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Early blood stream infection following allogeneic hematopoietic stem cell transplantation is a risk factor for acute grade III–IV GVHD in children and adolescents

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Abstract

Background: Graft-versus-host disease (GVHD) remains a major cause of mortality and morbidity in allogeneic hematopoietic stem cell transplantation (HSCT). In adults, early blood stream infection (BSI) and acute GVHD (AGVHD) have been reported to be related. The impact of BSI on risk for AGVHD, however, has not been assessed in pediatric patients.

Procedure: We conducted a retrospective analysis to test the hypothesis that early BSI (before day +30) predisposes allogeneic pediatric transplant patients to severe AGVHD. We analyzed 293 allogeneic HSCT performed at Children's Healthcare of Atlanta between 2005 and 2014 that met eligibility criteria.

Results: The cumulative incidence of acute grade III–IV GVHD at 100 days after HSCT was 17.1%. In multivariate analysis, risk for acute grade III–IV GVHD was associated with HLA-mismatched donor (hazard ratio [HR] = 4.870, $P < 0.001$), and BSI between day 0 and +30 prior to AGVHD (HR = 3.010, $P = 0.001$).

Conclusions: These results indicate that early BSI appears to be a risk factor for acute grade III–IV GVHD. Further research is needed to determine if the link is causal.

KEYWORDS

acute graft-versus-host disease, blood stream infection, hematopoietic stem cell transplantation, risk factor

1 | INTRODUCTION

Blood stream infection (BSI) and graft-versus-host disease (GVHD) are major causes of mortality and morbidity in allogeneic hematopoietic stem cell transplantation (HSCT).[1,2] Studies comprised largely or exclusively of adults and suggest that in addition to the direct harm caused by BSI, they may also cause morbidity and mortality indirectly by engendering acute GVHD (AGVHD).[3,4] The impact of early BSI on risk for AGVHD has not been closely examined in children and adolescents. We retrospectively assessed the risk for AGVHD from BSI in pediatric allogeneic HSCT recipients at our center over a 10-year period.

Abbreviations: AGVHD, acute GVHD; BSI, blood stream infection; CHOA, Children's Healthcare of Atlanta; CI, confidence interval; GVHD, graft-versus-host disease; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; MBI, mucosal barrier injury

2 | PATIENTS AND METHODS

2.1 | Eligibility

We retrospectively abstracted data on allogeneic transplants performed at the Aflac Cancer and Blood Disorders Center (Emory University and Children's Healthcare of Atlanta [CHOA]) between January 1, 2005 and December 31, 2014. We identified 329 allogeneic transplants in 301 patients during the study period. Eligible cases were defined as allogeneic transplants following myeloablative or reduced intensity conditioning regimen without primary/secondary engraftment failure. Therefore, we excluded 36 transplants from all 329 allogeneic transplants for the following reasons: neutrophil recovery occurring after day +30, transplant performed without conditioning, secondary graft failure between day +30 and +100. Two hundred ninety-three transplants were analyzed to assess if early BSI

following allo-HSCT could be at risk for acute grade III–VI GVHD. Age at HSCT ranged from 0 to 22 years old with a median of 9 years. Seventy-one (24.2%) patients had acute lymphoblastic leukemia, 66 (22.5%) had acute myeloid leukemia, 35 (11.9%) had sickle cell disease, 18 (6.1%) had myelodysplastic syndrome/juvenile myelomonocytic leukemia, chronic myelomonocytic leukemia, 18 (6.1%) had severe aplastic anemia, 12 (4.1%) had hemophagocytic lymphohistiocytosis, and 73 (24.9%) had various other diseases.

2.2 | Preparative regimens and supportive care

A total of 238 patients received myeloablative regimen while the rest had reduced-intensity regimen based on the definition proposed by Bacigalupo A et al.[5] Single cord blood (CB) unit was the standard approach, only a few (nine patients) received double CB units. Calcineurin inhibitor (cyclosporine or tacrolimus) in combination of methotrexate (bone marrow or peripheral blood stem cells) or mycophenolate mofetil (MMF, for CB unit) was used as GVHD prophylaxis. All patients received standard supportive care including antiviral (acyclovir) prophylaxis for positive HSV recipients, antifungal (fluconazole) prophylaxis, and preemptive CMV prophylaxis. No prophylactic antibiotics were used during the transplant course.

