (using the median as the cut-off point), gender, adverse cytogenetics, disease status at transplantation (CR1 vs. CR2), time from diagnosis to transplantation (using the median as the cut-off point), HLA disparity (1 locus vs. 2 loci vs. 3 loci), donor–recipient gender matching (female to male vs. others), donor–recipient relationship (maternal donors vs. other donors), ABO compatibility (matched vs. mismatched), HCT-CI (3 + vs. 0–2), and aGVHD (grades III to IV vs. others). Independent variables with $P > 0.1$ were sequentially excluded from the model. $P < 0.05$ was considered statistically significant. All reported *P*-values were based on two-sided hypothesis tests. Data analyses were primarily conducted with the Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, IL, USA), whereas the R software package (version 2.6.1) was used for competing risk analysis.

Results

General characteristics of cGVHD

The patient characteristics are showed in Table 1. Thirty-four patients experienced relapse, and 25 patients experienced NRM. A total of 169 patients experienced cGVHD. Mild, moderate, and severe cGVHD were observed in 66, 67, and 36 patients, respectively. The 8-year cumulative incidence of total cGVHD was 60.0% at 95% confidence intervals (CI), 54.2%–65.8%. The 8-year cumulative incidence of moderate to severe cGVHD and severe cGVHD were 36.4% (95% CI, 30.7%–42.1%) and 12.9% (95% CI, 9.0%–16.8%), respectively. The detail characteristics of cGVHD are summarized in Table 2.

Most of the 169 patients with cGVHD received corticosteroid (n = 123) and cyclosporine A (n = 118). The number of patients receiving multiple immunosuppressants ($\geqslant$ 3) were 7 (10.6%), 28 (41.8%), and 23 (63.9%) in mild, moderate, and severe cGVHD groups, respectively ($P < 0.001$). The median duration of immunosuppressive therapies was 228 (range, 73–378 days), 287 (145–891 days), 370 (120–2220 days), 625 (132–1578 days) for non-cGVHD, mild cGVHD, moderate cGVHD, and severe cGVHD patients, respectively ($P < 0.001$).

The 8-year cumulative incidence of relapse and NRM were 12.1% (95% CI, 8.3%–15.9%) and 9.0% (95% CI, 5.6%–12.4%), respectively. The 8-year probabilities of OS, DFS, and GRFS were 82.4% (95% CI, 77.9%–86.9%), 78.9% (95% CI, 74.1%–83.7%), and 47.1% (95% CI, 41.2%–53.0%), respectively.

Table 2 Characteristics of cGVHD after haplo-HSCT

Characteristics of cGVHD	N = 169
Time from cGVHD to haplo-HSCT, day (range)	100 (172–1023)
Severity of cGVHD, *n* (%)	
Mild	66 (39.1)
Moderate	67 (39.6)
Severe	36 (21.3)
Type of cGVHD, *n* (%)	
Classical cGVHD	155 (91.7)
Overlap syndrome	14 (8.3)
Site of cGVHD, *n* (%)	
Skin	145 (85.8)
Mouth	41 (24.3)
Eye	32 (18.9)
Liver	48 (28.4)
Gut	27 (16.0)
Lung	5 (3.0)
Joint	3 (1.8)
Number of sites, *n* (%)	
1	83 (49.1)
2	48 (28.4)
$\geqslant$3	38 (22.5)
Treatment of cGVHD	
Corticosteroid	123 (72.8)
Cyclosporine A	118 (69.8)
Methotrexate	52 (30.8)
Mycophenolate mofetil	35 (20.7)
Penicillamine	22 (13.0)
Tacrolimus	17 (10.1)
Azathioprine	9 (5.3)
Number of drugs	
0	8 (4.8)
1	20 (11.8)
2	83 (49.1)
$\geqslant$3	58 (34.3)

cGVHD, chronic graft-versus-host disease; haplo-HSCT, haploidentical hematopoietic stem cell transplantation.

Patient and disease characteristics and cGVHD

We identified the correlations among the following: patient characteristics (gender, age, and HCT-CI scores); disease characteristics (cytogenetics, disease status before haplo-HSCT, and time from diagnosis to haplo-HSCT); and cGVHD after haplo-HSCT. None of these factors was associated with cGVHD after haplo-HSCT (Table 3).

