Original Article

Title

High lymphocyte counts before antithymocyte globulin administration predict acute graft-versus-host disease

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Abstract

Antithymocyte globulin (ATG) reduces severe acute and chronic graft-versus-host disease (GVHD) in allogeneic peripheral blood stem cell transplantation (PBSCT). However, risk factors for severe acute GVHD in PBSCT using ATG remain to be determined. We conducted a single-center, retrospective study to analyze the association of acute GVHD requiring systemic corticosteroid (SC-aGVHD) with absolute lymphocyte counts (ALC) before the administration of ATG or conditioning in 53 patients with HLA-matched PBSCT using low-dose thymoglobulin (2 mg/kg) after myeloablative conditioning. The cumulative incidence of SC-aGVHD was 17.0% and ALC before ATG were significantly higher in patients with SC-aGVHD compared to that in patients without it (median, 0.15 × 10^9/L vs 0.06 × 10^9/L, *P* = 0.047). The cumulative incidence of SC-aGVHD was significantly higher in patients with high ALC before ATG (≥ 0.15 × 10^9/L) than in those with low ALC (38.5% vs 10.0%, *P* = 0.016). Non-relapse mortality (NRM) was also significantly higher in the high ALC before ATG group than the low ALC before ATG group (2-year NRM: 23.9% vs 6.0%, *P* = 0.048), leading to worse survival (2-year overall survival: 69.2% vs 83.5%, *P* = 0.039). Our study suggested that high ALC before ATG is a risk factor for SC-aGVHD.

Keywords

allogeneic hematopoietic stem cell transplantation, peripheral blood stem cell transplantation, antithymocyte globulin, graft-versus-host disease, absolute lymphocyte count

Introduction

Graft-versus-host disease (GVHD) is one of the main causes of transplant-associated morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). For prophylaxis of GVHD, antithymocyte globulin (ATG) is recommended, particularly in HSCT from unrelated donor or peripheral blood stem cell transplantation (PBSCT) [1], based on the results of a series of prospective randomized phase III studies [2-7]. However, the appropriate dose of ATG remains to be determined. Because a higher dose of ATG is a risk factor for infection or posttransplant lymphoproliferative disorder, a few recent studies have utilized lower doses of ATG [8-13]. Our previous pilot study showed that a total of 2 mg/kg of rabbit ATG (thymoglobulin) was sufficient to reduce naive T cells at day 28 after PBSCT [14]. In a prospective, multicenter, phase II study to evaluate the safety and efficacy of low-dose ATG in HLA-matched PBSCT, we showed that 2 mg/kg of thymoglobulin efficiently prevented severe acute and chronic GVHD, with 1.4% grade III to IV acute GVHD and 5.6% moderate to severe chronic GVHD [15].

Recent studies suggested an association between absolute lymphocyte counts (ALC) before transplantation and transplant outcomes [16-20]. In this study, we evaluated this association in PBSCT using low-dose ATG.

Materials and methods

Patients

We conducted a single-center, retrospective study to evaluate risk factors for acute

GVHD requiring systemic corticosteroids (SC-aGVHD) in patients who underwent HLA-matched PBSCT using low-dose ATG after myeloablative conditioning (MAC) between December 2013 and July 2019. Patients with active relapse and refractory disease at transplantation were excluded. We also analyzed the association of SC-aGVHD with white blood cell count (WBC) and ALC before the administration of ATG or conditioning. The study was performed in accordance with institutional ethical guidelines, including the World Medical Association Declaration of Helsinki, and was approved by the institutional review board (No. 015-0387).

Transplantation procedures

All patients received MAC regimens. A total of 2 mg/kg of thymoglobulin was administered on days -2 and -1 (1 mg/kg/day). GVHD prophylaxis consisted of a combination of tacrolimus (Tac) administered from day -1 and short-term methotrexate (day 1: 10 mg/m^2, days 3 and 6: 7 mg/m^2). Ursodeoxycholic acid and low-molecular-weight heparin were administered for veno-occlusive disease/sinusoidal obstruction syndrome prophylaxis, and granulocyte colony-stimulating factor was administered from day 5 until neutrophil engraftment. The prophylactic regimen for infection included levofloxacin, micafungin, and acyclovir.