2.3 | Definitions

BSI was diagnosed if one or more blood cultures were positive for a bacterial pathogen with the exceptions that two or more positive blood cultures were required (at least two times within 72 hr and/or two different sites) to meet the definition of a BSI for the following organisms: *Propionibacterium* spp., *Bacillus* spp., coagulase negative staphylococci, *Clostridium perfringens*, and *viridans group streptococci*. These organisms are frequently associated with contamination, sometimes to rates as high as 30%.[6–9] Early BSI in this study was defined as the first BSI episode occurring within day +30.

Blood cultures were investigated in response to a sign of infection, typically pyrexia. Date of engraftment was defined as the first day of at least three consecutive days of an absolute neutrophil count of $>0.5 \times 10^9$/l after HSCT. Organisms causing BSI were categorized into two groups according to the National Healthcare Society Network definitions[10]: mucosal barrier injury (MBI) associated organisms and non-MBI-associated organisms. Prophylactic antibacterial antibiotics were not routinely prescribed in this study population.

AGVHD was graded based on AGVHD staging system devised in 1994 at the Consensus Conference held in Keystone as a modified version of the Glucksberg scale.[11]

Data were analyzed as of January 31, 2016. The CHOA institutional review board approved this project and a waiver of informed consent was obtained.

2.4 | Statistical analysis

The main effect examined was the association of first BSI occurring between day 0 and +30 and subsequent acute grade III–IV GVHD. This analysis was based on a time-dependent competing risk model. More specifically, BSI preceding the diagnosis of AGVHD was considered a risk factor, whereas BSI occurring after the diagnosis of AGVHD was not. Patients who relapsed/died within day +100 were censored at the time of relapse/death. Secondary analyses, examining the association between acute grade III–IV GVHD and preceding MBI-associated BSI and between acute grade III–IV GVHD and preceding non-MBI-associated BSI, were performed. An analysis of risk for early MBI-associated BSI was also performed. A χ^2 test or Mann–Whitney U test was used to compare patients with or without BSI. Risk factors for acute grade III–IV GVHD were evaluated by univariate and multivariate analyses using the Cox regression model. A multivariate model was constructed by the backward stepwise method using threshold *P*-values of 0.15 for removal or additions to the model. Values of $P < 0.05$ were considered significant. Measures of association are expressed as hazard ratios (HRs) with a 95% confidence interval (CI). Cumulative incidence of AGVHD was calculated using the Gray method. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Tochigi, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of the R commander designed to add statistical functions frequently used in biostatistics.[12]

3 | RESULTS

3.1 | Baseline characteristics

Baseline patient, disease, and transplantation-related characteristics categorized by whether recipients developed acute grade III–IV GVHD are shown in Table 1. There was no difference in time to engraftment between cases transplanted with single CB unit (median, 17 days; range 7–30 days) and with double CB units (median, 17 days; range 9–25 days).

3.2 | Incidence of acute grade III–IV GVHD

A total of 50 patients developed acute grade III–IV GVHD by day +100 representing the cumulative incidence of 17.1% (95% CI: 12.7–21.3). The median day of diagnosis was 22 (range 8–74). The incidence of acute grade III–IV GVHD by different graft sources was 4.2% (5/119), 30% (6/20), 17.6% (13/74), and 32.5% (26/80) for matched-related BM/PBSC/CB, mismatched-related BM/PBSC, unrelated BM/PBSC, and unrelated CB, respectively. This may also be confounded by different GVHD prophylaxis regimens as cyclosporine and MMF was the primary GVHD prophylaxis for CB grafts.

Among the 50 patients, three had stage 4 skin GVHD, 47 had stage 2–4 gut GVHD, and eight patients had stage 2–4 liver involvement. There was no significant correlation between BSI and cumulative incidence of grade II-IV GVHD ($P = 0.11$). However, the cumulative incidence of acute grade III–IV GVHD was significantly higher in patients with preceding BSI than in those without (30.4% (95% CI: 18.7–40.5) vs. 13.0% (95% CI: 8.5–17.3), $P < 0.001$), as shown in Figure 1. The cumulative incidence of acute grade III–IV GVHD, in patients with preceding MBI-associated BSI, was 39.0% (95% CI: 22.1–52.3), which

