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ARTICLE

Associations between febrile neutropenia-related parameters and the risk of acute GVHD or non-relapse mortality after allogeneic hematopoietic stem cell transplantation

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Abstract

Infection and inflammation can induce acute graft-vs.-host disease (aGVHD). We hypothesized that febrile neutropenia early after allogeneic hematopoietic cell transplantation (HCT) would increase the risk of aGVHD and non-relapse mortality (NRM). We retrospectively evaluated the impact of fever, C-reactive protein (CRP) concentration and blood stream infection (BSI) early after HCT on the incidence of grade II–IV aGVHD and NRM in 227 patients. Within 7 days after HCT, 91 (40.1%) patients experienced fever for at least 2 days (early-FN group). BSI occurred in 27 (11.9%) patients and the maximum CRP concentration was 2.57 mg/dl in the median. In a multivariate analysis, early-FN (hazard ratio (HR) 1.81, $P = 0.007$) and older recipient age (HR 1.68, $P = 0.019$) were significantly associated with the incidence of grade II–IV aGVHD. High-CRP and BSI were not significant risk factors for grade II–IV aGVHD. On the other hand, high-CRP was significantly associated with the incidence of NRM (HR 2.67, $P = 0.004$) in a multivariate analysis. In conclusion, although fever, CRP elevation and BSI are considered to be closely related events, they had different effects on the incidence of aGVHD and NRM. The development of early-FN after HCT may predict the risk of aGVHD.

Introduction

Acute graft-vs.-host disease (aGVHD) remains a major cause of morbidity and mortality following allogeneic hematopoietic cell transplantation (HCT) [1–4]. Tissue damage due to the conditioning regimen, donor T-cell activation, and cellular and inflammatory effectors are considered to be important factors in the pathophysiology of aGVHD [5]. Inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)−1, are secreted from damaged tissue and play an important role in the process of aGVHD [6–9]. The influence of inflammatory cytokines on aGVHD can occur within the first week after HCT [10].

Febrile neutropenia (FN) is one of the most common complications caused by mainly bacterial or fungal infection during the pre-engraftment phase after HCT. Theoretically, FN can induce aGVHD through the elevation of inflammatory cytokines [11–14]. Indeed, FN-related events, such as the occurrence of blood stream infection (BSI) [15, 16] and the elevation of C-reactive protein (CRP) [17] have been reported to be associated with an increased risk of aGVHD. However, it is not clear whether an increase in body temperature itself is associated with aGVHD. In addition, the association between the use of antibiotics during the neutropenic period and aGVHD has not been elucidated. Previously, the use of prophylactic antibiotics against intestinal anaerobic bacteria was reported to reduce the risk of aGVHD [18, 19]. On the other hand, the alteration of the intestinal microbiota induced by the use of broad-spectrum antibiotics, such as carbapenem or piperacillin/tazobactam, was reported to

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be associated with an increased risk of GVHD-related mortality [20–24].

It is difficult to identify which factors are strongly associated with the occurrence of aGVHD. In addition, in many institutes, it is difficult to analyze the stool microbiota composition in daily practice. Therefore, we retrospectively evaluated the impact of various FN-related parameters, such as fever, CRP and BSI, during the first 7 days after HCT on the incidence of grade II-IV aGVHD and non-relapse mortality (NRM). We also evaluated the effect of the use of broad-spectrum antibiotics on the risk of aGVHD.

Patients and methods

Patients

We retrospectively reviewed the charts of 306 adult patients who underwent allogeneic HCT at Saitama Medical Center, Jichi Medical University between June 2007 and December 2016 and achieved engraftment without progression of the underlying disease before engraftment. Seventy-nine patients who had a history of prior allogeneic HCT or who received in-vivo T-cell depletion were excluded. The remaining 227 patients were included in this study. This study was approved by the Institutional Review Board of Saitama Medical Center, Jichi Medical University.

Conditioning regimens and immunosuppressive agents

The myeloablative conditioning regimen (MAC) mainly consisted of a combination of cyclophosphamide and either total body irradiation (TBI) 12 Gy or busulfan (3.2 mg/kg i.v. once daily for 4 days). A reduced-intensity conditioning regimen (RIC) consisted mainly of fludarabine-based regimens, such as fludarabine combined with busulfan or melphalan. GVHD prophylaxis included the continuous infusion of cyclosporine (CSA) ($n = 211$) or tacrolimus (TAC) ($n = 16$) combined with short-term methotrexate (MTX) (10–15 mg/m^2 on day 1, 7–10 mg/m^2 on days 3 and 6, and an optional dose on day 11). The dose of CSA was adjusted to maintain the blood CSA concentration between 450 and 550 ng/ml or between 350 and 450 ng/ml in standard-risk patients from an unrelated or related donor, respectively, or between 250 and 350 ng/ml in high-risk patients. The target concentration of TAC was 15 ng/ml. Disease risk was defined as previously described by Armand et al. [25] in 2014. For some diseases that were not included in the Armand Disease Risk index, we defined as follows: acute leukemias of ambiguous lineage and blastic plasmacytoid dendritic neoplasm were categorized same as ALL, and relapsed Langerhans cell sarcoma as high-risk.