Definitions

Acute GVHD was graded according to the consensus criteria [21], and chronic GVHD was graded according to the criteria of the National Institutes of Health consensus development project [22]. The onset of SC-aGVHD was defined as the first day of systemic corticosteroids for the treatment of acute GVHD. A hematopoietic cell transplantation-specific comorbidity index (HCT-CI) score was calculated according to the scoring system [23], and disease risk of the patients was determined according to the refined disease risk index (DRI), as previously described [24]. Peripheral blood cell counts were investigated on the first day or the day before the administration of ATG or conditioning. Overall survival (OS) was calculated from the day of PBSCT, with patients alive at the time of last follow-up censored. Progression-free survival (PFS) was calculated from the date of PBSCT until the date of disease recurrence or death from any cause, or last follow-up for patients without these events censored. Non-relapse mortality (NRM) was defined as death due to any cause other than relapse. Relapse was defined by hematological evidence of underlying disease.

Statistical analysis

Statistical analysis was performed using Mann–Whitney *U*-test for contingency data, Kaplan-Meier method and Log-rank test for PFS and OS, Gray's test for engraftment, acute and chronic GVHD, relapse and NRM, and in multivariate analysis, Fine and Gray competing risk regression model for SC-aGVHD. The cutoff value of each parameter was based on ROC-curve analysis. A value of $P < 0.05$ was used to determine statistical significance, and all analyses were performed with EZR (Saitama Medical Center, Jichi Medical University,

Japan), which is a graphic user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [25].

Results

Patients and transplant characteristics

Patient and transplant characteristics in this study are shown in Table 1. A total of 53 patients were included in this study, and the median patient age at the time of transplant was 50 years, ranging from 19 to 66 years. Diagnoses included acute myeloid leukemia (AML; n = 27. 50.9%), acute lymphoblastic leukemia (ALL; n = 12, 22.6%), myelodysplastic syndrome (MDS; n = 7, 13.2%), myeloproliferative neoplasm (MPN; n = 5, 9.4%), and malignant lymphoma (n = 2, 3.8%). At the time of transplantation, 40 patients (75.5%) were in complete remission, 41 patients (77.4%) were classified as intermediate risk by the refined DRI, and 30 patients (56.6%) had a score of 0 or 1 in HCT-CI. Further, 24 (45.3%) and 29 (54.7%) patients underwent PBSCT from related and unrelated donors, respectively. The conditioning regimen was either cyclophosphamide (CY) + total body irradiation (n = 13, 24.5%) or fludarabine (FLU) + BU based (n = 40, 75.5%). The median number of $CD34^+$ cells was 3.28 × 10^6/kg, ranging from 1.87 to 8.99 × 10^6/kg, and that of $CD3^+$ cells was 1.56 × 10^6/kg, ranging from 0.27 to 3.49 × 10^6/kg. Peripheral blood cell counts before the administration of ATG or conditioning were as follows: ALC before ATG: median, 0.84 × 10^9/L, range, 0 to 0.88 × 10^9/L; WBC before ATG: median, 2.10 × 10^9/L, range, 0.20 to 10.1 × 10^9/L; ALC before conditioning: median, 0.68 × 10^9/L, range, 0 to 2.30 × 10^9/L; WBC before the conditioning: median, 2.90 ×

10^9/L, and range 0.70 to 14.0 × 10^9/L.

Clinical outcomes

All patients achieved neutrophil engraftment with a median of 13 days, ranging from 11 to 20 days. The cumulative incidence of grade II to IV and grade III to IV acute GVHD at 100 days was 17.0% (95% CI, 8.3 to 28.3%) and 1.9% (95% CI, 0.1 to 8.9%, Supplemental fig 1a), and the cumulative incidence of all-grade chronic GVHD and moderate to severe GVHD at 2 years was 22.8% (95% CI, 12.0 to 35.7%) and 16.2% (95% CI, 7.4 to 28.0%, Supplemental fig 1b), respectively. The cumulative incidence of relapse was 28.0% (95% CI, 16.1 to 41.1%, Supplemental fig 1c) and that of NRM was 10.4% (95% CI, 3.7 to 21.1%, Supplemental fig 1d) after 2 years. With a median follow-up of 738 days, the 2-year OS was 79.9% (95% CI, 65.8 to 88.7%; Supplemental fig 1e) and the 2-year PFS was 61.7% (95% CI, 46.5 to 73.8%, Supplemental fig 1f).