TABLE 1 Baseline characteristics and distribution of risk factors for acute grade III–IV GVHD

Acute GVHD, grade III–IV	(+)	n = 50	(–)	n = 243	*P*-value
Age					0.213
≥10 years	19	(38.0%)	118	(48.6%)	
<10 years	31	(62.0%)	125	(51.4%)	
Sex					0.347
Female	24	(48.0%)	98	(40.3%)	
Male	26	(52.0%)	145	(59.7%)	
Race					0.241
Caucasian	17	(34.0%)	113	(46.5%)	
African American	24	(48.0%)	91	(37.4%)	
Other	9	(18.0%)	39	(16.0%)	
Primary diagnosis					0.207
Malignant disease	34	(68.0%)	140	(57.6%)	
Others	16	(32.0%)	103	(42.4%)	
Primary immunodeficiency	5	(10.0%)	20	(8.2%)	0.780
Experience of relapse including primary induction failure (only in patient with malignant disease)	16	(47.1%)	61	(43.6%)	0.848
Prior HSCT	4	(8.0%)	21	(8.6%)	1.000
BMT period					0.756
2005–2009	25	(50.0%)	113	(46.5%)	
2010–2014	25	(50.0%)	130	(53.5%)	
HLA compatibility					<0.001
Matched	17	(34.0%)	173	(71.2%)	
Mismatched	33	(66.0%)	70	(28.8%)	
Donor type					<0.001
Related	11	(22.0%)	128	(52.7%)	
Unrelated	39	(78.0%)	115	(47.3%)	
Stem cell source					<0.001
BM	23	(46.0%)	176	(72.4%)	–
PBSC	1	(2.0%)	10	(4.1%)	1.000
CB	26	(52.0%)	57	(23.5%)	<0.001
Combination of graft sources					<0.001
Matched-related BM/PBSC/CB	5	(10.0%)	114	(46.9%)	–
Mismatched-related BM/PBSC	6	(12.0%)	14	(5.8%)	0.001
Unrelated BM/PBSC	13	(26.0%)	61	(25.1%)	0.004
Unrelated CB	26	(52.0%)	54	(22.2%)	<0.001
Intensity of conditioning					0.704
Myeloablative	41	(82.0%)	192	(79.0%)	
Reduced intensity	9	(18.0%)	51	(21.0%)	
TBI for conditioning	27	(54.0%)	99	(40.7%)	0.116
GVHD prophylaxis					<0.001
CSA/sMTX	14	(28.0%)	147	(60.5%)	–
CSA/MMF	23	(46.0%)	54	(22.2%)	<0.001
CSA/mPSL	3	(6.0%)	12	(4.9%)	0.164
Others	10	(20.0%)	30	(12.3%)	0.011
BSI between day 0 and +30 (prior to acute GVHD)					<0.001

(Continues)

TABLE 1 (Continued)

Acute GVHD, grade III–IV	(+)	n = 50	(–)	n = 243	*P*-value
No	29	(58.0%)	195	(80.2%)	–
Yes	21	(42.0%)	48	(19.8%)	0.002
MBI-associated BSI	16	(32.0%)	25	(10.3%)	<0.001
Non-MBI associated BSI	5	(10.0%)	23	(9.5%)	0.555
Donor/recipient CMV serology					0.097
–/–	16	(32.0%)	89	(36.6%)	–
+/–	1	(2.0%)	27	(11.1%)	0.122
–/+	24	(48.0%)	83	(34.2%)	0.220
+/+	9	(18.0%)	44	(18.1%)	0.819
Recipient CMV status					0.087
Recipient–	17	(34.0%)	116	(47.7%)	
Recipient+	33	(66.0%)	127	(52.3%)	

GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; TBI, total body irradiation; CSA, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil; mPSL, methylprednisolone ; BSI, blood stream infection; MBI, mucosal barrier injury; CMV, cytomegalovirus.

