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Original Article

Effects of Gram-negative Rod Blood Stream Infection on Acute GVHD in Allogeneic Hematopoietic Stem Cell Transplantation: A Single-institute Analysis

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A bloodstream infection (BSI) is the most common serious infectious complication of hematopoietic stem cell transplantation (HSCT). BSI promotes an inflammatory state, which exacerbates acute graft-versus-host disease (GVHD). We investigated whether a Gram-negative rod bloodstream infection (GNR-BSI), which develops early after allo-HSCT, affected the onset or exacerbated acute GVHD in 465 patients who underwent allo-HSCT from 1995 through 2015 at a single institution. Eighty-eight patients (19%) developed BSI during the study period. Among the cultures, 50 (57%) were Gram-positive cocci (GPC) and 31 (35%) were GNR. Of the 465 patients, 187 (40%) developed acute GVHD of grade II or higher within the first 100 days post-allogeneic HSCT: 124 (27%) had acute GVHD grade II, 47 (10%) had grade III, and 16 (3%) had grade IV. Multivariate analysis revealed that GNR-BSI was a significant risk factor for grade II-IV acute GVHD (grade II-IV: hazard ratio [HR] 1.75, 95% confidence interval [CI] 1.03-2.97; grade III-IV: HR 2.37, 95% CI 1.03-5.43). These results suggest that GNR-BSI may predict the onset and exacerbation of acute GVHD.

Key words: blood stream infection, graft-versus-host disease, gram negative rods

A bloodstream infection (BSI) is the most common serious infectious complication of hematopoietic stem cell transplantation (HSCT) [1-3]. BSI can develop after the start of the pre-transplant treatment and before the peri-engraftment period as a result of immunodeficiency due to neutropenia and mucosal damage associated with the pre-transplant treatment, and can in turn induce graft-versus-host disease (GVHD). Both treatment and prophylaxis for GVHD are impacted by the presence of BSI.

BSI promotes an inflammatory condition, which exacerbates acute GVHD [4]. However, about 10 years ago, 2 studies reported that a BSI is a risk factor of acute GVHD [1,5]. In recent years, transplant sources and pre-transplantation treatments have widened to encompass cord blood (CB), human leukocyte antigen (HLA)-mismatched HSCT, and reduced-intensity stem cell transplantation; thus, it is necessary to consider both BSI and acute GVHD after HSCT in the new era.

Regarding the mechanism by which BSI causes acute GVHD, it has been proposed that lipopolysaccharides (LPS) produced by Gram-negative rods (GNR) contribute to the activation and expansion of donor-derived T cells via antigen-presenting cells, as pathogen-associated molecular patterns (PAMPs) [4,6,7].

In this study, we retrospectively investigated the cases of 465 patients who underwent allo-HSCT at a

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single institution to determine whether they developed GNR-BSI and, if so, whether GNR-BSI induced or exacerbated acute GVHD.

Patients and Methods

Study design and patient population. We retrospectively studied 523 consecutive allogeneic HSCT recipients whose HSCTs were performed at Okayama University Hospital from 1995 through 2015. The study setting is shown in Fig. 1. During HSCT, all patients were isolated in a room equipped with a laminar airflow system and received trimethoprim-sulfamethoxazole for prophylaxis of *Pneumocystis jirovecii*. Fluoroquinolone (mainly levofloxacin: 300-500 mg/day) was administered for antibiotic prophylaxis on a case-by-case basis in consideration of the risk of infection and the emergence of resistant bacteria. Fluconazole (200-400 mg/day) or micafangin (50-150 mg/day) was administered for prophylaxis of fungal infections. Acyclovir (200-1,000 mg/day) was also given as prophylaxis against herpes virus infection. Neutropenic fever was managed according to the guidelines [8, 9]. Granulocyte colony-stimulating factor (lenograstim 5 μg kg/day or filgrastim 300 $\mu g/m^2$) was given intravenously for 60 min, starting on day 1 or 5 after HSCT, and was continued until the absolute neutrophil count was $> 5 \times 10^9$/L.

We analyzed the data by means of a multivariate analysis, with the incidence of acute GVHD as the primary variable and the presence of GNR-BSI as the secondary variable. We compared the incidence of acute GVHD between the GNR-BSI(+) and GNR-BSI(−) groups. Other endpoints included the cumulative incidence of GNR-BSI and total BSI, and disease-free survival (DFS).