We usually administrate granulocyte-colony stimulating factor (G-CSF) for diseases other than myeloid malignancy from the next day after the last MTX administration. In cord blood transplantation, we use G-CSF from day + 1 after HCT. If the patients suffered life-threating infection, we administer G-CSF for patients with any background diseases.

Infection definitions, CRP measurement and antibiotic use

Patients were isolated in rooms equipped with a laminar air-flow system with high-efficiency particulate air filters at least until engraftment. As bacterial prophylaxis, oral fluoroquinolone was administered from the beginning of the conditioning regimens to engraftment. Oral fluconazole (200 mg/day) was mainly used as antifungal prophylaxis. Other agents such as itraconazole, voriconazole, liposomal amphotericin B or micafungin were sometimes selected for anti-mold prophylaxis at the discretion of the treating physician, preferentially in patients with a previous history of aspergillosis. Oral acyclovir (200 mg/day) was given as prophylaxis for herpes simplex or varicella zoster virus. These antifungal or antiviral prophylaxes were continued at least until the end of immunosuppressive therapy. Prophylaxis for *Pneumocystis jirovecii* infection consisted of oral trimethoprim-sulfamethoxazole or inhalation of pentamidine from engraftment to the end of immunosuppressive therapy. FN was treated with broad-spectrum antibiotics after blood and other cultures, if applicable, were obtained in accordance with published guidelines [26]. We defined body temperature ≥ 38 °C for at least 2 days within the first 7 days after HCT as "early FN". In order to exclude single isolated febrile episode caused by such as transfusion, drug reaction and other incidental events, we defined the threshold of early-FN for 2 days. We defined "early" as "until 7 days after HCT" according to the previous studies for biomarkers early after HCT [10, 27]. The cytomegalovirus (CMV) prevention strategy consisted of weekly antigenemia surveillance after engraftment and preemptive therapy with ganciclovir, valganciclovir or foscarnet. Stomatitis was assessed according to the Bearman's grading system [28].

We used the definition of BSI we previously reported [29]. Briefly, a definite BSI was defined as the isolation of a bacterial or fungal pathogen, except for common skin contaminants, from at least one blood culture. For common skin contaminants such as *Bacillus* spp and coagulase-negative staphylococci (CNS), detection of these pathogens from 2 separate blood cultures was required for the diagnosis of definite BSI.

In our institute, the serum CRP concentration was measured at least three times a week as a routine monitoring. In this study, we analyzed the maximum CRP concentration within the first 7 days after HCT.

Definitions of engraftment, GVHD grading and treatment

Neutrophil engraftment was defined as the first of three consecutive days with a neutrophil count of $>500 \times 10^6$/L. We excluded patients who did not achieve engraftment before day + 42 post-transplantation. Engraftment syndrome was defined as previously reported by Spitzer [30].

Acute GVHD was diagnosed and graded according to established criteria [31, 32]. For patients with grade I aGVHD, initial management was basically topical steroid therapy and calcineurin inhibitor at an optimized concentration without the administration of additional systemic corticosteroid. Patients with grade II–IV aGVHD received systemic corticosteroid therapy with prednisolone or methylprednisolone at an equivalent dose of basically 1 mg/kg per day. Some patients with grade II aGVHD limited to the skin were treated the same as those with grade I aGVHD [33].

Statistical analysis

Nominal and continuous variables were compared using Fisher's exact test and the Mann–Whitney *U* test, respectively. In the following analyses, continuous variables were transformed to binary variables by treating the median value as a threshold. The cumulative incidence of GVHD was estimated based on cause-specific hazard function. The cumulative incidence of NRM was evaluated using Gray's method, where progression of the underlying disease was considered to be a competing event. Overall survival (OS) was estimated using the Kaplan-Meier method. In multivariate analyses, the Cox proportional hazard regression model was used to identify risk factors associated with OS and GVHD, whereas a Fine-Gray proportional hazard model was used for the cumulative incidences of NRM. Recipient age, HLA mismatch, donor/recipient sex mismatch (female/male vs. others), recipient CMV serostatus, disease risk, underlying disease (acute leukemia vs. others), prior cycles of chemotherapy, conditioning regimen (MAC vs. RIC), TBI-containing regimens (TBI at >8 Gy) and the use of G-CSF were subjected to multivariate analyses and deleted in a stepwise manner. Factors with a *P*-value <0.1 in the univariate analysis were also subjected to a multivariate analysis. Statistical significance was defined as a two-tailed *P*-value <0.05. All statistical analyses were performed with EZR version 1.36 (Saitama Medical Center, Jichi Medical University, Accessed 23 February 2018, http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 3.3.2) [34]. More precisely, it is a modified version of R commander (version 2.3–2) that was designed to add statistical functions that are frequently used in biostatistics.

