Bone Marrow Transplantation (2011) 46, 379–384

www.nature.com/bmt

ORIGINAL ARTICLE

Air-leak syndrome following allo-SCT in adult patients: report from the Kanto Study Group for Cell Therapy in Japan

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We retrospectively investigated air-leak syndrome (ALS), including pneumothorax and mediastinal/s.c. emphysema, following allogeneic hematopoietic SCT. Eighteen patients (1.2%) developed ALS among 1515 undergoing SCT between 1994 and 2005 at the nine hospitals participating in the Kanto Study Group on Cell Therapy. The median onset of ALS was at 575 days (range: 105–1766) after SCT and 14 patients (77.8%) had experienced late onset noninfectious pulmonary complications (LONIPC) before ALS. Chronic GVHD (cGVHD) was the strongest risk factor for ALS (odds ratio 13.5, $P=0.013$ by multivariate analysis). Repeat SCT, male sex and age <38 years at the time of transplantation were also significant risk factors for ALS. Patients with ALS had a significantly worse survival rate than those without ALS (61.5 vs 14.9% at 3 years; $P=0.000$). The main cause of death was respiratory complications in 8 of the 18 patients. In conclusion, ALS is a rare complication of SCT that is more likely to occur in relatively young male patients with cGVHD and/or LONIPC. It is possible that better understanding and treatment of LONIPC may lead to prevention of ALS.

Bone Marrow Transplantation (2011) **46,** 379–384; doi:10.1038/bmt.2010.129; published online 31 May 2010
Keywords: air-leak syndrome; allo-SCT; late onset noninfectious pulmonary complications; chronic GVHD

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Received 14 October 2009; revised 12 April 2010; accepted 17 April 2010; published online 31 May 2010

Introduction

The survival of patients with hematological disorders who receive allo-SCT has shown continuing improvement due to the introduction of various innovative therapeutic approaches. However, organ dysfunction/damage and infection remain problematic for long-term survivors after SCT, especially those with chronic GVHD (cGVHD), and such complications affect both their quality of life and survival. Late onset noninfectious pulmonary complications (LONIPC) are one of the most common manifestations of organ damage during the late phase after allo-SCT,[1–5] and have been reported to be the major cause of death for patients in this phase. Unlike LONIPC, air-leak syndrome (ALS), which includes pneumothorax (PT), mediastinal emphysema (ME) and s.c. emphysema (SE), is a relatively rare complication of SCT.[6–9] Although patients with ALS after SCT had a fatal outcome according to some case reports or small-scale retrospective studies, the clinical features of post transplant ALS remain obscure. The purpose of this study was to clarify the characteristics and risk factors for ALS after SCT, as well as its effect on survival.

Materials and methods

Patients

We retrospectively surveyed 1515 patients aged ⩾15 years who received allo-SCT between January 1994 and March 2005 at nine hospitals participating in the Kanto Study Group on Cell Therapy (KSGCT) in Japan. Detailed clinical data were collected by reviewing the medical records of each institution, whereas baseline pretransplant and post transplant information on the patients was retrieved from the KSGCT database.

Definitions

Air-leak syndrome was diagnosed from chest X-ray films and/or computed tomography scans as follows: PT was diagnosed by detection of extra-alveolar air in the left and/or right hemithorax, ME was defined as the presence of extra-alveolar air in the mediastinal space and SE was defined as extra-alveolar air in the s.c. tissue. Iatrogenic ALS that occurred after procedures such as trans-bronchial lung biopsy or mechanical ventilation was excluded. cGVHD and LONIPC were diagnosed from previously reported criteria.[10,11]

The case report form for ALS patients included the following information: date of diagnosis of ALS, type of ALS (described above), initial symptoms (cough, dyspnea and chest pain), presence of active cGVHD at the onset of ALS (yes or no), type of cGVHD (limited or extensive), presence of LONIPC (bronchiolitis obliterans (BO), bronchiolitis obliterans with organizing pneumonia (BOOP) and interstitial pneumonia (IP)), time from LONIPC to ALS, immunosuppressive therapy at the onset of ALS (prednisolone (PSL), CYA, tacrolimus (FK) and others), treatment of ALS (drain, initiate or increase the dose of immunosuppressive therapy, decrease the dose of immunosuppressive therapy, pleurodesis, observation and others), response to treatment (improved, stable or worsened), outcome and cause of death.

Statistical analysis

To identify risk factors for ALS, we tested the following variables by univariate and multivariate analyses: recipient age and sex, stem cell source, conditioning regimen (conventional vs reduced intensity), use of TBI, number of SCT procedures (first vs second or more), GVHD prophylaxis (CYA based vs FK based), grade of acute GVHD (0–I vs II–IV) and cGVHD (none vs limited or extensive). Comparison of categorical variables was carried out by the χ^2-test or Fisher's exact test, whereas comparison of continuous variables was performed with Student's *t*-test. To evaluate the independence of potential risk factors for ALS, we performed multiple logistic regression analysis. In all analyses, $P<0.05$ was considered to indicate statistical significance. Survival curves after the occurrence of ALS were estimated by the Kaplan–Meier method and the survival of patients with ALS was compared to that of those without ALS by the log-rank test, treating the occurrence of ALS as a time-dependent variable. To determine whether ALS was an independent poor prognostic factor for long-term survival after SCT, we performed Cox proportional hazards analysis including the following variables: recipient age and sex, disease risk (standard risk diseases were acute leukemia in the first or second CR, aplastic anemia, refractory anemia, refractory anemia with ringed sideroblasts, CML in the first chronic phase, multiple myeloma in PR or CR and malignant lymphoma in the first or second CR, whereas all other diseases/states were considered to be high risk), stem cell source, type of conditioning regimen and use of TBI. Statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Clinical features of ALS

Air-leak syndrome was diagnosed in 18 patients (1.2% of all patients) after allo-SCT. Table 1 shows a summary of the baseline characteristics of these patients. Sixteen patients were men and the median age was 29.5 years. Grade II–IV acute GVHD occurred in 11 patients (61%) and all but one patient (94%) had cGVHD. The clinical presentation and outcome of ALS are shown in Table 2. The median time of onset was day 575 (range: days 105–1766) after SCT. ALS was classified as PT in seven patients, ME/SE in six patients and PT combined with ME/SE (mixed ALS) in five patients. At the onset of ALS, 16 patients had active cGVHD. Before ALS occurred, 14 patients had also experienced LONIPC, including 4 with BO, 4 with BOOP, 5 with IP and 1 with IP along with BOOP. At the diagnosis of ALS, LONIPC had resolved in two patients (nos. 5 and 18), but persisted in the remaining 12 patients. The median time from the diagnosis of LONIPC to the onset of ALS among the patients who had persistent LONIPC was 74 days (range: 3–1177 days). Seventeen patients (94%) had been treated with steroids and 13 patients (72%) were on steroids at the time of diagnosis of ALS. Treatment of ALS included drainage in 11 patients and pleurodesis in 3. ALS improved in 12 of 18 patients, remained stable in 2 patients and progressed despite treatment in 4 patients. The three patients (nos. 7, 12 and 13) who underwent pleurodesis all had progressive ALS (Table 2).

Eleven patients died at a median of 222 days after the occurrence of ALS (range: 6–944 days). Respiratory complications were the direct cause of death in eight patients, including ALS in four, BO in two and IP in two. Patient 10 did not have LONIPC before the occurrence of ALS, but BO appeared after improvement of ALS and became progressively worse. Two nonrespiratory deaths were attributable to relapse of the primary disease and one was due to multiple organ failure that was unrelated to ALS. Among the patients with PT or ME/SE, seven out of nine who responded to treatment are still alive, whereas all four patients whose initial therapy

Table 1 Summary of baseline characteristics of the patients with ALS

No. of patients (%)	18 (1.2%)
Sex (male/female)	16/2
Median age (range)	29.5 (16–53)
Primary disease (AML/ALL/CML/ML)	9/5/2/2
Disease status at HSCT (CR or CP/NR or BC)	14/4
Donor source (RBM/UBM/PB/CB)	6/8/4/0
Conditioning (myeloablative/nonmyeloablative)	17/1
TBI or TLI (yes/no)	15/3
No. of SCT (first/second or more)	5/13
GVHD prophylaxis (CYA based/FK based)	8/8
aGVHD (0–I/II–IV)	6/12
cGVHD (no/limited/extensive)	1/2/15

Abbreviations: aGVHD = acute GVHD; BC = blastic crisis; CB = cord blood; cGVHD = chronic GVHD; CP = chronic phase; FK = tacrolimus; ML = malignant lymphoma; NR = non remission; RBM = related BM; RPB = related PBSCs; TLI = total lymphoid irradiation; UBM = unrelated BM.