All data were collected retrospectively from medical records and entered into a database for research purposes. This study was approved by the Ethics Committee of Okayama University Hospital (approval number: 1701-510).

Definitions. Neutrophil engraftment was defined as an absolute neutrophil count $> 0.5 \times 10^9$/L for 3 consecutive days. Platelet engraftment was defined as a platelet count $> 20 \times 10^9$/L independent of transfusion. Primary graft failure was defined as profound, persistent pancytopenia and marrow hypoplasia without donor-derived cells on day 60 after HSCT, or the requirement for a second allogeneic HSCT. The diagnosis and grades of acute GVHD were defined by Glucksberg *et al.* [10].

The definition of BSI was adapted from the 2004 Centers for Disease Control and Prevention definition

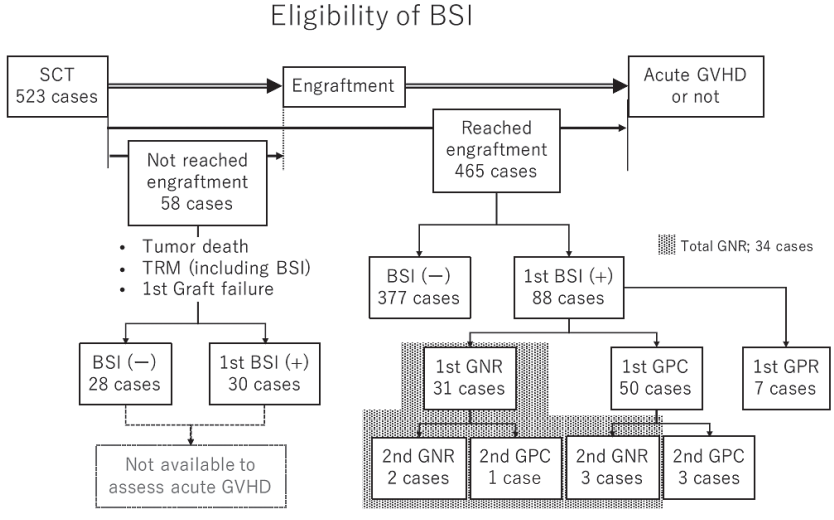

Fig. 1 Study setting. In total, 523 patients underwent consecutive allogeneic HSCT during the study period. Fifty-eight cases were excluded from the analysis because of engraftment failure. Therefore, 465 cases were included in the final analysis. Eighty-eight patients (19%) developed a BSI during the study period. We analyzed the total of 34 patients with GNR-BSI, which included 4 cases that had both GNR-BSI and GPC-BSI (shaded area).
SCT, stem cell transplantation; GVHD, graft-versus-host disease; TRM, treatment related mortality; BSI, blood stream infection; GNR, gram-negative rods; GPC, Gram-positive cocci.

for nosocomial infection [11]. In the case of bacteria not known to normally colonize the skin, such as Gram-negative bacilli, or certain pathogens such as *Staphylococcus aureus* or fungi, BSI was defined as at least one positive blood culture [12]. For bacteria that typically colonize the skin, such as coagulase-negative *Staphylococcus*, *Propionibacterium*, the *Streptococcus viridans* group, and non-JK strains of *Corynebacterium*, two consecutive positive blood cultures, 2 positive blood cultures within 72 h, or one positive blood culture and one positive intravascular catheter tip culture within 72 h constituted a BSI. All blood cultures were obtained in response to an indication of infection, usually fever (armpit temperature ≥ 37.5°C).

Statistical analysis. The results are presented as median values with ranges. The probabilities of acute GVHD, incidence of BSI by donor sources, and DFS rates were estimated using the Kaplan-Meier method, and the significance of differences among the curves was evaluated with prognostic factors on DFS. We used Cox regression proportional hazard analysis to assess the risk factors for grade II to IV or grade III to IV acute GVHD. The variables analyzed in this model were GNR-BSI, age (> 55 years vs. ≤ 54 years), donor type (BM, PB, CB, or Haplo), risk level (non-remission or second SCT), HLA category (> 2 vs. ≤ 1 locus mismatch), conditioning (myeloablative vs. non-myeloablative), antimicrobial prophylaxis, and diseases. These variables were selected because they were gatherable among the factors that could be involved in the development of BSI and acute GVHD in this retrospective setting. The chi-square test was performed to compare the proportions between two or more groups. A p-value < 0.05 was considered significant. All statistical analyses were performed using SPSS software (v. 23.0; IBM Corp, Armonk, NY, USA).

