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Occurrence and Severity of Donor Lymphocyte Infusion–Associated Chronic Graft-versus-Host Disease Influence the Clinical Outcomes in Relapsed Acute Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

The aim of this study was to investigate the occurrence and severity of chemotherapy plus donor lymphocyte infusion (Chemo-DLI)-associated chronic graft-versus-host disease (cGVHD) in a consecutive cohort of patients with acute leukemia who experienced relapse after allogeneic hematopoietic stem cell transplantation (n = 104). The 5-year cumulative incidence of complete remission after Chemo-DLI was 81.0% (95% CI, 73.3% to 88.7%) and 84.6% (95% CI, 74.5% to 94.7%) in the moderate and severe cGVHD groups, respectively, which was significantly higher than that of the mild cGVHD group at 40.9% (95% CI, 29.3% to 52.5%) and non-cGVHD group at 29.2% (95% CI 23.1% to 35.3%). The cumulative incidence of nonrelapse mortality was comparable between patients with and without cGVHD. The 5-year probabilities of progression-free survival after Chemo-DLI were 42.9% (95% CI, 26.2% to 70.2%) and 34.6% (95% CI, 15.3% to 78.2%) in the moderate and severe cGVHD groups, respectively, which were both significantly higher than those of the mild cGVHD group at 9.1% (95% CI, 2.4% to 34.1%) and non-cGVHD group at 8.3% (95% CI 3.3% to 21.3%). The 5-year probabilities of overall survival after Chemo-DLI were 56.7% (95% CI, 38.9% to 82.7%) and 43.1% (95% CI, 22.1% to 84.0%) in the moderate and severe cGVHD groups, respectively, which were both significantly higher than those of the mild cGVHD group at 9.1% (95% CI 1.8% to 47.1%) and non-cGVHD group at 14.9% (95% CI, 7.3% to 30.2%). Our observations highlight the close relationship between cGVHD and immune-mediated graft-versus-leukemia (GVL) effect in patients with relapse receiving Chemo-DLI; however, mild cGVHD may not be associated with a sufficiently strong GVL effect to induce remission and improve survival.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective treatment for acute leukemia. However, post-transplantation relapse remains 1 of the most important causes of transplant failure. Donor lymphocyte infusion (DLI), which aims to induce a graft-versus-leukemia (GVL) effect, is the most important immunotherapy for relapse after HSCT [1-4]. We also reported that chemotherapy plus DLI (Chemo-DLI) can significantly improve the outcomes of patients who relapsed after allo-HSCT compared with those received chemotherapy alone [5,6].

A previous study showed a close relationship between chronic graft-versus-host disease (cGVHD) and immune-mediated GVL effect after allo-HSCT [7]. In addition, we observed that Chemo-DLI–associated cGVHD correlated with lower relapse and better survival in patients who experienced relapse after allo-HSCT [5,6]. However, these studies only categorized patients as "with cGVHD" or "without cGVHD" after Chemo-DLI, and no further information regarding Chemo-DLI–associated cGVHD among these patients was provided [5,6]. Currently, the detailed characteristics of Chemo-DLI–associated cGVHD remain unknown.

In addition, several studies reported that severe cGVHD may be associated with higher risk of nonrelapse mortality (NRM) after allo-HSCT [8,9], and the lower risk of disease progression may be offset by increased NRM in severe cGVHD cases. However, previous studies used the Seattle staging

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system to categorize episodes of cGVHD as limited or extensive, which lack sufficient information regarding the severity of cGVHD [10]. Using the composite score in addition to the number of organs or sites involved, the new global scoring system proposed by National Institutes of Health (NIH) Consensus Conference provides more information on cGVHD severity [11]. Several studies observed that this global scoring was useful for predicting NRM and survival after allo-HSCT [7,12,13]. However, relatively few reports have examined the association between NIH global scoring and either relapse or survival after Chemo-DLI in patients who experienced relapse after allo-HSCT; furthermore, whether NIH global scoring can predict clinical outcomes after Chemo-DLI in these patients remains unknown. Therefore, the present study aimed to investigate the effect of Chemo-DLI–associated cGVHD on clinical outcomes in a cohort of patients with acute leukemia who experienced relapse after allo-HSCT, with focus on the efficacy of NIH global scoring in Chemo-DLI–associated cGVHD.

METHODS

Patients

This study included all consecutive patients with acute leukemia who relapsed after allo-HSCT at the Institute of Hematology, Peking University, Beijing, China, between June 2001 and July 2015. The final study cohort comprised 104 patients (Table 1). The last follow-up visits for endpoint analysis were conducted in May 2018. 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. Forty-seven patients for whom data were previously reported in 2016 [14] were followed further in this study.

Transplant Regimens

Preconditioning consisted of cytarabine, busulfan (days −8 to −6), cyclophosphamide (days −5 to −4), and simustine (day −3). Rabbit antithymocyte globulin was administered to the haploidentical related donor and unrelated donor groups [15,16]. In addition, patients received cyclosporine A, mycophenolate mofetil, and short-term methotrexate (MTX) as GVHD prophylaxis [17,18]. The details of transplant regimens are described in Supplementary Methods.

Chimerism Analysis

Quantitative chimerism analysis was performed using real-time PCR based on 29 sequence polymorphism system markers, described in detail by Qin et al. [19].

Protocol of Therapeutic Chemo-DLI

Post-transplantation immunosuppression was immediately tapered in subjects who relapsed after transplantation. Granulocyte colony-stimulating factor–mobilized peripheral blood stem cells were administered instead of the more common unstimulated donor blood lymphocytes. In our DLI protocol the dose of mononucleated cells for a single DLI infusion were 1.0 to 2.0×10^8 /kg, and single DLI doses were also defined as $CD3^+$ cells per kilogram of recipient weight 1.0 to 10.0×10^7/kg [20]. The doses of mononuclear cells, $CD3^+$ cells, and $CD34^+$ cells infused in each cycle are presented in Supplementary Table 1.