Table 2 Clinical presentation and outcome of 18 patients with ALS

Patient no.	*Time from SCT to ALS (days)*	*Type of ALS*	*Initial symptoms*			*Active cGVHD at onset of ALS/ type of cGVHD*	*Active LONIPC at onset of ALS*	*Time from LONIPIC to ALS (days)*	*Immuno-suppression at onset of ALS*	*Treatment of ALS*	*Response to therapy*	*Outcome (cause of death)*
			Cough	*Dyspnea*	*Chest pain*							
1	1332	PT (one side)	+	+	–	Yes/Limit	BOOP	264	—	Drainage	Improved	Alive
2	296	ME/SE	+	+	–	Yes/Ext	BO	36	PSL + FK	Observation	Improved	Alive
3	257	ME/SE	–	–	–	Yes/Ext	IP	20	—	Observation	Improved	Died (IP)
4	636	PT (both sides)	–	+	–	No	—		—	Drainage	Improved	Alive
5	600	PT (one side)	–	–	+	Yes/Ext	(BOOP; resolved)[a]		PSL + FK	Drainage	Improved	Alive
6	1766	ME/SE	–	–	–	Yes/Ext	BOOP	170	PSL + CYA	Increase IS	Improved	Alive
7	806	PT (both sides)	+	+	–	Yes/Ext	BOOP	112	PSL + FK	Drainage, increase IS, pleurodesis	Progressed	Died (ALS)
8	155	PT (one side)	+	+	+	Yes/Ext	BO	3	PSL + CYA	Drainage, increase IS	Improved	Died (primary disease)
9	145	Mixed	–	–	–	Yes/Ext	IP	13	PSL + FK	Observation	Improved	Died (primary disease)
10	185	PT (both sides)	–	–	+	Yes/Ext	—		PSL + FK	Drainage	Unchanged	Died (BO)
11	163	Mixed	–	–	+	Yes/Limit	IP	18	PSL + FK	Drainage	Improved	Died (IP)
12	584	Mixed	+	+	+	Yes/Ext	BO	170	PSL + FK	Drainage, increase IS, pleurodesis	Progressed	Died (ALS)
13	1378	Mixed	+	+	+	Yes/Ext	BOOP/IP	1177	PSL + FK	Drainages, increase IS, pleurodesis	Progressed	Died (ALS)
14	105	ME/SE	–	–	–	Yes/Ext	—		PSL + CYA	Observation	Improved	Alive
15	565	Mixed	–	+	–	Yes/Ext	—		PSL + CYA	Drainage	Improved	Died (MOF)
16	841	ME	+	+	–	Yes/Ext	BO	170	—	Start IS	Unchanged	Died (BO)
17	209	ME/SE	–	+	–	Yes/Ext	IP	23	PSL + CYA	Increase PSL	Progressed	Died (ALS)
18	1438	PT (one side)	–	+	+	No	(IP; resolved)[a]		—	Drainage	Improved	Alive

Abbreviations: ALS = air-leak syndrome; BO = bronchiolitis obliterans; BOOP = bronchiolitis obliterans with organizing pneumonia; Ext = extensive type; FK = tacrolimus; IP = interstitial pneumonia; IS = immunosuppressants; Limit = limited type; ME = mediastinal emphysema; mixed = PT with ME/SE; MOF = multiple organ failure; PSL = prednisolone; PT = pneumothorax; SE = s.c. emphysema.
[a]At the diagnosis of ALS, LONIPC had already resolved.

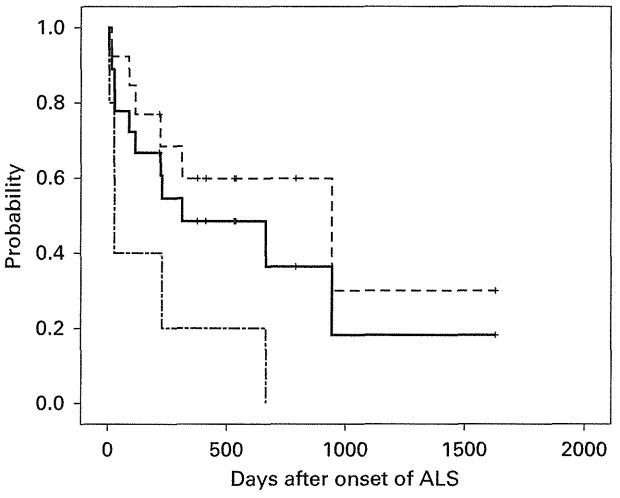

Figure 1 OS after the onset stratified according to the type of ALS. ——— OS of all 18 patients. ---- OS of patients with PT and ME/SE ($n=13$). -·-·- OS of patients with mixed ALS ($n=5$). Patients with PT and ME/SE showed better survival than those with mixed ALS ($P=0.017$).

failed eventually died of LONIPC and/or ALS. In contrast, all five patients with mixed ALS died regardless of their responses to treatment and three of them died of progressive lung disease.

For all 18 patients, OS at 1 year after the onset of ALS was $48.5 \pm 12.1\%$ and the 3-year survival rate was $18.2 \pm 14.6\%$ (Figure 1). Patients with PT and ME/SE showed better survival than did those with mixed ALS (59.8 vs 20.0% at 1 year and 29.9 vs 0% at 3 years, respectively; $P=0.017$; Figure 1). The patients without active LONIPC at the diagnosis of ALS ($n=6$) had a higher survival rate at 3 years than those with active LONIPC (53.3 vs 0%; $P=0.15$; data not shown).

Risk factors for ALS

Air-leak syndrome was always diagnosed more than 3 months after SCT in this study, so we compared the clinical features of patients with or without ALS who survived for more than 90 days after transplantation (1142 recipients). According to univariate analysis, ALS was significantly more frequent in recipients with cGVHD ($P=0.001$), those who received a second or subsequent SCT ($P=0.043$), younger recipients ($P=0.013$) and male recipients ($P=0.013$; data not shown). We also evaluated the risk factors for ALS by logistic regression analysis in 1047 recipients, after excluding 95 recipients (8.3%) because complete data were not available. The median age of the recipients was 38 years, so we divided them into two groups aged <38 and $\geqslant 38$ years. Chronic GVHD was identified as the strongest risk factor for ALS (odds ratio (OR), 13.48; $P=0.013$), whereas second or subsequent SCT (OR, 7.91; $P=0.021$), male sex (OR, 4.95; $P=0.038$), age <38 years (OR, 3.55; $P=0.033$) and FK-based GVHD prophylaxis (OR, 3.3; $P=0.025$) were also identified as independent risk factors (Table 3).

Table 3 Multivariate analysis of factors related to ALS

Variable	*HR*	*95% CI*	P-*value*
Recipient age ($\geqslant 38$)	3.55	1.11–11.37	0.033
Recipient sex, male	4.95	1.10–22.36	0.038
Donor source, unrelated	0.62	0.30–1.27	0.19
No. of SCT $\geqslant 2$	7.91	1.57–39.91	0.021
Reduced-intensity conditioning	0.22	0.02–2.49	0.219
TBI	0.96	0.26–3.54	0.953
GVHD prophylaxis with FK	3.30	1.17–9.36	0.025
Acute GVHD (grade II–IV)	1.51	0.56–4.10	0.193
Chronic GVHD	13.48	1.75–103.89	0.013

Abbreviation: FK, tacrolimus.

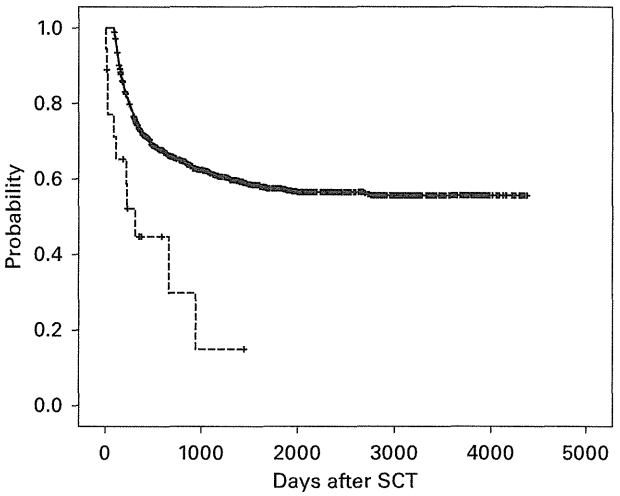

Figure 2 Kaplan–Meier curves for OS after SCT in patients with or without ALS. ——— ALS(−) ($n=1124$). ---- ALS(+) ($n=18$). Recipients with ALS showed significantly worse survival after SCT than those without ALS ($P=0.002$).

Impact of ALS on OS

The patients with ALS had significantly lower survival rates (44.7% at 1 year and 14.9% at 3 years) compared with those without ALS (72.7% at 1 year and 61.5% at 3 years; $P=0.002$; Figure 2). We analyzed factors associated with lower OS among 1079 recipients who lived for more than 90 days after allo-SCT by multivariate Cox regression analysis (63 patients (5.5%) were excluded because of incomplete data). ALS was identified as an independent predictor of worse survival after SCT (OR, 3.468; $P=0.001$), as was a high-risk disease status (OR, 2.851; $P=0.000$) and an age $\geqslant 38$ years (OR, 1.267; $P=0.016$; Table 4).

Discussion

All forms of thoracic air leak are defined as ALS according to Franquet *et al.*,[12] including spontaneous pneumomediastinum or pneumopericardium, SE, interstitial emphysema and spontaneous PT. There have been only a few reports about ALS associated with SCT. Recently, Toubai *et al.*[8] performed a single-institution retrospective study and found ALS in 5 out of 213 recipients (2.3%)

Table 4 Multivariate analysis of factors associated with worse OS after SCT

Variable	*HR*	*95% CI*	P-*value*
Recipient age (⩾38)	1.27	1.05–1.54	0.016
Recipient sex, male	0.89	0.73–1.08	0.231
Donor source, unrelated	1.07	0.88–1.30	0.528
Reduced-intensity conditioning	0.75	0.53–1.06	0.105
TBI	0.97	0.75–1.25	0.795
Disease status, high risk	2.85	2.34–3.48	0.000
ALS	3.47	1.63–7.40	0.001

Abbreviation: ALS = air-leak syndrome.

following allo-SCT, whereas Vogel *et al.*[9] reported ALS in 7 out of 300 recipients (2.3%). The incidence of ALS in the present study was slightly lower than in these previous studies. In this retrospective multicenter study, we tried to identify the characteristics, risk factors and prognosis of ALS after SCT, but the following limitations of our study must be considered. Unrecognized biases might have influenced the results of this retrospective study, especially as patients with asymptomatic ALS could not be detected. All cases of ALS were diagnosed more than 100 days after SCT in our series. Other studies and case reports have also shown that this complication occurs more than 100 days after SCT,[6–9] and all authors have agreed that ALS can be classified as a late complication of allo-SCT.