Results

Patient characteristics

The characteristics of the 227 patients are summarized in Table 1. The median age was 47 years and the underlying diseases included AML ($n = 97$), ALL ($n = 44$), MDS ($n = 30$), malignant lymphoma including adult T-cell leukemia/lymphoma ($n = 34$), CML ($n = 6$), multiple myeloma ($n = 9$), myeloproliferative neoplasm ($n = 3$) and others ($n = 4$). Early FN occurred more often in younger patients. MAC and TBI at more than 8 Gy were each strongly associated with fever. MAC was also associated with a high-CRP concentration. The median age of patients who received MAC was 38 (range: 15–62) in contrast to 60 (range: 19–68) in RIC. Sex, disease status, recipient CMV serostatus, the type of donor, stem cell source and chemotherapy prior to the HCT were not significantly associated with early FN or high-CRP concentration.

Febrile neutropenia

Within 7 days after HCT, 91 (40.1%) patients experienced fever for at least 2 days (early-FN group). The relationships between early FN and other FN-related parameters are shown in Supplementary Table S1. The maximum CRP concentration in the first 7 days after HCT was 2.57 mg/dl in the median (range: 0.03–33.36). Maximum CRP concentration was significantly higher in the early-FN group. We defined patients with a maximum CRP concentration in the first 7 days of 2.57 mg/dl or higher as the "high-CRP" group. In the high-CRP group, 67.5% of the patients were in the early-FN group ($P < 0.001$ vs no-early FN group). BSI occurred in 27 (11.9%) patients within 7 days after HCT. The most common isolate was CNS ($n = 17$), followed by gram-negative bacteria ($n = 8$) and Enterococci ($n = 3$) (Table 2). However, there was no significant difference in the occurrence of BSI between the no-early and early FN groups ($P = 0.10$). Other than BSI, 2 pneumonia, 1 urinary tract infection, 1 cellulitis and 1 clostridium difficile infection were documented. No obvious source of infection was detected in the remaining 59 patients. Carbapenem or piperacillin/tazobactam (PIPC/TAZ) and glycopeptide antibiotics were used more often in the early FN group before engraftment (both

