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ORIGINAL ARTICLE

Blood eosinophilia as a marker of favorable outcome after allogeneic stem cell transplantation

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Summary

Eosinophilia is observed in a variety of disorders including acute and chronic graft-versus-host disease (GVHD). The clinical records of 237 patients who underwent allogeneic stem cell transplantation (allo-SCT) were retrospectively reviewed. Eosinophilia, defined as a relative eosinophil count >4% within the first 100 days, was observed in 135 patients (57%). The incidence of grades II–IV acute GVHD was significantly higher in patients without eosinophilia than in those with eosinophilia (68% vs. 43%; $P < 0.001$). The incidence of chronic GVHD was significantly higher in patients without eosinophilia than in those with eosinophilia (73% vs. 56%; $P = 0.011$). Relapse rate was similar between patients with and without eosinophilia (33% vs. 27%; $P = 0.438$). The probability of nonrelapse mortality was 10% in patients with eosinophilia, which was significantly lower than that in patients without eosinophilia (31%; $P < 0.001$), and the overall survival (OS) at 3 years was 67% in patients with eosinophilia, which was significantly higher than that in patients without eosinophilia (51%; $P = 0.003$). Multivariate analysis identified older age, high-risk disease, acute GVHD, sex disparity between patient and donor, and the absence of eosinophilia as significant factors for reduced OS. These data lead us to conclude that eosinophilia after allo-SCT may serve as a favorable prognostic marker.

Introduction

Eosinophils are cells that play an important role in protecting their host against infectious pathogens, particularly parasites, while mediating allergic syndromes including asthma and drug reactions. Interleukin (IL)-5 is the most important cytokine that contributes to the activation, proliferation, terminal differentiation and mobilization of eosinophils. IL-3 and macrophage colony-stimulating factor also have complementary effects with IL-5 on eosinophil maturation *in vitro* [1].

Eosinophilia is observed in a variety of systemic disorders, and it can also occur in type 2 $CD4^+$ T-helper cell (Th2)-mediated reactions such as allograft rejection after solid organ transplantation. There have been several reports, suggesting that eosinophilia in the graft or blood could be a prognostic marker of acute rejection [2–14]. Barnes *et al.* [13] evaluated the impact of eosinophilia in 101 liver transplant recipients, and have shown its positive predictive value of 82% for acute cellular rejection. Additionally, blood eosinophilia has been observed in both children and adults with chronic graft-versus-host disease (GVHD) [15–19]. Chronic GVHD is suggested to be a Th2-mediated reaction [20]. Also there have been reports demonstrating an association between blood eosinophilia and the presence of acute GVHD, which is considered to be a Th1-mediated reaction [21–23]. However, to date, few studies have examined the clinical significance of eosinophilia in large numbers of recipients after allogeneic stem cell transplantation (allo-SCT) for hematologic disorders. Sato *et al.* [24] recently reported that the incidence of acute and chronic GVHD does not

correlate with the presence of blood eosinophilia after allo-SCT in children; however, they observed a significant association between blood eosinophilia and survival after allo-SCT.

Therefore, we carried out the present retrospective study to assess whether blood eosinophilia after allo-SCT for hematologic disorders is associated with acute and/or chronic GVHD, relapse, nonrelapse mortality (NRM), and overall survival (OS) in adults.

Patients and methods

Patients

The clinical records were retrospectively collected for 254 adult patients (age ≥ 15) who underwent allo-SCT at Keio University Hospital for a variety of hematologic diseases between April 1997 and March 2005. Seventeen patients were excluded from the present analysis because of death before engraftment ($n = 9$) or inadequate laboratory data ($n = 8$), leaving a total of 237 evaluable subjects. Aplastic anemia, acute leukemia in first or second remission, Philadelphia chromosome-positive chronic myelogenous leukemia (CML) in first or second chronic phase, chemotherapy-sensitive lymphoma, and myelodysplastic syndrome (MDS) without excess of blasts were considered to be standard-risk diseases. Acute leukemia not in remission, adult T-cell leukemia/lymphoma, CML in third or later chronic phase, accelerated phase, and blast phase, chemotherapy-refractory lymphoma, multiple myeloma, MDS with excess blasts, and primary myelofibrosis were considered to be high-risk diseases.