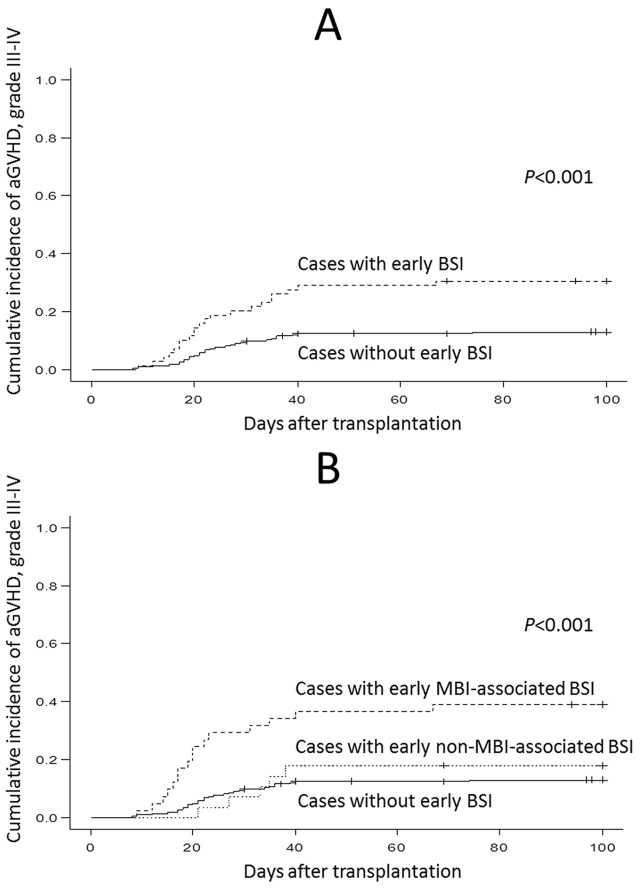

FIGURE 1 Cumulative incidence of acute grade III–IV GVHD at 100 days after transplantation (A) Cumulative incidence of acute grade III–IV GVHD in cases with BSI was 30.4% (95% CI: 18.7–40.5), which was significantly higher compared to cases without BSI (13.0%, 95% CI: 8.5–17.3) ($P < 0.001$). (B) Cumulative incidence of acute grade III–IV GVHD in cases with MBI-associated BSI was 39.0% (95% CI: 22.1–52.3), which was significantly higher compared to cases with non-MBI-associated BSI (17.9%, 95% CI: 2.4–30.9) and cases without BSI (13.0%, 95% CI: 8.5–17.3) ($P < 0.001$). GVHD, graft-versus-host disease; BSI, blood stream infection; MBI, mucosal barrier injury

was significantly higher than that in patients with preceding non-MBI-associated BSI (17.9%, 95% CI: 2.4–30.9) ($P < 0.001$). Meanwhile, the cumulative incidence of stage 2–4, GI-GVHD, in patients with preceding MBI-associated BSI was 33.1% (95% CI: 16.6–46.3), which was higher than that in patients with preceding non-MBI-associated BSI (17.9% (95% CI: 2.4–30.9)), although the difference was not significant ($P = 0.128$). The median duration between the diagnosis of BSI and acute grade III–IV GVHD development was 12 days (range 5–44).

3.3 | Multivariate analysis

In a multivariate analysis, using all BSI and other factors extracted from univariate analysis including HLA-mismatched donor, unrelated donor, CB as stem cell source, total body irradiation (TBI) for conditioning and recipient CMV seropositivity, HLA-mismatched donor (HR = 4.870, 95% CI: 2.510–9.450, $P < 0.001$), and preceding BSI between day 0 and +30 (HR = 3.010, 95% CI: 1.520–5.970, $P = 0.002$) were associated with acute grade III–IV GVHD. In an analysis using MBI-associated BSI rather than all BSI, MBI-associated BSI between day 0 and +30 (HR = 3.840, 95% CI: 1.750–8.420, $P = 0.001$), HLA-mismatched donor (HR = 3.480, 95% CI: 1.690–7.170, $P = 0.001$), and unrelated donor (HR = 2.24, 95% CI: 1.010–4.960, $P = 0.048$) were associated.