Characteristics of SC-aGVHD and impacts on transplant outcomes

The cumulative incidence of SC-aGVHD at 100 days was 17.0% (95% CI, 8.3 to 28.3%, Fig 1a). Gradings of SC-aGVHD were as follows: grade IV in 1 patient, grade II in 6 patients, and grade I in 2 patients. Skin involvement was observed in 6 patients, gut in 5 patients, and liver in 4 patients. Systemic corticosteroids were administered to 2 patients with grade I acute GVHD because of severe clinical manifestations. In patients with SC-aGVHD, the 2-year NRM rates were significantly higher compared to those without it (48.9%; 95% CI, 11.4 to

79.0% vs 2.6%; 95% CI, 0.2 to 11.9%; *P* < 0.01, Fig 1b), whereas relapse rates were equivalent between the two groups (11.1%; 95% CI, 0.5. 40.9% vs. 29.9%; 95% CI, 16.3 to 44.7%; *P* = 0.47, Fig 1c). OS was significantly lower in patients with SC-aGVHD than that in patients without SC-aGVHD; the 2-year OS was 40.0% (95% CI, 9.8 to 69.7%) in patients with SC-aGVHD and 90.0% (95% CI, 75.5 to 96.1%) in those without it (*P* < 0.01, Fig 1d).

Association of acute GVHD with peripheral blood cell counts

We evaluated the association of SC-aGVHD with WBC or ALC before the administration of ATG or conditioning. ALC before ATG was significantly higher in patients with SC-aGVHD compared to that in patients without it (median, 0.15 × 10^9/L vs. 0.06 × 10^9/L, *P* = 0.047, Fig 2a). The other indices were equivalent between the groups (median WBC before ATG: 1.50 × 10^9/L vs. 2.10 × 10^9/L, *P* = 0.89, Fig 2b; median ALC before the conditioning: 0.75 × 10^9/L vs. 0.67 × 10^9/L, *P* = 0.21, Fig 2c; median WBC before the conditioning: 2.50 × 10^9/L vs. 3.30 × 10^9/L, *P* = 0.38, Fig 2d). The cumulative incidence of SC-aGVHD was significantly higher in patients with high ALC before ATG (≥ 0.15 × 10^9/L) than in those with low ALC (38.5% vs. 10.0%, *P* = 0.016, Fig 2e). Particularly in unrelated donor transplants, increased risk of SC-aGVHD in patients with high ALC before ATG was observed compared to that in related donor transplant (≥ 0.15 × 10^9/L: 45.5% vs. < 0.15 × 10^9/L: 11.1%, *P* = 0.04). The other indices were not associated with the incidence of SC-aGVHD (WBC before ATG: ≥ 1.50 × 10^9/L: 16.1% vs. < 1.50 × 10^9/L: 18.2%, *P* = 0.87, Fig 2f; ALC before the conditioning:≥ 1.00 × 10^9/L: 33.3% vs. < 1.00 × 10^9/L: 12.2%, *P* = 0.077, Fig 2g; WBC before the conditioning: ≥

2.90 × 10^9/L: 11.1% vs. < 2.90 × 10^9/L: 23.1%, *P* = 0.23, Fig 2h). ALC before ATG also affected the grade of acute GVHD; ALC before ATG was significantly higher in patients with grade II to IV compared to that in patients with grade I (median, 0.29 × 10^9/L vs. 0.09 × 10^9/L, P = 0.022, Fig 2i). Remarkably, the 2-year NRM was significantly higher in the high ALC before ATG (≥ 0.15 × 10^9/L) group than that in the low ALC before ATG (< 0.15 × 10^9/L) group (23.9%; 95% CI, 5.2 to 50.2% vs. 6.0%; 95% CI, 1.0 to 17.7%; *P* = 0.048, Fig 2j), whereas relapse rates were equivalent between the two groups (24.2%; 95% CI, 5.1% to 50.9% vs. 28.8%; 95% CI, 15.3% to 43.8%; *P* = 0.72, Fig 2k). The two-year OS was also worse in the high ALC before ATG group than that in the low ALC before ATG group (69.2%; 95% CI, 37.3 to 87.2% vs. 83.5%; 95% CI, 66.8% to 92.3%; *P* = 0.039, Fig 2l). Similar results were obtained for grade II to IV acute GVHD (Supplemental fig 2).