Results

Patient characteristics. In total, 523 patients underwent consecutive allogeneic HSCT during the study period. Fifty-eight cases were excluded from the analysis because of engraftment failure. Therefore, data on 465 cases were entered into the final analysis (Fig. 1). Their characteristics are shown in Table 1. The median age of all patients was 50 years (range: 15-73 years). The major underlying disease was acute myeloid leukemia in 169 cases (36%), myelodysplastic syndrome in 70 cases (15%), acute lymphoblastic leukemia in 70 cases (15%), and malignant lymphoma in 105 cases (23%). The high-risk allogeneic HSCT group included 165 cases (36%) of non-remission. No significant differences in characteristics were observed between the BSI and non-BSI groups.

Allogeneic sources of stem cells included bone marrow (BM) in 193 cases (42%), peripheral blood (PB) stem cells including haploidentical (Haplo) donors in 187 cases (40%), and umbilical CB in 85 cases (18%). A myeloablative conditioning regimen was administered in 237 cases (51%), and a reduced-intensity conditioning or non-myeloablative conditioning regimen was administered in 228 cases (49%). In the majority of patients, acute GVHD prophylaxis consisted of cyclosporine + methotrexate (206 cases, 45%) or tacrolimus + methotrexate (163 cases, 35%). A total of 334 cases (72%) received antimicrobial prophylaxis. A significant difference in antimicrobial prophylaxis was observed between the BSI and non-BSI groups ($p = 0.001$).

Bacterial BSI. Eighty-eight patients (19%) developed a BSI during the study period (Table 2). BSI was defined to exclude contamination as described in the Patients and Methods section. Median onsets of both total and GNR-BSI were 6 days (range: 4-336 days) after HSCT. In total, 97 blood isolates were obtained from the BSI episodes of the 88 individuals. Among these cultures, 50 (57%) were Gram-positive cocci (GPC). Of the GPC, 50% (or 28% of the total number of isolates) were *Staphylococcus epidermidis*; 35% (n = 31) were GNR. Of the GNR, 32% (or 11% of the total number of isolates) were *Escherichia coli*. Moreover, GNR was detected as the second BSI in 3 patients. On the other hand, one patient developed GPC-BSI after the occurrence of GNR-BSI. A second BSI was defined as BSI developing after the initial BSI was clinically recovered. We analyzed a total of 34 patients with GNR-BSI, which included 4 cases that had both GNR-BSI and GPC-BSI (Fig. 1, shaded area).

In the overall BSI group, CB and Haplo donors were the most common donor sources (cumulative incidence: CB, 28.0%; Haplo, 24.5%; BM, 17.4%; PB, 13.5%: CB vs. PB, $p = 0.015$). The same tendency was seen in the GNR-BSI group (cumulative incidence: Haplo, 14.0%; CB, 11.3%; BM, 5.9%; PB, 5.7%: Haplo vs. BM, $p = 0.043$).

The median follow-up time of this study was 272 days (range: 10-7,223 days). The 5-year DFS rate was

