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Clinical Impacts of Using Serum IL-6 Level as an Indicator of Cytokine Release Syndrome after HLA-Haploidentical Transplantation with Post-Transplantation Cyclophosphamide

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HLA-haploidentical allogeneic hematopoietic cell transplantation with post-transplantation cyclophosphamide (PT/Cy-haplo) is widely used because of such advantages as low procedure cost, high probability of finding a suitable donor, and donor availability at short notice. Cytokine release syndrome (CRS), resulting from bidirectional alloreaction between host and donor, occurs frequently in recipients of PT/Cy-haplo, especially when peripheral blood is used. Severe and life-threatening instances of CRS have been reported. The clinical significance of CRS remains unclear, however. Here we used serum IL-6 level as a surrogate marker of CRS to evaluate the impact of outcomes in 65 consecutive patients receiving PT/Cy-haplo at our institution. Our results indicate that active disease status, high Hematopoietic Cell Transplantation-Specific Comorbidity Index score, and very severe CRS are significantly related to peak serum IL-6 level. In our cohort, high peak serum IL-6 level and severe CRS were significantly associated with the development of grade III or IV acute graft-versus-host disease (GVHD). High peak serum IL-6 level was identified a significant risk factor for poor 3-year overall survival. Our results suggest that even transient CRS following PT/Cy-haplo may contribute to poor survival owing to an increase in severe acute GVHD.

INTRODUCTION

Allogeneic hematopoietic cell transplantation is the sole available treatment with the potential to cure various hematologic malignancies with a poor prognosis. The efficacy and safety of T cell-replete HLA-haploidentical allogeneic hematopoietic cell transplantation using post-transplantation cyclophosphamide (PT/Cy-haplo) have been reported previously [1–8]. PT/Cy-haplo, which is in wide use worldwide owing to its low cost and ease of administration, shows potential as a platform for HLA-haploidentical transplantation.

Initially, PT/Cy-haplo was performed using bone marrow (BM) as a common stem cell source. However, granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs) are now widely used as an alternative stem cell source [4–7]. Compared with the use of BM, PT/Cy-haplo using PBSCs is associated with an increased incidence of graft-versus-host disease (GVHD) but a decreased incidence of relapse in patients with leukemia [9].

Furthermore, compared with BM, PT/Cy-haplo with PBSC is associated with a higher rate of noninfectious fever shortly after graft infusion [4,6,7]. These noninfectious fevers are mediated by elevated levels of cytokines such as IL-6, IL-8, and IL-10 [7]. This phenomenon, termed the "haploimmunostorm" [7,10], is known to resemble cytokine release syndrome (CRS) [11–13]. Host-donor alloreactive T cells are activated and proliferate in response to the dual allogeneic antigen challenge immediately after graft infusion of PT/Cy-haplo [10]. Thus, CRS in PT/Cy-haplo is considered to result from a bidirectional alloreaction between host and donor occurring immediately after transplantation. Activated alloreactive T cells can be selectively eliminated by PT/Cy on day 3 or day 4 after transplantation [10]; however, excessive release of cytokines may lead to severe GVHD and/or failure to engraft, or to rejection altogether. Interestingly, it has been reported that cytokine release shortly after infusion of HLA-haploidentical donor cells may have an antitumor effect [14]. However, the clinical significance of CRS has been insufficiently elucidated, and the impact of CRS following PT/Cy-haplo on final outcomes remains unclear [11,12].

Although based on a small cohort study, the usefulness of serum IL-6 levels as a surrogate marker of CRS severity has

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been reported [11]. In the present study, we investigated the impact of CRS on final outcomes after PT/Cy-haplo, using serum IL-6 level as a surrogate marker.

METHODS

Patients and Data Collection

Patients who underwent PT/Cy-haplo between August 2012 and January 2017 at Osaka City University Hospital were considered eligible for this study. Most of the patients were treated using a single protocol, and the remainder were treated using a uniform clinical approach based on the single protocol. According to the eligibility criteria of the protocol, all patients were at least 15 years of age and had a hematologic malignancy. Detailed clinical data were collected retrospectively from the institutional database. To evaluate cytokine data, serum samples were collected prospectively on days 0, 1, 3, 5, and 7 after signed informed consent was obtained. This study was approved by the Human Subjects Review Committee at Osaka City University.

Transplantation Procedure

The busulfan (Bu)-based conditioning regimen consisted of 15 mg/m^2 fludarabine and 2.0 g/m^2 cytarabine twice daily on days -11 and -10, and 30 mg/m^2/day fludarabine with 3.2 mg/kg/day i.v. Bu on days -6 to -3 (.8 mg/kg every 6 hours for 16 doses, given over 4 days). Pharmacokinetics-directed dose adjustment of Bu was not used. The melphalan (Mel)-based conditioning regimen was similar to the Bu-based regimen but with 100 mg/m^{2}Mel on day -2 instead of i.v. Bu on days -6 to -3. In accordance with the study protocol, patients who received PT/Cy-haplo before July 2013 received 2.0 mg/kg antithymocyte globulin (ATG) on days -8 and -7 to facilitate engraftment, as described previously [7]. G-CSF-mobilized T cell-replete peripheral blood grafts were infused on day 0. All patients received uniform GVHD prophylaxis with PT/Cy, tacrolimus, and oral mycophenolate mofetil. In patients who did not develop GVHD, mycophenolate mofetil was discontinued on day +40 and tacrolimus was tapered starting on day +60 to +100, with discontinuation by day +180. PT/Cy was administered at a dose of 25 mg/kg on days +3 and +4. The dose of PT/Cy was reduced from the original dose out of concern that PT/Cy could hinder graft-versus-leukemia/tumor effects and result in a higher relapse rate in patients with high-risk disease [15]. G-CSF was initiated on the day after completion of PT/Cy treatment. Supportive care after PT/Cy-haplo was provided as reported previously [7].