We evaluated the risk factors for acute SC-aGVHD from pre-transplant parameters, including age, sex, DRI, HCT-CI, stem cell source, conditioning, $CD34^+$ cells, $CD3^+$ cells, and ALC before ATG. In univariate analysis, ≥ 57 years of recipient age (HR, 4.52; 95% CI, 1.14 to 18.0; *P* = 0.03) and ≥ 0.15 × 10^9/L of ALC before ATG (HR, 4.60; 95% CI, 1.28 to 16.5; *P* = 0.02) were identified as risk factors for SC-aGVHD. In multivariate analysis, ALC before ATG was only identified as a significant risk factor for SC-aGVHD (HR, 3.857; 95% CI, 1.161–12.82; *P* = 0.028, Table 2).

We also evaluated the association between chronic GVHD and ALC before the administration of ATG. The cumulative incidences of both all-grade chronic GVHD and moderate to severe chronic GVHD were suggested to be higher in patients with high ALC

before ATG (≥ 0.26 × 10^9/L) than in those with low ALC, whereas ALC before ATG was equivalent between patients with chronic GVHD and those without it (data not shown).

Discussion

In this study, we evaluated the association between ALC before ATG and SC-aGVHD. Recent studies have suggested an association between ALC before transplantation and transplant outcomes. Kennedy et al. evaluated the outcome of unrelated HSCT using 3 different doses of thymoglobulin, i.e., 10 mg/kg, 7.5 mg/kg, and 5 mg/kg, and showed that low ALC on the day of ATG administration was associated with an increased risk of death, whereas high ALC was associated with a lower mortality risk [18]. In unrelated HSCT using a total of 60 mg/kg anti-T lymphocyte globulin (ATLG), Soiffer et al. also showed that ATLG administration negatively affected OS and PFS in patients with low ALC [17]. The discrepancy between these results and our results might be due to the different doses and preparation of ATG used. As ATG also binds to B cells and NK cells, low-dose ATG may be insufficient to deplete T cells in the presence of high ALC at the time of ATG administration, leading to an increased risk of developing GVHD. While, high dose ATG may be too immunosuppressive in low ALC before ATG, leading to an increased risk of infection or relapse [26]. Recent studies have evaluated the ALC before the administration of ATG or conditioning in HSCT using low-dose ATG [19,20]. However, the association between ALC and post-transplant outcome was unclear. To our knowledge, our study is the first to demonstrate that a high ALC before ATG was a risk factor for SC-aGVHD, leading to increased NRM and worse survival

in HLA-matched PBSCT using low-dose ATG. Our study suggests that ALC before ATG is a superior marker to ALC before conditioning for predicting the onset of severe acute GVHD. ALC before conditioning may be less accurate in predicting ALC at the time of administration of ATG because of the difference in intensity of lymphodepletion in each conditioning regimen. Based on these results, individualized therapy using dose modification of ATG based on ALC before ATG may be a promising strategy to reduce the incidence of severe acute GVHD. A recent study also showed that in unrelated HSCT, an optimum exposure of ATG (8 mg/kg) was associated with favorable survival in PBSCT; excessive exposure to ATG led to higher relapse-related mortality, and insufficient exposure increased non-relapse mortality and grade III to IV acute GVHD compared with optimum exposure [16].

Our study has several limitations that should be considered when reviewing the results. Importantly, it is a retrospective design with a small number of patients. ATG preparation is known to have differential activity and depletion power *in vivo*; therefore, it is unclear whether our results using thymoglobulin can be obtained with other ATG preparations. The association of chronic GVHD with ALC before ATG was not elucidated in this study. Nevertheless, our data highlight the possibility of individualized ATG therapy. High ALC before ATG is suggested as a risk factor for SC-aGVHD. In future, larger prospective studies should be conducted to confirm our findings.

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Conflict of Interest statement

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Figure legends

Fig 1. The cumulative incidence of SC-aGVHD (a). The cumulative incidence of NRM (b), relapse (c), and Kaplan-Meier plots of OS (d) after PBSCT using low-dose ATG in patients with SC-aGVHD (*dashed lines,* n = 9) and without it (*solid lines,* n = 44).