Table 1 Patient characteristics

	N (%)	Total 465 (100)	BSI (+) 88 (19)	BSI (−) 377 (81)	*P* value	GNR BSI (+) 34 (7)	GNR BSI (−) 431 (93)	*P* value
Age	Median	50	49	50		47.5	50	
	Range	15–73	19–72	15–73		19–66	15–73	
Sex (%)	Male	279 (60)	57 (65)	222 (59)	0.310	23 (68)	256 (59)	0.344
	Female	186 (40)	31 (35)	155 (41)		11 (32)	175 (41)	
Disease (%)	Acute myeloid leukemia	169 (36)	42 (48)	127 (34)	0.122	16 (47)	153 (35)	0.835
	Myelodysplastic syndromes	70 (15)	9 (10)	61 (16)		5 (15)	65 (15)	
	Acute lymphoblastic leukemia	70 (15)	9 (10)	61 (16)		4 (12)	66 (15)	
	Malignant lymphoma	105 (23)	19 (22)	86 (23)		6 (18)	99 (23)	
	Multiple myeloma	5 (1)	2 (2)	3 (1)		1 (3)	4 (1)	
	Myeloproliferative neoplasms	19 (4)	1 (1)	18 (5)		0 (0)	19 (4)	
	Aplastic anemia	16 (3)	5 (6)	11 (3)		2 (6)	14 (3)	
	Solid tumors	8 (2)	0 (0)	8 (2)		0 (0)	8 (2)	
	Other	3 (1)	1 (1)	2 (1)		0 (0)	3 (1)	
High risk (%)	Non-remission	165 (35)	32 (36)	133 (35)	0.874	13 (38)	152 (35)	0.742
Donor Source (%)	Bone marrow	193 (42)	34 (39)	159 (42)	0.116	11 (32)	182 (42)	0.228
	Peripheral blood	144 (31)	21 (24)	123 (33)		9 (26)	135 (31)	
	Cord blood	85 (18)	22 (25)	63 (17)		8 (24)	77 (18)	
	Haplo-identical	43 (9)	11 (13)	32 (8)		6 (18)	37 (9)	
Cycle (%)	≧2nd	76 (16)	19 (22)	57 (15)	0.139	9 (26)	67 (16)	0.097
HLA (%)	≧2/6 mismatch	90 (19)	23 (26)	67 (18)	0.074	8 (24)	82 (19)	0.522
Conditioning (%)	MAC	237 (51)	43 (49)	194 (51)	0.661	14 (41)	223 (52)	0.236
	RIC/NMA	228 (49)	45 (51)	183 (49)		20 (59)	208 (48)	
Pre-antibacterial agents (%)		334 (72)	51 (58)	283 (75)	**0.001**	16 (47)	318 (74)	**0.001**
GVHD prophylaxis	CSP+MTX	206 (44)	40 (45)	166 (44)	0.949	15 (44)	191 (44)	0.851
	CSP+MMF	27 (6)	4 (5)	23 (6)		0 (0)	27 (6)	
	CSP+mPSL	7 (2)	2 (2)	5 (1)		1 (3)	6 (1)	
	TCR+MTX	163 (35)	29 (33)	134 (36)		11 (32)	152 (35)	
	TCR+MMF	15 (3)	2 (2)	13 (3)		1 (3)	14 (3)	
	TCR+mPSL	39 (8)	11 (13)	28 (7)		6 (18)	33 (8)	
	Other	8 (2)	0 (0)	8 (2)		0 (0)	8 (2)	

HLA, human leukocyte antigen; GVHD, graft-versus-host disease; BSI, blood stream infection; GNR, gram-negative rods; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; NMA, non-myeloablative conditioning; CSP, cyclosporin; MTX, methotrexate; MMF, mycophenolate mofetil; mPSL, methylprednisolone; TCR, tacrolimus.

significantly higher in the non-BSI group than the overall BSI group (42.5% vs. 28.7%, $p=0.006$, Fig. 2A). The 5-year DFS rate was significantly higher in the non-GNR-BSI group than the GNR-BSI group (41.5% vs. 18.9%, $p=0.002$, Fig. 2B).

Acute GVHD. Of the 465 patients, 187 (40%) developed acute GVHD of grade II or higher post-allogeneic HSCT: 124 (27%) had grade II, 47 (10%) had grade III, and 16 (3%) had grade IV. The median times to development of grade II to IV and grade III to IV acute GVHD were 30 days (range 7-144) and 29 days (range 11-144), respectively. Of the 88 patients who developed BSI, 48 patients went on to develop acute GVHD; among the 48 patients with acute GVHD, the median BSI onset was day 6 after HSCT (range: 4-72) and the median acute GVHD onset was day 30 after HSCT (range: 11-121).

