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RESEARCH ARTICLE

Positive stool culture could predict the clinical outcomes of haploidentical hematopoietic stem cell transplantation

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Abstract **We aimed to identify the effect of positive stool cultures (PSCs) on the clinical outcomes of patients undergoing haploidentical hematopoietic stem cell transplantation (haplo-HSCT) (n = 332). PSCs were observed in 61 patients (PSC group, 18.4%). Enterobacteriaceae in stool specimens was associated with a higher risk of bloodstream infection, and *Candida* in stool specimens was related to a higher risk of platelet engraftment failure. The cumulative incidence of infection-related mortality 1 year after haplo-HSCT in the PSC group was higher than that of the patients who showed persistently negative stool cultures (NSC group; 19.2% vs. 8.9%, P = 0.017). The probabilities of overall survival (71.4% vs. 83.8%, P = 0.031) and disease-free survival (69.6% vs. 81.0%, P = 0.048) 1 year after haplo-HSCT for the PSC group were significantly lower than those for the NSC group, particularly for patients who had *Candida* in their stool specimens. In multivariate analysis, *Candida* in stool specimens significantly increased the risk of mortality and was associated with poorer survival. Our results showed that PSC influenced the clinical outcomes after haplo-HSCT, particularly those who had *Candida* in their stool specimens.**

Keywords haploidentical; hematopoietic stem cell transplantation; stool culture; *Candida*

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective post-consolidative and curative option for patients with hematological malignances. China has a shortage of human leukocyte antigen (HLA)-matched sibling donors and unrelated donors [1]. As such, hematopoietic stem cell transplantation using an HLA-haploidentical donor (haplo-HSCT) becomes a viable alternative to transplant procedures [2–4], and it has been the largest source of allo-HSCT donors in China since 2013 [5,6]. However, severe complications, such as engraftment failure, acute graft-versus-host disease (aGVHD), and infection, remarkably influence the clinical outcomes of haplo-HSCT [7,8].

The human gastrointestinal tract harbors a highly diverse microbial population, and the composition of intestinal microbiota has been implicated in several human diseases, such as rheumatoid arthritis [9], malnutrition, kwashiorkor development [10], obesity [11], and diabetes [12]. Several studies have also investigated how intestinal microbiota affected allo-HSCT outcomes [13,14]. The intestinal microbiota of many patients undergoing haplo-HSCT may be altered during transplantation because of the intense conditioning and the use of prophylactic or therapeutic antibiotics. Positive stool cultures (PSCs) can reflect the dominant microorganisms in the gastrointestinal tract because most of haplo-HSCT recipients routinely received bacterial decontamination; however, studies have yet to identify the role of stool cultures in haplo-HSCT recipients.

In this retrospective study, we aimed to identify the effect of microorganisms in stool cultures on the clinical outcomes of haplo-HSCT recipients.

Materials and methods

Patients

Consecutive patients who underwent non-T cell-depleted haplo-HSCT from January 2016 to November 2017 at the

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Peking University Institute of Hematology (PUIH) were enrolled if they underwent a comprehensive fecal culture analysis, which targeted all bacteria and fungi in the gastrointestinal tract during the pre-engraftment phase. A total of 332 patients were enrolled (Table 1). The endpoint of the last follow-up was on March 31, 2018. The Institutional Review Board of Peking University approved this study, and informed consent was obtained from all of the subjects and donors. This study was conducted in accordance with the *Declaration of Helsinki*.