Measurement of Serum IL-6

Blood samples were collected and centrifuged at 1500 rpm for 30 minutes at 4°C. Serum was aliquoted in 1-mL cryogenic vials and frozen at -80 °C until needed for cytokine measurement. Before measuring serum IL-6, the frozen vials were thawed completely and then vortexed and centrifuged to remove particles. Serum IL-6 level was measured by a chemiluminescent enzyme immunoassay using a 2-step sandwich method with an IL-6 measurement cartridge (Human IL-6 CLEIA; Fujirebio, Tokyo, Japan). In brief, the serum sample was first incubated with mouse anti-human IL-6 monoclonal antibody-conjugated ferrite particles (primary antibodies). Then, following aspiration and washing, alkaline phosphatase-labeled mouse anti-human IL-6 monoclonal antibodies (secondary antibodies) were added, followed by incubation. Finally, AMPPD [3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt] was added as a chemiluminescent substrate, following aspiration and washing. Alkaline phosphatase in the sample degrades AMPPD, causing chemiluminescence. The intensity of chemiluminescence was quantified via a luminometer. All IL-6 measurements were performed automatically with a chemiluminescent enzyme immunoassay system (LUMIPULSE G1200; Fujirebio). The minimum detection limit was .2 pg/mL, and the maximum measurable level was 1000 pg/mL.

Definitions

Acute and chronic GVHD were defined and graded according to standard criteria [16,17]. CRS was defined and graded according to the criteria described by Lee et al [13]. Manifestations occurring within 7 days after PT/Cy-haplo were included. Samples in which pathogenic microbes had been detected were excluded. Although this grading system was designed for CRS induced by chimeric antigen receptor (CAR) T cell therapy, we used this system in the present study to assess the severity of the immune reaction following stem cell infusions, termed "CRS following PTCy-haplo," along with serum IL-6. The Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) score was calculated as described previously [18]. The time to neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of $>500/\mu$L. The time to platelet engraftment was defined as the first day of a platelet count $>20{,}000/\mu$L without transfusion.

We stratified the patients by disease status at transplantation as follows: remission was defined as hematologic complete remission (CR) for patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia; as CR as assessed by computed tomography (CT) scan for patients with malignant lymphoma; as refractory anemia or refractory cytopenia with multilineage dysplasia for patients with myelodysplastic syndrome; and as CR as assessed by CT scan and laboratory data for patients with adult T cell leukemia/lymphoma. Active disease was defined as nonhematologic CR for patients with AML, ALL, and chronic myelogenous leukemia; as non-CR as assessed by CT for patients with malignant lymphoma; as refractory anemia with excess blasts for patients with myelodysplastic syndrome; and as non-CR as assessed by CT scan and laboratory data for patients with adult T cell leukemia/lymphoma. We also divided AML and ALL patients in the "remission" group into 2 subgroups based on minimal residual disease (MRD) at transplantation; MRD-positive CR was defined as hematologic CR with MRD detected by flow cytometry, cytogenetics, or molecular markers.

Table 1
Patient and Transplantation Characteristics

Characteristic	Value
Patient age, yr, median (range)	48 (17-68)
Sex, male/female, n	40/25
Underlying disease, n (%)	
Acute myelogenous leukemia	39 (60)
Acute lymphoblastic leukemia	10 (15)
Myelodysplastic syndrome	4 (6)
Chronic myelogenous leukemia	2 (3)
Malignant lymphoma	8 (12)
Adult T cell leukemia/lymphoma	2 (3)
Disease status at transplantation, n (%)	
Remission	28 (43)
Active	37 (57)
HCT-CI score, n (%)	
0	31 (48)
1	3 (5)
2	11 (17)
≥3	20 (31)
Conditioning regimen, n (%)	
Bu-based	10 (15)
Mel-based	55 (85)
ATG use, n (%)	
Yes	15 (23)
No	50 (77)
HLA disparity (antigen), n (%)	
4/8	35 (54)
5/8	24 (37)
6/8	5 (8)
7/8	1 (2)
Donor-recipient CMV serostatus, n (%)	
Positive/positive	35 (54)
Positive/negative	11 (17)
Negative/positive	13 (20)
Negative/negative	4 (6)
Unknown	2 (3)
ABO mismatch, n (%)	25 (38)
Sex mismatch, n (%)	
Female to male	18 (28)
Other	47 (72)
Infused cell dose, median (range)	
CD3$^+$ cells $\times$ 10^8/kg	1.7 (.6-7.3)
CD34$^+$ cells $\times$ 10^6/kg	4.4 (2.1-30.1)
CRS severity, n (%)	
Grade 0	2 (3)
Grade 1	39 (60)
Grade 2	23 (35)
Grade 3	1 (2)

CMV, cytomegalovirus; CRS, cytokine release syndrome.