Table 3 Risk factors for cGVHD after haplo-HSCT

Variables	Total cGVHD				Moderate to severe cGVHD			
	Univariable analysis		Multivariate analysis		Univariable analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Patient characteristics								
Gender								
Male	1				1			
Female	1.08 (0.79–1.46)	0.644			1.02 (0.69–1.52)	0.912		
Age								
<median	1				1			
⩾median	1.02 (0.75–1.38)	0.897			1.02 (0.69–1.50)	0.934		
HCT-CI scores								
0–2	1				1			
⩾3	0.69 (0.42–1.14)	0.148			0.71 (0.37–1.37)	0.308		
Disease characteristics								
Disease status before haplo-HSCT								
CR1	1				1			
CR2	0.86 (0.51–1.44)	0.562			0.94 (0.49–1.81)	0.859		
Adverse cytogenetics								
No	1				1			
Yes	1.10 (0.54–2.25)	0.785			0.84 (0.31–2.28)	0.728		
Time from diagnosis to HSCT								
<6 months	1				1			
⩾6 months	0.89 (0. 64–1.22)	0.463			0.87 (0.58–1.32)	0.517		
Donor characteristics								
HLA disparity								
1 locus	1				1			
2 loci	1.60 (0.88–2.92)	0.124			2.01 (0.85–4.79)	0.113		
3 loci	2.03 (1.14–3.63)	0.017			2.67 (1.16–6.18)	0.022		

(*Continued*)

Variables	Total cGVHD				Moderate to severe cGVHD			
	Univariable analysis		Multivariate analysis		Univariable analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Donor type								
Others	1				1			
Maternal donors	1.22 (0.86–1.73)	0.267			1.64 (1.07–2.52)	0.022		
Donor–recipient sex matched								
Others	1				1			
Female donor/male recipient	1.06 (0.74–1.52)	0.745			1.21 (0.77–1.90)	0.409		
ABO matched								
Matched/minor mismatched	1				1			
Major/major-minor mismatched	1.03 (0.76–1.40)	0.829			0.77 (0.52–1.15)	0.201		
aGVHD after haplo-HSCT								
None	1		1		1		1	
Grades I to II	1.38 (0.99–1.92)	0.058	1.36 (0.98–1.90)	0.070	1.56 (1.00–2.43)	0.048	1.52 (0.98–2.37)	0.063
Grades III to IV	2.49 (1.52–4.09)	<0.001	2.40 (1.46–3.93)	0.001	3.55 (1.96–6.42)	<0.001	3.09 (1.69–5.66)	< 0.001
Infection after haplo-HSCT								
Cytomegalovirus infection								
None	1				1			
Cytomegaloviremia	1.10 (0.81–1.49)	0.537			1.17 (0.79–1.73)	0.433		
Cytomegalovirus disease	1.59 (0.78–3.24)	0.199			1.48 (0.60–3.63)	0.394		
Epstein-Barr virus infection								
None	1				1			
Epstein-Barr viremia	1.19 (0.56–2.54)	0.652			1.41 (0.57–3.46)	0.456		
PTLD	1.46 (0.46–4.58)	0.519			1.56 (0.38–6.35)	0.533		

aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CR, complete remission; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; PTLD, posttransplant lymphoproliferative disorders.

Donor characteristics and cGVHD

We identified the correlations between donor characteristics (HLA disparity, donor–recipient relation, donor–recipient sex matched, and ABO compatibility) and cGVHD after haplo-HSCT. The patients who had three loci mismatched had higher cumulative incidence of total cGVHD (8 years: 66.0% vs. 53.7%, $P = 0.031$) and moderate to severe cGVHD (8 years: 42.4% vs. 30.1%, $P = 0.036$), respectively, compared with patients who had one to two loci mismatched (Table 3, Fig. 1A and 1B). The patients who had maternal donors had higher cumulative incidence of moderate to severe cGVHD (8 years: 49.2% vs. 32.9%, $P = 0.024$) than the patients who had other donors (Table 3, Fig. 1C).