Patients also received antileukemic chemotherapy 48 to 72 hours before DLI. The chemotherapy before DLI included aclacinomycin 10 mg/m^{2}d for 5 d and Ara-C 100 mg/m^{2}d for 5 d (AA), fludarabine 30 mg/m^{2}d for 5 d, Ara-C 1.0 g every 12 h for 10 doses, and G-CSF 300 ug/d for 6 d (FLAG), or harringtonine 2 mg/m^{2}d for 5 d, aclacinomycin 10 mg/ m^{2}d for 5 d, and Ara-C 100 mg/m^{2}d for 5 d (HAA) for patients with acute myeloid leukemia and MTX or cyclophosphamide 800 mg/m^{2}d for 2 d, vincristine 1 mg/m^{2}d for a single dose, daunorubicin 40 mg/m^{2}d for 3 d, and prednisone 60 mg/d for 7 d (CODP) for patients with acute lymphoblastic leukemia (see Supplementary Methods) [20,21]. Patients received immunosuppressive drugs such as cyclosporine A or MTX to prevent GVHD after DLI. Patients receiving DLIs from an HLA-identical sibling donor (ISD) received GVHD prophylaxis for 2 to 4 weeks, whereas those receiving DLI from a haploidentical related donor received GVHD prophylaxis for 6 weeks at the discretion of the attending physicians (which usually depended on the patient's GVHD status after DLI) [22].

Patients who achieved complete remission (CR) after Chemo-DLI received multiple consolidation Chemo-DLI performed every 3 months to prevent second relapse, as previously reported in detail (Figure 1 and Supplementary Methods) [6]. For patients who did not achieve CR after Chemo-DLI, a second DLI was administered for those without GVHD. The treatment of acute GVHD (aGVHD) and cGVHD after Chemo-DLI was according to the common international criteria [23,24].

Table 1
Patient Characteristics

Characteristics	No. of Patients (%) or Median (range)
Patient age, yr	26 (5-58)
Patient gender	
Male	54 (51.9)
Female	50 (48.1)
Diagnosis	
AML	63 (60.6)
ALL	41 (39.4)
Donor type	
HLA-haploidentical related donor	64 (61.5)
Unrelated donor	5 (4.8)
HLA-identical sibling donor	35 (33.7)
HLA-A, -B, or -DR mismatches	
0-1	48 (46.2)
2-3	56 (53.8)
Donor gender	
Male	61 (58.7)
Female	43 (41.3)
Donor–recipient sex match	
Female to male	26 (25.0)
Others	78 (75.0)
ABO match	
Matched	66 (63.5)
Minor mismatched	18 (17.3)
Major mismatched	15 (14.4)
Major–minor mismatched	5 (4.8)
Disease status at transplantation	
CR	101 (97.1)
Non-CR	3 (2.9)
Site of relapse	
Bone marrow relapse alone	84 (80.8)
Bone marrow and extramedullary relapse	1 (1.0)
Extramedullary relapse alone	19 (18.2)
CNSL	
Yes	6 (5.8)
No	98 (94.2)
Chimerism at the time of relapse	
Full-donor chimerism	80 (76.9)
Partial-donor chimerism	24 (23.1)
Chemotherapy	
AA	35 (33.6)
FLAG	5 (4.8)
HAA	23 (22.1)
MTX	14 (13.5)
CODP	27 (26.0)
cGVHD before Chemo-DLI intervention	
None	61 (58.7)
Mild	31 (29.8)
Moderate	12 (11.5)
Interval from HSCT to relapse, day	235 (39-2405)
Interval from first CR to relapse, day	373 (83-2503)
Interval from CR after Chemo-DLI to second relapse, day	240 (64-1450)
Blast count in BM before Chemo-DLI, %	22 (6-95)
MNCs in a single Chemo-DLI, $\times 10^8$/kg	1.0 (.9-2.0)
$CD3^+$ cells in a single Chemo-DLI, $\times 10^7$/kg	3.2 (1.3-6.9)
$CD34^+$ cells in a single Chemo-DLI, $\times 10^6$/kg	.58 (.35-2.18)
Duration of follow-up of survivors, days	1689 (868-2000)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CNSL, central nervous system leukemia; BM, bone marrow; MNC, mononuclear cell; PR, partial remission.

Treatment for cGVHD after Chemo-DLI

The treatment for cGVHD was performed in accordance with the common international criteria [23]. Mild cGVHD was mostly treated with topical treatments and supportive agents. Moderate to severe cGVHD was treated with prednisone (1 mg·kg^{-1} per day), and cyclosporine A was adjusted to maintain blood concentration > 150 ng/mL. Second- or third-line immunosuppressive therapies such as mycophenolate mofetil, MTX, penicillamine, azathioprine, or tacrolimus were administered in cases of steroid-refractory cGVHD.

Definitions and Assessments

The diagnosis of GVHD was in accordance with the common international criteria [25]. Relapse was defined as the presence of morphologic evidence of

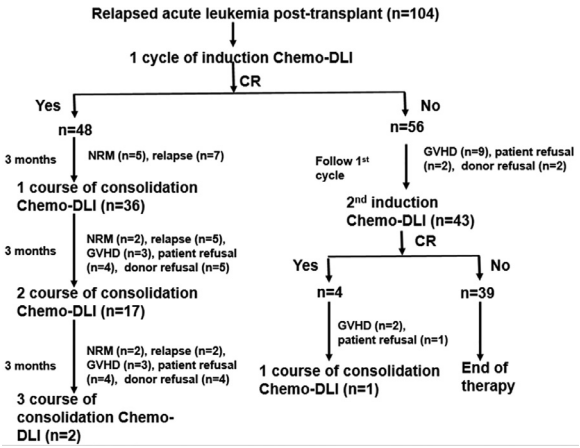

Figure 1. Flow diagram of therapeutic Chemo-DLI.

disease in samples from peripheral blood, bone marrow, or extramedullary sites or by the recurrence and sustained presence of pretransplantation chromosomal abnormalities. Patients who showed minimal residual disease were not classified as relapsed cases. NRM was defined as death without disease progression or relapse. Progression-free survival (PFS) was defined as the CR period without relapse. Overall survival (OS) events were defined as death from any cause. Early relapse was defined as relapse within 6 months after haploidentical HSCT, and late relapse after haploidentical HSCT was defined as relapse $\geq$ 6 months after haploidentical HSCT.