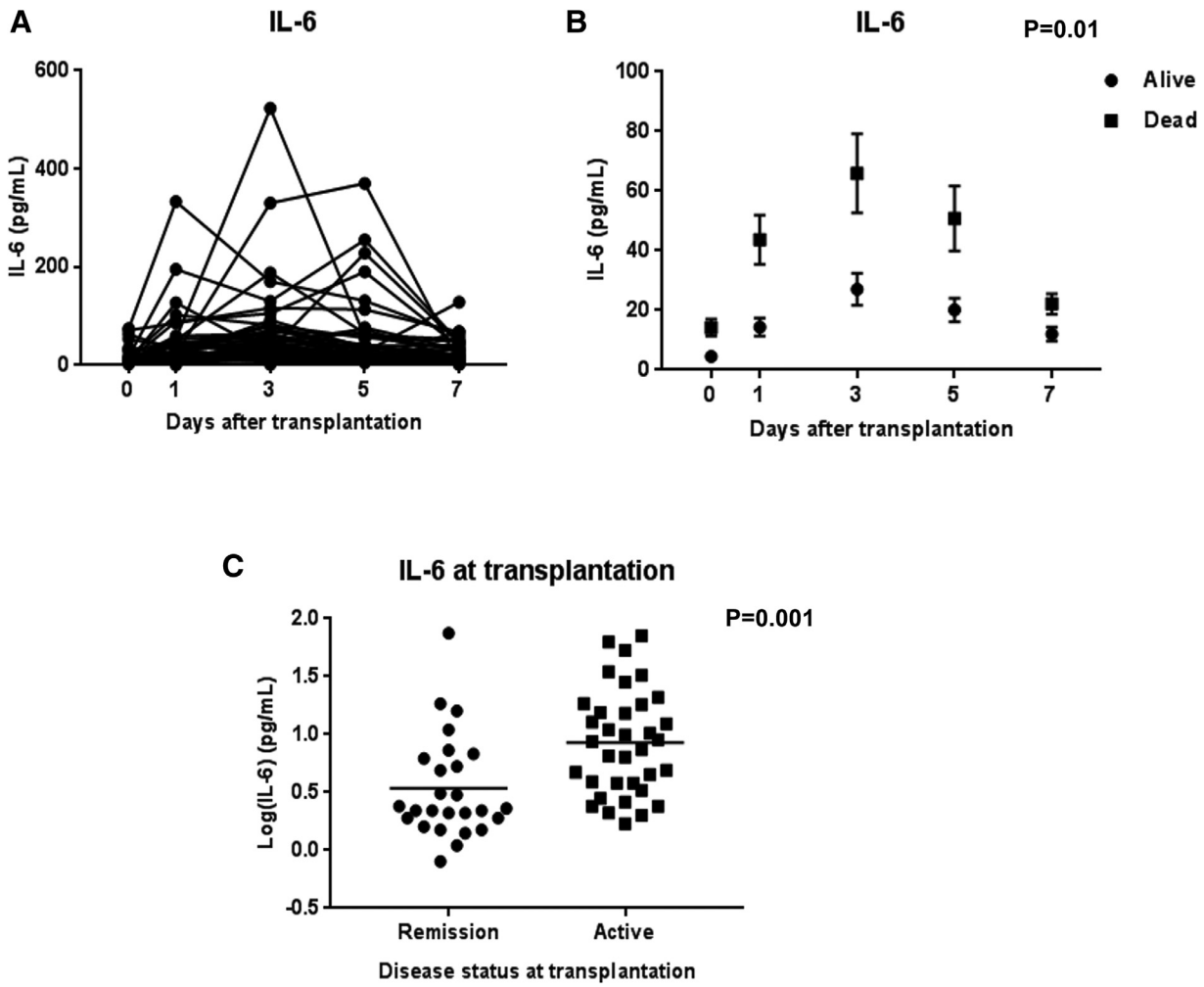

Figure 1. Serum IL-6 levels before and after PT/Cy-haplo. (A) Serum IL-6 levels on each day from day 0 to day 7 following PT/Cy-haplo (n = 65). (B) Daily mean serum IL-6 levels from day 0 to day 7, following PT/Cy-haplo in the 2 IL-6 level groups of patients who died before 3 years and those who were alive at that time (alive, n = 30; dead, n = 35). Error bars indicate SEM. (C) Comparison of log-transformed serum IL-6 levels at baseline in the 2 groups categorized by disease status at transplantation (remission, n = 28; active, n = 37).

Statistical Analysis

According to the European Society for Blood and Marrow Transplantation statistical guidelines, subsequent transplantation was not censored but failed [19]. Overall survival (OS) was calculated using the Kaplan-Meier method and compared statistically using the log-rank test. Cumulative incidence curves were used to analyze engraftment, nonrelapse mortality (NRM), relapse mortality, and GVHD to accommodate competing risks. Any deaths without engraftment were considered competing events for engraftment. Any deaths, subsequent transplantations, or donor lymphocyte infusions without GVHD were competing events for GVHD. NRM and relapse mortality were mutually competing. Gray's test was used to compare cumulative incidences.

Simple or multiple linear regression analyses were used to assess factors affecting peak serum IL-6 levels between days 1 and 7 following PT/Cy-haplo. Cox proportional hazards regression was used for OS, and the Fine and Gray proportional subdistribution hazard regression model was used for analysis of NRM, relapse mortality and GVHD. Factors associated with a P value $<.05$ by univariate analysis and considered clinically relevant were included in multivariate analysis.

The peak values of serum IL-6 levels between days 1 and 7 following PT/Cy-haplo were used in the analysis. For the purposes of this study, serum IL-6 concentrations equal to or greater than the median value of 42.2 pg/mL (range, 4.6 to 523 pg/mL) were defined as high. Log-transformed serum IL-6 absolute value served as a continuous variable. Baseline serum IL-6 levels were compared using the 2-sample t test. Repeated-measures analysis of variance was used to assess the difference in the degree of change in serum IL-6 level following PT/Cy-haplo among groups divided by survival status at 3 years.

All statistical analyses and generation of graphs were performed using EZR version 1.37 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [20] or Prism version 7.03 (GraphPad Software, La Jolla, CA).

RESULTS

Patient and Transplantation Characteristics

Seventy-three patients underwent PT/Cy-haplo at our institution during the study period. Eight patients declined the agreement associated with the cytokine analysis study; thus, a total of 65 consecutive patients were available for both clinical studies and serum IL-6 analyses. Patient and transplantation characteristics are summarized in Table 1. One patient with a high HCT-CI score in each conditioning regimen group did not receive cytarabine to reduce regimen-related toxicity. The median infused numbers of $CD3^+$ T cells and $CD34^+$ T cells were 1.7×10^8/kg (range, .6-7 to 3×10^8/kg) and 4.4×10^6/kg (range, 2.1 to 30.1×10^6/kg), respectively. No patients had severe (grade 4) CRS, and no patients were treated with the IL-6 receptor antagonist tocilizumab. The median duration of follow-up in survivors was 1016 days (range, 49 to 2033 days).