Fig 2. ALC (a) or WBC (b) before the administration of ATG, and ALC (c) or WBC (d) before the conditioning in patients with or without SC-aGVHD. The cumulative incidence of SC-aGVHD in patients with high ALC before ATG (≥ 0.15 × 10^9/L, *dashed lines,* n = 13) or low ALC before ATG (*solid lines,* n = 40) (e), high WBC before ATG (≥ 1.50 × 10^9/L, *dashed lines,* n = 31) or low WBC before ATG (*solid lines,* n = 22) (f), high ALC before the conditioning (≥ 1.00 × 10^9/L, *dashed lines,* n = 12) or low ALC before the conditioning (*solid lines,* n = 41) (g), and high WBC before the conditioning (≥ 2.90 × 10^9/L, *dashed lines,* n = 27) or low WBC before the conditioning (*solid lines,* n = 26) (h). ALC before the administration of ATG in patients with grade I or grade II to IV acute GVHD (i). The cumulative incidence of NRM (j),

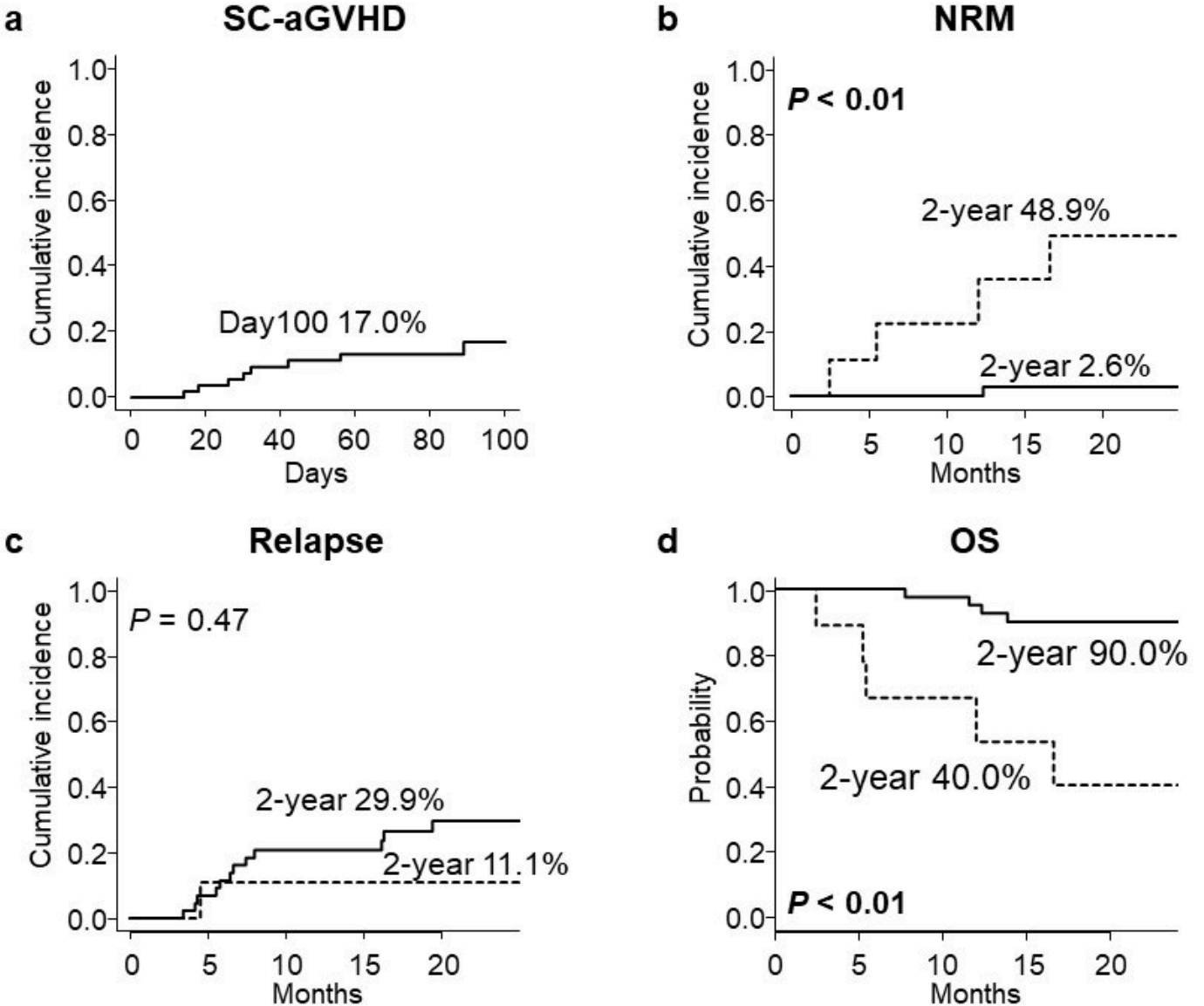
relapse (k), and Kaplan-Meier plots of OS (l) in patients with high ALC before ATG (*dashed lines*) or low ALC before ATG (*solid lines*).