Table 1 Patient characteristics

Variable	Negative stool cultures group (n = 271)	Positive stool cultures group (n = 61)	P value
Gender of patients, male/female	161/110	34/27	0.599
Median age at transplantation, year (range)	30 (2–67)	27 (7–63)	0.123
Adult/children	227/44	52/9	0.775
Diagnosis, no. (%)			
Acute myeloid leukemia	106 (39.1)	20 (32.8)	0.773
Acute lymphoblastic leukemia	76 (28.0)	20 (32.8)	
Myelodysplastic syndrome	37 (13.7)	10 (16.4)	
Severe aplastic anemia	32 (11.8)	5 (8.2)	
Chronic myeloid leukemia	6 (2.2)	2 (3.2)	
Others	14 (5.2)	4 (6.6)	
Chemotherapy before HSCT, no. (%)	198 (73.1)	45 (73.8)	0.910
Median cycles before HSCT, no. (range)	4 (1–18)	4 (1–14)	0.228
Disease status at transplantation, no. (%)			
Standard risk	259 (95.6)	61 (100.0)	0.133
High risk	12 (4.4)	0 (0.0)	
Disease risk index before transplantation, no. (%)			
Low risk	52 (19.2)	10 (16.4)	0.363
Intermediate risk	187 (69.0)	40 (65.6)	
High risk	28 (10.3)	11 (18.0)	
Very high risk	4 (1.5)	0 (0.0)	
No. of HLA-A, -B, and -DR mismatch, no. (%)			
3/6	229 (84.5)	48 (78.7)	0.462
4/6	33 (12.2)	10 (16.4)	
5/6	9 (3.3)	3 (4.9)	
Previous HSCT, no. (%)[a]	3 (1.1)	0 (0.0)	1.000
Donor–recipient sex match, no. (%)			
Female–male	39 (14.4)	8 (13.1)	0.796
Others	232 (85.6)	53 (86.9)	
Donor–recipient relations, no. (%)			
Father	123 (45.4)	31 (50.8)	0.207
Mother	12 (4.4)	5 (8.2)	
Sibling	55 (20.3)	14 (23.0)	
Child	74 (27.3)	9 (14.8)	
Others	7 (2.6)	2 (3.2)	
Donor–recipient blood type, no. (%)			
Matched	142 (52.4)	29 (47.5)	0.660
Major mismatched	55 (20.3)	13 (21.3)	
Minor mismatched	58 (21.4)	13 (21.3)	
Major and minor mismatched	16 (5.9)	6 (9.9)	
Median mononuclear cells, $\times 10^8$/kg (range)	8.1 (4.6–13.2)	8.0 (5.2–11.4)	0.463
Median $CD4^+$ counts, $\times 10^7$/kg (range)	1.1 (0.1–4.2)	1.0 (0.2–2.3)	0.870
Median $CD34^+$ counts, $\times 10^6$/kg (range)	2.3 (0.5–8.2)	2.4 (0.3–7.6)	0.264

(*Continued*)

Variable	Negative stool cultures group (n = 271)	Positive stool cultures group (n = 61)	P value
Previous broad-spectrum antibiotics before stool cultures, no. (%)			
Carbapenem	91 (33.6)	22 (36.1)	0.711
Other β-lactam antibiotics	128 (47.2)	22 (36.1)	0.113
Tigecycline	8 (3.0)	4 (6.6)	0.244
Vancomycin/teicoplanin/linezolid	33 (12.2)	13 (21.3)	0.062
Multiple broad-spectrum antibiotics (⩾2)	110 (40.6)	23 (37.7)	0.678
Oral mucositis during preconditioning treatment, no. (%)			
None	223 (82.3)	53 (86.9)	0.647
Grades I to II	40 (14.8)	6 (9.8)	
⩾Grade III	8 (2.9)	2 (3.3)	
Gastrointestinal tract toxicities during preconditioning treatment, no. (%)			
⩾Grade II	64 (23.6)	11 (18.0)	0.637
Cumulative dose of corticosteroid before stool cultures after haplo-HSCT, no. (prednisone, mg/kg)	4.9 (0.3–126.0)	5.1 (0.6–33.8)	0.483
Median duration of follow-up in survivors, day (range)	434 (61–765)	391 (64–756)	0.351

HLA, human leukocyte antigen.
[a] All of the three patients received autologous stem cell transplantation.