Table 2
Factors Affecting Peak Serum IL-6 Levels from Day 1 to Day 7 after PT/Cy-Haplo

Variable	Peak Serum IL-6 Level			
	Simple Linear Regression Analysis		Multiple Linear Regression Analysis	
	β (95% CI)	*P* Value	β (95% CI)	*P* Value
ABO mismatch (vs match)	-.0 (-.2 to .2)	.67		
Age ≥48 (vs <48) yr	-.1 (-.3 to .1)	.43		
ATG yes (vs no)	-.0 (-.2 to .2)	.95		
Bu-based (vs Mel-based)	-.0 (-.3 to .2)	.80		
$CD3^+$ cells ≥1.7 (vs <1.7) × 10^8/kg	.0 (-.2 to .2)	.68		
$CD34^+$ cells ≥4.4 (vs <4.4) × 10^6/kg	-.2 (-.3 to .0)	.12		
CMV serostatus: D^-/R^+ (vs other)	-.1 (-.3 to .2)	.57		
Active disease (vs remission)	**.2 (.0-.4)**	**.03**	**.2 (.0-.3)**	**.047**
Donor age ≥33 (vs <33) yr	.1 (-.1 to .3)	.37		
HCT-CI ≥3 (vs 0-2)	**.4 (.2-.6)**	**.0004**	**.3 (.1-.5)**	**.004**
HLA disparity				
1/8-3/8 antigen mismatch	Reference			
4/8 antigen mismatch	-.1 (-.3 to .1)	.52		
Female to male (vs other)	.1 (-.2 to .3)	.58		
CRS severity: grade 2 or greater (vs 0 or 1)	**.3 (.1-.5)**	**<.0001**	**.3 (.1-.5)**	**.0005**
Female (vs male)	-.0 (-.2 to .2)	.92		
Disease type				
Myeloid malignancy	Reference			
Lymphoid malignancy	-.1 (-.3 to .2)	.59		

Significant values are in bold type.
D indicates donor; R, recipient.

Factors Affecting Peak Serum IL-6 Level from Day 1 to Day 7 after PT/CY-Haplo

Serum IL-6 levels were elevated during the first 1 to 5 days following graft infusion but decreased by day 7 in most cases (Figure 1A). The median peak serum IL-6 level between days 1 and 7 following PT/Cy-haplo were significantly higher in patients who died before 3 years compared with patients who were alive at that time ($P = .01$) (Figure 1B). The same trend was observed for body temperature and CRP readings as well (Supplementary Figure 1). Baseline serum IL-6 levels were significantly higher in patients with active disease at transplantation compared with patients who were in remission at transplantation ($P = .001$) (Figure 1C). Both simple linear regression and multiple linear regression analyses indicated that active disease status at transplantation, high HCT-CI score, and severe CRS were significantly associated with high peak serum IL-6 level (Table 2). Among patients with AML and ALL, there were significant differences among MRD-negative CR, MRD-positive CR, and nonhematologic CR groups (log IL-6, .43 versus .69 versus .92 pg/mL; $P = .009$). Because we defined CRS as the manifestations within 7 days after transplantation, we could not assess the impact of cytomegalovirus reactivation on CRS or serum IL-6 levels in this study.

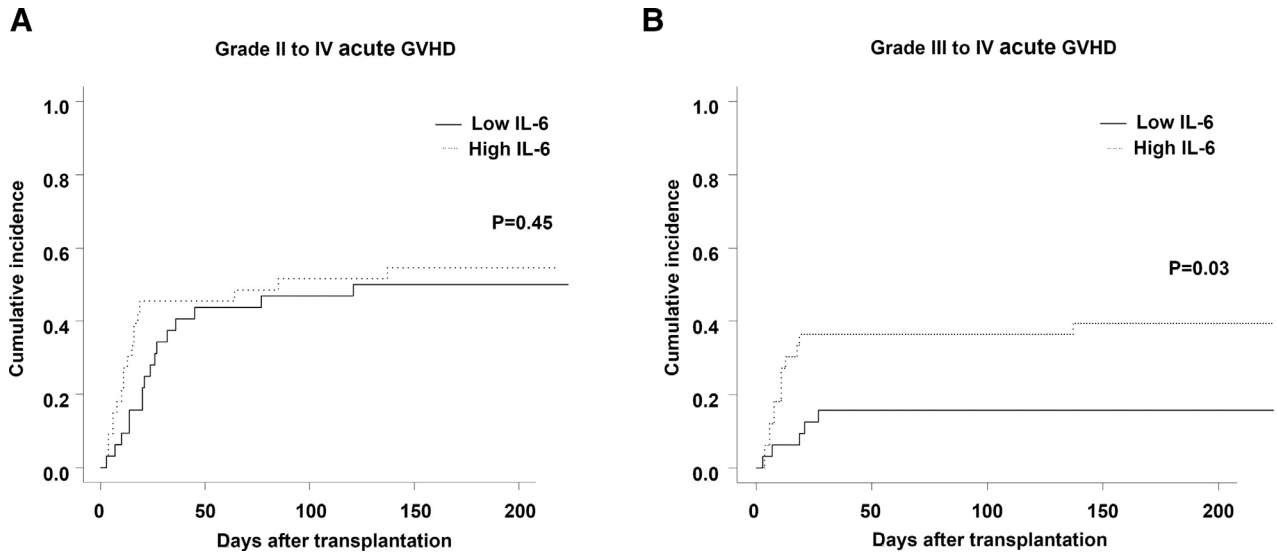

Figure 2. Association between incidence of acute GVHD and serum IL-6 level. (A) Grade II-IV GVHD and (B) grade III-IV GVHD in the 2 study groups. There was no significant difference in the cumulative incidence of grade II-IV acute GVHD at 180 days between the high and low serum level IL-6 groups (54.5% versus 50.0%; $P = .45$). The cumulative incidence of grade III-IV acute GVHD at 180 days was significantly higher in the high serum IL-6 group compared with the low serum IL-6 group (39.4% versus 15.6%; $P = .03$).