BSI and acute GVHD of grade II or higher. A

Table 2 Etiology of blood stream infection

	Etiology of BSI	1st BSI N	1st BSI %	2nd BSI N	2nd BSI %	Resistant	N	%
GNR	*Escherichia coli*	10	11.4	1	1.1			
	Enterobacter spp.	3	3.4	0	0.0			
	Klebsiela pneumoniae	7	8.0	0	0.0	ESBL	1	1.1
	Pseudomonas aeruginosa	6	6.8	2	2.3	MDRP	0	0.0
	Stenotrophomonas maltophillia	2	2.3	2	2.3			
	Other GNR	3	3.4	0	0.0			
	Total GNR	31	35.2	5	5.7			
GPC	*Staphylococcas aureus*	4	4.5	1	1.1	MRSA	5	4.5
	Staphylococcus epidermidis	25	28.4	2	2.3	MRCNS	3	3.4
	Other *staphylococcus* spp.	4	4.5	0	0.0			
	Streptococcus spp.	7	8.0	0	0.0			
	Enterococcus spp.	9	10.2	1	1.1			
	Other GPC	1	1.1	0	0.0			
	Total GPC	50	56.8	4	4.5			
GPR	*Bacillus* spp.	4	4.5	0	0.0			
	Corynebacterium spp.	2	2.3	0	0.0			
	Listeria spp.	1	1.1	0	0.0			
	Total GPR	7	8.0	0	0.0			

BSI, blood stream infection; GNR, gram-negative rods; GPC, gram-positive cocci; ESBL, extended spectrum β-lactamases; MDRP, multi-drug resitant *psedomonas aeruginosa*; MRSA, methicillin-resistant *Staphylococcus aureus*; MRCNS, methicillin-resistant coagulase negative staphylococci.

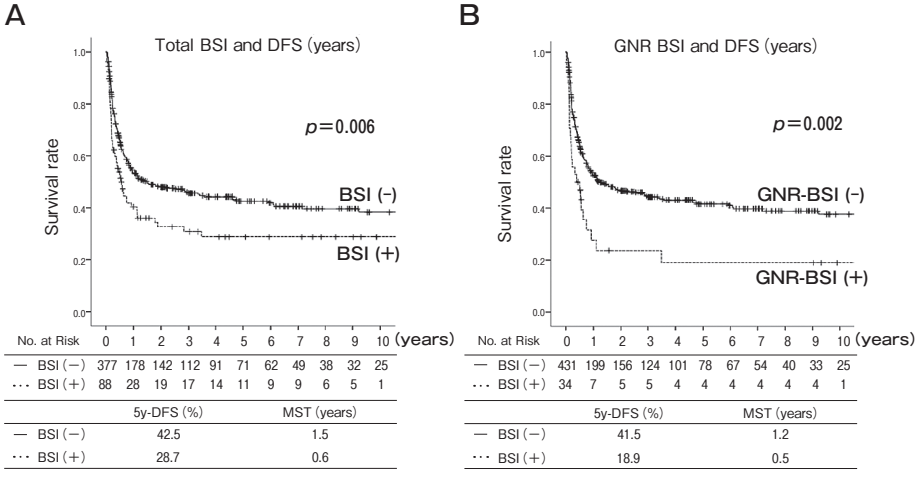

Fig. 2 The 5-year disease-free survival according to the blood stream infection. The 5-year DFS rate was significantly higher in the non-BSI group than the overall BSI group (42.5% vs. 28.7%, $p=0.006$, Figure 2**A**). The 5-year DFS rate was significantly higher in the non-GNR-BSI group than the GNR-BSI group (41.5% vs. 18.9%, $p=0.002$, Figure 2**B**). The DFS rate was estimated using the Kaplan-Meier method, and the significance of differences among the curves was evaluated with the log rank test.
BSI, blood stream infection; DFS, disease-free survival; GNR, gram-negative rods.

BSI occurring before acute GVHD was the primary predictor of interest. Thus, patients with BSI that occurred after acute GVHD of grade II or higher were censored, and only BSIs that occurred before acute GVHD of grade II or higher were assessed.

We visualized the relationship between BSI and acute GVHD of grade II or higher via a Kaplan-Meier analysis (Fig. 3). The cumulative incidence rates of

acute GVHD of grade II or higher in the non-BSI and BSI groups were 41.1% and 47.5%, respectively ($p=0.516$, Fig. 3A). The cumulative incidence rates of acute GVHD of grade II or higher in the non-GNR-BSI group and the GNR-BSI group were 40.8% and 63.8%, respectively, which represented a trend towards significance ($p=0.067$, Fig. 3B).

The multivariate analysis revealed that GNR-BSI and BM as a stem cell source were significant risk factors for acute GVHD of grade II or higher (GNR-BSI: hazard ratio [HR] 1.75, 95% confidence interval [CI] 1.03-2.97, $p=0.037$; BM as a stem cell source: HR 1.50, 95% CI 1.04-2.17; Table 3).