Transplant regimen

The major preconditioning treatment for patients with hematologic malignancies consisted of the administration of cytarabine (4 g/(m^2/day), from days −10 to −9, and day 0 as the first day of donor cell infusion), busulfan (3.2 mg/(kg/day) administered intravenously on days −8 to −6), cyclophosphamide (CY, 1.8 g/(m^2/day), from days −5 to −4), and semustine (250 mg/m^2, on day −3). This treatment also included rabbit antithymocyte globulin (ATG, thymoglobulin, 2.5 mg/(kg/day) and rabbit ATG from Imtix Sangstat, Lyon, France; from days −5 to −2) [15]. For patients with severe aplastic anemia, the preconditioning treatment included the administration of busulfan (3.2 mg/(kg/day) administered intravenously from days −7 to −6), CY (50 mg/(kg/day), from days −5 to −2), and ATG (thymoglobulin, 2.5 mg/(kg/day); rabbit ATG from Imtix Sangstat, Lyon, France; from days −5 to −2). All of patients received cyclosporine A (CSA), mycophenolate mofetil, and short-term methotrexate for aGVHD prophylaxis. All of the patients received granulocyte colony-stimulating factor-mobilized, fresh, and unmanipulated bone marrow (G-BM) cells and peripheral blood (G-PB) stem cells [16,17].

Infection prevention regimen

All of the patients were hospitalized in rooms with high-efficiency particulate arresting (HEPA) air filters for 4–5 weeks from day –10 until the time at which neutrophil recovery was achieved. All of the patients received antibiotics for gastrointestinal decontamination before and during the period the patient had neutropenia. In particular, fluoroquinolone was orally given to adults, and an injection liquid of gentamicin was orally given to children. The patients received trimethoprim-sulfamethoxazole to prevent *Pneumocystis jirovecii* infection from days –10 to + 180. The patients who did not have an invasive fungal infection (IFI) before they underwent haplo-HSCT received fluconazole (n = 57) or itraconazole capsules (n = 140) to prevent IFI from days –10 to + 75. Conversely, the patients who had an IFI before they had haplo-HSCT were given itraconazole injection (n = 28), voriconazole (n = 58), posaconazole (n = 1), or echinocandins (n = 48) to prevent the recrudescence of IFI from days –10 to + 100. Acyclovir (200–400 mg) was also orally administered against herpes simplex virus and varicella zoster virus. It was administered twice daily from day + 1 until the time CSA was discontinued. Ganciclovir (5 mg/kg) was administered intravenously twice daily from days –9 to –2 to achieve prophylaxis against cytomegalovirus infection. The infection surveillance and treatment protocols used at our institute were described in detail in a previous study [18].

Stool specimen analysis

After the patients received haplo-HSCT, the surveillance cultures of microorganisms, including bacteria and fungi, in stool specimens were prepared during the pre-engraftment period, that is, from day −10 until the neutrophil engraftment. The target microorganisms were all bacteria and fungi in the gastrointestinal tract. The routine culturing of strictly anaerobic bacteria was not performed because

processing specimens immediately after they were collected is impossible. Stool specimens were collected into sterile containers and inoculated in Columbia blood agar with a sterile swab within 2 h of collection. The Columbia blood agar plate was incubated for 18–24 h at 35 °C. If any of the bacteria and fungi did not grow for 24 h, the incubation time was extended to 48 h. Subsequently, up to five colonies of any bacteria or fungi were subcultured and isolated for further identification. The identified species were confirmed through matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF, Bruker Autoflex, Bruker Daltonik, Bremen, Germany). Antimicrobial susceptibility was tested in a clinical microbiology laboratory by using a Vitek 2 compact automated system (bioMérieux, Marcy l'Etoile, France).