GVHD and Engraftment

In the whole cohort, the cumulative incidences of overall grade II-IV and grade III-IV acute GVHD at 180 days and chronic GVHD at 3 years were 52.3%, 27.7%, and 8.7%, respectively. There was no significant difference in the cumulative incidence of grade II-IV acute GVHD between the high and low serum IL-6 level groups (54.5% versus 50.0%; $P = .45$) (Figure 2A). The cumulative incidence of grade III-IV acute GVHD was significantly higher in the high serum IL-6 group (39.4% versus 15.6%; $P = .03$) (Figure 2B). Univariate analysis showed that high serum IL-6 level and CRS severity were associated with the development of grade III-IV acute GVHD (Table 3). Two individual models for multivariate analysis were constructed to avoid multicollinearity between serum IL-6 level and CRS severity. Multivariate analysis confirmed high serum IL-6 level and severe CRS as independent risk factors for the development of grade III-IV acute GVHD (hazard ratio [HR], 4.6 [$P = .0008$] and 3.2 [$P = .022$], respectively) (Table 3). The cumulative incidence of chronic GVHD at 3 years was also slightly higher in the high IL-6 group compared with the low IL-6 group, but the difference did not reach statistical significance (13.1% versus 4.0%; $P = .17$) (Supplementary Figure 2). As we reported previously, the cumulative incidence of chronic GVHD is low in the setting of our procedure [7]. There were also only 6 patients who had chronic GVHD in this cohort. The cumulative incidence of grade III-IV acute GVHD was significantly lower in the patients who received an ATG-containing regimen compared with those who did not (6.7% versus 34.0%, $P = .04$); however, there were no significant differences in peak IL-6 level ($P = .95$) or the cumulative incidence of grade II-IV GVHD (53.3% versus 52.0%; $P = .93$), or chronic GVHD (14% versus 6.8%, $P = .37$) irrespective of ATG use.

There was no significant difference in the cumulative incidence of neutrophil engraftment between the 2 serum IL-6 level groups (low versus high, 93.8% versus 90.9%; $P = .53$) (Figure 3A). Although the cumulative incidence of platelet engraftment was lower in the high serum IL-6 group when analyzed at the population level (low versus high, 87.5% versus 73.5%; $P = .03$) (Figure 3B), no significant differences were observed at the subgroup level among patients in remission at transplantation (n = 28; low versus high, 88.2% versus 90.9%; $P = .89$) (Supplementary Figure 3). This suggests that the low incidence of platelet engraftment in the high serum IL-6 group may be due to the confounding effect caused by the inclusion of more patients with active disease at transplantation. In addition, there is the possibility that patients with thrombocytopenia have higher serum IL-6 levels to drive platelet production, given IL-6's critical role in thrombopoiesis [21].

OS, NRM, and Relapse Mortality

The 3-year NRM was borderline significantly higher in the high serum IL-6 group compared with the low serum IL-6 group (low versus high, 7.1% versus 26.1%; $P = .055$) (Figure 4A), whereas there was no significant difference in 3-year relapse mortality between the 2 groups (low versus high, 48.5% versus 60.7%; $P = .26$) (Figure 4B). The 3-year OS was significantly better in the low serum IL-6 group (low versus high; 43.6% versus 12.1%; $P = .004$) (Figure 4C). Multivariate Cox proportional hazard regression analysis identified high serum IL-6 level along with active disease at transplantation as an independent risk factor for survival (HR, 2.2; 95% confidence interval [CI], 1.0 to 4.7; $P = .049$) (Table 4). A high infused $CD3^+$ T cell dose was identified as an independent risk factor for 3-year NRM, although it had no association with serum IL-6 level or the cumulative incidence of grade III-IV acute GVHD. Relapse mortality and OS were significantly higher in the active disease group compared with the remission group (Table 4 and Supplementary Table 1). Furthermore, the subgroup analysis of patients with AML and ALL found a significant difference among the MRD-negative CR, MRD-positive CR, and nonhematologic CR groups in 3-year relapse mortality (29.3% versus 57.8% versus 77.3%; $P = .006$), with no significant differences in the 3-year NRM (22.7% versus 0% versus 18.2%; $P = .37$). Because only 2 patients died without disease relapse within 100 days after transplantation, we could not assess the impact of IL-6 on very early NRM.

Table 3
Univariate and Multivariate Analysis for Risk Factors of 180-Day Grade III-IV Acute GVHD

Variable	HR (95% CI)	*P* Value
Univariate analysis		
ABO mismatch (vs match)	.6 (.2-1.6)	.31
Age ≥48 (vs <48) yr	1.1 (.4-2.7)	.86
ATG yes (vs no)	.2 (.0-1.3)	.088
Bu-based (vs Mel-based)	.3 (.0-2.1)	.22
$CD3^+$ cells ≥1.7 (vs <1.7) × 10^8/kg	1.3 (.5-3.2)	.60
$CD34^+$ cells ≥4.4 (vs <4.4) × 10^6/kg	1.0 (.4-2.4)	.92
CMV serostatus: D-/R+ (vs other)	1.7 (.6-4.6)	.31
Active disease (vs remission)	1.1 (.4-2.9)	.79
Donor age ≥33 (vs <33) yr	1.4 (.6-3.6)	.47
HCT-CI score ≥3 (vs 0-2)	1.5 (.6-3.8)	.38
HLA disparity		
1/8-3/8 antigen mismatch	Reference	
4/8 antigen mismatch	.7 (.3-1.9)	.49
CRS severity: grade 2 or greater (vs 0 or 1)	2.8 (1.1-7.0)	.02
High IL-6 (vs low)	3.0 (1.1-8.1)	.03
IL-6*	3.5 (1.4-8.4)	.006
Female to male (vs other)	.7 (.2-2.2)	.58
Female (vs male)	.9 (.4-2.3)	.90
Disease type		
Myeloid malignancy	Reference	
Lymphoid malignancy	1.4 (.6-3.6)	.43
Multivariate analysis		
Model 1		
ATG yes (vs no)	.1 (.0-1.2)	.07
$CD3^+$ cells ≥1.7 (vs <1.7) × 10^8/kg	.8 (.3-2.2)	.71
$CD34^+$ cells ≥4.4 (vs <4.4) × 10^6/kg	1.5 (.6-3.7)	.42
HLA disparity		
1/8-3/8 antigen mismatch	Reference	
4/8 antigen mismatch	.8 (.3-2.1)	.65
Female to male (vs other)	.5 (.1-1.8)	.29
IL-6*	**4.6 (1.9-11.3)**	**.0008**
Model 2		
ATG yes (vs no)	.1 (.0-1.2)	.07
$CD3^+$ cells ≥1.7 (vs <1.7) × 10^8/kg	.8 (.3-2.0)	.60
$CD34^+$ cells ≥4.4 (vs <4.4) × 10^6/kg	1.4 (.6-3.3)	.51
HLA disparity		
1/8-3/8 antigen mismatch	Reference	
4/8 antigen mismatch	.7 (.2-2.2)	.52
Female to male (vs other)	.5 (.1-2.4)	.40
CRS severity: grade 2 or greater (vs 0 or 1)	**3.2 (1.2-8.7)**	**.02**

Significant values are in bold type.
* Serum IL-6 concentrations were log-transformed.