3.4 | Microbiology and risk for early BSI

The incidence of BSI by day +30 was 23.5% (69/293 cases). Organisms causing BSI are shown in Table 2. *α-Streptococcus* (n = 17) was the most common isolate. Fourteen of these were *viridans group streptococcus*. In five cases, two organisms were detected. MBI-associated organisms were responsible for 56.5% (39/69) of all BSI. MBI-associated BSI were more common in patients receiving TBI for conditioning than in those receiving non-TBI regimens (61.0% vs. 40.1%, HR = 2.340, 95% CI: 1.190–4.590, $P = 0.014$). The majority of patients receiving TBI (81.7% [103/126]) and non-TBI-based conditioning (77.8% [130/167]) received myeloablative regimens. There were no other baseline

TABLE 2 Organisms causing BSI between day 0 and +30 following allogeneic HSCT

Gram-positive cocci	51
α-Streptococcus	17
Viridans group streptococci[a]	14
Streptococcus mitis[a]	1
Streptococcus oralis[a]	1
Streptococcus penumoniae	1
Staphylococcus epidermidis	9
Staphylococcus aureus	9
Enterococcus faecalis[a]	5
Staphylococcus hominis	4
Staphylococcus warneri	2
Enterococcus faecium[a]	1
Staphylococcus auricularis	1
Staphylococcus hemolyticus	1
Staphylococcus capitis	1
β-Hemolytic streptococcus[a]	1
Gram-negative bacilli	24
Klebsiella pneumoniae[a]	5
Escherichia coli[a]	4
Enterobacter cloacae[a]	3
Stenotrophomonas maltophilia	2
Capnocytophaga spp.[a]	2
Pseudomonas aeruginosa	2
Haemophilus influenzae	1
Citrobacter freundii[a]	1
Bacteroides distasonis[a]	1
Neisseria sicca	1
Chryseobacterium indologenes	1
Serratia marcescens[a]	1

Two organisms were detected in two patients at the same time:
– *Viridans group streptococci*[a] + *Escherichia coli*[a].
– *Klebsiella pneumoniae*[a] + *Enterobacter cloacae*[a].
– *Stenotrophomonas maltophilia* + *Chryseobacterium indologenes.*
– *Viridans group streptococci*[a] + *Staphylococcus hominis.*
– *Streptococcus pneumoniae* + *Haemophilus influenza.*
[a]Organisms categorized in "mucosal barrier injury (MBI) associated" organisms according to the National Healthcare Safety Network (NHSN) definition.[9]
BSI, blood stream infection; HSCT, hematopoietic stem cell transplantation.

patient, disease, or treatment-related factors significantly associated with MBI-associated BSI.

4 | DISCUSSION

The results of our study examining the relationship between early BSI and AGVHD in children and adolescents suggest that, as in adults,[3] early BSI is a risk factor for severe AGVHD. Our results further suggest that only MBI-associated BSI is related to severe AGVHD. In the adult study, it was not studied whether the incidence of AGVHD differs depending on the types of bacteria identified in blood culture.[3]

It is plausible that systemic inflammation engendered by BSI could contribute to the development of AGVHD[13] by priming host-specific donor T cells, much the same way that gut inflammation does.[14,15]

On the other hand, the relationship between BSI and AGVHD may not be causal. It is conceivable that they are linked by the third confounding factor, namely GI mucositis and attendant bacterial translocation.[16] Our finding that only MBI-associated BSI is related to severe AGVHD is consistent with this supposition. A limitation of our study is that we did not assess mucositis. Another potential confounder is intestinal dysbiosis. A growing body of research has implicated changes in GI microbiota, especially loss of bacterial diversity, in the development of both GI GVHD and BSI.[17–21] Treatment of febrile neutropenia in allo-HSCT recipients using broad-spectrum antibiotic administration has been reported to be associated with increased GVHD frequency and GVHD-related mortality.[17–19] Analysis of stool specimen from allo-HSCT recipient was reported to be associated with perturbation of gut microbial composition in human patients and mice.[22] We did not evaluate the actual use of broad-spectrum antibiotics in our study. Studies on the use of antibiotics and changes in GI microbiota and concomitant changes in the causal organisms of bloodstream infection and the relationship with GI GVHD are expected in the future.

Although it might be an indirect effect related to mucositis or intestinal dysbiosis, BSI, especially MBI-associated BSI, was one of the risks of severe AGVHD. Further research involving a larger, multicenter cohort, careful assessment of mucositis and perturbations in the microbiota is needed to confirm our findings and explore these potential etiological links.

5 | CONCLUSION

Our results suggest that early MBI-associated BSI is a risk factor for subsequent AGVHD.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

H.S., J.A.H., M.Q., K-Y.C., and J.H. planned the study, interpreted the data, and wrote the manuscript. K.A. performed data quality assurance. J.N. and B.W. helped with data interpretation.

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