BSI and acute GVHD of grade III or higher. We visualized the relationship between BSI and acute GVHD of grade III or higher *i.e.*, severe acute GVHD) via a Kaplan-Meier analysis (Fig. 4). The cumulative incidence rates of acute GVHD of grade III or higher in

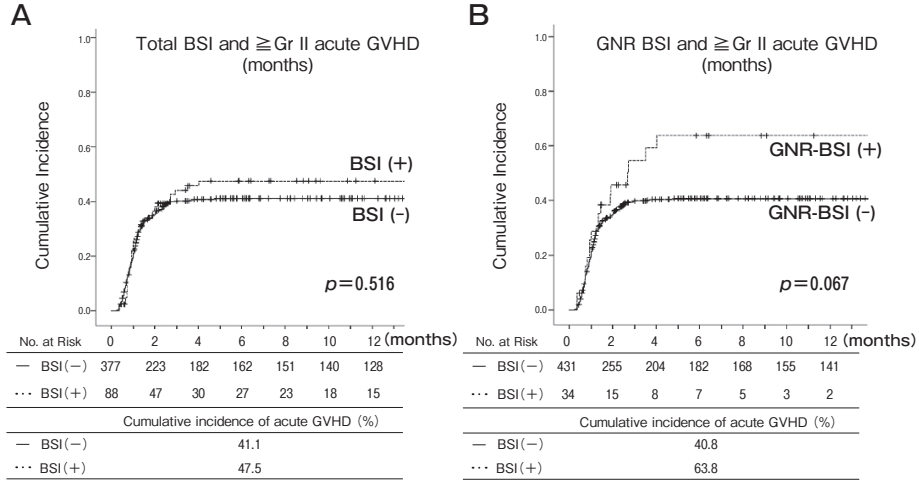

Fig. 3 Cumulative incidence of grade II or higher acute GVHD according to the blood stream infection. Cumulative incidence of acute GVHD of grade II or higher in the non-BSI and BSI groups (**A**) and the non-GNR-BSI and GNR-BSI groups (**B**). Cumulative incidence was estimated using the Kaplan-Meier method and the significance of differences among the curves was evaluated with the log rank test.
BSI, blood stream infection; GVHD, graft-versus-host disease; GNR, gram-negative rods.

Table 3 Multivariate analysis of risk factors for grade II–IV acute GVHD

Variable	Hazard ratio [95% CI]	*P* value
GNR-BSI	**1.75 [1.03–2.97]**	**0.037**
Age (>55 years vs. ≤54 years)	0.99 [0.71–1.40]	0.969
Donor type		
Peripheral blood	1.00 [reference]	0.117
Bone marrow	**1.50 [1.04–2.17]**	**0.029**
Cord blood	0.94 [0.49–1.80]	0.861
Haplo-identical	0.88 [0.35–2.25]	0.793
Risk level (non-remission or 2nd HSCT)	1.23 [0.89–1.70]	0.207
HLA category (>2 vs. ≤1 locus mismatch)	0.80 [0.39–1.64]	0.549
Conditioning (myeloablative vs. non-myeloablative)	0.99 [0.69–1.40]	0.935
Antimicrobial prophylaxis	0.99 [0.69–1.44]	0.963
Disease		
Solid tumor	1.00 [reference]	0.481
Acute myeloid leukemia	2.06 [0.48–8.77]	0.330
Myelodysplastic syndromes	1.81 [0.42–7.89]	0.429
Acute lymphoblastic leukemia	1.60 [0.36–7.09]	0.533
Malignant lymphoma	1.93 [0.45–8.19]	0.374
Myeloproliferative neoplasms	4.39 [0.88–21.35]	0.071
Aplastic anemia	0.97 [0.17–5.57]	0.975

CI, confidence interval; GNR, gram-negative rods; BSI, blood stream infection; HSCT, hematopoietic stem cell transplantation; HLA, human leukocyte antigen.

the non-BSI and BSI groups were 12.9% and 19.2%, respectively ($p=0.185$, Fig. 4A). The cumulative incidence rates of acute GVHD of grade III or higher in the non-GNR-BSI and GNR-BSI groups were 13.1% and 27.1%, respectively ($p=0.035$, Fig. 4B).

The multivariate analysis revealed that only GNR-BSI was a significant risk factor for acute GVHD of grade III or higher (HR 2.37, 95% CI 1.03-5.43, $p=0.041$; Table 4).

Discussion

The present study revealed that GNR-BSI was a significant risk factor for grade II-IV acute GVHD in a multivariate analysis.