Definitions and assessments

The following subjects were defined as low risk: (1) subjects with acute leukemia were at a standard risk if they were in first or second complete remission (CR); (2) subjects with lymphoma or multiple myeloma were at a standard risk if they were in first or second CR, partial remission, or had a stable disease; (3) subjects with chronic myeloid leukemia in the first chronic phase; (4) subjects with myelodysplastic syndrome with $< 20\%$ BM blasts; and (5) subjects with nonmalignant hematologic disorders (severe aplastic anemia, $n = 37$; adrenoleukodystrophy, $n = 2$; and mucopolysaccharidoses, $n = 1$). The other subjects were at a high risk [7]. Disease risk index during diagnosis was reported in accordance with the criteria of Armand *et al*. [19], and a nonmalignant hematology disorder was defined as a low risk in the present study. Neutrophil engraftment was defined as the 1st day an absolute neutrophil count of $\geqslant 0.5 \times 10^9$ cells/L was observed for 3 consecutive days, and platelet engraftment denoted the 1st day a platelet count of $\geqslant 20 \times 10^9$ cells/L was observed for 7 consecutive days without transfusion. aGVHD was diagnosed in accordance with the internationally accepted criteria [20]. Relapse was defined by the morphologic evidence of disease in the peripheral blood, marrow, or extramedullary sites or by the recurrence and sustained presence of pretransplantation chromosomal abnormalities. Patients who showed a minimal residual disease (MRD) were not considered to have relapsed. Nonrelapse mortality (NRM) was defined as death because of any cause in the first 28 days post-HSCT or death without evidence of disease recurrence beyond day 28. Infection-related mortality (IRM) was defined as death resulting from infection. Overall survival (OS) was defined as the time from transplantation to death because of any cause. Disease-free survival (DFS) was defined as survival in continuous CR.

Statistical analysis

Data were censored at the time of death or the last available follow-up. Continuous variables were compared using a Mann–Whitney *U* test, whereas categorical variables were compared using χ^2 and Fisher's exact tests. Survival probabilities were estimated using the Kaplan–Meier method. Competing risk analyses were performed to calculate the cumulative incidence of engraftments, aGVHD, relapse, and NRM. Gray's test was conducted to assess differences between the groups [21]. Potential prognostic factors for outcomes were evaluated through multivariate analysis via Cox proportional hazard regression with a forward stepwise model selection approach. Cox proportional hazard models included the following variables: sex of the recipient, age (< 18 years vs. $\geqslant 18$ years), underlying disease (hematologic malignancy vs. nonmalignant hematologic disorder), matching of sex between donor and recipient (female to male vs. others), donor–recipient relationship (mother to child vs. others), donor–recipient blood type (major mismatched/major and minor mismatched vs. others), HLA disparity (0–1 vs. 2–3), disease risk index before haplo-HSCT (low risk, intermediate risk, high risk, and very high risk), previous broad-spectrum antibiotic exposure (yes vs. no), and microorganisms in stool specimens. Independent variables with $P > 0.1$ were sequentially excluded from the model, and those with $P < 0.05$ were considered to be statistically significant. All reported *P* values were based on two-sided hypothesis tests. Data analyses were primarily conducted with SPSS (SPSS Inc., Chicago, IL, USA), whereas competing risk analysis was performed with the R software package (v. 2.6.1).

Results

Results of stool cultures

A total of 1440 stool specimens were obtained from 332 patients during the pre-engraftment phase after they received haplo-HSCT. PSCs were observed in 61 patients (18.4%; PSC group), and the characteristics were comparable with those of patients who had persistently negative stool cultures (NSC group) (Table 1). The median time from transplantation to obtaining positive stool result was $+1$ day (range, -9 to $+18$ days, and day 0 as the 1st day of donor cell infusion), 27 before and 34 after donor cell infusion, respectively. The most common bacterium identified from stool specimens was *Escherichia coli* followed by *Enterococcus faecium*, *Enterococcus faecalis*, and *Klebsiella pneumoniae* (Table 2). Sixteen specimens of Enterobacteriaceae were positive for extended-spectrum β-lactamases (ESBL; *Escherichia coli*, 12; *Klebsiella*