Transplant procedures

The typing of human leukocyte antigen (HLA)-A and HLA-B antigens was performed by using standard serologic techniques. The typing of HLA-DRB1 alleles was performed by using high-resolution DNA techniques.

For the conditioning regimen, myeloablative regimens including total body irradiation (TBI; 12Gy) combined with cyclophosphamide (120 mg/kg) with or without high-dose cytarabine and busulfan (16 mg/kg)-cyclophosphamide (120 mg/kg), or reduced-intensity regimens were chosen according to the protocols available during the study period [25–28]. The reduced-intensity stem cell transplantation (RIST) included fludarabine (125 mg/m^2) and melphalan (140 mg/m^2) with or without low-dose TBI [29]. For GVHD prophylaxis, cyclosporine A (CsA) at a dose of 3 mg/kg/day or tacrolimus at a dose of 0.03 mg/kg/day was administered by continuous i.v. infusion starting on day −1. Calcineurin inhibitors were not selected in a random fashion, but according to the type of donor: CsA was chosen for HLA-matched related donors, and tacrolimus for unrelated donors, HLA-mismatched donors, and cord blood units. The dose was adjusted to maintain the blood concentrations of calcineurin inhibitors within the therapeutic range (200–400 ng/mL for CsA, and 10–20 ng/mL for tacrolimus). Methotrexate (MTX) was given at a dose of 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3 and 6 for HLA-matched-related transplantation and cord blood transplantation. In cases of unrelated or HLA-mismatched donor transplantation, 10 mg/m^2 of MTX was added on day 11. MTX on day 6 or 11 was omitted in those cases in which a patient developed severe mucositis, renal impairment or ascites. Intravenous CsA and tacrolimus were switched to oral administration when the patient was able to eat. CsA and tacrolimus were discontinued by 6 months post-SCT provided that there was no clinical evidence of active GVHD.

With respect to the grading and treatment of GVHD, acute GVHD was graded according to the established criteria [30]. Acute GVHD (grade II or higher) was treated initially with prednisolone (PSL) at a dose of at least 1 mg/kg/day. Calcineurin inhibitors were continued during GVHD treatment unless there was toxicity. After 2 weeks of treatment, an attempt was made to decrease the dose of PSL. Patients who showed no response to PSL or in whom PSL could not be reduced underwent salvage therapy with methyl-PSL pulse (1 g for 3 days) with or without antithymocyte globulin. Chronic GVHD was graded according to the established criteria for patients who survived 100 days after allo-SCT [31]. Initial treatment for extensive chronic GVHD consisted of PSL alternating with CsA or tacrolimus every other day for at least 9 months. Tissue biopsy samples were obtained to confirm the diagnosis of GVHD whenever clinically feasible.

During supportive care, all patients were treated in a room with laminar air flow from day −14 to engraftment. Antibacterial and antifungal prophylaxis consisted of oral ciprofloxacin at a dose of 600 mg/day and fluconazole at a dose of 200 mg/day, and antiviral prophylaxis consisted of i.v. acyclovir at a dose of 750 mg/day from day −3 to day 14; oral acyclovir at a dose of 1000 mg/day was used in RIST patients. Oral sulfamethoxazole/trimethoprim was administered from day −21 to −8 for the prophylaxis of *Pneumocystis carinii pneumonia*. Granulocyte colony-stimulating factor was given i.v. at a dose of 5 μg/kg starting on day 1 until engraftment, which was defined as an absolute neutrophil count of 0.5×10^9/l for three consecutive days. As pre-emptive therapy for cytomegalovirus (CMV) infection, ganciclovir at a dose of 5–10 mg/kg was started when cytomegalovirus antigenemia became positive after allo-SCT. Intravenous immunoglobulin was given to maintain IgG > 500 mg/dl.

Eosinophilia

Peripheral complete blood cell counts (CBCs) were performed daily until engraftment, then two or three times a week until day 100. After engraftment, microscopic examination of blood smears was performed on each CBC. Eosinophilia was defined as a relative eosinophil count (REC) >4% within the first 100 days after allo-SCT [13,17].