We confirmed that cGVHD, second or subsequent SCT, male sex, age <38 years and FK-based GVHD prophylaxis were independent risk factors for ALS. Several case reports have indicated that ALS following SCT is associated with severe cGVHD or noninfectious pulmonary complications such as BO.[6,9,13,14] In our series, 17 out of 18 recipients with ALS experienced cGVHD, and this is the first report to confirm statistically that the occurrence of ALS is strongly associated with cGVHD. It has also been reported that cGVHD is a significant risk factor for the development of LONIPC.[1–4] In this series, most ALS patients also had LONIPC based on cGVHD. Although the mechanism leading to chronic pulmonary GVHD is unknown, it is thought that host-reactive donor T cells cause injury to the lungs.[15] Continuous inflammation due to cGVHD may lead to fibrotic change of the peripheral airways that decreases lung compliance. Chronic GVHD appears to cause the progression of LONIPC, resulting in the occurrence of ALS. Our recipients who received a second or subsequent SCT would have suffered pulmonary damage by the conditioning regimen, which could have contributed to the development of ALS, although the mechanism of lung injury differs from that of cGVHD. According to a review of the literature,[6,7,13,14,16,17] ALS occurred after SCT in 17 men and 6 women with a median age of 30 years (range: 8–51 years). Our study confirmed by multivariate analysis that younger (<38 years old) men have a high risk of developing ALS after SCT. FK-based GVHD prophylaxis is generally chosen if stem cells are obtained from high-risk donors for GVHD, such as unrelated or HLA-mismatched related donors. This might explain the association between FK-based GVHD prophylaxis and development of ALS in our study.

The survival rate of patients after the occurrence of ALS was significantly impaired (18.2 ± 14.6% at 3 years), and the prognosis was related to the type of ALS (mixed ALS vs others). Alveolar rupture can occur because of an elevated intra-alveolar pressure, following damage to the alveolar walls, or for both reasons.[9] The subtypes of ALS reflect the extent of pulmonary/thoracic tissue damage, rather than different pathophysiological processes. This may explain the very poor outcome of mixed ALS. It is well known that the prognosis of secondary PT in patients with COPD is worse compared with idiopathic PT because it usually takes longer to reexpand the lung after a chest tube is inserted and failure of treatment is common.[16] It can be suggested that the onset of ALS in recipients with LONIPC leads to more severe lung tissue damage and a poor prognosis.

In conclusion, we were able to identify a subgroup of SCT recipients with a high risk of developing ALS, namely younger (<38 years) men with cGVHD, second or subsequent SCT and/or FK-based GVHD prophylaxis. As ALS is rare and its etiology is multifactorial, trials of new treatments are not feasible. A more promising strategy may be to improve our understanding and treatment of LONIPC, which should then lead to prevention of ALS.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We thank Satoshi Morita and Tetsuji Kaneko from Yokohama City University Medical Center for the expert statistical advice. We also thank all of the dedicated physicians and paramedical staff at the participating transplantation centers who contributed data to this study.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Eosinophilia, Regardless of Degree, is Related to Better Outcomes after Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract

Objective Several recent studies report that, after allogeneic hematopoietic cell transplantation (allo-HCT), eosinophilia is a favorable factor for transplant outcomes. However, whether the degree of eosinophilia influences transplant outcomes is yet to be established.

Methods We studied 144 patients with hematological malignancy who received allo-HCT at our institution. The stem cell sources were bone marrow in 84 patients, peripheral blood stem cells in 32 patients, and cord blood in 28 patients. One hundred and twelve patients underwent myeloablative conditioning and 49 patients had high-risk disease. We performed semi-landmark analysis to examine the influence of eosinophilia.

Results Eosinophilia developed at a median of 47 days after transplantation in 63 patients (44%). The patients with eosinophilia showed significantly better overall survival (OS) and a lower relapse rate at three years, compared to those without eosinophilia (66% vs 55%, p=0.04 and 30% vs 50%, p=0.002). On analysis following division into groups with mild (500-1,500×10^6/L) and hyper- (>1,500×10^6/L) eosinophilia, three-year OS and relapse rates were 68% and 65% (p=0.92), and 31% and 28% (p=0.90), respectively. On multivariate analysis, eosinophilia was significantly associated with lower relapse rates [HR: 0.5 (95% CI: 0.3-0.9); p=0.01] and the same trend was preserved in the analysis of the mild and hyper-eosinophilic groups.

Conclusion The results suggest that eosinophilia after allo-HCT was associated with better OS and a lower relapse rate, regardless of the levels. The mechanism of this effect is still unclear, and requires study of the pathophysiological process to clarify the relationship between the higher levels of eosinophilia after allo-HCT and organ infiltration.

Key words: eosinophilia, allogeneic hematopoietic cell transplantation, relapse, overall survival

(Intern Med 51: 851-858, 2012)
(DOI: 10.2169/internalmedicine.51.6726)

Introduction

Increased blood eosinophil counts may be caused by many kinds of disease, such as allergy, infection, collagen diseases, pulmonary diseases, and malignant disorders (1). In general, the term "eosinophilia" is defined as a blood eosinophil count that exceeds 500×10^6/L (2). Three levels of severity of eosinophilia have been defined as follows: mild 500-1,500×10^6/L, moderate 1,500-5,000×10^6/L, and severe > 5,000×10^6/L (2). Hypereosinophilia, which refers to eosinophil levels above 1,500×10^6/L, may intrinsically cause tissue and organ damage, regardless of the underlying etiology (2). Recently, several studies have reported that eosinophilia was

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Received for publication October 12, 2011; Accepted for publication January 6, 2012
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related to better outcomes in allogeneic hematopoietic cell transplantation (allo-HCT) (3-6). Better overall survival (OS) in patients with eosinophilia after allo-HCT was shown to be a result of the lower relapse rates (3), lower non-relapse mortality (NRM) (4), and both lower relapse rates and NRM (5). Recently, another study reported that eosinophilia still affected transplant outcomes in the subgroup of patients with acute graft-versus-host disease (GVHD) (6), but no effect was seen in those patients that underwent cord blood transplantation (7). Previous studies reported that activated tissue eosinophils were implicated in the pathogenesis of GVHD (8, 9). Kim et al discussed the possibility that late eosinophilia might simply be a marker of Th2 cytokine activation and that activation of the Th2 pathway correlates with the recovery of humoral immunity, a component in the pathogenesis of chronic GVHD (5). In addition, unlike mild chronic GVHD, severe chronic GVHD resulted in poor outcomes (10). From this perspective, higher eosinophilia may enhance GVHD and thus worsen the prognosis. However, to our knowledge, no reports have addressed the question of the extent to which blood eosinophilia influences the outcome of allo-HCT. In this study, we therefore investigated how the existence and degree of eosinophilia after allo-HCT influenced transplant outcomes.

Materials and Methods

Patients

We retrospectively evaluated 182 consecutive patients with hematological malignancy who underwent allogeneic stem cell transplantation at our institute between January 2004 and April 2011. We excluded 18 patients who died before neutrophil engraftment, and 12 patients who underwent haplo-identical stem cell transplantation. We also excluded eight patients who died or experienced disease relapse or progression before the median onset of eosinophilia, to allow semi-landmark analysis. Ultimately, 144 patients were included in this analysis.

As it was difficult to obtain informed consent for this retrospective study, we made the context of this study known to the public by putting up a notice at our hospital and on our website in accordance with the ethical guidelines for epidemiological research compiled by both the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare in Japan. This retrospective study was approved by the Institutional Review Board of our institute.

Transplantation procedures

Myeloablative and reduced-intensity conditioning were used in 111 and 33 patients, respectively. We used the definition of conditioning intensity from previous reports (11, 12). Myeloablative conditioning was used in 111 patients, with total body irradiation of 12 Grays in 42 patients and busulfan (Bu)-based conditioning in 62 patients. In contrast, in the 33 patients who underwent reduced-intensity conditioning, Bu-based conditioning was used in 20 patients, melphalan-based conditioning in 10 patients, and cyclophosphamide-based conditioning was used in two patients.

For acute GVHD prophylaxis, cyclosporine A was used alone in 10 patients, cyclosporine A and short-term methotrexate (sMTX) were used in 112 patients, cyclosporine A and mycophenolate mofetil (MMF) were used in five patients, tacrolimus was used alone in three patients, tacrolimus and sMTX were used in five patients, tacrolimus and MMF were used in eight patients, and no treatment was given to one patient. Antithymocyte globulin was used in just two patients. In 116 patients transplanted from either a related peripheral blood stem cell/ bone marrow donor or unrelated bone marrow donor, 93 pairs (sibling: 33; unrelated: 60) were matched for six of six HLA-A, B, and DRB1 alleles, and 23 pairs (related: 8; unrelated: 15) were matched for five of six of these alleles. In 28 patients transplanted with cord blood, seven pairs were serologically matched for five of six at HLA-A, B, and DR loci; 20 pairs were matched for four of six; and one pair was matched for three of six loci. Acute and chronic GVHD were evaluated based on established criteria (13, 14). In general, grade II to IV acute GVHD was treated initially with prednisolone or methylprednisolone (1-2 mg/kg/day), except for grade II acute GVHD involving just the skin, following CBT.

Eosinophilia was defined as an absolute eosinophil count of $\geq 500\times10^6$/L on microscopy of peripheral blood on at least two consecutive days after neutrophil engraftment, except for eosinophilia that occurred after relapse or progression of the original disease. We analyzed the influence on transplant outcome of not only the presence of eosinophilia, but also the degree of eosinophilia. The group with eosinophilia (63 patients) was stratified into two groups, the mild eosinophilia group (peripheral blood eosinophil count: 500-1,500$\times10^6$/L, 35 patients) and the hypereosinophilic group (more than 1,500$\times10^6$/L, 28 patients) based on the maximum eosinophil count after transplantation.