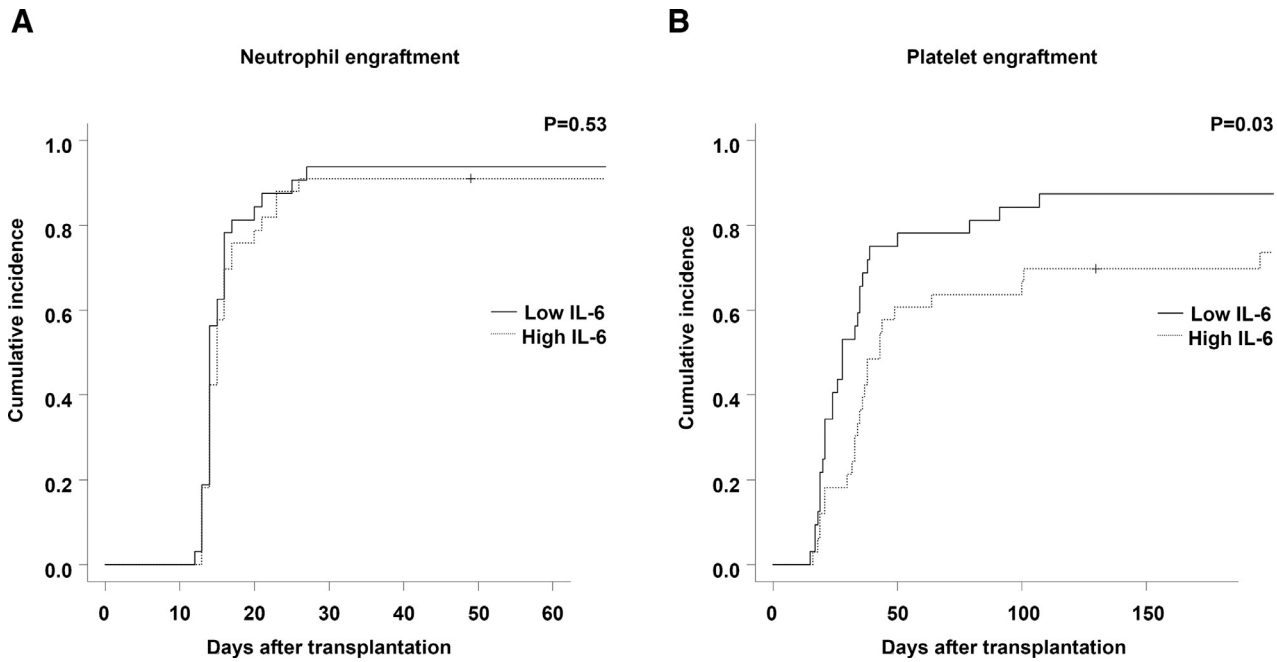

Figure 3. Association between engraftment and serum IL-6 level. (A) Neutrophil engraftment and (B) platelet engraftment in the high serum IL-6 group compared with the low serum IL-6 group.

We also analyzed the data to evaluate the degree of elevation from baseline serum IL-6 between days 1 and 7 after PT/Cy-haplo (ie, ΔIL-6). Only 61 patients were included in this subgroup analysis, since serum IL-6 data at day 0 were missing for 4 patients. The 3- year NRM was significantly higher in the high ΔIL-6 group than that in the low ΔIL-6 group (31.5% versus 7.6%; $P = .029$), whereas there was no significant difference in 3-year relapse mortality between the 2 groups (low versus high, 39.8% versus 45.8%; $P = .74$) (Supplementary Figure 4A and B). The 3-year OS was significantly higher in the low ΔIL-6 group (41.8% versus 13.3%; $P = .009$) (Supplementary Figure 4C). Based on the multivariate proportional subdistribution hazard regression analysis, high ΔIL-6 level was identified as an independent risk factor for NRM (HR, 4.1; 95% CI, 1.5 to 10.9; $P = .006$) (Supplementary Table 1).

DISCUSSION

The results of this study indicate that high levels of serum IL-6 derived from CRS immediately following Bu- or Mel-based conditioning for PT/Cy-haplo may adversely affect 3-year OS by increasing 3-year NRM frequency, which is associated with an increase in severe acute GVHD. In most patients, treatment with PT/Cy-haplo eliminated noninfectious fever immediately; however the underlying mechanism of IL-6 production is unclear. The transient surge in serum IL-6 levels might be driven by the residual host monocytes activated by the conditioning regimen and residual active disease, as well as the infusion of high T cell doses in the graft.

Life-threatening complications caused by CRS include cardiac, renal, and hepatic dysfunction; adult respiratory distress syndrome; neurologic toxicity; and disseminated intravascular coagulation from capillary leaks and/or vasodilatory shock [13]. IL-6 has been identified as a central mediator of toxicity in CRS, and serum IL-6 level has been correlated with severe CRS [11–13]. The IL-6 receptor antagonist tocilizumab is reportedly effective for treating severe CRS following PT/Cy-haplo [11], suggesting that serum IL-6 level may be useful as a surrogate marker of CRS severity. However, because serum IL-6 level is also affected by disease status, there is a concern that it might not accurately reflect the severity of CRS [13]. Indeed, even in our study, serum IL-6 levels before transplantation were significantly higher in patients not in remission compared with those in remission. Furthermore, disease status before allogeneic hematopoietic cell transplantation significantly affected the peak levels of IL-6 (Figure 1C; Table 2). However, in our study, irrespective of the effects of disease status on baseline serum IL-6 level, serum IL-6 level after PT/Cy-haplo was a more powerful predictor of outcome according to the CRS grading system.