Statistic analysis

Chi-squared analysis or Fisher's exact test ($m \times n$) was used to assess the differences in categorical variables between two groups. For the comparison of continuous variables between two groups, the Mann–Whitney *U*-test was used. Overall survival, relapse, acute GVHD and NRM outcomes were based on the Kaplan–Meier estimates, and the differences between groups were compared by using the log-rank statistic [32,33]. Overall survival was defined as the interval between the date of allo-SCT and the date of death or last contact. For OS, death due to any cause was considered an event; surviving patients were censored at the last follow-up and relapse was calculated by using cumulative incidence curves to accommodate competing risks. For GVHD, death from any cause was considered the competing event; patients surviving without GVHD were censored at the last follow-up. For relapse, NRM was the competing event; patients alive and in remission were censored at the last follow-up evaluation. For NRM, relapse was the competing event; patients alive and in remission were censored at the last follow-up evaluation. Multivariate Cox regression analysis was used to identify the independent risk factors associated with OS [34]. The following pre-transplant patient characteristics were analyzed for their potential prognostic value with respect to each of the outcomes: age, sex, risk of disease, stem cell source, conditioning regimen, GVHD prophylaxis, sex disparity, type of donor, and presence of acute GVHD and chronic GVHD [16,35–45]. All tests were two-sided and the type 1 error rate was fixed at 0.05. The Statistical Package for Social Scientists (SPSS 13.0; SPSS, Inc., Chicago, IL, USA) was used for data management and analysis by the Kaplan–Meier and Cox methods.

Results

Patient characteristics and eosinophilia

The clinical characteristics of our 237 evaluable patients are shown in Table 1. Of these patients, 230 had hematologic malignancies and seven had aplastic anemia. Blood eosinophilia was observed in 135 patients (57%, Eo group), while it was not observed in 102 patients (Non-Eo group). The median time to the onset of eosinophilia was 39 days after allo-SCT (range: 13–87 days). Eosinophilia was observed in 1–28 samples (median, six samples) per patient. One hundred twenty-five patients (92.5%) had more than two samples with eosinophilia. There were no significant differences in patient characteristics, including sex, age, disease and disease status, time from diagnosis to allo-SCT, stem cell source, conditioning, GVHD prophylaxis, sex disparity, or type of donor, between the Eo and Non-Eo groups, nor was there any difference in frequency of blood sampling between the two groups.

Acute and chronic GVHD

The incidence of grades II–IV acute GVHD for the Eo and Non-Eo groups were 43% and 69%, respectively ($P < 0.001$; Table 2). The estimates of cumulative incidence of grades II–IV acute GVHD were significantly higher in the Non-Eo group than in the Eo group ($P < 0.001$; Fig. 1). The difference in the estimates of cumulative incidence of grades II–IV acute GVHD between the Eo and Non-Eo groups was similarly significant if eosinophilia was defined as an REC >4% within the first 50 days after allo-SCT (38% vs. 68%; $P < 0.001$). The median time to the onset of acute GVHD was 23 days (range: 6–83 days) in the Eo group, and 16 days (range: 6–58 days) in the Non-Eo group. Of 85 recipients with acute GVHD and eosinophilia after allo-SCT, acute GVHD preceded eosinophilia by a median of 21 days (range: 1–80 days) in 42 patients, and eosinophilia preceded acute GVHD by a median of 47 days (range: 2–75 days) in 41 patients. In the remaining two patients, eosinophilia and acute GVHD developed concurrently. With respect to affected organs, the incidence of skin GVHD was similar between the two groups; however, that of liver GVHD was significantly lower in the Eo group ($P = 0.004$; Table 2). Although the incidence of gut GVHD between the two groups was not statistically significant, there was a trend toward higher incidence in the Non-Eo group. In 15 of 58 (26%) patients in the Eo group and 41 of 70 (59%) patients in the Non-Eo group, acute GVHD was resistant to standard doses of PSL and the patients required salvage therapy; this proportion was also significantly lower in the Eo group ($P = 0.022$). Of the 58 patients with eosinophilia and grades II–IV acute GVHD, 26 patients received systemic steroids for acute GVHD after developing the eosinophilia. In 22 (85%) of the 26 patients, eosinophilia was no longer observed after glucocorticoid administration was initiated.