Table 1 Patient characteristics

Variables		Early FN[a]		*P*	High-CRP[b]		*P*
		No $n = 136$	Yes $n = 91$		No $n = 113$	Yes $n = 114$	
Age, year, median (range)	47 (15–68)	53 (16–68)	40 (15–68)	<0.001	50 (15–68)	45 (18–68)	0.05
Sex				0.27			1
Male	138 (60.8%)	87 (64.0%)	51 (56.0%)		69 (61.1%)	69 (60.5%)	
Female	89 (39.2%)	49 (36.0%)	40 (44.0%)		44 (38.9%)	45 (39.5%)	
Sex mismatch				0.52			0.64
Female to male	53 (23.3%)	34 (25.0%)	19 (20.9%)		28 (24.8%)	25 (21.9%)	
Other	174 (76.7%)	102 (75.0%)	72 (79.1%)		85 (75.2%)	89 (78.1%)	
Disease				0.03			0.41
Acute leukemia	141	76 (55.9%)	65 (71.4%)		67 (59.3%)	74 (64.9%)	
Others	86	60 (44.1%)	26 (28.6%)		46 (40.7%)	40 (35.1%)	
Disease risk				0.96			0.29
Low	22 (9.7%)	14 (10.3%)	8 (8.8%)		14 (12.4%)	8 (7%)	
Intermediate	127 (55.9%)	76 (55.9%)	51 (56.0%)		64 (56.6%)	63 (55.3%)	
High	78 (34.4%)	46 (33.8%)	32 (35.2%)		35 (31%)	43 (37.7%)	
CMV serostatus				1			0.74
Positive	182 (80.2%)	108 (79.4%)	74 (81.3%)		91 (80.5%)	91 (79.8%)	
Negative	43 (18.9%)	26 (19.1%)	17 (18.7%)		20 (17.7%)	23 (20.2%)	
NA	2 (0.9%)						
Relation to donor				0.56			0.26
Related	72 (31.7%)	41 (30.1%)	31 (34.1%)		40 (35.4%)	32 (28.1%)	
Unrelated	155 (68.3%)	95 (69.9%)	60 (65.9%)0		73 (64.6%)	82 (71.9%)	
HLA mismatch				0.49			0.13
Match	139 (61.2%)	86 (63.2%)	53 (58.2%)		75 (66.4%)	64 (56.1%)	
Mismatch	88 (38.8%)	50 (36.8%)	38 (41.8%)		38 (33.6%)	50 (43.9%)	
Stem cell source				0.20			1
BM	144 (63.4%)	88 (64.7%)	56 (61.5%)		72 (63.7%)	72 (63.2%)	
PB	69 (30.4%)	37 (27.2%)	32 (35.2%)		34 (30.1%)	35 (30.7%)	
CB	14 (6.2%)	11 (8.1%)	3 (3.3%)		7 (6.2%)	7 (6.1%)	
Conditioning				<0.001			<0.001
MAC	144 (63.4%)	68 (50%)	76 (83.5%)		59 (52.2%)	85 (74.6%)	
RIC	83 (36.6%)	68 (50%)	15 (16.5%)		54 (47.8%)	29 (25.4%)	
TBI regimen				<0.001			<0.001
Yes	130 (57.3%)	54 (39.7%)	76 (83.5%)		50 (44.2%)	80 (70.2%)	
No	97 (42.7%)	82 (60.3%)	15 (16.5%)		63 (55.8%)	34 (29.8%)	
GVHD prophylaxis				0.003			0.62
CSA	211 (93.0%)	121 (89.0%)	90 (98.9%)		104 (92.0%)	107 (93.9%)	
TAC	16 (7.0%)	15 (11.0%)	1 (1.1%)		9 (8.0%)	7 (6.1%)	
Chemotherapy cycles 6 months before HCT				0.21			0.50
<3	89 (39.2%)	58 (42.6%)	31 (34.1%)		47 (41.6%)	42 (36.8%)	
≥3	138(60.8%)	78 (57.4%)	60 (65.9%)		66 (58.4%)	72 (63.2%)	

CRP C-reactive protein, *CMV* cytomegalovirus, *NA* not applicable, *HLA* human leukocyte antigen, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood, *MAC* myeloablative conditioning, *RIC* reduced-intensity conditioning, *TBI* total body irradiation, *HCT* hematopoietic cell transplantation

[a]Early FN is defined as body temperature ≥38 °C for at least 2 days during the first 7 days.

[b]High-CRP is defined as the maximum CRP concentration ≥ 2.57 mg/dl during the first 7 days

$P < 0.001$). Stomatitis was more prominent in the early FN group ($P < 0.001$). Only 1 patient developed engraftment syndrome during the first 7 days. The median days to engraftment were exactly the same between patients with early-FN or no (21 days vs 21 days, $P = 0.88$). There was no association between the use of G-CSF during the first 7 days and the development of early FN ($P = 1$).

Acute GVHD

The cumulative incidences of all grade aGVHD, grade II–IV and III–IV aGVHD at day 100 were 77.2%, 40.8%, and 14.8%, respectively. In the univariate analysis, the early-FN group showed a significantly higher incidence of grade II–IV aGVHD than the no-early FN group (48.7% vs 35.5%, $P = 0.03$) (Fig. 1a, Table 3). High-CRP (46.3% vs. 35.3%, $P = 0.06$) (Fig. 1b), BSI (44.4% vs 40.3%, $P = 0.67$) (Fig. 1c) and use of antibiotics such as carbapenem or PIPC/TAZ (42% vs 30.7%, $P = 0.29$) and glycopeptide (42.4% vs 33.4%, $P = 0.37$) were not significantly associated with grade II–IV aGVHD. In the multivariate analysis, early FN (HR 1.81, 95% CI 1.17–2.78, $P = 0.007$) and older recipient age (HR 1.68, 95% CI 1.09–2.59, $P = 0.019$) were significantly associated with grade II–IV aGVHD. The cumulative incidences of grade II-IV aGVHD at day 100 according to the duration of fever during the first 7 days were as follows: 35.5% in 0–1 days ($n = 136$), 46.8% in 2–3 days ($n = 54$), and 51.5% in 4 ≤ days ($n = 37$). We examined the impact of early FN on the development of aGVHD according to each organ system. In the multivariate analyses, early FN was also a significant risk factor for stage 3–4 skin aGVHD (HR 2.14, 95% CI 1.31–3.48, $P = 0.002$) and a borderline significant risk factor for liver aGVHD of any stage (HR 2.68, 95% CI 0.99–7.28, $P = 0.05$), but not for gut aGVHD (HR 0.85, 95% CI 0.45–1.59, $P = 0.67$). High-CRP and early BSI were not associated with any of aGVHD with specific organ involvement in the multivariate analyses. The patient with engraftment syndrome did not developed grade II–IV aGVHD later.