Statistical Analysis

Data were censored at the time of the last available follow-up. Demographic and clinical characteristics were compared using chi-square and Fisher's exact tests for dichotomous variables and the Mann-Whitney U test for continuous variables. Survival probabilities were estimated using the Kaplan-Meier method. Competing risk analyses were used to calculate the cumulative incidence of cGVHD and NRM using the Gray test to determine the differences between the groups [26]. A landmark analysis was performed to assess effects of Chemo-DLI on each outcome, and the post-transplantation day of Chemo-DLI was defined as the landmark day.

Potential prognostic factors for clinical outcomes after Chemo-DLI were evaluated by multivariate analysis using Cox proportional hazards regression with a forward-stepwise model selection approach. Factors included in the regression model were patient age, sex, disease (acute lymphoblastic leukemia versus acute myeloid leukemia), disease status before allo-HSCT (standard risk versus high risk), donor–recipient sex match (female to male versus others), ABO-matched (matched versus mismatched), HLA disparity (ISD versus alternative donor), interval from first CR to relapse (using the median value as the cut-off point), time from HSCT to relapse (early relapse versus late relapse), blast count in the bone marrow before Chemo-DLI (using the median value as the cut-off point), cGVHD after Chemo-DLI (no versus yes), cGVHD severity (non-cGVHD/mild cGVHD versus moderate cGVHD versus severe cGVHD), and the number of involved organs (0 versus 1 versus > 1).

Independent variables showing $P > .1$ were sequentially excluded from the model, and the level of significance was set at $P < .05$. All reported P values were based on 2-sided tests. Data analyses were primarily conducted using SPSS software (SPSS Inc., Chicago, IL), whereas the R software package (version 2.6.1; http://www.r-project.org) was used for competing risk analysis.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. Twenty children and 84 adults were enrolled. Seventy-nine patients (76.0%) received more than 1 Chemo-DLI, and 59, 18, and 2 patients received 2, 3, and 4 cycles of Chemo-DLI, respectively. Eight (7.7%), 31 (29.8%), 26 (25.0%), and 8 (7.7%) patients experienced grades I, II, III, and IV aGVHD, respectively, after Chemo-DLI, and the cumulative incidence of aGVHD was 62.5% for grades II to IV and 32.7% for grades III to IV (Table 2, Supplementary Figure 1). In total, 56 patients (53.8%) experienced cGVHD after Chemo-DLI, and mild, moderate, and severe cGVHD were observed in 22 (21.1%), 21 (20.2%), and 13 (12.5%) patients, respectively. The characteristics of cGVHD are shown in Table 3.

Table 2
Characteristics of aGVHD after Chemo-DLI

Characteristics of aGVHD	No. of Cases (%)
Severity of aGVHD after Chemo-DLI	
None	31 (29.8)
Grade I	8 (7.7)
Grade II	31 (29.8)
Grade III	26 (25.0)
Grade IV	8 (7.7)
No. of organs	
0	31 (29.8)
1	44 (42.3)
>1	29 (27.9)
Organs involved	
Skin	52 (50.0)
Liver	17 (16.3)
Digestive tract	38 (36.5)
The organ combinations with aGVHD	
Skin, digestive tract	14 (13.5)
Skin, liver	7 (6.7)
Digestive tract, liver	3 (2.9)
Skin, digestive tract, liver	5 (4.8)

The cumulative incidence of cGVHD at 5 years after Chemo-DLI was 53.9% (95% confidence interval [CI], 49.0% to 58.7%). The cumulative incidence of mild, moderate, and severe cGVHD at 5 years after Chemo-DLI was 21.2% (95% CI, 19.6% to 22.7%), 20.2% (95% CI, 18.8% to 21.6%), and 12.5% (95% CI, 11.7% to 13.3%), respectively. The cumulative incidence of NRM at 5 years after Chemo-DLI was 17.3% (95% CI, 17.29% to 17.35%). The probabilities of PFS and OS at 5 years after Chemo-DLI were 18.8% (95% CI, 12.5% to 28.2%) and 25.7% (95% CI, 18.1% to 36.5%), respectively.

Impact of cGVHD before Relapse on cGVHD after Chemo-DLI

In total, 43 patients (41.3%) had experienced cGVHD before relapse, and mild, moderate, and severe cGVHD were observed in 31 (29.8%), 12 (11.5%), and 0 (.0%) patients, respectively; however, all of these patients showed that cGVHD had been

Table 3
Characteristics of cGVHD after Chemo-DLI

Characteristics of cGVHD	No. of Cases (%)
Severity of cGVHD after Chemo-DLI	
None	48 (46.2)
Mild	22 (21.1)
Moderate	21 (20.2)
Severe	13 (12.5)
No. of organs	
0	48 (46.2)
1	26 (25.0)
>1	30 (28.8)
Organs involved	
Skin	47 (45.2)
Liver	24 (23.1)
Mouth	21 (20.2)
Eyes	6 (5.8)
Digestive tract	5 (4.8)
Lung	2 (1.9)
Nail	1 (1.0)
The most common organ combinations with cGVHD	
Skin, liver	8 (7.7)
Skin, mouth	7 (6.7)
Skin, liver, mouth	4 (3.8)
Type of cGVHD	
Overlap syndrome	7 (12.5)
Classic cGVHD	49 (87.5)