pneumoniae, 3; *Proteus mirabilis*, 1), and none of the specimens of *Staphylococcus* were methicillin resistant. The most common yeast identified from stool specimens was *Candida albicans* followed by *Candida glabrata* and *Saccharomyces cerevisiae.* The detection rate of *Candida* was comparable between patients receiving azole and echinocandin antifungal agents for IFI prophylaxis (7.0% vs. 14.6%, $P = 0.088$). Multiple pathogens ($\geqslant 2$ pathogens) were identified in 15 patients, and most of them were identified as Enterobacteriaceae plus *Enterococcus* ($n = 7$) or *Enterococcus* plus *Candida* ($n = 4$) in stool specimens.

Table 2 Microorganisms in stool specimens

	Type of organism	Number
Enterobacteriaceae	*Escherichia coli*	22
	Klebsiella pneumoniae	4
	Proteus mirabilis	2
Enterococcus	*Enterococcus faecalis*	5
	Enterococcus faecium	9
Other bacteria	*Pseudomonas aeruginosa*	2
	Streptococcus salivarius	1
Candida	*Candida albicans*	16
	Candida parapsilosis	1
	Candida glabrata	7
	Candida tropicalis	1
	Candida krusei	1
	Saccharomyces cerevisiae	2

Effect of PSC on bloodstream infection (BSI) and other infections

BSI

A BSI was observed in 63 patients after transplantation. The most common pathogen was *E. coli* ($n = 18$), followed by *K. pneumoniae* ($n = 10$), *Staphylococcus epidermidis* ($n = 7$), *E. faecium* ($n = 5$), *Staphylococcus hemolyticus* ($n = 4$), *Pseudomonas aeruginosa* ($n = 4$), *Staphylococcus hominis* ($n = 3$), and *Stenotrophomonas maltophilia* ($n = 2$). A total of 25 specimens of Enterobacteriaceae had ESBL (*E. coli*, 16; *K. pneumoniae*, 9), and all of the specimens of *Staphylococcus* were methicillin resistant. Two patients presented *Trichosporon asahii* BSI. The BSI rate was comparable between PSC and NSC groups (24.6% vs. 17.7%, $P = 0.216$). The patients whose stools had Enterobacteriaceae had a higher rate of BSI than those without Enterobacteriaceae in stool specimens (37.5% vs. 17.5%, $P = 0.027$). The patients whose stools had *E. coli* seemed to have a higher rate of *E. coli* BSI than those without *E. coli* in stool specimens (13.6% vs. 4.8%, $P = 0.107$). Similarly, the patients whose stools contained *K. pneumoniae* seemed to have a higher rate of *K. pneumoniae* BSI than those without *K. pneumoniae* in stool specimens (25.0%% vs. 2.7%, $P = 0.116$). In general, the patients whose stools had Enterobacteriaceae had a higher rate of Enterobacteriaceae BSI than those without Enterobacteriaceae in stool specimens (16.7% vs. 7.8%, $P = 0.132$). However, the patients whose stools had *Enterococcus* (20.0% vs. 18.9%, $P = 1.000$) or *Candida* (18.5% vs. 19.0%, $P = 1.000$) did not have a higher rate of BSI. The patients with *Enterococcus* in their stools did not also have a higher rate of *Enterococcus* BSI (0.0% vs. 1.9%, $P = 1.000$). The patients with *Candida* in their stools did not have a higher rate of fungal BSI (0.0% vs. 0.7%, $P = 1.000$).