Post-transplant complications and cGVHD

aGVHD and cGVHD

The patients who had grades III to IV acute graft-versus-host (aGVHD) had higher cumulative incidence of total cGVHD (8 years: 88.0% vs. 50.4%, $P < 0.001$) and moderate to severe cGVHD (8 years: 68.0% vs. 27.0%, $P < 0.001$), respectively, compared with the non-aGVHD group. The patients who had grades III to IV aGVHD also had higher incidence of total cGVHD (8 years: 88.0% vs. 62.8%, $P = 0.012$) and moderate to severe cGVHD (8 years: 68.0% vs. 38.6%, $P = 0.006$), respectively, compared with the patients with grades I to II aGVHD (Fig. 2A and 2B).

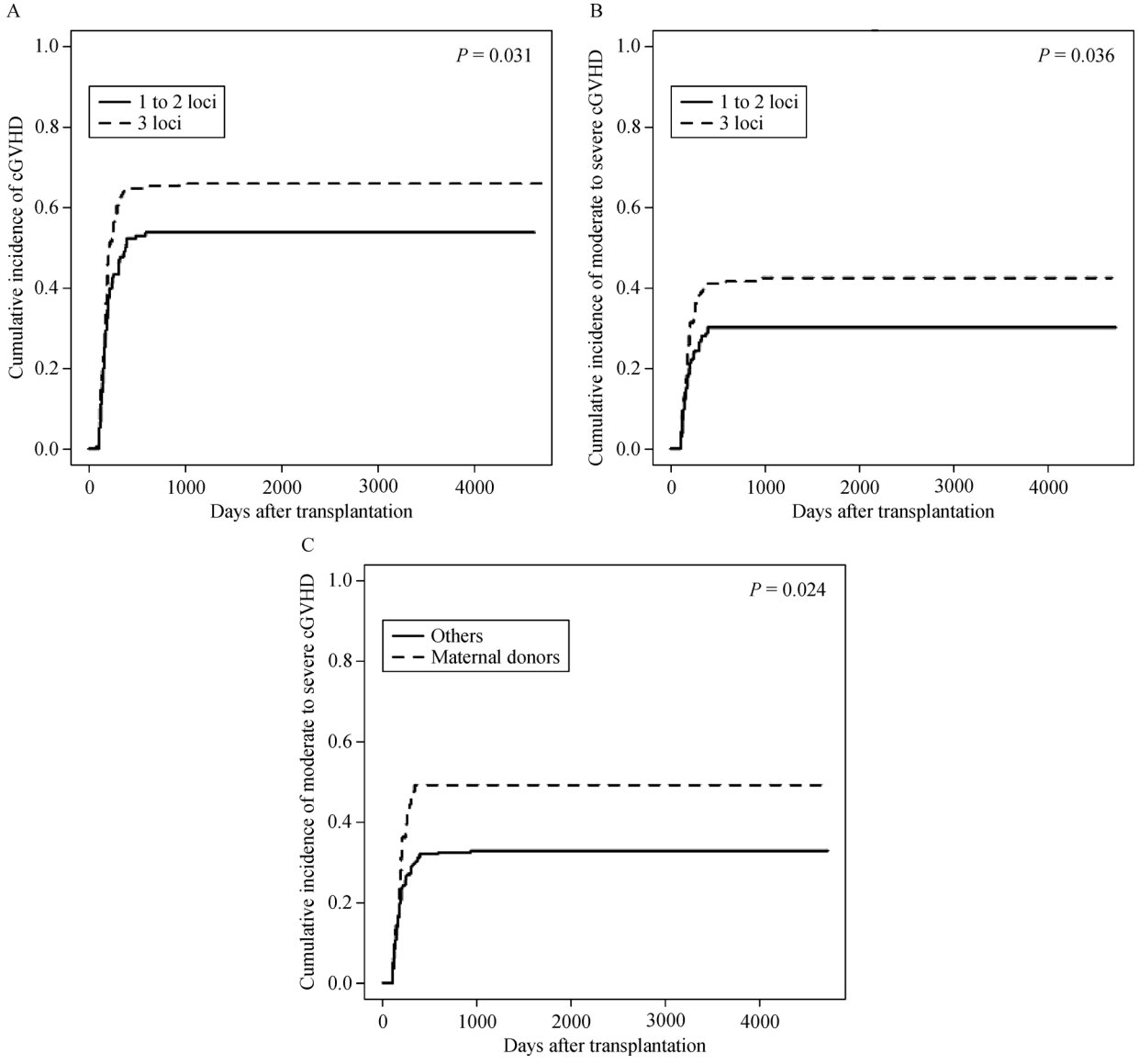

Fig. 1 cGVHD according to HLA disparity and donor–recipient relation. (A) HLA disparity and total cGVHD; (B) HLA disparity and moderate to severe cGVHD; (C) Donor–recipient relationship and moderate to severe cGVHD. cGVHD, chronic graft-versus-host disease.