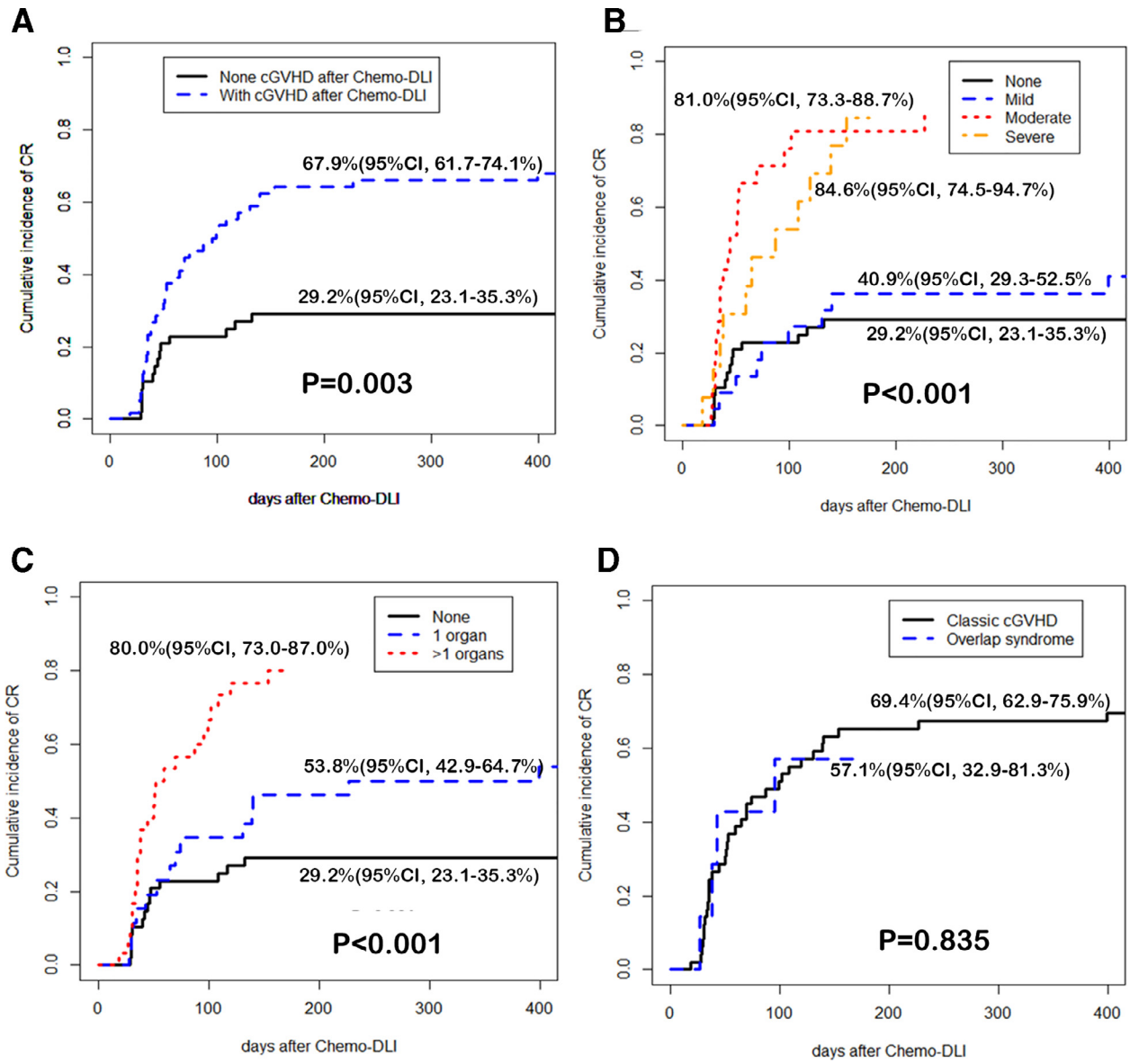

Figure 2. Impact of cGVHD on CR after Chemo-DLI. (A) Cumulative incidence of CR in patients with and without cGVHD after Chemo-DLI. (B) Cumulative incidence of CR in patients with none, mild, moderate, and severe cGVHD after Chemo-DLI. (C) Cumulative incidence of CR in patients with non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ after Chemo-DLI. (D) Cumulative incidence of CR in patients with overlap syndrome and classic cGVHD after Chemo-DLI. The cumulative incidence of CR at 5 years after Chemo-DLI is shown.

controlled when they received Chemo-DLI. The cumulative incidence of total and severe cGVHD at 5 years after Chemo-DLI was 69.8% versus 42.6% ($P = .008$) and 16.3% versus 9.8% ($P = .336$), respectively, in patients with and without cGVHD before relapse (Supplementary Figure 2A,B). The NRM, PFS, and OS at 5 years after Chemo-DLI were comparable between patients with and without cGVHD before relapse (Supplementary Figure 3A-C).

Impact of cGVHD on CR after Chemo-DLI

In total, 52 patients (50%) achieved CR after Chemo-DLI. The cumulative incidence of CR at 5 years after Chemo-DLI was 50% (95% CI, 45.1% to 54.9%). The cumulative incidence of CR at 5 years after Chemo-DLI was significantly higher in the cGVHD group compared with the non-cGVHD group ($P = .003$, Figure 2A). The cumulative incidence of CR at 5 years after Chemo-DLI in the moderate and severe cGVHD groups were significantly higher than those of the mild cGVHD and non-cGVHD groups ($P < .001$, Figure 2B). The cumulative incidence of CR at 5 years after Chemo-DLI was 29.2%, 53.8%, and 80.0% in non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ group, respectively ($P < .001$, Figure 2C). The cumulative incidence of CR at 5 years after Chemo-DLI was comparable between patients with overlap syndrome and classical cGVHD, respectively ($P = .835$, Figure 2D). In the multivariate analysis moderate and severe cGVHD were significantly associated with higher CR after Chemo-DLI (Table 4) (Supplementary Figure 4).