Table 2 Microorganisms isolated from blood within 7 days after HCT

Isolated micro-organisms	No. of Episodes
Gram-positive	
Coagulase-negative Staphylococcus	17
Enterococcus spp.	3
Corynebacterium spp.	2
Bacillus spp.	3
Gram-negative	
Escherichia coli	2
Pseudomonas aeruginosa	1
Acinetobacter spp.	2
Sphingomonas paucimobilis	1
Enterobacter cloacae	1
Morganella morganii	1

HCT hematopoietic cell transplantation

NRM, OS and cause of death

The cumulative incidence of NRM at 1 year was 12.4% (95% CI 8.5–17.0%). In the univariate analysis, high-CRP was associated with an increased risk of NRM than low-CRP (17.5% vs 7.1%, $P = 0.005$), while early FN and early BSI were not significantly associated with NRM (Fig. 2a–c). In the multivariate analysis, older recipient age (HR 2.64, $P = 0.004$), older donor age (HR 2.16, $P = 0.03$), HCT-specific comorbidity index ≥ 3 (HR 4.30, $P < 0.001$), grade III–IV aGVHD (HR 2.47, $P = 0.009$) and high-CRP (HR 2.67, $P = 0.004$) were identified as significant risk factors for NRM (Table 4).

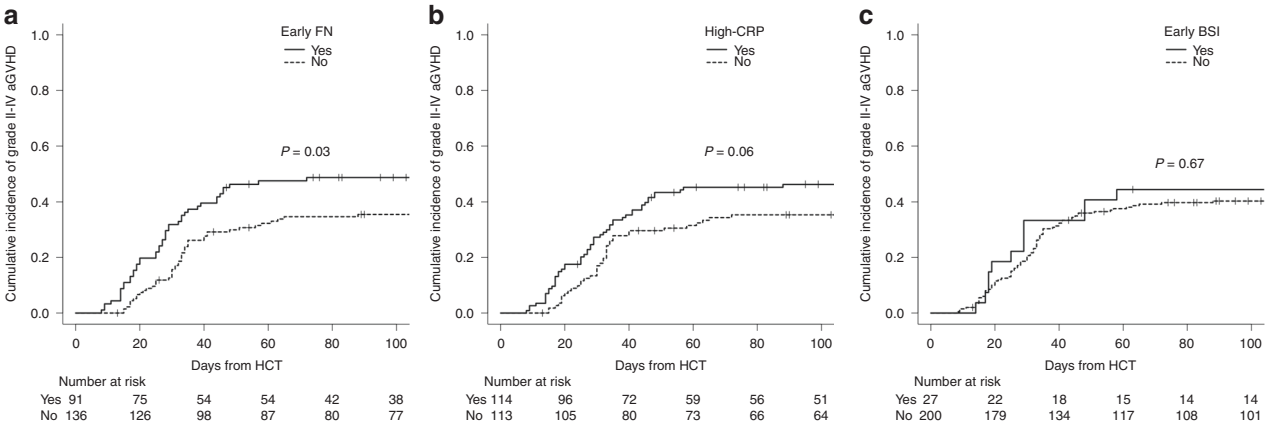

Fig. 1 Cumulative incidence of grade II–IV acute GVHD grouped according to the presence of high fever for at least 2 days within the first 7 days after HCT (**a**), maximum CRP concentration within the first 7 days (**b**), and BSI episode within the first 7 days (**c**)

Table 3 Factors for grade II–IV acute GVHD in univariate and multivariate analyses