Statistics

To compare patient characteristics, the Chi-square and Wilcoxon tests were used for categorical and metric variables, respectively. To evaluate the effect of eosinophilia on OS, relapse, and non-relapse mortality (NRM), we employed "semi-landmark plots" (5). In patients who developed eosinophilia, the day of onset after transplant was defined as the landmark day. On the other hand, in patients who did not develop eosinophilia, day 47 after transplant, which was the median onset day for eosinophilia, was defined as the landmark day. OS, relapse and NRM were calculated based on the interval from the landmark day. OS analyses were performed by the Kaplan-Meier method, and a log-rank test was employed to assess the difference between two groups. Cumulative incidences of NRM and relapse were analyzed under the assumption that they represented competing risks,

Table 1. Patient Characteristics

	Eosinophilia-	Eosinophlia+	p
No. of patients, n	81	63	
Median recipient age at transplantation, years (range)	47(16-69)	42(17-64)	0.28
Recipient sex, n (male/female)	47/34	33/30	0.50
Disease, n			0.19
Acute myeloid leukemia	31	24	
Acute lymphoid leukemia/ Lymphoblastic leukemia	14 (Ph+:4, LBL:1)	17(Ph+:7, LBL:1)	
Myelodysplastic syndrome	10	8	
Chronic myeloid leukemia	4	4	
Acute leukemia of ambiguous lineage	1	3	
Aggressive Lymphoma	17	4	
Adult T cell leukemia/lymphoma	10	3	
Diffuse large B cell lymphoma	2	1	
NK/T cell lymphoma	1	0	
Peripheral T cell lymphoma	2	0	
Anaplastic large cell lymphoma	2	0	
Indolent Lymphoma	4	2	
Follicular lymphoma	3	2	
Mantle cell lymphoma	1	0	
Chronic active EB virus infection	0	1	
Disease status, n			0.12
Standard	49	46	
High-risk	32	17	
Cytomegalovirus serostatus, n			0.17
Recipient-/Donor-	4	7	
Other	77	56	
Sex mismatch, n			0.26
Female donor to male recipient	16	8	
Other	65	55	
ABO blood type incompatibility, n			0.02
Match	35	40	
Mismatch	46	23	
Stem cell source, n			0.13
Bone marrow	53	31	
Peripheral blood	16	16	
Cord blood	12	16	
HLA matching (at A, B, DR allele), n			0.49
Sibling match	19	18	
Other	62	45	
GVHD prophylaxis, n			0.30
Cyclosporine A-based	70	57	
Tacrolimus-based	11	5	
Anti-thymocyte globulin +/-	1/81	1/63	0.85
Intensity of Conditioning regimen, n			0.86
Myeloablative	62	49	
Reduced-intensity	19	14	
Conditioning regimen, n			
Total body irradiation-associated	26	26	0.26
Busulfan-containing	54	34	0.12
Melphalan-containing	7	6	0.85
Fludarabine-containing	32	28	0.55
Acute GVHD, n			0.27
Nil/0	20	17	
I	11	9	
II	33	18	
III	15	19	
IV	2	0	
Systemic Steroid therapy, n			0.37
no	31	31	
yes	50	32	
Chronic GVHD, n	76 (NE:5)	62 (NE:1)	0.88
nil	39	31	
limited	14	10	
extensive	23	21	

Ph+, Philadelphia chromosome-positive; LBL, lymphoblastic leukemia; GVHD, graft versus host disease; NE, not evaluated.

and compared using Gray's test. For NRM, relapse was the competing event; for relapse, death without relapse was the competing event. The Cox proportional hazards model was used to test the statistical significance of several potential prognostic factors for OS, NRM, and relapse. Two factors, acute GVHD and systemic steroid therapy, were treated as time-dependent co-variates. We defined statistical significance as a *P* value of less than 0.05. Statistical analyses were performed using R version 2.11.1.

Results

Patient characteristics and eosinophilia

Patient characteristics are summarized in Table 1. The median age at transplantation was 45.5 years (range: 16-69).

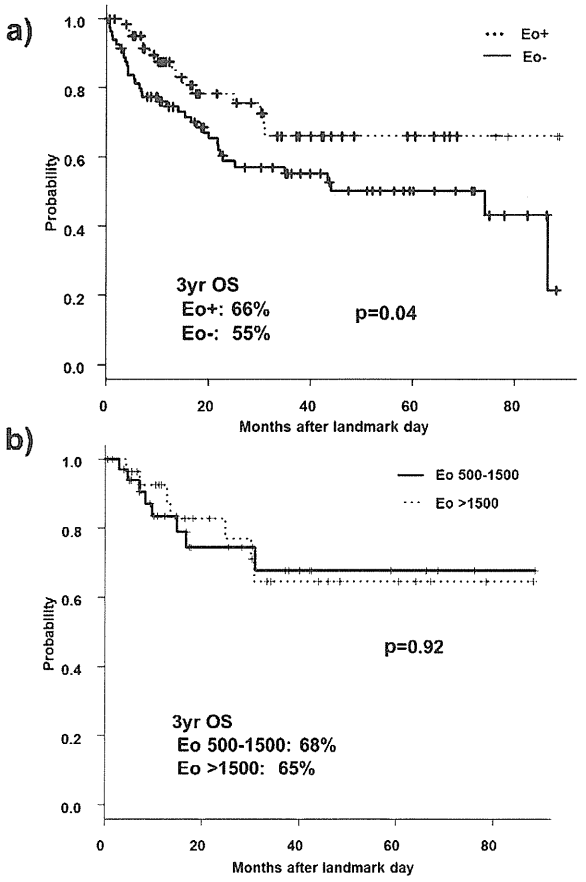

Figure 1. (a) Overall survival curves for patients with and without eosinophilia. (b) Overall survival curves for patients with mild (500-1,500×10⁶/L) and hyper- (>1,500×10⁶/L) eosinophilia.

These patients included 55 individuals with acute myeloid leukemia (AML); 31 with acute lymphoblastic leukemia (ALL) or lymphoblastic lymphoma (LBL) (ALL: 29, LBL: 2); four with acute leukemias of ambiguous lineage; eight with chronic myeloid leukemia (CML); 18 with myelodysplastic syndrome (MDS); 21 with aggressive lymphoma (adult T-cell leukemia/lymphoma: 13; diffuse large B-cell lymphoma: 3; peripheral T-cell lymphoma: 2; NK/T-cell lymphoma: 1; anaplastic large cell lymphoma: 2); six with indolent lymphoma (follicular lymphoma: 5 and mantle cell lymphoma: 1); and one with chronic, active Epstein-Barr virus infection (CAEBV). We considered the following patients to be at a standard disease risk: acute leukemia in the first or second complete remission (CR), CML in the first or second chronic phase, MDS in the absence of refractory anemia with excess blasts, indolent lymphoma (any status), aggressive lymphoma in complete remission, or CAEBV. All other patients were considered to be at high risk. Of 144 patients, 63 (44%) developed eosinophilia (Eo+). In the non-eosinophilia (Eo-) group, ABO blood type matched to a significantly lesser degree than in the Eo+ group (43% and 63%, p=0.02). The remaining factors did not differ between the two groups. The use of systemic steroid therapy (prednisolone or methylprednisolone≥0.5 mg/kg), and the incidence of acute and chronic GVHD were also not significantly different between the two groups. There was also no significant difference in the severity of chronic GVHD (no, limited and extensive chronic GVHD) between the mild and hyper-Eo+ sub-groups (p=0.67). The median time of onset of Eo+ was 47 days (range: 21-1,026) after allo-HCT, but eosinophilia tended to occur later after CBT (81.5 days) than after BMT and PBSCT (42 and 43.5 days, respectively) (p=0.05). The median maximum eosinophil count of Eo+ was 1,403×10⁶/L (range: 516-10,010×10⁶), and there was no difference among those with BMT, PBSCT, or CBT (1,280, 1,210 and 1,740×10⁶, respectively, p=0.47). Of 144 patients, 51 patients (35%) died during the follow-up period and the median follow-up period of survivors was 1,072 days (range: 129-2,737) after the landmark day.

Relationship between eosinophilia and overall survival

The Eo+ group had a significantly better outcome (3 year OS: 66% in Eo+ group and 55% in Eo- group, p=0.04) (Fig. 1). In addition, in the analysis that divided the Eo+ group into mild and hyper sub-groups, outcomes were similar (68% and 65%, p=0.92). On univariate analysis of OS, four factors had a significant influence: older age {[HR: 1.3 (95% confidence interval (CI): 1.03-1.6), p=0.03], high-risk disease [HR: 1.8 (95% CI: 1.1-3.2), p=0.03], Eo+ [HR: 0.5 (95%CI: 0.3-0.98), p=0.04],respectively}, and systemic steroid therapy [HR: 1.9 (95% CI: 1.1-3.3), p=0.03] (Table 2). On multivariate analysis, only two factors, older age and systemic steroid therapy, remained significant [HR: 1.3 (95% CI: 1.01-1.6), p=0.04 and HR: 1.8 (95% CI: 1.03-3.2), p=0.04]. Eo+ showed a tendency toward better OS, although this failed to achieve significance [HR: 0.6 (95%CI: 0.3-1.1), p=0.11]. On multivariate investigation that distinguished between the mild and hyper-Eo+ sub-groups, both sub-groups had a better OS than the Eo- group, but not significantly so [HR: 0.7 (95% CI: 0.3-1.4), p=0.29 and HR: 0.6 (95% CI: 0.2-1.2), p=0.15, respectively].

Correlation between eosinophilia and relapse or progression

As with OS, the Eo+ group had a significantly better outcome (three-year relapse or progression rate: 30% in the Eo+ group and 50% in the Eo- group, p=0.002) (Fig. 2). Again, in the sub-analysis of the mild Eo+ and hyper-Eo+ sub-groups, both sub-groups had similar rates of relapse or progression (three-year relapse or progression rate: 31% in mild Eo+ group and 28% in hyper-Eo+ group, p=0.90). On univariate analysis, three factors were significant: female donor to male recipient [HR: 2.6 (95% CI: 1.4-4.7), p=0.002], high risk of disease [HR: 2.5 (95% CI: 1.5-4.3), p=0.0006] and Eo+ (HR: 0.4 (95% CI: 0.2-0.7, p=0.002) (Table 2). On multivariate analysis, these three factors remained significant [female donor to male recipient: HR: 2.5 (95% CI: 1.4-4.5), p=0.003, high risk of disease: HR: 2.4 (95% CI: 1.4-4.1),

Table 2. Uni- and Multivariate Analyses of Clinical Risk Factors for OS, NRM, and Relapse