Impact of cGVHD on NRM after Chemo-DLI

Among the 52 patients who achieved CR, 9 patients (9/25, 17.3%) died of NRM, and the causes of NRM are summarized in Supplementary Table 2. The cumulative incidence of NRM at 5 years after Chemo-DLI was comparable between cGVHD and non-cGVHD groups ($P = .767$, Figure 3A). The cumulative

Table 4
Multivariate Analysis for 5-year Clinical Outcomes after Chemo-DLI.

Outcomes	HR (95%CI)	P
CR		
cGVHD severity after Chemo-DLI		
None/Mild	1	<0.001
Moderate	5.195 (2.573-10.492)	<0.001
Severe	3.215 (1.375-7.514)	0.007
PFS		
cGVHD severity after Chemo-DLI		
None/Mild	1.000	0.005
Moderate	0.439 (0.228-0.846)	0.014
Severe	0.302 (0.118-0.771)	0.012
Other significant factors		
Duration from HSCT to relapse		
Early relapse	1	
Late relapse*	0.494 (0.295-0.827)	0.007
OS		
cGVHD severity after Chemo-DLI		
None/Mild	1.000	0.001
Moderate	0.312 (0.145-0.671)	0.003
Severe	0.234 (0.082-0.662)	0.006
Other significant factors		
Duration from HSCT to relapse*		
Early relapse	1	
Late relapse*	0.484 (0.282-0.829)	0.008
Blast count in BM before Chemo-DLI#		
<22%	1	
≥22%	1.807 (1.066-3.062)	0.028

* Duration from HSCT to relapse (using the 6 months as the cut-off point);
# Blast count in BM before Chemo-DLI (using the median value as the cut-off point)Factors included in the multivariate analysis were patient age, sex, disease (ALL vs. AML), disease status before allo-HSCT (standard-risk vs. high-risk), donor-recipient sex-matched (female to male vs. others), ABO-matched (matched vs. mismatched), HLA disparity (ISD vs. alternative donor), interval from CR1 to relapse (using the median value as the cut-off point), time from HSCT to relapse (early relapse vs late relapse), blast count in BM before Chemo-DLI (using the median value as the cut-off point), cGVHD after Chemo-DLI (no vs. yes), cGVHD severity (non-cGVHD/mild cGVHD vs. moderate cGVHD vs. severe cGVHD), and the number of involved organs (0 vs. 1. vs. > 1).CR, complete remission; Chemo-DLI, chemotherapy plus donor lymphocyte infusion; cGVHD, chronic graft-versus-host disease; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; OS, overall survival; PD, progressive disease; PFS, progression-free survival.

incidence of NRM at 5 years after Chemo-DLI was comparable among mild, moderate, and severe cGVHD groups ($P = .663$, Figure 3B). The cumulative incidence of NRM at 5 years after Chemo-DLI was comparable among the non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ groups ($P = .899$, Figure 3C). The cumulative incidence of NRM at 5 years after Chemo-DLI was comparable between overlap syndrome and classic cGVHD ($P = .326$, Figure 3D). In multivariate analysis no factors were associated with increased NRM risk after Chemo-DLI.

Impact of cGVHD on PFS and OS after Chemo-DLI

The probabilities of PFS at 5 years after Chemo-DLI were significantly higher in the cGVHD group compared with the non-cGVHD group ($P = .001$, Figure 4A). The probabilities of PFS at 5 years after Chemo-DLI in the moderate and severe cGVHD groups were both significantly higher than those of mild cGVHD and non-cGVHD groups ($P < .001$, Figure 4B). The probabilities of PFS at 5 years after Chemo-DLI were 8.3%, 15.4%, and 38.5% in non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ groups, respectively ($P = .001$, Figure 4C). The probabilities of PFS at 5 years after Chemo-DLI were comparable between overlap syndrome and classical cGVHD ($P = .196$, Figure 4D). In the multivariate analysis moderate and severe cGVHD and late relapse were significantly associated with higher PFS after Chemo-DLI (Table 4) (Supplementary Figure 4).

The probabilities of OS at 5 years after Chemo-DLI were significantly higher in the cGVHD group compared with the non-cGVHD group ($P = .004$, Figure 5A). The probabilities of OS at 5 years after Chemo-DLI in the moderate and severe cGVHD groups were both significantly higher than those of mild cGVHD and non-cGVHD groups ($P < .001$, Figure 5B). The probabilities of OS at 5 years after Chemo-DLI were 14.9%, 20.2%, and 47.8% in non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ groups, respectively ($P = .003$, Figure 5C). The probabilities of OS at 5 years after Chemo-DLI were comparable between patients with overlap syndrome and classic cGVHD ($P = .395$, Figure 5D). In the multivariate analysis moderate and severe cGVHD, late relapse, and a lower blast count in the bone marrow before Chemo-DLI were significantly associated with higher OS after Chemo-DLI (Table 4) (Supplementary Figure 4).

DISCUSSION

In the present study we observed that the occurrence of cGVHD can significantly improve the clinical outcomes of patients who relapsed and received Chemo-DLI after allo-HSCT. In addition, moderate and severe cGVHD were associated with better survival. Thus, our first observation was that the severity of cGVHD had a significant impact on Chemo-DLI outcomes in patients who relapsed after allo-HSCT.

There were several methods for acute leukemia patients who experienced after allo-HSCT, such as chemotherapy, DLI alone, and second transplantation. Yan et al. [5] reported the clinical outcomes of Chemo-DLI were significantly superior to those of chemotherapy alone. Some authors reported that the OS of patients who experienced relapse and received DLI alone and second transplantation was about 20% [27] and 14% to 30% [28-30], respectively. The OS rate in the present study was 25.7%, which suggested that Chemo-DLI was effective for acute leukemia patients who experienced relapse after allo-HSCT.

In the present study mild, moderate, and severe cGVHD were observed in 21.1%, 20.2%, and 12.5% of patients, respectively; these findings were similar to those observed in patients who did not receive Chemo-DLI after allo-HSCT [7]. In addition, we observed that skin and liver were the most common targets of cGVHD, which was also in accordance with the results of studies that did not enroll patients receiving DLI [31-34].