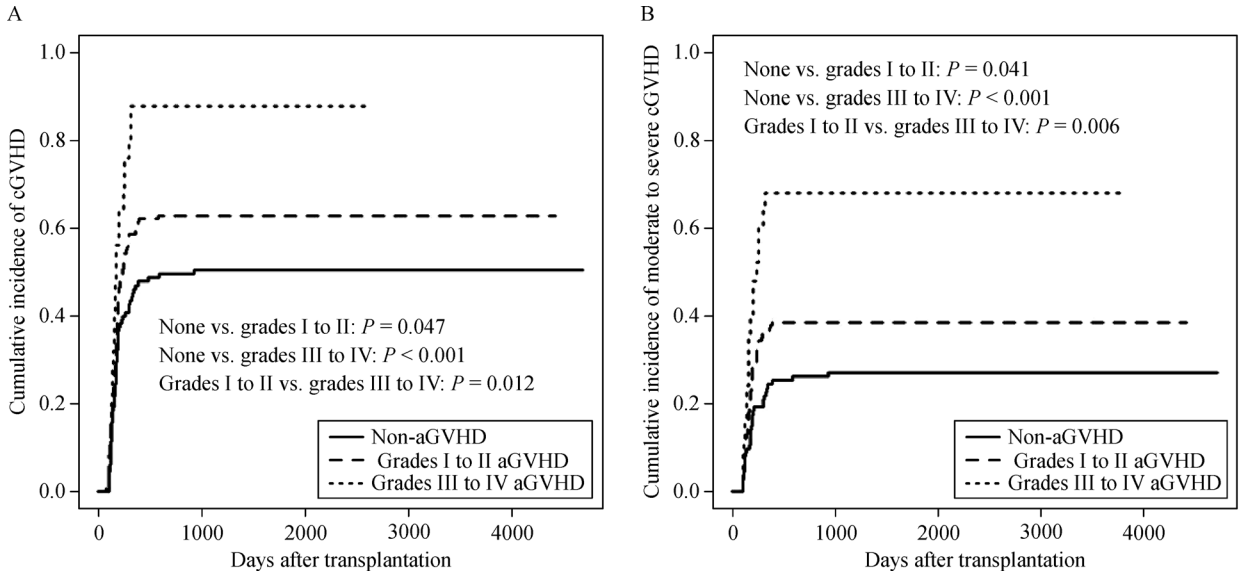

Fig. 2 cGVHD according to aGVHD severity. (A) aGVHD and total cGVHD; (B) aGVHD and moderate to severe cGVHD. aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

Virus infection and cGVHD

We tried to identify the correlations between post-transplant viral infection (cytomegalovirus and Epstein-Barr virus) and cGVHD after haplo-HSCT. They were not associated with cGVHD after haplo-HSCT (Table 3).

Multivariate analysis for risk factors of cGVHD

In multivariate analysis, grades III to IV aGVHD were the only independent risk factors for total cGVHD and moderate to severe cGVHD (Table 3). However, multivariate analysis failed to determine any risk factors for severe cGVHD (data not shown).

cGVHD and clinical outcomes after haplo-HSCT

Patients who had mild cGVHD showed lower relapse, and those who had mild and moderate cGVHD showed better OS and DFS compared with those without cGVHD. The NRM rates were comparable among the groups. Severe cGVHD did not decrease relapse and improve survival compared with those without cGVHD (Fig. 3A – 3D).