The incidence rates of chronic GVHD in the Eo and Non-Eo groups were 56% and 73%, respectively

Table 1. Patient characteristics.

	Total	Eosinophilia (+), no. (%)	Eosinophilia (–), no. (%)	*P*-value
Number	237	135	102	
Gender				
Male	129	71 (53)	58 (57)	0.513
Female	108	64 (47)	44 (43)	
Age (year)				
Median, range	41, 16–62	41, 16–62	41, 18–61	0.134
Disease				
AML	70	39 (29)	31 (30)	0.096
ALL	39	16 (12)	23 (23)	
CML	41	22 (16)	19 (19)	
MDS	40	28 (21)	12 (12)	
NHL	25	19 (14)	6 (6)	
MM	9	6 (4)	3 (3)	
AA	7	2 (2)	5 (5)	
ATL	3	2 (2)	1 (1)	
MF	3	1 (1)	2 (2)	
Disease status*				
Standard-risk	113	58 (43)	55 (54)	0.094
High-risk	124	77 (57)	47 (46)	
Interval from diagnosis to allo-SCT (d)				
Median	371	338	406	0.063
Range	76–6014	85–3665	76–6014	
Stem cell source				
BM	182	98 (73)	84 (82)	0.203
PB	40	26 (19)	14 (14)	
CB	15	11 (8)	4 (4)	
Conditioning				
TBI-based	168	89 (66)	79 (77)	0.159
BU-based	20	13 (10)	7 (7)	
Flu-based	49	33 (24)	16 (16)	
GVHD prophylaxis				
CsA alone	11	6 (4)	5 (5)	0.876
CsA-based†	97	58 (43)	39 (38)	
Tacrolimus alone	4	2 (2)	2 (2)	
Tacrolimus-based†	125	69 (51)	56 (55)	
Duration of G-CSF after allo-SCT (d)				
Median	21	20	23.5	0.159
Range	5–95	5–78	12–95	
Donor				
Sex disparity				
Male to male	94	52 (39)	42 (41)	0.88
Female to female	57	35 (26)	22 (22)	
Male to female	51	29 (22)	22 (22)	
Female to male	35	19 (14)	16 (16)	
Age (year)				
Median	33	34	32	0.114
Range	0–64	0–64	0–62	

($P = 0.014$; Table 2). The estimates of cumulative incidence of chronic GVHD were significantly higher in the Non-Eo group than in the Eo group ($P = 0.011$; Fig. 2). The median time to the onset of chronic GVHD was 116 days (range: 69–727 days) in the Eo group, and 120 days (range: 59–276 days) in the Non-Eo group.

Relapse and NRM

In the 230 patients with hematologic malignancies, the cumulative incidence of relapse was similar between the Eo and Non-Eo groups (33% vs. 27%, respectively; $P = 0.438$; Fig. 3). On the other hand, the cumulative NRM was 10% for the Eo group compared with 31% for the

Table 1. Continued

	Total	Eosinophilia (+), no. (%)	Eosinophilia (–), no. (%)	*P*-value
Relationship				
HLA-matched sibling donor	89	57 (42)	32 (31)	0.128
HLA-mismatched related donor	7	4 (3)	3 (3)	
HLA-matched unrelated donor	114	56 (42)	58 (57)	
HLA-mismatched unrelated donor	27	18 (13)	9 (9)	

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic disease; NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; AA, aplastic anemia; ATL, adult T-cell leukemia; MF, myelofibrosis; BM, bone marrow; PB, peripheral blood; CB, cord blood; TBI, total body irradiation; BU, busulfan; Flu, fludarabine; GVHD, graft-versus-host disease; CsA, cyclosporine A; G-CSF, granulocyte colony-stimulating factor; allo-SCT, allogeneic stem cell transplantation.

*Standard-risk, acute leukemia in first or second remission, Philadelphia chromosome-positive chronic myeloge-nous leukemia in first or second chronic phase, chemotherapy-sensitive lymphoma, and myelodysplastic syndrome without excess of blasts; high-risk, all other diseases.

†Methotrexate was omitted because of severe mucositis, renal impairment or ascites (17 in the eosinophilia group; 21 in the noneosinophilia group; $P = 0.165$).