Certain studies showed that severe cGVHD may increase NRM risk [35]. Arai et al. [36] observed that the NRM rate was 32% for patients with severe cGVHD and was only 3% and 9% for patients with mild and moderate cGVHD, respectively ($P < .0001$). However, several studies reported that even severe cGVHD was not associated with an increased risk of NRM [7,37]. Yan et al. [38] reported that of the 305 patients who died after unmanipulated haploidentical HSCT in Peking University Institute of Hematology (PUIH), GVHD was cited as the cause of death in only 19 patients (6.2%).

The idea that severe cGVHD can improve survival by decreasing the relapse rate in patients who relapse after allo-HSCT is still controversial [39,40]. Saillard et al. [35] observed that mild and moderate cGVHD were associated with better survival than the severe form due to higher NRM among the latter patients, although they had a comparable relapse risk. In our previous studies (in which patients who received DLI were excluded), only mild and moderate cGVHD were significantly associated with improved disease-free survival (DFS), but no significant effect of severe cGVHD on DFS was observed. In addition, among patients who received Chemo-DLI because of

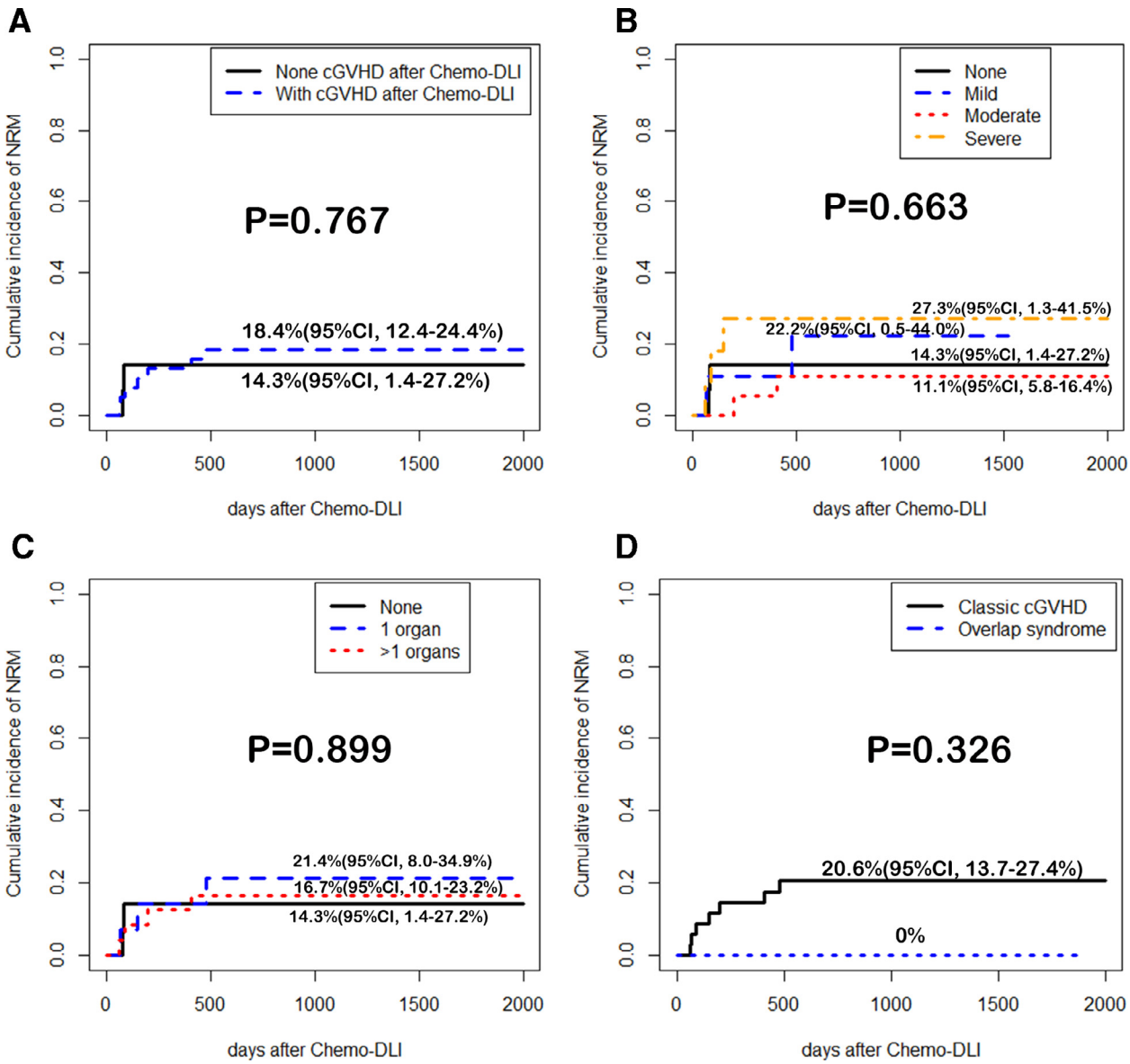

Figure 3. Impact of cGVHD on NRM after Chemo-DLI. (A) Cumulative incidence of NRM in patients with and without cGVHD after Chemo-DLI. (B) Cumulative incidence of NRM in patients with none, mild, moderate, and severe cGVHD after Chemo-DLI. (C) Cumulative incidence of NRM in patients with non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ after Chemo-DLI. (D) Cumulative incidence of NRM in patients with overlap syndrome and classic cGVHD after Chemo-DLI. The cumulative incidence of NRM at 5 years after Chemo-DLI is shown.

minimal residual disease positivity after allo-HSCT, both mild and moderate cGVHD were significantly associated with reduced relapse and improved DFS; however, the role of severe cGVHD in relapse or DFS appeared to diminish. In the present study mild cGVHD did not improve the clinical outcomes, and only moderate and severe cGVHD after Chemo-DLI could significantly improve survival in patients who relapsed after allo-HSCT. Terwey et al. [37] reported that severe cGVHD was also associated with superior OS due to lower relapse incidence. We suggested that for patients who relapsed after allo-HSCT, the tumor burdens were higher in those who were only minimal residual disease positive, and the leukemic cells could be multidrug resistant. Thus, mild cGVHD may not be strong enough to eradicate leukemic cells. Furthermorewe also observed that similar effect of Chemo-DLI associated cGVHD with higher cumulative incidence of CR and better PFS in patients with either early relapse (<6 months) or late relapse (>6 months) (Supplementary Figures 5 and 6). As mentioned above, severe cGVHD did not increase the risk of NRM after Chemo-DLI. Thus, despite the high incidence of severe cGVHD after Chemo-DLI, the lower risk of disease progression cannot be offset by NRM in severe cGVHD cases.