Prognostic factor	Univariate		Multivariate					
			Model1		Model 2		Model 3	
	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
OS								
Older age (by 10 years)	0.03	1.3 (1.03-1.6)	0.04	1.3 (1.01-1.6)	0.16	1.2 (0.9-1.5)	0.01	1.4 (1.1-1.8)
Female donor to male recipient	0.07	1.9 (0.9-3.5)						
High-risk disease	0.03	1.8 (1.1-3.2)	0.08	1.7 (0.9-2.9)	0.21	1.5 (0.8-2.7)	0.19	1.5 (0.8-2.8)
Eosinophilia	0.04	0.5 (0.3-0.98)	0.11	0.6 (0.3-1.1)				
Mild eosinophilia	0.13	0.6 (0.3-1.2)			0.29	0.7 (0.3-1.4)		
Hyper eosinophilia	0.11	0.5 (0.2-1.2)					0.15	0.6 (0.2-1.2)
Acute GVHD, grade II to IV	0.08	1.7 (0.9-3.0)						
Systemic steroid therapy	0.03	1.9 (1.1-3.3)	0.04	1.8 (1.03-3.2)	0.04	1.9 (1.02-3.5)	0.048	1.9 (1.01-3.5)
Cord blood transplantation	0.95	1.0 (0.5-2.3)						
Myeloablative conditioning	0.24	1.6 (0.7-3.4)						
Donor except for HLA matched sibling donor	0.3	1.4 (0.7-2.7)						
ABO blood type-mismatched donor	0.16	1.5 (0.9-2.6)						
Donor and/ or recipient CMV seropositivity	0.24	2.3 (0.6-9.5)						
Relapse incidence								
Older age (by 10 years)	0.40	1.1 (0.9-1.3)						
Female donor to male recipient	0.002	2.6 (1.4-4.7)	0.003	2.5 (1.4-4.5)	0.045	2.0 (1.01-3.8)	0.0004	3.1 (1.6-5.7)
High risk disease	0.0006	2.5 (1.5-4.3)	0.002	2.4 (1.4-4.1)	0.02	2.0 (1.1-3.5)	0.001	2.6 (1.4-4.6)
Eosinophilia	0.002	0.4 (0.2-0.7)	0.01	0.5 (0.3-0.9)				
Mild eosinophilia	0.02	0.4 (0.2-0.9)			0.05	0.5 (0.2-1.01)		
Hypereosinophilia	0.02	0.4 (0.2-0.8)					0.07	0.5 (0.2-1.1)
Acute GVHD, grade II to IV	0.88	1.0 (0.6-1.6)						
Systemic steroid therapy	0.90	1.0 (0.6-1.8)	0.75	1.1 (0.6-1.9)	0.93	1.0 (0.6-1.8)	0.56	1.2 (0.7-2.1)
Cord blood transplantation	0.28	0.6 (0.3-1.5)						
Myeloablative conditioning	0.76	0.9 (0.5-1.7)						
Donor other than HLA matched sibling donor	0.23	1.5 (0.8-2.9)						
ABO blood type-mismatched donor	0.10	1.6 (0.9-2.6)						
Donor and/ or recipient CMV seropositivity	0.19	2.6 (0.6-10.6)						
NRM								
Older age (by 10 years)	0.006	2.1 (1.2-3.5)	0.004	2.1 (1.2-3.5)	0.01	2.0 (1.2-3.5)	0.005	2.2 (1.1-22.8)
Female donor to male recipient	0.52	0.5 (0.1-3.9)						
High risk disease	0.63	1.3 (0.4-3.8)						
Eosinophilia	0.11	0.4 (0.1-1.2)	0.13	0.4 (0.1-1.3)				
Mild eosinophilia	0.20	0.4 (0.1-1.7)			0.19	0.4 (0.1-1.6)		
Hypereosinophilia	0.25	0.4 (0.1-1.9)					0.36	0.5 (0.1-2.3)
Acute GVHD, grade II to IV	0.03	5.2 (1.2-23.1)	0.02	5.6 (1.3-24.9)	0.03	9.8 (1.3-75.5)	0.04	5.0 (1.1-22.8)
Systemic steroid therapy	0.03	4.2 (1.2-14.9)						
Cord blood transplantation	0.25	2.0 (0.6-6.2)						
Myeloablative conditioning	0.39	1.9 (0.4-8.4)						
Donor other than HLA matched sibling donor	0.54	1.5 (0.4-5.3)						
ABO blood type-mismatched donor	0.20	2.0 (0.7-5.6)						

OS, overall survival; NRM, non-relapse mortality; HR, hazard ratio; CI, confidence interval; GVHD, graft versus host disease; RIC, reduced intensity conditioning; HLA, human leukocyte antigen; CMV, cytomegalovirus

p=0.002) and Eo+: HR: 0.5 (95% CI: 0.3-0.9, p=0.01), respectively]. In the multivariate study of the mild and hyper-Eo+ sub-groups, both sub-groups had a tendency towards lower relapse than did the Eo- group [HR: 0.5 (95% CI: 0.2-1.01), p=0.05 and HR: 0.5 (95% CI: 0.2-1.1), p=0.07].

Influence of eosinophilia on NRM

Outcomes in the Eo+ group were similar to those in the Eo- group (three-year NRM: 10% in the Eo+ group and 14% in the Eo- group, p=0.22) (Fig. 3). In the analysis that distinguished mild and hyper-Eo+ sub-groups, each outcome was also similar (three-year NRM: 10% in the mild Eo+ sub-group and 10% in the hyper-Eo+ sub-group, p=0.98). On univariate analysis, three factors, older age, grade II to IV acute GVHD and systemic steroid therapy, were associated with a significantly higher NRM [older age: HR: 2.1 (95% CI: 1.2-3.5), p=0.006; grade II to IV of acute GVHD: HR: 5.2 (95% CI: 1.2-23.1), p=0.03; and systemic steroid therapy: HR: 4.2 (95% CI: 1.2-14.9), p=0.03]. Eo+ showed a tendency to better NRM, but not significantly so [HR: 0.4 (95% CI: 0.1-1.2), p=0.11] (Table 2). On multivariate analysis, the same tendency was shown. In the multivariate study

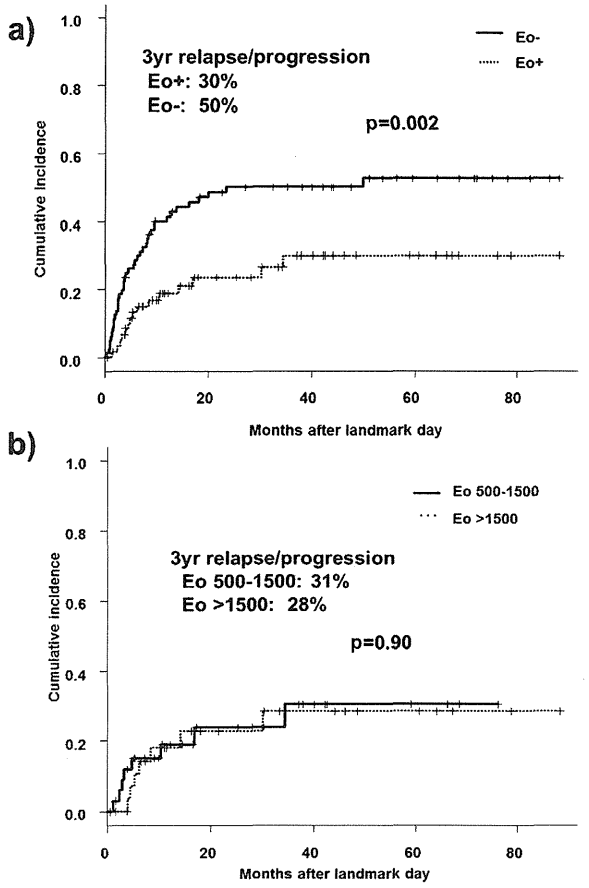

Figure 2. (a) Cumulative relapse incidences for patients with and without eosinophilia. (b) Cumulative relapse incidences for patients with mild (500-1,500×10^6/L) and hyper- (>1,500×10^6/L) eosinophilia.

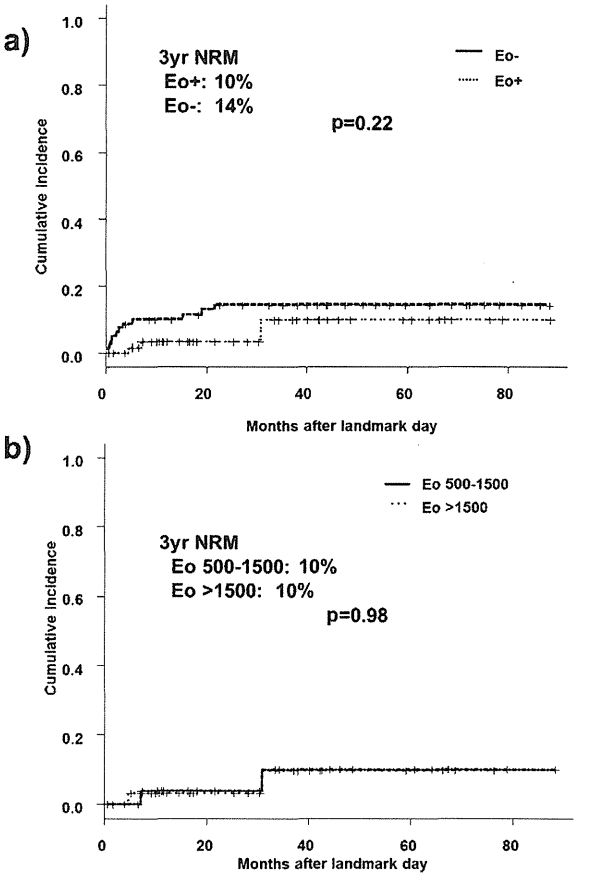

Figure 3. (a) Cumulative non-relapse mortality for patients with and without eosinophilia. (b) Cumulative non-relapse mortality for patients with mild (500-1,500×10^6/L) and hyper- (>1,500×10^6/L) eosinophilia.

that distinguished between the mild and hyper-Eo+ subgroups, both groups showed a trend towards lower NRM, compared with the Eo- group, but significance was not achieved [HR: 0.4 (95% CI: 0.1-1.6), p=0.19 and HR: 0.5 (95% CI: 0.1-2.3), p=0.36, respectively].