Immune recovery after haplo-HSCT

The recoveries of $CD3^+$, $CD4^+$, $CD8^+$ T, and $CD19^+$ B cells were summarized in Supplementary Table S1. On days 365 after transplantation, the immune recoveries of the severe cGVHD group were significantly worse than those of non-cGVHD and mild cGVHD groups.

cGVHD after DLI

Twenty-eight patients received MRD-directed DLI after haplo-HSCT. Twenty-one, 5, and 2 patients received 1, 2, and 3 cycles of DLI, respectively. Nineteen (67.9%) patients experienced cGVHD after DLI. Skin was the most commonly involved organ ($n = 16$), followed by mouth ($n = 7$), liver ($n = 6$), eye ($n = 5$), lung ($n = 3$), and the gastrointestinal tract ($n = 3$). Seven patients showed the involvement of more than three organs. Two, 10, and 7 patients were categorized into mild, moderate, and severe cGVHD groups, respectively. The 8-year cumulative incidence of total cGVHD and moderate to severe cGVHD was 67.9% (95% CI, 49.1%–86.7%) and 60.7% (95% CI, 41.4%–80.0%), respectively. In the multivariate analysis, only time from diagnosis to transplantation was associated with cGVHD after DLI (HR = 0.32, 95%CI 0.10 – 0.99, $P = 0.048$), and the other variables (including aGVHD before DLI) were not associated with cGVHD after DLI. None of the variables were associated with moderate to severe cGVHD after DLI. In addition, cycles and doses of DLI were not associated with cGVHD after DLI (data not shown).

Discussion

In the present study, we observed that severe aGVHD, maternal donors, and HLA disparity were risk factors for cGVHD in univariate analysis, and severe aGVHD was the independent risk factor for cGVHD and moderate to severe