Huang et al. [41] developed a modified DLI, which included the use of granulocyte colony-stimulating factor–mobilized peripheral blood progenitor cells and the short-term immunosuppressive agents after Chemo-DLI. A previous study observed that the infusion of donor granulocyte colony-stimulating factor–mobilized peripheral blood progenitor cells showed an improved antileukemic effect compared with traditional DLI [42]. Yan et al. [43] reported that prophylaxis for 6 to 8 weeks after Chemo-DLI not only reduced incidence of severe GVHD, but also reduced incidence of re-relapse and improved OS and DFS in patients developing hematologic

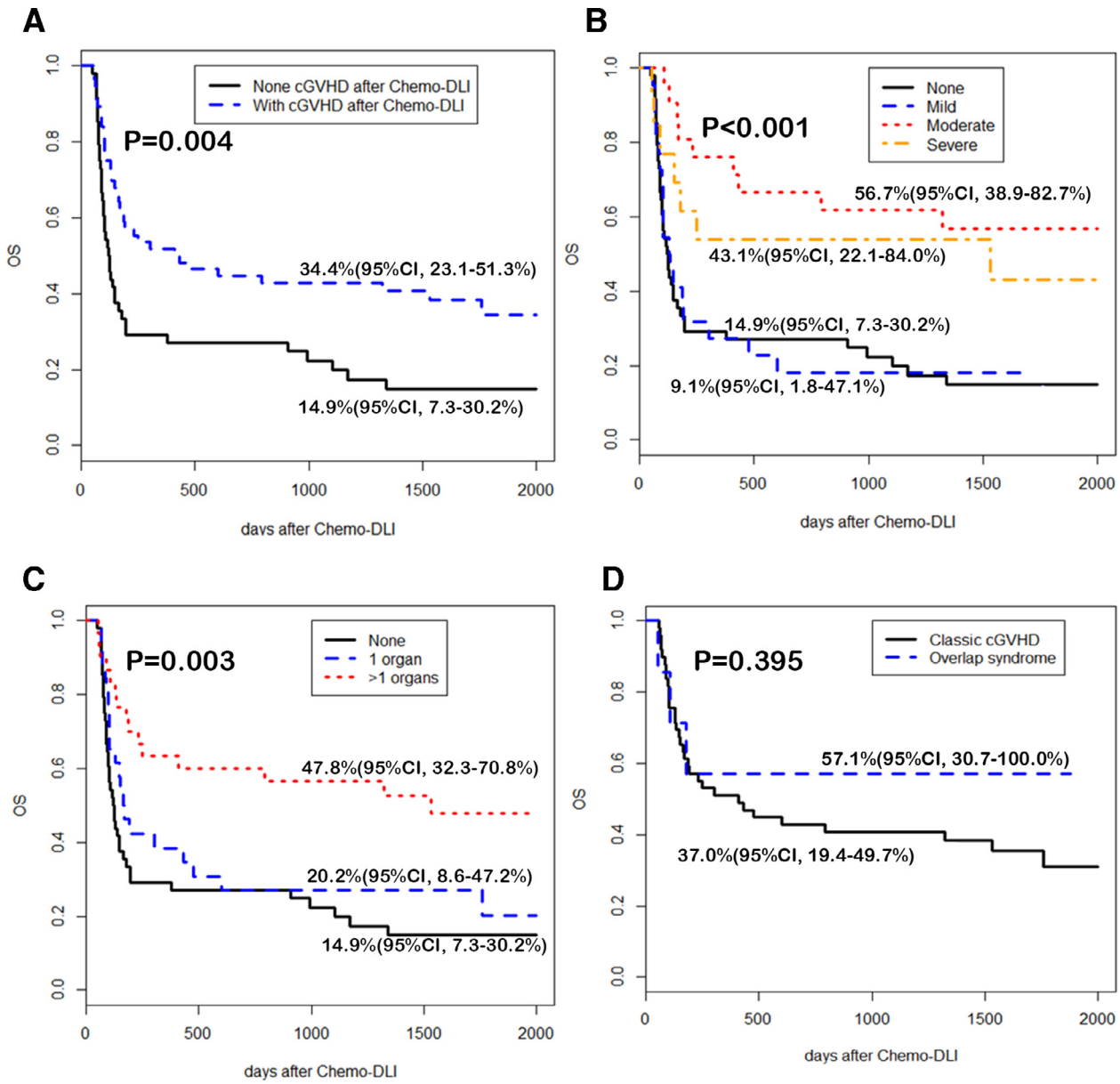

Figure 4. Impact of cGVHD on PFS after Chemo-DLI. (A) PFS in patients with and without cGVHD after Chemo-DLI. (B) PFS in patients with none, mild, moderate, and severe cGVHD after Chemo-DLI. (C) PFS in patients with non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ after Chemo-DLI. (D) PFS in patients with overlap syndrome and classic cGVHD after Chemo-DLI. The PFS at 5 years after Chemo-DLI is shown.

relapse post-transplantation. The 5-year NRM rate was 15.4% in the present study, which was similar to our previous studies (6.0% to 14%) [5,20,21]. Thus, it is safe for patients who experienced relapse after allo-HSCT.