Supplemental fig 1. The cumulative incidence of grade II to IV (*solid lines*) and grades III to IV (*dashed lines*) acute GVHD (a), all-grade chronic GVHD (*solid lines*) and moderate to severe (*dashed lines*) chronic GVHD (b), relapse (c), NRM (d), Kaplan-Meier plots of OS (e) and PFS (f) after PBSCT using low-dose ATG.

Supplemental fig 2. ALC (a) and WBC (b) before the administration of ATG; ALC (c), and WBC (d) before conditioning in patients with or without grade II to IV acute GVHD. ALC before ATG in patients with grade II to IV acute GVHD was significantly higher compared to those without it (median, 0.18 × 10^9/L vs. 0.63 × 10^9/L, P = 0.01). The other indices were equivalent between the groups (WBC before ATG; median, 2.50 × 10^9/L vs. 2.00 × 10^9/L, P = 0.42, ATG before conditioning; median, 0.75 × 10^9/L vs. 0.67 × 10^9/L, P = 0.32, WBC before conditioning; median, 2.70 × 10^9/L vs 3.30 × 10^9/L, P = 0.67). The cumulative incidence of grade II to IV acute GVHD in patients with high ALC before ATG (≥ 0.15 × 10^9/L, *dashed lines,* n = 13) or low ALC before ATG (*solid lines,* n = 40) (e), high WBC before ATG (≥ 1.50 × 10^9/L, *dashed lines,* n = 31) or low WBC before ATG (*solid lines,* n = 22) (f), high ALC before the conditioning (≥ 1.00 × 10^9/L, *dashed lines,* n = 12) or low ALC before conditioning (*solid lines,* n = 41) (g), and high WBC before conditioning (≥ 2.90 × 10^9/L, *dashed lines,* n = 27) or low WBC before conditioning (*solid lines,* n = 26) (h). The cumulative incidence of grade II to IV

acute GVHD was significantly higher in patients with high ALC before ATG (≥ 0.15 × 10^9/L) than in those with low ALC (≥ 0.15 × 10^9/L: 53.8% vs. < 0.15 × 10^9/L: 5.0%, $P < 0.01$). The other indices were not associated with the incidence of grade II to IV acute GVHD (WBC before ATG; ≥ 1.50 × 10^9/L: 22.6% vs. < 1.50 × 10^9/L: 9.1%, $P = 0.18$, ATG before conditioning; ≥ 1.00 × 10^9/L: 33.3% vs. < 1.00 × 10^9/L: 12.2%, $P = 0.08$, WBC before the conditioning; ≥ 2.90 × 10^9/L: 14.8% vs. < 2.90 × 10^9/L: 19.2%, $P = 0.64$).