Variable	n	Cumulative incidence of aGVHD II-IV at day + 100 (95% CI), %	Univariate *P*	HR (95% CI)	Multivariate *P*
Recipient age			0.11		
<47	112	36.8 (28.6–46.5)		1	Reference
≥47	115	44.8 (36.1–54.6)		1.68 (1.09–2.59)	0.019
Donor age			0.07		
<37	108	35.9 (27.6–45.9)			
≥37	119	45.2 (36.7–54.7)			
Donor/Recipient sex			0.69		
Female to male	53	42.4 (30.3–56.9)			
Other	174	40.3 (33.4–48.1)			
CMV serotatus			0.65		
Positive	182	40.4 (33.6–48)			
Negative	43	44.3 (31–60.3)			
Disease			0.34		
Acute leukemia	141	39.4 (31.8–48)			
Other	86	43.2 (33.3–54.5)			
Disease risk			0.60		
Low	22	50.4 (31.8–72.3)			
Intermediate	127	41.4 (33.3–50.5)			
High	78	37.1 (27.3–49.1)			
Relation to donor			0.37		
Related	72	35.2 (25.4–47.5)			
Unrelated	155	43.5 (36–51.8)			
HLA			0.20		
Match	139	37.9 (30.4–46.6)			
Mismatch	88	45.4 (35.5–56.5)			
Chemotherapy cycles 6 months before HCT			0.70		
<3	89	37.7 (28.4–48.7)			
≥3	138	42.8 (35–51.6)			
Conditioning regimen			0.53		
Myeloablative	144	40.9 (33.3–49.5)			
Reduced intensity	83	40.5 (30.8–52)			
TBI regimen			0.71		
Yes	130	40.7 (33.2–49.2)			
No	97	31.8 (24.5–40.7)			
Use of G-CSF during the first 7 days			0.16		
No	184	43 (36.2–50.6)			
Yes	43	31.2 (19.5–47.7)			
Early FN[a]			0.03		
No	136	35.5 (28–44.3)		1	Reference
Yes	91	48.7 (38.9–59.4)		1.81 (1.17–2.78)	0.007
High-CRP[b]			0.06		
No	113	35.3 (27.2–45.1)			
Yes	114	46.3 (37.6–55.9)			
BSI during the first 7 days			0.67		

Table 3 (continued)

Variable	n	Cumulative incidence of aGVHD II-IV at day + 100 (95% CI), %	Univariate *P*	HR (95% CI)	Multivariate *P*
No	200	40.3 (33.8–47.6)			
Yes	27	44.4 (28.2–64.8)			
Glycopeptide use for FN			0.37		
No	39	33.4 (21–50.5)			
Yes	188	42.4 (35.6–49.9)			
Carbapenem or PIPC/TAZ use for FN			0.29		
No	23	30.7 (16–53.9)			
Yes	204	42 (35.5–49.2)			

GVHD graft-vs.-host disease, *CI* confidence interval, *HR* hazard ratio, *CMV* cytomegalovirus, *HLA* human leukocyte antigen, *HCT* hematopoietic cell transplantation, *TBI* total body irradiation, *G-CSF* granulocyte-colony stimulating factor, *CRP* C-reactive protein, *FN* febrile neutropenia, *PIPC/TAZ* piperacillin/tazobactam

[a]Early FN is defined as body temperature ≥ 38 °C for at least 2 days during the first 7 days during the first 7 days

[b]High-CRP is defined as the maximum CRP concentration ≥ 2.57 mg/dl during the first 7 days

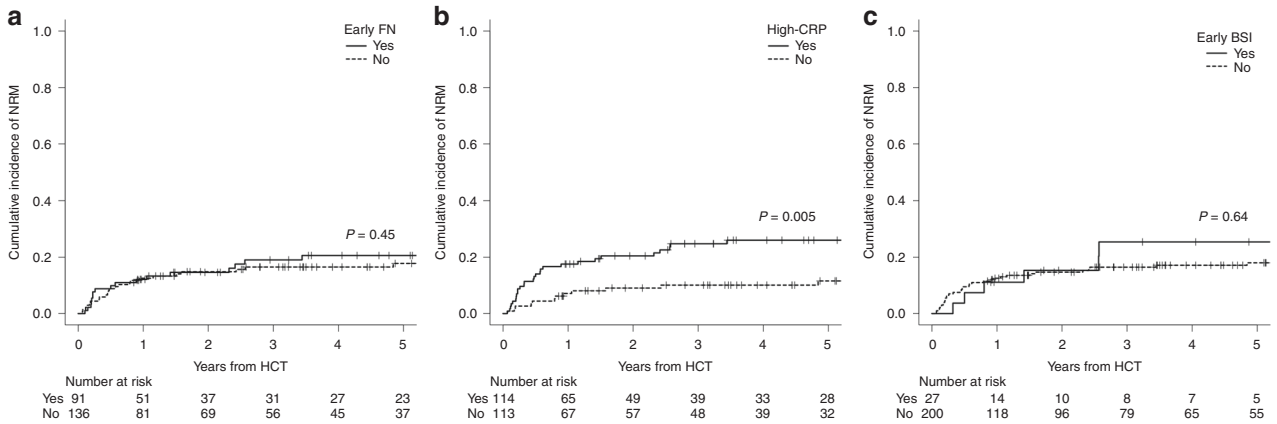

Fig. 2 Cumulative incidence of NRM grouped according to the presence of high fever for at least 2 days within the first 7 days after HCT (**a**), maximum CRP concentration within the first 7 days (**b**), and BSI episode within the first 7 days (**c**)