Discussion

In this study, we found that: 1) eosinophilia after allo-HCT was significantly associated with a lower incidence of relapse and tended to improve OS, and 2) differences in the degree of eosinophilia had no influence on the results. To our knowledge, four studies indicate better outcome after allo-HCT if eosinophilia is present (3-6). Two previous studies reported that eosinophilia after allo-SCT correlated with lower relapse rates (3, 5), and three studies showed that eosinophilia after allo-SCT was associated with lower NRM (4-6). On the other hand, one study found no relationship between eosinophilia and transplant outcome (7). Of these five studies, just two reported median maximum eosinophil counts, which were 840.5×10^6/L (3) and 1,180×10^6/L (7). However, no study has investigated the relationship between the grade of eosinophilia and transplant outcome, despite the concern that hypereosinophilia may cause tissue and organ damage, regardless of the underlying etiology (2). In this study, therefore, we planned to address this specific point. We undertook the analysis, taking into consideration the potential for confounding between eosinophilia and the use of systemic steroids, especially with GVHD treatment, because eosinophilia is influenced by systemic steroid therapy. The median number of days after transplantation at which eosinophilia was detected was later than the median onset of acute GVHD [47 (range: 21-1,026) versus 19 (range: 3-133) days after transplantation, respectively], and in Eo+ patients with grade II to IV acute GVHD, many cases showed eosinophilia (33 of 37 patients) after the occurrence of acute GVHD. Therefore, if anything, we were concerned about a tendency to underestimate the incidence of eosinophilia, related to systemic steroid use for acute GVHD, grades II to IV. However, there was no difference in the incidence of eosinophilia at any grade of acute GVHD [none or grade I: 26 of 57 patients (46%); grades II to IV: 37 of 87 patients (43%)]. We speculate that systemic steroid use did not necessarily decrease eosinophilia, although it might account for the observed delay. On multivariate analysis, we included systemic steroid use in order to

analyze its influence on eosinophilia, so as to compensate for potential confounding effects. As a result, we believe we were able to minimize the influence of confounding effects in this study.

One previous study reported that there was no correlation between eosinophilia and transplant outcome in CBT (7). However, in the present study, we also found favorable outcomes in CBT, as with BMT and PBSCT (data not shown); we therefore included cases with CBT in this analysis. We cannot easily explain why our results differ, but outcomes may depend on differences in transplant conditions, such as patients' ages, disease status, conditioning regimens, and the timing of systemic steroid therapy.

Eosinophilia after allo-SCT may reflect the recovery of Th2/Tc2 cells. Th2 cytokines including IL-4, IL-5 and IL-10 are produced by CD4 Th2/ CD8 Tc2 cells and appear to be important in the process of chronic GVHD (15), which was reported to be related to graft-versus-tumor (GVT) effect (16). Therefore, eosinophilia after allo-HCT may lead to fewer relapses and improved OS via the activation of the GVT effect. The present study also showed that eosinophilia after allo-HCT was associated with a trend towards lower NRM, although not significantly so. Th2 pathway activation possibly causes a decline in Th1 pathway activity, which plays an important role in the deleterious effects of acute GVHD, and thereby correlates with lower NRM (3-5).

Regarding the degree of eosinophilia, our results showed no difference between the mild and hyper-eosinophilia subgroups as regards both transplant outcome and severity of acute and chronic GVHD. In their review, Roufosse and Weller discuss that we should watch and wait if hypereosinophilia is asymptomatic, and that we must look for organ involvement at regular intervals and thereby determine the clinical urgency for use of an eosinophil-lowering agent (2). Another study determined that the extent of tissue eosinophilic infiltration varies and usually does not correlate with peripheral blood eosinophilia (17). Moreover, several studies reported an inhibitory effect of calcineurin inhibitors on eosinophilic infiltration (18-20). We speculate that where there is blood eosinophilia after allo-HCT, tissue infiltration may not correlate well with higher blood eosinophilia because of the use of immunosuppressive drugs. Further consideration of organ infiltration is needed to make a clinical decision as to whether we should use steroids to treat marked and persistent eosinophilia after transplantation.

This study has the following limitations: 1) it is a retrospective study from a single institution; 2) the patients encompass a variety of diseases, disease statuses, donor sources, conditioning regimens and approaches to GVHD prophylaxis.

In conclusion, eosinophilia after allo-HCT may be associated with better OS and a lower relapse rate, regardless of the extent of eosinophilia. Higher degrees of eosinophilia may not have an adverse influence on transplant outcome. Further studies are necessary to clarify the relationship between the degree of blood eosinophilia and organ infiltration after allo-HCT, with investigation of the pathophysiological processes involved.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science, Sports, and Culture, and a grant from the Japanese Ministry of Health, Welfare, and Labour.

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日本アフェレシス学会雑誌 31(3):209-215, 2012

総　説

非血縁者間末梢血幹細胞採取

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Unrelated Peripheral Blood Stem Cell Harvest—Current Status and Future Direction—

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Summary Allogeneic peripheral blood stem cell transplantation (PBSCT) as well as allogeneic bone marrow transplantation (BMT) is a curative treatment strategy for patients with hematopoietic disorders. PBSCT is associated with more rapid hematopoietic recovery than BMT. Although PBSC harvest is feasible without the risk of anesthesia or invasive multiple bone marrow aspirations, the other concern is the short-term and long-term safety. In a nation-wide prospective survey of the Japan Society for Hematopoietic Cell Transplantation and other worldwide registry data, PBSC harvest was mostly tolerated as well as BM collection. Since the first unrelated PBSCT in 2011, 4 transplants have been performed. Both patients and donors have more therapeutic options by the use of PBSC for unrelated donor transplantation. However, some problems (work-sharing, apheresis center, management of post-transplantation, cryopreservation, etc.) should be solved.

Key words : peripheral blood stem cell harvest, unrelated, Japan marrow donor program, granulocyte colony-stimulating factor, apheresis center

1. はじめに

末梢血幹細胞移植（PBSCT）は骨髄移植（BMT）より20年あまり遅れた1990年代後半から積極的な臨床応用が行われ，わが国でも2000年4月より健康保険適応が承認された（造血細胞移植学会の全国調査では1992年より報告がみられる）ことで血縁者間の幹細胞ソースとして普及し，2011年の日本造血細胞移植学会の全国調査では，血縁ドナーの過半数が末梢血幹細胞を提供している（図1）．非血縁者間PBSCTに関しては，米国骨髄バンク（NMDP）で1999年より開始し，2010年の統計では18歳以上の成人の非血縁ドナーからの移植の76%を占めるに至っており（図2）[1]，World Marrow Donor Association（WMDA）のデータでも1998年以降，非血縁BMTが約3,000例/年で維持しているのに対し，非血縁PBSCTは着実に増加しており[2]，標準医療として確立されている．一方，わが国ではドナーの安全性の確認を重視し，非血縁ドナーへの適応拡大については慎重な対応がなされてきた．日本造血細胞移植学会における10年にわたるドナーフォローアップ事業の結果[3]や海外の報告[4]から安全性において骨髄採取に劣るものではないことが確認され，骨髄移植推進財団（PBSCT委員会），日本造血細胞移植学会，日本輸血・細胞治療学会，厚生労働科学研究免疫アレルギー疾患等予防・治療研究事業「同種末梢血幹細胞移植を非血縁者間で行う場合等の医学，医療，社会的基盤に関する研究」班（宮村耕一班長）が協力し準備を行った結果，2010年10月より過去に非血縁骨髄採取を実施したドナーのみを対象にし，非血縁PBSCTが開始され，1例目が2011年3月に実施された．現在は初回ドナーに対しても適応が拡大され，2012年4月までに4例が実施されている．

2. 末梢血幹細胞採取マニュアル

造血幹細胞を提供するドナーは，献血同様，自由意思による健康なボランティアであるため，安全には十分配慮し，また，退院後すぐに社会生活に復帰できるように負担を最小限にする必要がある．日本造血細胞移植学会および日本輸血・細胞治療学会によって作成，改訂された「同種末梢血幹細胞移植のための健常人ドナーからの末梢血幹細胞動員・採取に関するガイドライン」（第4版）[5]をもとに，骨髄バンクではさらに厳しい基準で「末梢血幹細胞採取マニュアル」（暫定