In this study, CRS grade did not have a significant impact on NRM, relapse mortality, or survival following PT/Cy-haplo. Using the same grading system as used in our study, Raj et al [12] demonstrated that grade ≥ 2 CRS (compared with grade <2) was not associated with differences in OS or NRM following PT/Cy-haplo. The severity of CRS and timing of CRS onset appeared to be dependent on the type of cell therapy or drugs used. CRS severity criteria reported in previous studies [13,22] were used to develop a CRS severity grading system associated with monoclonal antibodies and adoptive targeted cell therapies, such as CAR T cells. In those studies, patients with severe CRS induced by CD19 CAR T cell therapy had plasma or serum IL-6 levels in the range of 10^2 to 10^3 pg/mL, higher than the values found in the present study. This CRS severity classification system was not specific for HLA-haploidentical transplantation, however [13]. Moreover, a specific procedure used in PT/Cy-haplo was intervention against CRS using high-doses of Cy. Therefore, we concluded that the existing CRS severity classification was not a sufficiently useful outcome indicator for the purpose of our study.

A high infused $CD3^+$ T cell dose was identified as an independent risk factor for 3-year NRM. Although we could not detect any correlations with serum IL-6 level or the cumulative incidence of grade III-IV acute GVHD, a high infused $CD3^+$ T cell dose was associated with a high incidence of

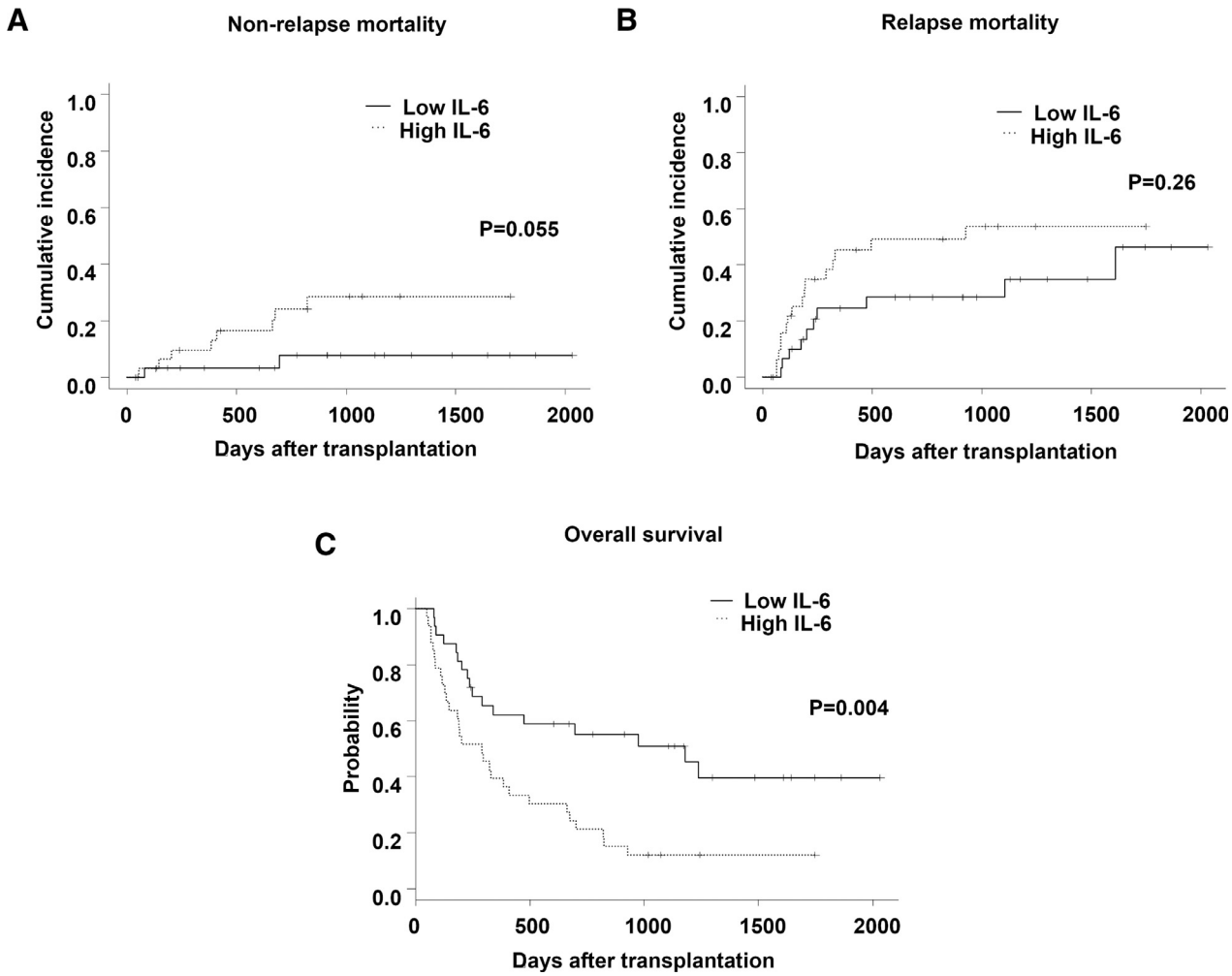

Figure 4. Association between outcomes and serum IL-6 levels. (A and B) Cumulative incidences of 3-year NRM (A) and 3-year relapse mortality (B) in the 2 study groups. (C) Three-year OS curves for the 2 study groups. NRM at 3 years was borderline significantly higher in the high serum IL-6 level group than that in the low serum IL-6 level group (low versus high, 7.1% versus 26.1%; $P = .055$).There was no significant difference in relapse mortality at 3 years between the 2 groups (low versus high, 48.5% versus 60.7%; $P = .26$). OS at 3 years was significantly better in the low serum IL-6 level group (43.6% versus 12.1%; $P = .004$).

steroid-refractory acute GVHD (high dose versus low dose, 28.1% versus 6.2%; $P = .03$), which could have contributed to the worse NRM. In addition, a tendency toward a correlation between higher $CD3^+$ T cell dose and higher IL-6 level was identified, with significantly elevated serum IL-6 level at a $CD3^+$ T cell dose $>3.5 \times 10^8$ /kg. Therefore, a very high dose of $CD3^+$ T cells may have a significant impact on elevated serum IL-6 level. We cannot definitively draw this conclusion, however, because only 4 of our patients received a $CD3^+$ T cell dose $>3.5 \times 10^8$ /kg.