This study has certain limitations. Although it is a large study of Chemo-DLI in patients who relapsed after allo-HSCT, the number of cGVHD patients was relatively small, which might influence the accuracy of our results. Second, different chemotherapy protocols were used in the present study, which may influence the interpretation of the results. However, chemotherapy may be auxiliary to DLI because chemotherapy alone did not achieve satisfactory outcomes [5]. Additional large multicenter studies may be able to further confirm our results and identify the association between cGVHD and the clinical outcomes of HSCT. Furthermore, we observed that more than 70% patients showed complete chimerism before Chemo-DLI. It may be because we used the peripheral blood for chimerism analysis. Using bone marrow and $CD34^+$ cell separation for quantitative chimerism analysis may further improve the sensitivity. We observed that aGVHD after Chemo-DLI was not associated with the cGVHD after Chemo-DLI. It is somewhat to our surprise because aGVHD was 1 of the most important risk factors for the occurrence of cGVHD [44,45]. We suggested that patients who experienced aGVHD after Chemo-DLI should receive immunotherapies until the aGVHD was controlled. However, for patients without GVHD, immunotherapies should be discontinued within 6 to 8 weeks after Chemo-DLI, which may also induce cGVHD. However, the association between aGVHD and cGVHD after Chemo-DLI should be further identified in prospective studies.

In summary, our findings highlight the close relation between cGVHD and the immune-mediated GVL effect in

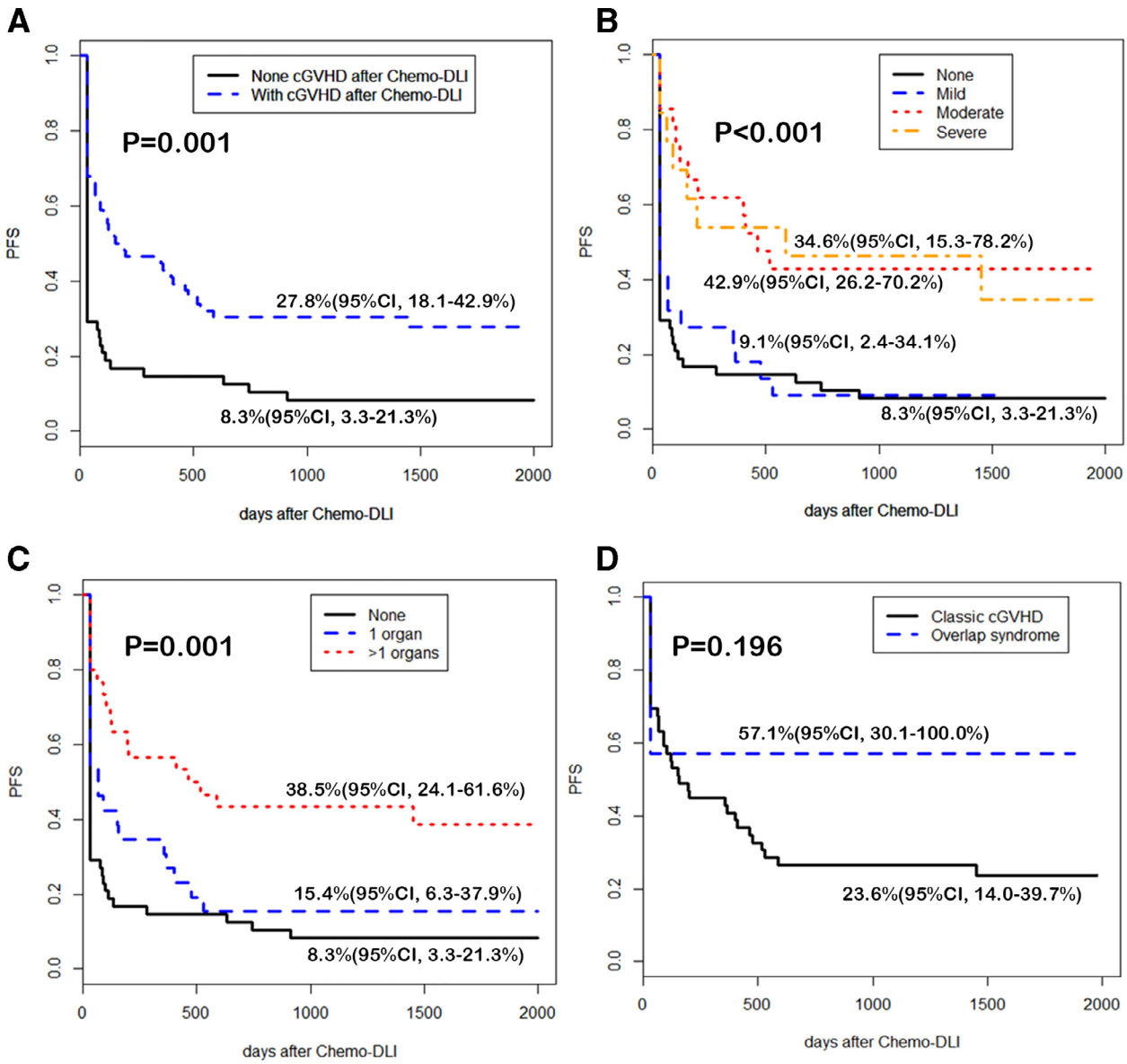

Figure 5. Impact of cGVHD on OS after Chemo-DLI. (A) OS in patients with and without cGVHD after Chemo-DLI. (B) OS in patients with none, mild, moderate, and severe cGVHD after Chemo-DLI. (C) OS in patients with non-cGVHD, cGVHD involving 1 organ, and cGVHD involving more than 1 organ after Chemo-DLI. (D) OS in patients with overlap syndrome and classical cGVHD after Chemo-DLI. The OS at 5 years after Chemo-DLI is shown.

patients who relapsed and received Chemo-DLI after allo-HSCT. However, mild cGVHD may not be associated with a strong enough GVL effect to induce remission and improve survival.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.11.024.

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