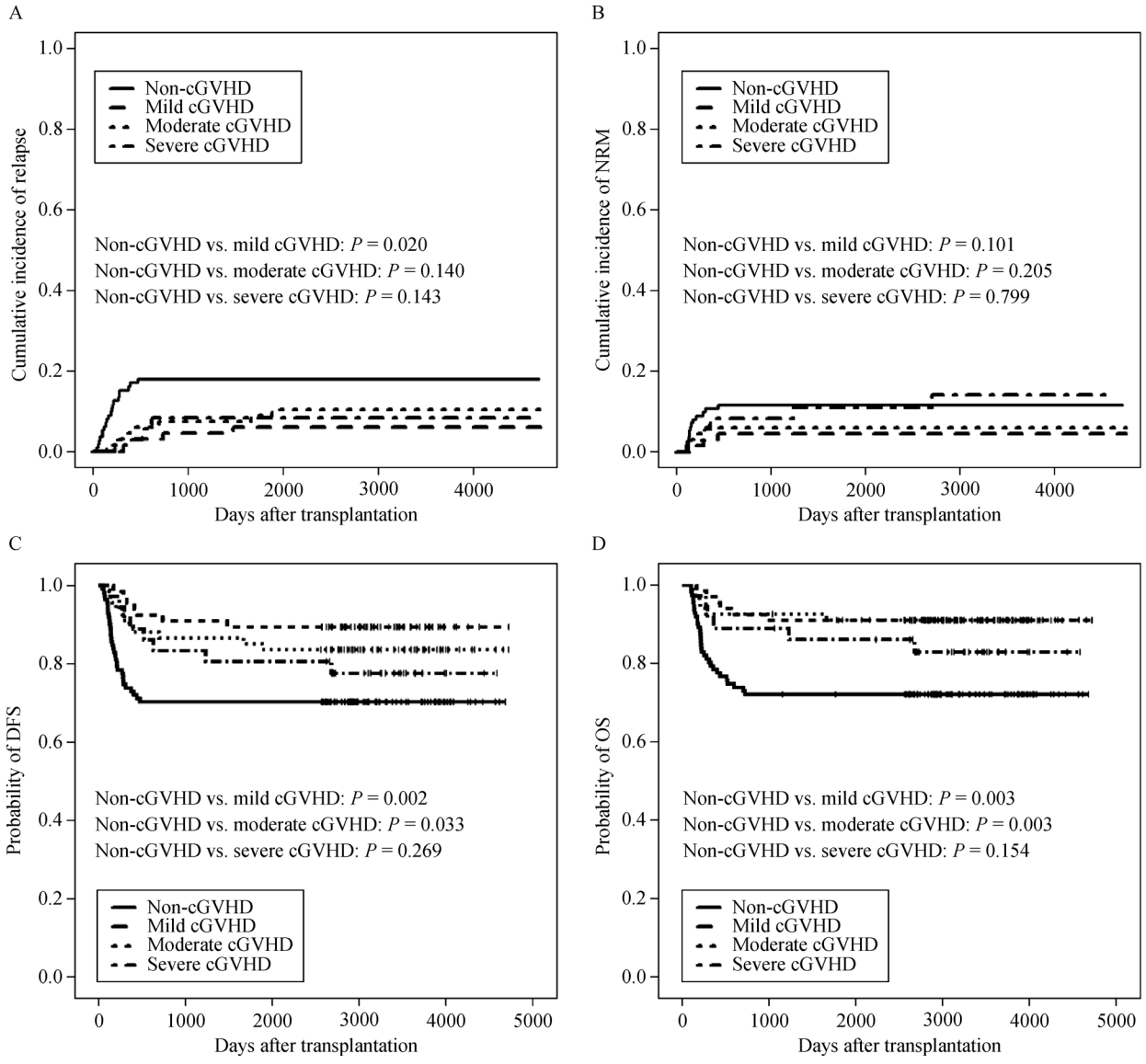

Fig. 3 Clinical outcomes according to cGVHD grading. (A) relapse; (B) non-relapse mortality; (C) disease-free survival; (D) overall survival. cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; NRM, non-relapse mortality; OS, overall survival.

cGVHD in multivariate analysis. To our knowledge, this is the first study to identify the risk factors for cGVHD in a disease-specific population of AML patients. It provides an opportunity to explore the up-to-date undefined association between aGVHD and cGVHD after unmanipulated haplo-HSCT.

Although 60% of the patients had cGVHD after haplo-HSCT, most of them had mild or moderate cGVHD. Severe cGVHD was observed in only 12.9% of the patients. Arai *et al*. [31] reported that only severe cGVHD was critical for the increased risk of NRM in cGVHD patients. The lower risk of relapse was not offset by increased NRM in patients with cGVHD after haplo-HSCT [9]. In addition, the 8-year probability of GRFS in the current study was 47.1%, which was similar to the results of haplo-HSCT performed with post-transplant cyclophosphamide [32,33].

Grades III to IV aGVHD was the independent risk factor for cGVHD and moderate to severe cGVHD after unmanipulated haplo-HSCT. Sohn *et al*. [21] reported that severe aGVHD (grades III to IV) ($P = 0.022$) was a significant independent factor that predicted a higher overall incidence of progressive- or quiescent-type cGVHD in ISDs and URD HSCT recipients. In addition, Flowers *et al*. [34] observed that grades III to IV aGVHD showed a statistically significant association with an increased risk of NIH cGVHD (HR = 1.42; 95% CI 1.14 – 1.77). Grube *et al*. [35] reported that a prior history

of grades II–IV aGVHD increased the risk of cGVHD in recipients of single umbilical cord blood transplantation (HR = 2.4, 95% CI, 1.0 – 6.0) and double umbilical cord blood transplantation (HR = 2.0; 95% CI, 1.3 – 3.2). In addition, grades II to IV aGVHD was independent risk factors for moderate-to-severe cGVHD [36]. Thus, preventing severe aGVHD was among the most important methods to prevent cGVHD after haplo-HSCT. Wang *et al*. [37] reported that maternal donors were associated with more aGVHD ⩾ grade II compared with paternal donors in haplo-HSCT; this finding is similar to that obtained by Tamaki *et al*. [38]. In addition, we reported that the incidence of grades III–IV aGVHD was higher in the 6 mg/kg ATG group than in the 10 mg/kg ATG group (16.1% vs. 4.5%, $P = 0.005$), thereby suggesting that a lower dose of ATG in conditioning regimen exposes patients to a higher risk of severe acute GVHD [39]. In addition, in a controlled, randomized, open-label trial (NCT01607580), Chang *et al*. [40] observed that patients were categorized as low risk or high risk for aGVHD according to the bone marrow allogeneic graft CD4:CD8 ratios. Low-dose corticosteroid prophylaxis can significantly decrease the incidence of grades II to IV aGVHD in high-risk patients (21% vs. 48%, $P < 0.001$), which was comparable with those of the low-risk patients (21% vs. 26%, $P = 0.43$). In the extension study, corticosteroid prophylaxis can also decrease the incidence of moderate to severe cGVHD in high-risk patients after haplo-HSCT (42% vs. 20%; $P = 0.010$) [41]. Thus, all these factors should be considered in haplo-HSCT recipients.