Table 2. Relationship between eosinophilia after allogeneic stem cell transplantation and GVHD.

	Eosinophilia (+), no. (%)	Eosinophilia (–), no. (%)	*P*-value
Acute GVHD			
No. of evaluable patients	135	102	
Grade			<0.001
0–I	77 (57)	32 (32)	
II–IV	58 (43)	70 (69)	
Affected [o]rgan			
Skin	75 (56)	73 (72)	0.872
Gut	35 (26)	45 (44)	0.089
Liver	4 (3)	16 (16)	0.004
Chronic GVHD			
No. of evaluable patients	129	85	
Grade			0.014
Limited	12 (9)	11 (13)	
Extensive	60 (47)	51 (60)	

GVHD, graft-versus-host disease.

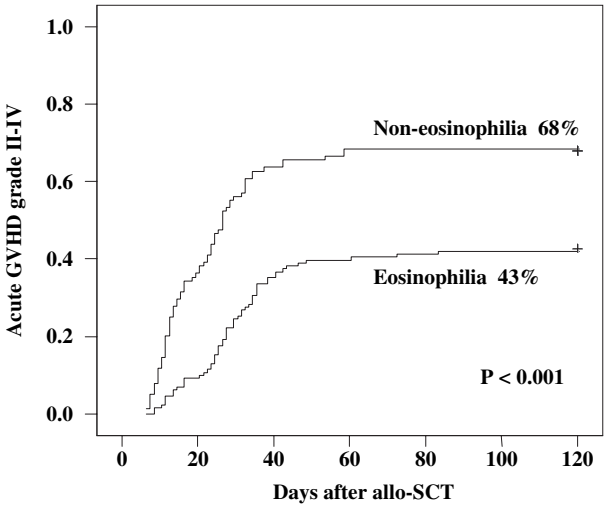

Figure 1 Cumulative incidence of grades II–IV acute graft-versus-host disease (GVHD) for patients with and without eosinophilia after allogeneic stem cell transplantation allo-SCT. Patients with post-transplant eosinophilia showed a significantly lower incidence of grades II–IV acute GVHD compared to those without eosinophilia (43% vs. 68%, $P < 0.001$).

Non-Eo group ($P < 0.001$; Fig. 4). The primary cause of death in the Eo group was recurrence of primary disease, while acute GVHD and infection in addition to recurrence contributed primarily to death in the Non-Eo group ($P = 0.008$; Table 3).

Overall survival and prognostic analysis

A total of 154 patients (65%) were alive at the time of analysis with a median follow-up period of 41.3 months. Overall survival was significantly better in the Eo group than in the Non-Eo group, with an estimated OS at 3 years of 67% for the Eo group and 51% for the Non-Eo group ($P = 0.003$; Fig. 5).

Univariate analysis showed the following variables to be significant for reduced survival: age above 40 years ($P = 0.011$), high-risk disease ($P < 0.001$), and the presence of grades II–IV acute GVHD ($P < 0.001$), and the absence of eosinophilia after allo-SCT (Table 4). To further assess the influence on OS of eosinophilia after allo-SCT, we performed multivariate Cox regression analysis, including

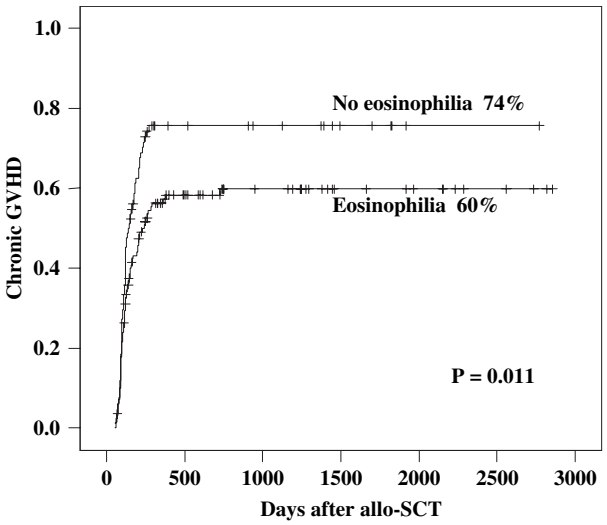

Figure 2 Cumulative incidence of chronic graft-versus-host disease (GVHD) for patients with and without eosinophilia after allogeneic stem cell transplantation. Patients with eosinophilia showed a significantly lower incidence of chronic GVHD compared with those without eosinophilia (60% vs. 74%, $P = 0.011$).