Acute GVHD is a well-known risk factor for post-transplant infection; however, few clinical studies have investigated the effect of BSI on the development of acute GVHD. Poustsiaka *et al.* reported that early BSI

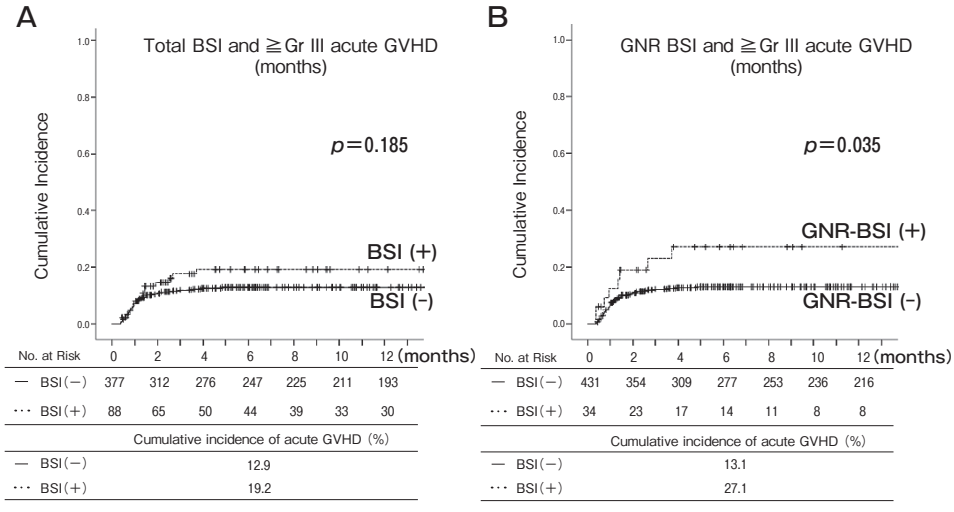

Fig. 4 Cumulative incidence of grade III or higher acute GVHD according to the blood stream infection. Cumulative incidence of acute GVHD of grade II or higher in the non-BSI and BSI groups (**A**) and non-GNR-BSI and GNR-BSI groups (**B**). Cumulative incidence was estimated using the Kaplan-Meier method and the significance of differences among the curves was evaluated with the log rank test.
BSI, blood stream infection; GVHD, graft-versus-host disease; GNR, gram-negative rods.

Table 4 Multivariate analysis of risk factors for grade III-IV acute GVHD

Variable	Hazard ratio [95% CI]	*P* value
GNR-BSI	**2.37 [1.03–5.43]**	**0.041**
Age (>55 years vs. ≤54 years)	0.85 [0.47–1.56]	0.601
Donor type		
Peripheral blood	1.00 [reference]	0.990
Bone marrow	1.11 [0.56–2.20]	0.773
Cord blood	1.12 [0.39–3.27]	0.830
Haplo-identical	1.20 [0.28–5.07]	0.806
Risk level (non-remission or 2nd HSCT)	1.56 [0.88–2.77]	0.132
HLA category (>2 vs. ≤1 locus mismatch)	0.59 [0.19–1.82]	0.362
Conditioning (myeloablative vs. non-myeloablative)	1.05 [0.56–1.98]	0.875
Antimicrobial prophylaxis	0.80 [0.40–1.58]	0.516
Disease		
Solid tumor	1.00 [reference]	0.988
Acute myeloid leukemia	0.86 [0.10–7.01]	0.884
Myelodysplastic syndromes	0.99 [0.12–8.42]	0.989
Acute lymphoblastic leukemia	0.86 [0.10–7.51]	0.889
Malignant lymphoma	1.11 [0.14–8.94]	0.919
Myeloproliferative neoplasms	2.37 [0.22–25.62]	0.477
Aplastic anemia	0.53 [0.03–9.26]	0.666

CI, confidence interval; GNR, gram-negative rods; BSI, blood stream infection; HLA, human leukocyte antigen.

was independently associated with an increased risk of subsequent grade II-IV acute GVHD [13]. Their report was published in 2011, and 93% of patients were transplanted from 6/6 HLA-matched donors. In contrast, 19% of our patients were transplanted from donors mismatched for ≥2 HLA loci (Haplo or CB donors). Nonetheless, despite differences in the era and donor sources between the previous study and our investigation, both studies found an association between BSI and acute GVHD.