In addition, we observed that paternal donors significantly decreased the risk of extensive cGVHD after haplo-HSCT compared with that of the maternal donors (HR = 0.68, $P = 0.008$) [42]. In the other cohort that included patients with AML, ALL, chronic myelogenous leukemia, and myelodysplastic syndrome, non-maternal donors were associated with a significantly lower risk of moderate to severe cGVHD after haplo-HSCT compared with maternal donors (HR = 0.48, $P = 0.001$) [15]. In this study on patients with AML, we observed that maternal donors were associated with a higher risk of moderate to severe cGVHD after haplo-HSCT in univariate analysis, although it did not achieve statistical significance in multivariate analysis.

We observed that two loci mismatched did not significantly increase the risk of cGVHD compared with one locus mismatched. Three loci mismatched was associated with a higher risk of total cGVHD and moderate to severe cGVHD in univariate analysis, but it lost its significance in multivariate analysis. Previous studies reported that aGVHD and cGVHD rates were both higher in haplo-HSCT recipients compared with those of the ISD HSCT recipients, thereby suggesting that HLA disparity was associated with GVHD [7,43]. However, Wang *et al*. [37] and Huo *et al*. [44] reported the lack of association between any HLA mismatch and cGVHD after haplo-HSCT with ATG. Similarly, Lorentino *et al*. [45] reported that for the cGVHD, no association with mismatch at any of the HLA loci could be found, neither in posttransplant cyclophosphamide nor in ATG regimen. Likewise, no association with cGVHD was found with the cumulative number of HLA locus mismatches on the unshared haplotype.

Higher patient age and higher ratio of female donors to male recipients were associated with cGVHD in previous studies [46–48], but we did not observe the associations between these two factors and cGVHD in the present study. The median age of recipients in the present study was 26 years old, and more than 20% of them were children. In addition, nearly 50% (30/61) of the female donors for the male recipients were maternal donors. Thus, we could not further identify the independent influence of older age and the ratio of female donors to male recipients on cGVHD after haplo-HSCT in the present study.

This study had certain limitations. First, although it is a relatively large study that identified the risk factors for cGVHD in ATG-based haplo-HSCT recipients with AML, it is a single-center designed study, and the number of cGVHD patients was relatively small, thereby possibly affecting the accuracy of our results. No risk factors for the severe cGVHD were observed in the present study, which might also be due to the small number of severe cGVHD patients. The risk factors for severe cGVHD, particularly the association between severe aGVHD and severe cGVHD after haplo-HSCT, should be further studied. Additional large, prospective registry-based studies may be able to further confirm our results and identify the risk factors for cGVHD after haplo-HSCT. In addition, this is an observational study, and immortal bias was inevitable. Thus, we should be cautious of the effect of the positive correlation between the cGVHD and GVL, because cGVHD is mostly diagnosed in patients who survived in continuous CR beyond 3 months after transplantation.

In summary, our findings highlighted the close relationship between severe aGVHD and cGVHD in AML patients receiving unmanipulated haplo-HSCT. These findings highlight the need to improve the prevention and treatment of severe aGVHD in AML patients who receive unmanipulated haplo-HSCT.

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Compliance with ethics guidelines

Meng Lv, Xiaohui Zhang, Lanping Xu, Yu Wang, Chenhua Yan, Huan Chen, Yuhong Chen, Wei Han, Fengrong Wang, Jingzhi Wang, Kaiyan Liu, Xiaojun Huang, and Xiaodong Mo declare no potential financial conflict of interest related to this manuscript. Informed consent was obtained from all patients or their guardians. The study was conducted in accordance with the *Declaration of Helsinki*. The study protocol was approved by the Ethics Committee of Peking University People's Hospital.

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