Fig. 1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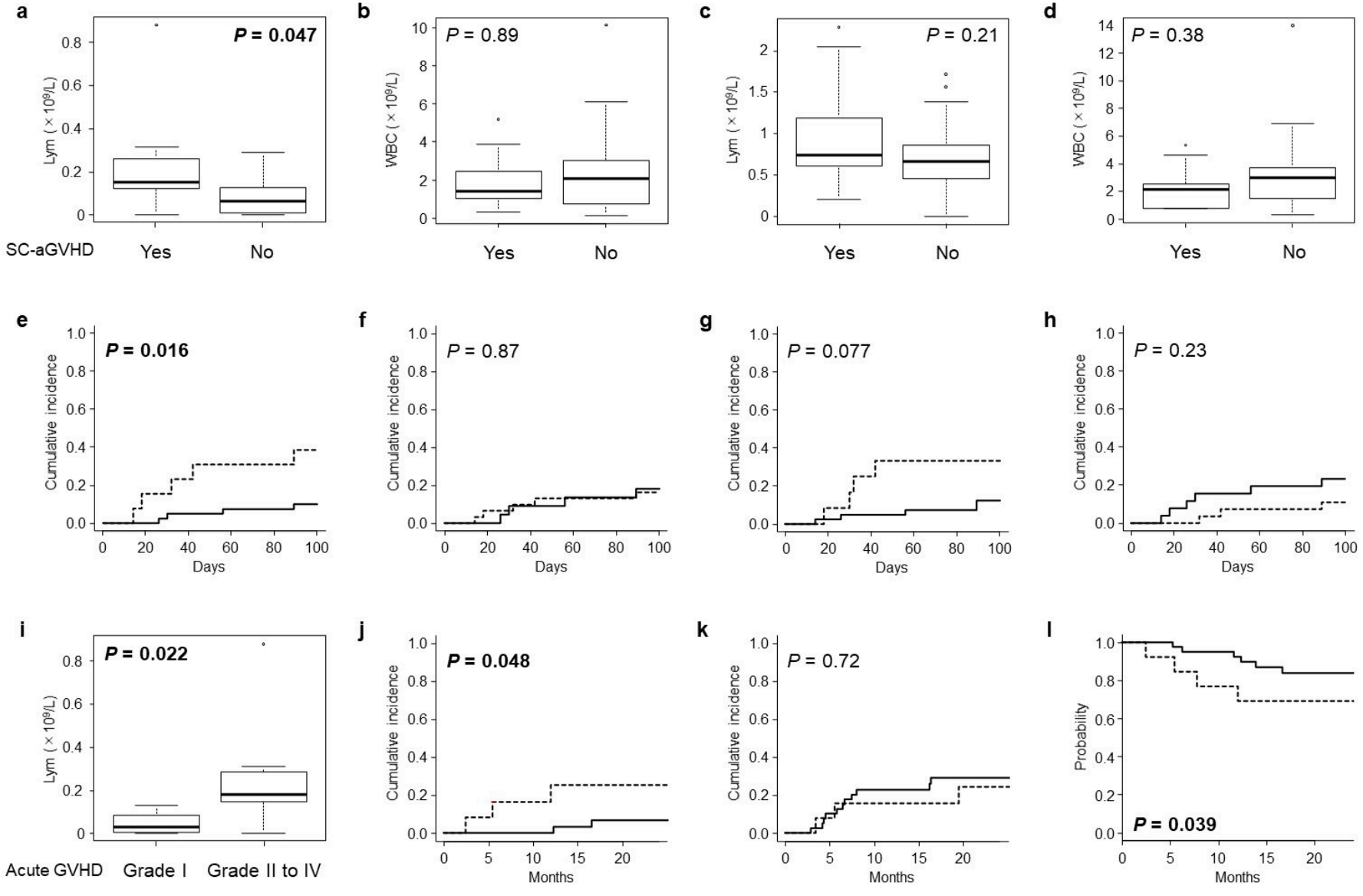
SC-aGVHD
Cumulative incidence
1.0
0.8
0.6
0.4
0.2
0.0
Day100 17.0%
0
20
40
60
80
100
Days
b
NRM
Cumulative incidence
P < 0.01
2-year 48.9%
2-year 2.6%
0
5
10
15
20
Months
c
Relapse
Cumulative incidence
P = 0.47
2-year 29.9%
2-year 11.1%
0
5
10
15
20
Months
d
OS
Probability
2-year 90.0%
2-year 40.0%
P < 0.01
0
5
10
15
20
Months

Fig. 2


a
P = 0.047
Lym (×10⁹/L)
SC-aGVHD
Yes
No
b
P = 0.89
WBC (×10⁹/L)
Yes
No
c
P = 0.21
Lym (×10⁹/L)
Yes
No
d
P = 0.38
WBC (×10⁹/L)
Yes
No
e
P = 0.016
Cumulative incidence
Days
f
P = 0.87
Cumulative incidence
Days
g
P = 0.077
Cumulative incidence
Days
h
P = 0.23
Cumulative incidence
Days
i
P = 0.022
Lym (×10⁹/L)
Acute GVHD
Grade I
Grade II to IV
j
P = 0.048
Cumulative incidence
Months
k
P = 0.72
Cumulative incidence
Months
l
Probability
P = 0.039
Months