The OS at 1 year was 71.7% (95% CI 65.3–77.1%). In the univariate and multivariate analyses, neither high-CRP nor early BSI was significantly associated with OS (Fig. 3b, c). Early FN was not associated with OS in the univariate analysis ($P = 0.16$), but significantly associated with worse OS in the multivariate analysis (HR 1.74, 95% CI 1.15–2.64, $P = 0.009$). In the multivariate analysis, older recipient age (HR 1.70, $P = 0.02$), older donor age (HR 2.05, $P < 0.001$), higher disease risk (HR 2.08, $P < 0.001$), grade III–IV aGVHD (HR 2.70, $P < 0.001$) and cGVHD (HR 0.26, $P < 0.001$) were significantly associated with OS (Table 4).

Causes of death stratified according to maximum CRP concentration within the first 7 days after HCT are summarized in Supplementary Table S2. Patients in the high-CRP group died more often due to infection than to GVHD.

Discussion

This retrospective analysis of 227 patients revealed some important findings regarding the risk for aGVHD and NRM in terms of the relationship with FN. First, FN during the first 7 days after HCT significantly increased the risk of grade II–IV aGVHD, while neither high-CRP, BSI nor the use of antibiotics such as carbapenem or PIPC/TAZ and glycopeptide was significantly associated with grade II–IV aGVHD. Second, high-CRP within the first 7 days after HCT was associated with an increased risk of NRM, while neither early FN nor BSI was significantly associated with NRM.

Fever is the manifestation of an acute-phase response to infection or other tissue damage [13]. This response involves many inflammatory cytokines and proteins. CRP is an acute-phase protein that is downstream of IL-6 [35].

Although elevation of the CRP concentration has been reported to be associated with infectious diseases and NRM [36–39], previous studies have disagreed on the association between elevation of the CRP concentration and the risk of aGVHD [17, 40]. The roles of other biomarkers in the diagnosis and risk stratification for GVHD have been extensively studied to date [41]. For example, the elevation of TNF receptor 1 within the first week has been associated with the risk of aGVHD in previous studies [10, 27, 42]. However, it can be difficult to assess these biomarkers in daily practice. Our study suggests that fever—the surrogate marker for inflammation that is the easiest and least costly to evaluate—can also predict the risk of grade II–IV aGVHD. In addition, our results suggest that fever and elevation of the CRP concentration do not reflect the same phenomenon in terms of the inflammatory response. While fever was significantly associated with grade II–IV aGVHD, high-CRP was not. In general, the acute-phase response is a common, but not an essential, element of the febrile response [43]. While IL-6 reportedly stimulates the full spectrum of acute-phase proteins including CRP seen in inflammatory states in human, IL-1 as well as TNFα only a moderate effect on the positive acute phase proteins as reviewed by Heinrich et al. [35]. On the other hand, both IL-1 and IL-6 can induce febrile response [44]. Administration of TNFα also reportedly induces fever via cyclooxygenease-2 upregulation in an animal model [45]. Indeed, IL-1 inhibition decreased the severity of GVHD in animal models [46, 47]. Detailed investigation in these differences may lead to a development of novel prophylactic or therapeutic strategy for aGVHD. Similar to the CRP concentration, BSI was not associated with an increased risk of aGVHD in our study. Bacterial components, especially lipopolysaccharide, have been reported to induce the inflammatory response and subsequent GVHD [48]. The lack of a significant association between the incidence of early BSI and aGVHD might be partly due to the widespread use of prophylactic antibiotics, which has decreased the incidence of BSI [49].

The use of broad-spectrum antibiotics, such as carbapenem or PIPC/TAZ, in the neutropenic period was not associated with the increased risk of grade II–IV aGVHD in our study. The prophylactic and therapeutic use of broad-spectrum antibiotics is performed as routine practice in stem cell transplantation to reduce the risk of infectious events, inflammation, especially in the intestinal tract, and subsequent mortality. On the other hand, intestinal bacteria play important roles not only as infectious pathogens but also as components of the immune system [50, 51]. Recently, the

Table 4 Multivariate analyses for NRM and OS

Outcomes and variables	HR	95% CI	*P*
NRM			
Older recipient	2.64	1.37–5.10	0.004
Older donor	2.16	1.09–4.31	0.03
HCT-CI ≥ 3	4.30	2.25–8.20	<0.001
High-CRP[b]	2.67	1.37–5.20	0.004
Grade III–IV acute GVHD	2.47	1.26–4.87	0.009
OS			
Older recipient	1.70	1.11–2.62	0.02
Older donor	2.05	1.37–3.09	<0.001
Higher disease risk	2.08	1.44–2.99	<0.001
Early FN[a]	1.74	1.15–2.64	0.009
Grade III–IV acute GVHD	2.70	1.64–4.46	<0.001
Chronic GVHD	0.26	0.17–0.40	<0.001