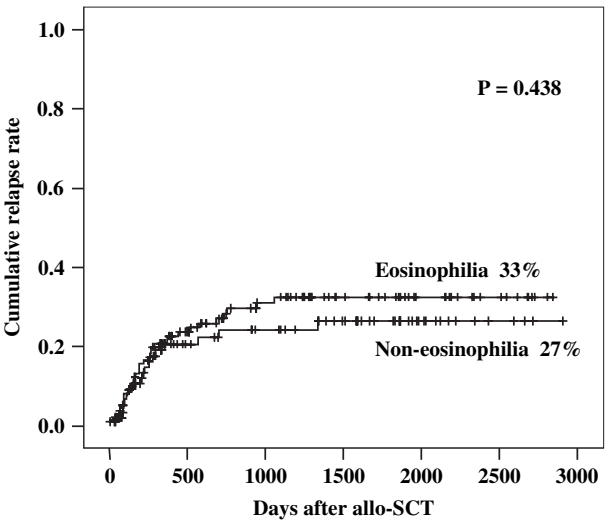

Figure 3 Cumulative relapse rate for patients with and without eosinophilia after allogeneic stem cell transplantation in a population of patients with hematologic malignancies. A comparison of patients with post-transplant eosinophilia with those without eosinophilia revealed no significant difference in the cumulative relapse rate (33% vs. 27%, $P = 0.438$).

all variables that were found to be significant variables in the univariate analysis, as well as sex disparity between recipient and donor. Under this analysis, age above 40 years [$P = 0.019$; relative risk (RR) 1.704], high-risk disease ($P < 0.001$; RR 3.666), grades II–IV acute GVHD ($P = 0.054$, RR 1.604), sex disparity ($P = 0.022$, RR 2.008), and the absence of eosinophilia after allo-SCT ($P = 0.001$, RR 0.457) remained as significant factors for reduced OS.

We also carried out a subgroup analysis, dividing the two groups according to the degree of eosinophilia (REC > 4% vs. >10%), which showed no significant differences of the incidence of acute GVHD, relapse rate or OS between the patients with eosinophilia after allo-SCT and those without eosinophilia (data not shown).

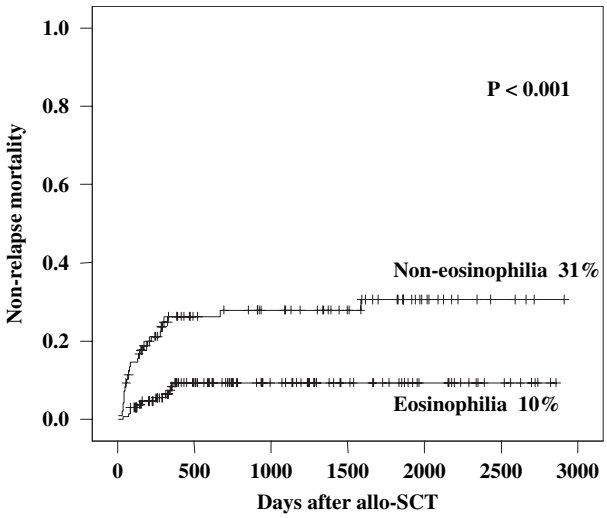

Figure 4 Cumulative nonrelapse mortality (NRM) for patients with and without eosinophilia after allogeneic stem cell transplantation. Patients with post-transplant eosinophilia showed a significantly lower NRM compared to those without eosinophilia (10% vs. 31%, $P < 0.001$).

Table 3. Causes of death.

	Eosinophilia (+), no. (%)	Eosinophilia (–), no. (%)	*P*-value
No. of death	37	46	
Relapse	25 (68)	17 (37)	0.008
Nonrelapse causes	12 (32)	29 (63)	
Acute GVHD	3	11	
Chronic GVHD	3	6	
Infection	4	8	
Organ failure	2	4	

GVHD, graft-versus-host disease.