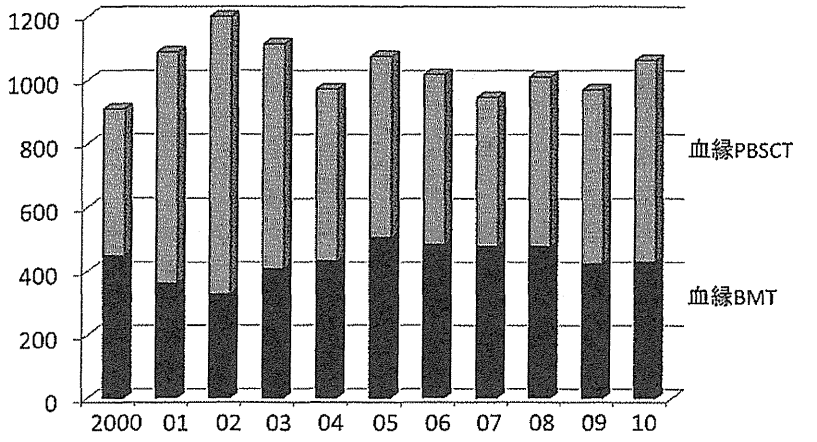

図1　わが国の幹細胞ソース別血縁移植件数の推移

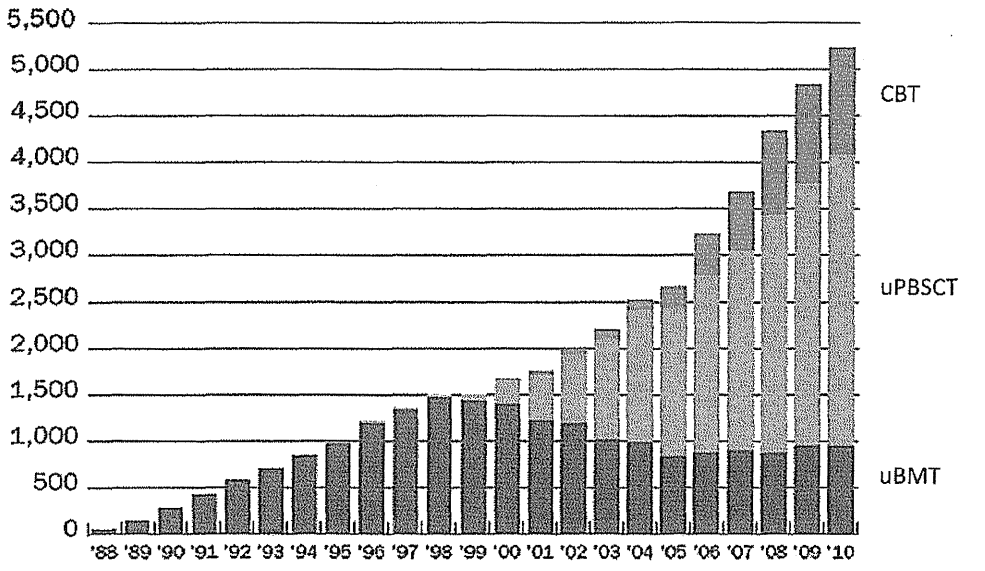

図2　NMDP の幹細胞ソース別非血縁移植件数の推移

版)[6]を作成し，これに基づいて非血縁末梢血幹細胞採取が実施されている．

2.1 施設認定

表1に骨髄バンクで定めた末梢血幹細胞採取施設認定基準を示す．施設からの申請に対してドナー安全委員会が中心となってサイトビジットを実施し，2012年4月時点で36施設が認定されているが，まだまだ足りない現状である．ドナーの安全を考慮し，認定施設へ通院可能な時間的制約をもうけているため，すべてのドナーが末梢血幹細胞採取を選択できるわけではない．骨髄バンクの認定施設（160施設207医師）を対象に，研究班が実施した施設調査アンケートの結果（125施設144医師が回答）によると，採取施設の負担，特に医師の負担が，採取施設が増えない大きな要因である．現在，移植を実施する施設は採取も実施す

表1　非血縁者間末梢血幹細胞採取施設認定基準

1. JMDP の非血縁者間骨髄採取施設認定基準と DLI 採取施設基準を満たすこと．
2. 「(改訂) 同種末梢血幹細胞移植のための健常人ドナーからの末梢血幹細胞動員・採取に関するガイドライン」(日本造血細胞移植学会) の実施施設の適格性を満たすこと．
3. 迅速に CD34 陽性細胞数が測定できる体制が確立されていること．
4. 施設において下記の(1)(2)を満たすこと．
   (1) 過去に末梢血幹細胞採取術を 30 例以上経験している医師が採取責任医師となること．
   あるいは過去に末梢血幹細胞採取術を 10 例以上経験している医師が採取責任医師となり，かつ施設として少なくとも末梢血幹細胞採取術を 30 回以上実行した経験を有すること．
   (2) 下記の a. b. のいずれかを満たすこと．
   a. 過去 2 年以内に末梢血幹細胞採取術を 5 例以上（うち 3 例以上健常人から）実施していること．
   b. 過去 1 年以内に末梢血幹細胞採取術を 3 例以上（うち 2 例以上健常人から）実施していること．

表2　G-CSFの減量・中止基準

症状	程度	副作用判定基準 (Grade)	G-CSF投与量調整
骨痛	自制不能	4	50%減量 24時間改善されなければ，投与中止
頭痛	自制不能	4	50%減量 24時間改善されなければ，投与中止
嘔気	経口による飲食物摂取不可能	≧3	投与中止
嘔吐	24時間で2〜5回嘔吐	2	50%減量
	24時間で6回以上嘔吐	≧3	投与中止
身体反応	痛みもしくは腫れを伴う炎症・静脈炎		50%減量 24時間改善されなければ，投与中止

る「ギブ・アンド・テイク」で運用されているが，骨髄バンクのアンケート調査では血液内科医が2〜3名しかいない施設も多く，地域によっては年間数例の骨髄採取しか実施されていない施設もある．ドナーの安全性を考慮した場合，ある程度の採取数を維持しておくことも必要であるが，血縁もしくは自家の末梢血幹細胞採取時の成分採血装置のセッティングを臨床工学士が行っているところは48%しかなく，多くの施設では医師に負担がかかっている現状がわかる．また，血管穿刺に関しても人工透析や献血のようにアフェレシスナースが担当しているところはほとんどなく，末梢血幹細胞採取の件数が増えることで，少人数で重篤な血液疾患の治療を行い，多忙な血液内科医に対する負担が，さらに多くなることが懸念されている．このような現状から，末梢血幹細胞採取のセンター化，拠点化が理想的であるが，全国に存在するドナーの利便性を考慮すると実際には難しい状況である．今後，アフェレシスセンターの実現に向けて，日本赤十字血液センターを含めた全体の議論が必要である．

2.2　適格性判定基準

ドナーの年齢は骨髄採取と共通で20〜55歳とし，採取責任医師は「ドナー適格性判定基準」に従って適格性を複数の医師で確認する．多くの項目は，骨髄採取，末梢血幹細胞採取に共通であるが，整形外科関連疾患や悪性高熱症などは骨髄提供の場合のみ不適格を検討する項目で，逆に，脂質異常症やG-CSF製剤に対するアレルギーや上肢である程度太い血管が確保できないドナーなどは末梢血幹細胞提供の場合のみ不適格を検討する項目である[7]．

2.3　末梢血幹細胞動員

G-CSFの投与を通院で行うか入院で行うかは，ドナーの都合や施設の事情等を考慮し，個々の施設が判断する．G-CSF（フィルグラスチム400 μg/m^{2}またはレノグラスチム10 μg/kg）を1日1回または2分割で連日皮下注射する．白血球数が50,000/μLを超えた場合は50%減量し，75,000/μLを超えた場合は中止する．血小板数が100,000/μLを切った場合は50%減量し，50,000/μLを切った場合は中止する．自制不能なGrade 4の骨痛や頭痛が出現した場合は50%減量し，嘔気・嘔吐が強い場合も減量，中止を行う（表2）．G-CSF投与により脾臓が腫大し，破裂した報告もあることから触診やTraube三角の打診，必要に応じて腹部エコーで確認する．

2.4　末梢血幹細胞採取

G-CSF投与4または5日目に血球分離装置を用いてアフェレシスを実施する．G-CSF投与30分後に，一過性に好中球は減少し，1時間後に回復，その後増加を続け，4〜8時間後にピークとなるため，アフェレシス開始はG-CSF投与後3時間以降が望ましい．

（1）　アフェレシスは抗凝固剤としてACD-A液を用い，両側肘静脈を確保して行う．非血縁ドナーでは両側肘静脈の確保が困難な場合，ドナー不適格であるが，当日やむを得ない場合は，大腿静脈にアクセスを行う．なお，数日間留置する場合は，透析用のダブルルーメン・カテーテルを使用する．

（2）　採取のための処理血液量は1回あたり200 mL/kg（上限量250 mL/kg），血流速度50〜80 mL/分で体外循環を行い，必要な細胞を採取する．アフェレシスの所要時間は3〜4時間前後となる．非血縁ドナーの場合，アフェレシス中は必ず医師が常時監視し，血管迷走神経反射，クエン酸中毒，不整脈，心虚血症状，穿刺部位の出血・血腫などの合併症に細心の注意を払う．

（3）　フローサイトメトリーを用いて当日中に

CD34陽性細胞を測定し，患者体重あたり2.0×10^6個を目標とし，1日目に達しない場合は2日目も採取する．幹細胞の動員が不十分なpoor mobilizerも0.5%程度みられるが，3日以上の採取は行わない．

2.5 凍結の可否

採取できるCD34陽性細胞数をG-CSF投与前にあらかじめ予測することは困難であり，血縁の場合はpoor mobilizerに備えて，移植前処置開始以前に末梢血幹細胞を採取し，凍結保存して細胞数を確認してから移植する施設も多いが，骨髄移植同様に前処置開始後に採取を行い，凍結せず，移植している施設もあり，特に問題は起こっていない．NMDPでも一般的には凍結せずに移植が行われている．現時点では，骨髄バンクでは骨髄移植との整合性もあり，原則凍結を認めていないが，必要量以上が採取できた場合は，一部を凍結保存し，ドナーリンパ球輸注（DLI）などに使用することが可能である．この際には，日本輸血・細胞治療学会および日本造血細胞移植学会により策定された「院内における血液細胞処理のための指針」を遵守することが適切である[8]．なお，凍結することは，ドナーにとっては時間的な余裕と提供機会の増加が得られ，患者にとっては病状悪化時の「なだれ込み移植」ではなく，ベストのタイミングの移植が調整できるため，双方にメリットがある場合もある．しかし，凍結された骨髄の一部が使用されず，廃棄されていることも事実であり，ドナーの善意を無駄にしない方策について，骨髄を含めた凍結の基準について検討する必要がある．

3. ドナー有害事象

日本造血細胞移植学会のホームページに2000年4月～2005年3月の同種PBSCTドナーフォローアップ事業による有害事象報告（2010年9月13日現在），2005年4月以後の血縁造血幹細胞ドナーフォローアップ事業による有害事象報告（2012年4月24日現在）が公開されている[3]．

3.1 短期有害事象

日本造血細胞移植学会にはG-CSFやアフェレシスとの因果関係に関わりなく採取後2カ月以内に起こった重篤有害事象として97件が報告されている．最も多いのは血小板減少で19件が報告され，1.9万/μLまで低下した例もある．血小板が80,000/μLよりも減少した場合は，自己多血小板血漿を作製してドナーに輸注することが望ましい．また，このような場合は，2回目のアフェレシスによるPBSC採取の中止を考慮する必要がある．

自己多血小板血漿分離方法（操作は非開放系で実施）を以下に示す．

（1） 分離用バッグを無菌接合機でPBSCバッグと接続する．

（2） 遠心条件：遠心（約300～1,100 G）4～5分，20～24℃

（3） 多血血小板血漿（PRP）とペレット（血小板以外の細胞成分；幹細胞分画/若干の赤血球）に分離．

（4） バッグを分離スタンドにかけ上清（PRP）を分離バッグに移す．

（5） PBSCのバッグを良くもみ血漿中に細胞を浮遊させ経静脈投与する．

その他，アフェレシスに伴うものとして，迷走神経反射や低カルシウム血症（抗凝固剤として用いるACD-A液による），血管外漏出，留置針の体内迷入，カテーテル感染，穿刺部の皮下出血なども散見される．低カルシウム血症は，カルシウム液の持続注入（グルコン酸カルシウム5～10 mL/hr）によって予防可能であるが，アフェレシス中はドナーの観察を十分に行って初期症状の把握に努め，早めに対処することを心がけることが肝腎であり，医師による常時監視が求められる．また，G-CSF投与を中止しなければならないような重篤な健康被害はまれとされるが，狭心症様発作，発熱，間質性肺炎，急性虹彩炎や痛風性関節炎など炎症の増悪なども報告されている（表3）．