NRM non-relapse mortality, *OS* overall survival, *CRP* C-reactive protein, *HCT-CI* hematopoietic cell transplantation-specific comorbidity index, *GVHD* graft-vs.-host disease

[a]Early FN is defined as body temperature ≥ 38 °C for at least 2 days during the first 7 days

[b]High-CRP is defined as the maximum CRP concentration ≥ 2.57 mg/dl during the first 7 days

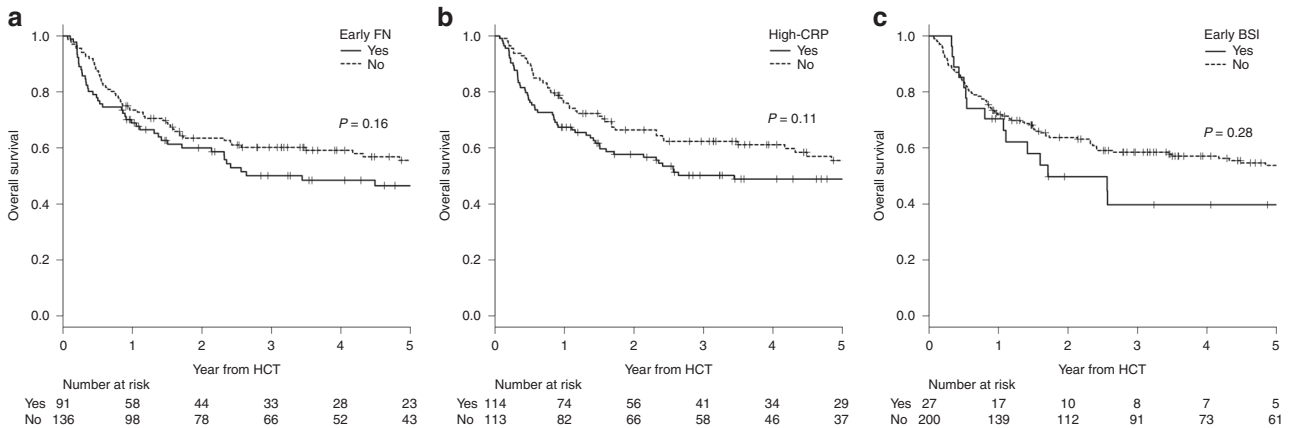

Fig. 3 Overall survival grouped according to the presence of high fever for at least 2 days within the first 7 days after HCT (**a**), maximum CRP concentration within the first 7 days (**b**), and BSI episode within the first 7 days (**c**)

relationship between the change in the intestinal microbiota and the risk of GVHD has been vigorously studied [20, 23, 52]. Several studies have reported that the use of broad-spectrum antibiotics induced intestinal microbiota disruption and increased the risk of GVHD-related mortality [20, 23, 24]. However, broad-spectrum antibiotics are generally used for patients with febrile neutropenia or prominent mucositis, or who are otherwise severely ill. Therefore, it is very difficult to identify the influence of each particular parameter on the risk of aGVHD. In addition, analysis of the intestinal microbiota is not available in daily practice. In our study, early FN more significantly predicted grade II–IV aGVHD than the use of broad-spectrum antibiotics in a multivariate analysis. In the future, analysis of the intestinal microbiota for patients with early FN may reveal more precise information about the risk of aGVHD.

This study had several limitations. First, this was a single-institution retrospective study that included patients with heterogeneous backgrounds. Second, because many patients received carbapenem or PIPC/TAZ, we may not have been able to sufficiently assess the effect of antibiotics against anaerobic bacteria. Third, we could not analyze the intestinal microbiota. Although we regarded the use of broad-spectrum antibiotics as a surrogate parameter for a change in the intestinal microbiota, it would be preferable to assess the composition of the microbiota directly. Fourth, we could not evaluate inflammatory biomarkers other than CRP.

In conclusion, while FN during the first 7 days after HCT significantly increased the risk of grade II–IV aGVHD, neither high-CRP, BSI nor the use of broad-spectrum antibiotics was significantly associated with grade II–IV aGVHD. In contrast, while high-CRP significantly increased the risk of NRM, FN and BSI did not. The exact difference in the mechanism between the febrile response and the acute-phase response in the pre-engraftment phase may lead to a development of novel prophylactic or therapeutic strategy for aGVHD. A larger prospective study is warranted to assess the effects of fever itself, a change in inflammatory biomarkers, the use of antibiotics and a change in the intestinal microbiota on the risk of GVHD and NRM.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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