Discussion

The reported incidence of blood eosinophilia after allo-SCT ranges from 10% to 39% [15,17–19, 24]. In this study, the incidence of eosinophilia within 100 days after allo-SCT was 57% in all recipients. This is somewhat higher than the rates reported in previous studies.

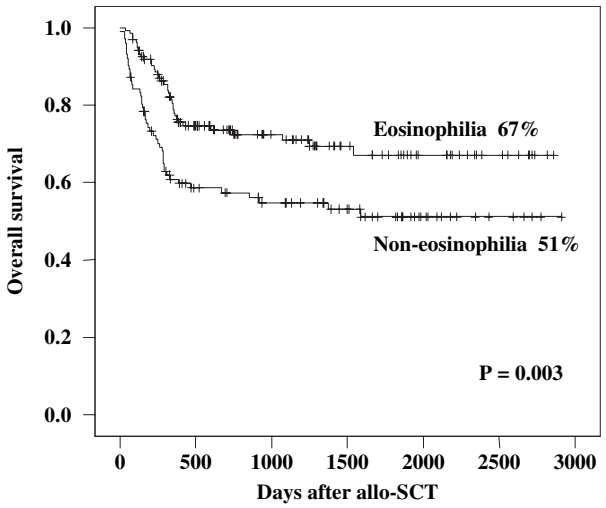

Figure 5 Overall survival (OS) for patients with and without eosinophilia after allogeneic stem cell transplantation. Patients with post-transplant eosinophilia showed a significantly higher OS than those without eosinophilia (67% vs. 51%, $P = 0.003$).

However, among the reports, a precise comparison is difficult because of the difference in the definition of eosinophilia [13,15–17,19,22,24]. In this study, eosinophilia was defined as peripheral blood eosinophil percentage >4% according to the recent reports [13,17].

The present study showed that the incidence and severity of acute GVHD were significantly lower in patients with eosinophilia, when compared with those in noneosinophilia patients. Because of the difference in the incidence and severity of acute GVHD, the effect of systemic steroid given for the treatment of acute GVHD on eosinophilia should be taken into consideration. However, some reports have already shown a predictive or diagnostic value of eosinophilia for acute GVHD [22,23], while one study failed to demonstrate any definite relationship between eosinophilia and acute GVHD [24]. The results of our study are not consistent with those of the previous studies. Current evidence suggests that Th1-type inflammatory response, characterized by the release of IL-2, IL-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, is predominantly involved in the development of acute GVHD [46,47], while eosinophilia is primarily observed in Th2 reactions. It is possible that acute GVHD-induced Th1 reactions are offset by Th2 reactions reflected by eosinophilia. Regulatory T cells have been shown to mediate their regulatory function by producing a distinct profile of immunosuppressive cytokines, including IL-10 [48]. Weston *et al.* [49] showed that a higher capacity of donor cells producing IL-10 is strongly associated with both lower incidence and lower severity of acute GVHD. Furthermore, Lin *et al.* [50] showed that the presence of the IL-10/-592*A allele in the patient and of the IL-10RB/238*G allele in the donor is associated with a significantly reduced risk of severe acute GVHD. Neither detailed cytokine profiles nor evidence of cytokine gene polymorphism was available in this study, but we speculate that the association between eosinophilia and low incidence of acute GVHD observed in this study may reflect the immunosuppressive role of Th-2 cytokines, including IL-10. In addition, responsiveness to first-line steroid therapy for acute GVHD was better in patients with eosinophilia than in those without, which supports our speculation. To the best of our knowledge, this is the first report demonstrating the association of responsiveness to therapy for acute GVHD with eosinophilia.

It has been earlier reported that recipients with chronic GVHD after allo-SCT frequently develop eosinophilia [15–19]. This coexistence of eosinophilia with chronic

Table 4. Factors associated with overall survival after allogeneic stem cell transplantation.