Our results indicated that GNR-BSI was more strongly associated with severe acute GVHD and DFS than the other types of BSI. Both exogenous and endogenous "danger signal proteins" are released from damaged tissues and abnormal intestinal microbial colonies after conditioning. In addition, BSI caused by gut translocation of colonized bacteria is another critical source of PAMPs after allo-HSCT [13]. Experimental data showed that PAMP and damage-associated molecular pattern proteins can function either independently or synergistically to initiate GVHD [14]. The putative mechanism of acute GVHD after BSI is that LPS produced by GNR promotes activation of dendritic cells as PAMPs [4,6,7]. In the activation of immune cells through a complex signaling cascade, toll-like receptors (TLRs) play an important role in recognizing PAMPs [15]. In addition to TLRs, various nucleotide-binding and oligomerization domain (NOD)-like receptors are essential for the recognition of PAMPs, and have the ability to initiate and support robust immune responses through the formation of inflammasomes and the activation of nuclear factor-kappa B, interferon regulatory factor, and mitogen-activated protein kinase pathways [16]. However, a child and adolescent study suggested that only a mucosal barrier injury associated with BSI is related to severe acute GVHD [17]. Kameda *et al.* showed that while BSI is not a significant risk factor for grade II-IV acute GVHD, early febrile neutropenia after HSCT may be a risk factor [18]. Although further investigation is needed, this is nevertheless the first study to demonstrate an association between GNR-BSI and acute GVHD.

The microbiological spectrum of the BSI cases in our study accords with the literature, with Gram-positive bacteria being predominantly involved [1-3,19,20]. Bacterial invasion, such as by *Enterococcus* spp., of skin flora due to long-term placement of a catheter or a BSI in the intestinal tract, is often the cause of infection. Our study showed that *S. epidermidis* was a highly prevalent Gram-positive bacterium, but as only cases confirmed twice were treated, the likelihood of contamination was low, suggesting the possibility of catheter-related BSI.

In this study, CB and Haplo donors tended to be more strongly associated with total BSI and GNR-BSI. We speculate that CB transplantation results in more long-term cytopenia compared to other donor sources; patients in the present series who underwent Haplo transplantation were more likely to have infections or re-transplantations, resulting in a higher incidence of BSI. Consequently, the 5-year DFS was significantly shorter in both the total BSI and GNR-BSI groups.

Our study showed that antibiotic prophylaxis was more prevalent in the non-BSI group than the BSI group. Although antimicrobial prophylaxis has been suggested to reduce the incidence of BSI, our multivariate analysis showed that it did not reduce the rate of acute GVHD. This might have been related to the small number of non-antimicrobial prophylaxis cases. Further study is needed to clarify whether antibiotic prophylaxis reduces BSIs, and thus the likelihood of acute GVHD.

Engelhard *et al.* reported recommendations for the prevention of bacterial infection after hematopoietic cell transplantation [21]. They considered that patients with severe hypogammaglobulinemia (that is, IgG < 400 mg per 100 mL) may be indicated for intravenous immunoglobulin prophylaxis.

Several limitations of this study should be discussed. First, this was a single-center, retrospective analysis. We could not analyze the relationship among the onset of BSI, duration and dose of GVHD prophylaxis and acute GVHD. In addition, there may have been biases due to a variety of uncontrolled factors, such as underlying disease. There may also have been a study bias related to progress in this field, such as improvements in GVHD prophylaxis and the diagnostic sensitivity of sepsis. Moreover, patients who died during the pre-engraftment period were excluded from the study because acute GVHD could not be assessed. In general, cases of pre-engraftment mortality usually develop BSI. Although additional analyses were performed on all 523 allo-HSCT patients with presymptomatic death due to acute GVHD, sufficiently powered analyses could not be performed (data not shown). Finally, our study included four cases who developed both GPC-BSI and

GNR-BSI. In such cases, GNR may not be a direct factor in acute GVHD and should be interpreted with caution. In future studies, it will be necessary to include more cases.

In conclusion, GNR-BSI was a significant risk factor for grade II or higher acute GVHD in a multivariate analysis. The findings of this study may help in predicting the onset and exacerbation of acute GVHD. In the future, a larger analysis of registry data will be needed to clarify the relationship between these two serious complications in the hope of improving the outcomes of allo-HSCT.

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