海外では，末梢血幹細胞採取によるドナーの死亡例が，表4に示すように脳血管障害，心不全，心筋梗塞，硬膜下出血，鎌状赤血球貧血クライシス，心停止以外に技術的な問題による合併症も含め合計12例報告されており，特に，高齢者や動脈硬化などの合併症を有している例は注意を要する．さらに，白血球の急激な増加で脾臓が破裂した症例も報告されている[9]．わが国では学会のガイドラインが遵守され，適格基準を逸脱するようなドナーは施設の倫理委員会の承認を求められた．全例が日本造血細胞移植学会に登録され，開始当初はガイドラインを逸脱したドナーに対しては，確認のための問い合わせがくるほど慎重にすすめてきたおかげもあり，ドナーの死亡事故は起こっていない．

軽度な健康被害としては，腰痛，胸痛，骨痛，背部痛，関節痛，筋肉痛，肝機能異常（GOT，GPT，LDH，ALP上昇），発疹，紅斑，悪心，嘔吐，発熱，頭痛，倦怠感，動悸，尿酸値上昇，血清クレアチニン

表3　2000年4月から2005年3月までに日本造血細胞移植学会ドナー登録センターに報告された血縁者間末梢血幹細胞採取ドナーの血縁者間末梢血幹細胞ドナー急性期比較的重篤健康被害

	初回のG-CSF投与日から起算した	
	発症日	消退日
明らかに重篤19（/3,264＝0.58%）		
間質性肺炎（2）	Day 3〜Day 25	Day 6〜Day 70
狭心症様発作（4）	Day 2〜Day 4	Day 4〜Day 6
腹水，心嚢液貯留，全身浮腫	Day 7	Day 9
くも膜下血腫（手術）	Day 23	Day 48
後腹膜血腫/貧血（手術）	Day 4	Day 25
深部静脈血栓症	Day 14	—
胆石胆嚢炎/痛風発作（手術）	Day 2	Day 19
発熱または感染症（5）	Day 2〜Day 7	Day 12〜Day 32
血痰	Day 3	Day 5
椎間板ヘルニア（手術）	Day 7	Day 62
出血性胃潰瘍	Day 8	Day 16

（ ）内は症例数.

表4　末梢血幹細胞提供ドナーにおける死亡症例（すべて海外）

	年齢	発生時期	死因	併存症
血縁	61歳	採取4日後	心不全	高血圧，冠動脈疾患
血縁	57歳	帰宅24時間以内	脳卒中	
血縁	64歳	動員終了後	心筋梗塞	冠動脈疾患
血縁	73歳	採取数日後	脳血管障害	高血圧，狭心症
血縁	67歳	採取2日後	硬膜下血腫	心筋梗塞，大動脈瘤手術
血縁	47歳	G-CSF投与4日目	鎌状赤血球症発作	鎌状赤血球症
血縁	未報告	未報告	脳血管障害	
血縁	50歳	カテーテル抜去後	空気塞栓（技術ミス）	
血縁	43歳	不明（採取15日後死亡）	心停止	高血圧
血縁	52歳	不明（採取17日後死亡）	心停止	喫煙
血縁	27歳	採取時	心停止（技術ミス）	
非血縁	21歳	カテーテル挿入時	出血（技術ミス）	

値上昇，CRP値上昇などが報告されているが，いずれもG-CSF投与終了後2〜3日以内で消失する．G-CSF投与により多くのドナーで骨痛が出現するが，一過性でアセトアミノフェン等の鎮痛薬で対応可能である．ただし，アスピリン製剤は使用すべきでない．

3.2　中長期的な有害事象

中長期に関しては生活習慣によるものや偶発症もあるため，G-CSF投与や末梢血幹細胞採取との因果関係を明らかにすることは困難であるが，因果関係の有無は別として56件が報告されている．このうち，採取後14カ月目に発症した急性骨髄性白血病の事例[10]はG-CSFとの因果関係が懸念されたため，日本造血細胞移植学会は「健康被害特別調査委員会」を設置し，情報開示のあり方，事務局の危機管理体制，善後策について検討が行われた．その結果，この事例におけるG-CSFと白血病発症の因果関係については，「健常者に短期間G-CSFを投与しただけで白血病が発症する可能性は医学的には考えられないが，完全に否定することはできない」という見解が示された．その後のわが国の調査では，骨髄ドナーでの血液腫瘍発生は2/5,921例（白血病2例），末梢血幹細胞ドナーでは1/3,262例（白血病1例）で有意差はないものと考えられた．米国NMDPの非血縁末梢血幹細胞ドナー2,408例の調査でも血液腫瘍は慢性リンパ性白血病が1例のみ[11]で骨髄性白血病の事例はなかったが，血縁ドナーの全例調査はわが国以外になかったため，Koderaらの提案によりヨーロッパ骨髄移植学会（EBMT）を中心に51,024例の後方視的解析が行われた結果，骨髄ドナー27,770人中8例に血液悪性腫瘍（AMLは2例），末梢血幹細胞ドナー23,254人中12

例に血液悪性腫瘍（AMLは1例）が発生しており，骨髄ドナーでは0.4/10,000人年，末梢血幹細胞ドナーでは1.2/10,000人年と計算された[4]．年齢および性別によって血液悪性腫瘍発症率は異なる（20～24歳で0.9/10,000，30～39歳で1.3～1.6/10,000人年，55～59歳で6.3/10,000人年）が，一般の発症率よりも低値であった．以上から，骨髄採取と比較して末梢血幹細胞採取において特に白血病などの発症が増えることはないとの結論に至ったが，リスクがあるとする報告[12]もある．さらに長期の影響に関しては未知数であることから，今後もドナーのフォローアップ体制が重要である．

4. コーディネート

4.1 コーディネートの流れ

ドナーに骨髄採取・末梢血幹細胞採取の2つの方法について説明し，ドナーの自由意思を尊重しつつ，患者にとって最良の移植ができるようなシステムを構築することが必要である．再生不良性貧血などはGVL効果を必要とせず，慢性GVHDのより少ない骨髄移植を希望することが多いと思われるが，ドナーに患者の要望（BMTかPBSCTか）を伝えることにより，ドナーの意思誘導になる可能性も懸念されており，ドナーが患者希望を知りたいかどうかを確認し，その希望があれば伝えることが適切であるとされた．現在のコーディネートの概要は以下のようにまとめられる．

（1） ドナー登録時には，ドナーは提供ソースの希望を選択しない．

（2） 患者登録時には，患者は要望を申請する．

（3） 確認検査面談の際に，コーディネーターはドナーに骨髄提供・PBSC提供の両方について説明を行ってドナーが希望しない提供方法があればここで確認しておく．この段階で，患者側の要望は一律にドナーに伝えることはしないが，ドナーが患者の要望を知りたいかどうかを聞いて，希望があればこれを伝える．患者の要望が変わる可能性があることも説明する．

（4） ドナー選定時に，患者側はドナーの選択（希望しない提供方法があるかどうか）および，適格性を踏まえて選定を行う．

（5） 最終同意面談では，ドナーが提供すると決めた方法のみ説明し，それについて本人と家族の同意を確認する．最終同意後の変更は原則として認めない．

現在までに8例の末梢血幹細胞移植が実施されたが，いずれも骨髄移植に比べて移植に至るまでの期間は短縮している．ただし，末梢血幹細胞採取認定施設数が十分ではなく，対象となるドナーは限定的であり，患者がPBSCT認定施設で移植予定，かつ，BMTとPBSCTのどちらも希望していること，HLAアリルフルマッチであること，ドナーが採取認定施設に通院可能であることが条件となり，それ以外は，従来通りの骨髄のみのコーディネートとなる．

4.2 骨髄採取と末梢血幹細胞採取の比較

骨髄採取と末梢血幹細胞採取はそれぞれ全く異なる手技で，有害事象も異なっており，ドナーの負担を単純に比較することはできない．肉体的な侵襲は，穿刺回数にも依存するが，骨髄採取のほうが強く，採取後，回復までの時間も長い[13]．また，採取時に尿道カテーテルを挿入する施設では，特に男性ドナーで尿道痛などの不快感が強い．一方，末梢血幹細胞採取では，覚醒状態で3～4時間あまりの採取に対する精神的なストレス，不安を訴えるドナーもある．また，肘静脈での採取が困難な場合，やむを得ず大腿静脈にブラッドアクセスを挿入する必要があるが，特に若い女性の場合にはストレスになると思われる．現在，非血縁ドナーにおいて骨髄採取と末梢血幹細胞採取に関するSF-36を用いた観察研究が実施されている．

5. ドナーサンクスカード運動

骨髄バンクのドナーは見ず知らずの患者に骨髄や末梢血幹細胞を提供するボランティアである．この「命の贈り物」をもらった患者は，お互いのプライバシーを侵害しないように無記名でお礼の気持ちを手紙に託すことができる．しかし，様々な事情のため，実際にドナーにお礼の手紙が届くのは50%程度しかない．ドナーサンクスカード運動は，移植に携わるチームが移植医療を支えてくださっているドナーへ感謝の意を伝えようと始まった．採取施設のスタッフとして，提供していただいたドナーの方に，採取終了後サンクスカードをお渡しする．また，移植施設のスタッフとして，移植を受ける患者のために骨髄や末梢血幹細胞を提供してくださったドナーの方には，採取施設の主治医に手紙をことづけ，患者・ドナーのプライバシーを侵害しないようにドナーの方に渡してもらう．平成23年9月15日発行の「MONTHLY JMDP」[14]に，「お手紙交換のルール変更について：移植施設の医師・医療スタッフのお立場でドナーの方にお手紙をご準備いただける場合は，当財団を通さずに採取施設のスタッフへ直接お渡しいただいて構いません（お手紙