This study has some limitations that need to be addressed. First, blood samples for cytokine were collected prospectively, but clinical data were collected retrospectively, and the sample size was small. Furthermore, the PT/Cy dose of 25 mg/kg administered twice was lower than the conventional dose of 50 mg/kg administered twice, and thus the rate of acute GVHD was higher compared with that with the conventional dose [7]. However, we believe that the use of a lower PT/Cy dose might have helped reveal the profound effect of serum IL-6 on CRS. In addition, in this study, the contribution of high serum IL-6 level to the suppression of relapse/progression was not clear. However, as mentioned earlier, serum IL-6 levels were statistically significant in patients who did not experience remission. Because a larger number of nonremission cases were included in the high serum IL-6 level group, the suppressive effect of relapse/progression might have been underestimated. Further prospective studies are needed to evaluate these relationships.

In conclusion, in this study, high serum cytokine levels accompanying CRS following PT/Cy-haplo were associated with severe GVHD, leading to worse survival. Recent studies on PT/Cy-haplo using nonmyeloablative conditioning at Johns Hopkins Hospital demonstrated that mild grade II GVHD (also termed “good GVHD”) was significantly related to a lower incidence of relapse and better survival [23], whereas severe acute GVHD was associated with a poor prognosis. Therefore, early detection of severe CRS leading to effective, early intervention may require the establishment of a readily available method for rapidly monitoring IL-6 levels, along with a more useful CRS severity assessment system specific to PT/Cy-haplo.

Table 4
Univariate and Multivariate Analysis for Predictive Factors of 3-Year NRM, Relapse Mortality, and OS

Variable	NRM				Relapse Mortality				OS			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
ABO mismatch (vs match)	2.6 (.8-9.0)	.13			1.0 (.5-2.1)	.90			1.3 (.7-2.4)	.34		
Age ≥48 (vs <48) yr	.9 (.3-3.1)	.91			1.4 (.7-2.8)	.29			1.4 (.8-2.6)	.23	1.6 (.9-3.0)	.12
ATG yes (vs no)	1.3 (.4-5.0)	.68			1.1 (.5-2.1)	.88			1.0 (.5-2.0)	.91		
Bu-based (vs Mel-based)	2.6 (.7-10.2)	.18			.6 (.2-1.9)	.41			1.0 (.5-2.3)	.91		
$CD3^+$ cells ≥1.7 (vs <1.7) × 10^8/kg	**9.5 (1.2-73.3)**	**.03**	**9.8 (1.2-77.8)**	**.03**	.6 (.3-1.1)	.10			1.2 (.7-2.2)	.50		
$CD34^+$ cells ≥4.4 (vs <4.4) × 10^6/kg	2.5 (.6-9.3)	.19			.6 (.3-1.2)	.14			.8 (.5-1.5)	.55		
CMV serostatus: D^-/R^+ (vs other)	2.6 (.8-8.8)	.12			**.3 (.1-1.0)**	**.047**	.3 (.1-1.1)	.063	.5 (.2-1.2)	.14		
Active disease (vs remission)	2.0 (.5-7.4)	.32			**2.7 (1.3-5.6)**	**.007**	**2.6 (1.3-5.3)**	**.01**	**3.2 (1.7-6.2)**	**.0003**	**2.6 (1.3-5.0)**	**.006**
Donor age ≥33 (vs <33) yr	2.2 (.6-8.0)	.25			1.1 (.5-2.1)	.83			1.6 (.9-2.8)	.15		
HCT-CI score ≥3 (vs 0-2)	**5.9 (1.6-22.5)**	**.008**	3.6 (.7-17.5)	.11	1.2 (.6-2.4)	.67			**2.3 (1.3-4.2)**	**.006**	1.3 (.7-2.6)	.41
HLA disparity												
1/8-3/8 antigen mismatch	1 (reference)				1 (reference)				1 (reference)			
4/8 antigen mismatch	.6 (.2-2.2)	.41			1.1 (.6-2.2)	.72			.8 (.4-1.5)	.48		
CRS grade 2-5 (vs 0 and 1)	1.2 (.3-4.1)	.78			1.6 (.6-2.4)	.69			1.3 (.7-2.3)	.43		
High IL-6 (vs low)	4.2 (.9-19.4)	.07			1.5 (.8-2.9)	.24			**2.3 (1.3-4.3)**	**.006**		
IL-6*	**3.2 (1.1-10.1)**	**.03**	2.4 (.6-9.5)	.22	1.8 (1.0-3.4)	.07			**2.7 (1.5-5.2)**	**.002**	**2.2 (1.0-4.7)**	**.049**
Female to male (vs other)	.2 (.0-1.7)	.16			1.8 (.9-3.5)	.08			1.0 (.6-2.0)	.82		
Female (vs male)	1.5 (.4-5.0)	.52			.8 (.4-1.7)	.58			.9 (.5-1.6)	.70		
Disease type												
Myeloid malignancy	1 (reference)				1 (reference)				1 (reference)			
Lymphoid malignancy	1.5 (.4-5.4)	.50			1.0 (.5-1.9)	.95			1.1 (.6-2.0)	.86		

Significant values are in bold type.

* Serum IL-6 concentrations were log-transformed.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.06.003.

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