		Multivariate		
	Univariate *P*-value	Relative risk	95% confidence interval	*P*-value
Age (≤40 vs >40)	0.011	1.704	1.092, 2.658	0.019
Gender (male vs. female)	0.101	–	–	–
Disease status (low-risk vs. high-risk)	0.001	3.666	2.230, 6.026	<0.001
Stem cell source (BM vs. PB vs. CB)	0.921	–	–	–
Conditioning (RIST vs. conventional)	0.829	–	–	–
GVHD prophylaxis (CsA vs. tacrolimus)	0.615	–	–	–
Donor type (related vs. unrelated)	0.722	–	–	–
Donor age (≤40 vs. >40)	0.376	–	–	–
Sex disparity (female-female vs. other)	0.052	2.008	1.105, 3.647	0.022
Acute GVHD (0-I vs. II-IV)	0.001	1.604	0.993, 2.593	0.054
Chronic GVHD (No vs. Yes)	0.883	–	–	–
Eosinophilia (No vs. Yes)	0.003	0.457	0.289, 0.722	0.001

BM, bone marrow; PB, peripheral blood; CB, cord blood; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; CsA, cyclosporine A.

GVHD has been associated with the exacerbation of chronic GVHD [19]. In our study, the incidence of chronic GVHD, which arose after 100 days after allo-SCT, was significantly lower in patients with eosinophilia compared with that in noneosinophilia patients. Because of the difference in the timing of evaluating eosinophilia, it is impossible to compare the results of our study with those of other studies. However, it is notable that an association of the absence of eosinophilia within 100 days after allo-SCT with the subsequent development of chronic GVHD has been shown in our study.

In this study, we demonstrated a favorable impact of eosinophilia within 100 days after allo-SCT on OS. In multivariate analyses, eosinophilia after allo-SCT as well as younger age and lower disease risk at the time of transplant, the absence of acute GVHD, and sex match between recipient and donor were found to be significant factors for better OS after allo-SCT. We also demonstrated a decrease in NRM in recipients with eosinophilia after allo-SCT compared to those without eosinophilia. We believe that the reduction in the severity and refractoriness to PSL of acute GVHD contributed to a low NRM and a better OS. To the best of our knowledge, there has been only one previous study investigating the relationship between post-transplant eosinophilia and survival after allo-SCT with an adequate number of patients [24]. Sato *et al.* [24] found that the occurrence of eosinophilia after allo-SCT was associated with a decrease in post-transplant relapse and improved OS in children with hematologic disorders. They speculated that the increase in serum IL-12 after allo-SCT promoted the graft-versus-tumor effect. In addition, Przepiorka *et al.* [16] state that eosinophilia was more frequent in patients with low-risk chronic GVHD than in those with high-risk chronic GVHD (47% vs. 12%). In their study, platelet count was used to categorize the risk of chronic GVHD as high (platelet count $<100 \times 10^9$/l) or low (platelet count exceeding 100×10^9/l), which correlated significantly with survival [16]. Therefore, post-transplant eosinophilia could be a favorable prognostic factor for chronic GVHD and possible survival. In contrast, significant difference in relapse rate between recipients with eosinophilia and those without eosinophilia was not observed in this study. It has been reported that the graft-versus-tumor effect is scarcely associated with acute GVHD in adults [51–54]. Thus, the significant reduction of acute GVHD in patients with post-transplant eosinophilia may not have contributed to the increase in relapse rate. The other possible explanation is that Th2 and type 2 $CD8^+$ T cytotoxic cells, which secrete type 2 anti-inflammatory cytokines possessing suppressive effects on GVHD, while accelerating graft-versus-tumor reactions, could be involved in post-transplant eosinophilia [55,56].

In conclusion, we have demonstrated that patients with eosinophilia which arose within 100 days after allo-SCT possessed a significantly higher OS and lower incidence of both acute and chronic GVHD and NRM compared to those without eosinophilia. These data led us to conclude that eosinophilia after allo-SCT may serve as a favorable prognostic marker. However, as this is a single center study of Japanese patients, these results should be re-evaluated in a multicenter setting, and the effect of ethnicity should also be examined. Further prospective studies, including detailed cytokine profiling, are essential for an understanding of the pathophysiologic process behind post-transplant eosinophilia.

Authorship

YA performed the study, analyzed data, wrote and revised the paper, and finally approved the paper. TM designed the study, and wrote and revised the paper. TN, TS, RY equally contributed to the data collection and analysis. YI designed the study. SO designed the study, revised the paper, and finally approved the paper.

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