Other infections

The rate of central nervous system infection (8.3% vs. 1.6%, $P = 0.084$) and skin infection (12.5% vs. 3.6%, $P = 0.071$) of the patients whose stools were positive for Enterobacteriaceae was higher than that of the patients without Enterobacteriaceae in stool specimens. The patients whose stools presented *Enterococcus* had a higher rate of gut infection (20.0% vs. 5.4%, $P = 0.053$) than those without *Enterococcus* in the stool specimens. The patients with *Candida* detected in their stools had a significantly higher rate of pulmonary infection (77.8% vs. 54.4%, $P = 0.019$) than those without *Candida* in the stool specimens (Supplementary Table 1). None of the patients manifested invasive candidiasis in the present study, and the patients whose *Candida* specimens were isolated from their stools did not experience a higher rate of other IFIs than those without *Candida* in the stool specimens (3.7% vs. 3.6%, $P = 1.000$).

Effect of PSC on engraftment and aGVHD

The neutrophil engraftments occurred at a median of 13 (range, 8–26 days) and 14 days (range, 2–30 days) in NSC and PSC groups ($P = 0.749$), respectively, whereas platelet engraftments occurred at a median of 20 (range, 7–120 days) and 21 days (range, 8–210 days) in NSC and PSC groups, respectively ($P = 0.263$). The neutrophil and platelet engraftments were comparable between the NSC and PSC groups (Table 3); however, the 100-day cumulative incidence of platelet engraftment in patients with *Candida* in the stool specimens (66.7%, 95% CI 48.1%–85.3%) was lower than that in patients without *Candida* in the stool specimens (87.9%, 95% CI 84.2%–91.6%; $P = 0.043$). The presence of *Candida* in the stool specimens was associated with a high risk of platelet engraftment failure (Table 3).

The occurrence of grades II to IV and grades III to IV aGVHD was comparable between the NSC and PSC groups, respectively. Enterobacteriaceae, *Enterococcus*, and *Candida*, which were isolated from stool specimens, did not result in an increased risk of aGVHD (Table 3).

Effect of PSC on relapse, NRM, IRM, DFS, and OS

The cumulative incidence of relapse 1 year after haplo-HSCT was comparable between the NSC (6.3%, 95% CI 3.0%–9.6%) and PSC (8.1%, 95% CI 0.3%–15.9%; P = 0.594; Fig. 1A; Table 4) groups. The causes of NRM are presented in Supplementary Table 2. The cumulative incidence of NRM 1 year after haplo-HSCT in the PSC group (22.2%, 95% CI 10.8%–33.6%) tended to be higher than that of the NSC group (12.7%, 95% CI 8.5%–16.9%; P = 0.059; Fig. 1B; Table 4). The probability of IRM 1 year after haplo-HSCT in the PSC group (19.2%, 95% CI 8.2%–30.2%) was significantly higher than that in the NSC group (8.9%, 95% CI 5.1%–12.7%; P = 0.017; Fig. 1C; Table 4).

The probabilities of OS and DFS 1 year after haplo-HSCT were lower in the PSC group than in the NSC group (OS: PSC, 71.4%, 95% CI 58.8%–84.0%; NSC, 83.8%, 95% CI 79.1%–88.5%, P = 0.031; DFS: PSC, 69.6%, 95% CI 56.8%–82.4%; NSC, 81.0%, 95% CI 75.9%–86.1%, P = 0.048; Fig. 1D and 1E; Table 4).

Enterobacteriaceae and *Enterococcus* in the stool specimens were not associated with relapse, NRM, and survival; conversely, *Candida* in the stool specimens was significantly related to higher IRM and lower survival rate than those without *Candida* detected in their stool (Fig. 2A–2C; Table 4). In multivariate analysis, the presence of *Candida* in the stool specimens significantly increased the risk of NRM and IRM and was associated with a poor OS and DFS (Table 5).

The patients with hematologic malignancies and who had PSC or *Candida* detected in their stools showed poor survival rates. Likewise, the patients with nonmalignant hematologic disorders and who had PSC or *Candida* in their stools had poor survival rates (Supplementary Figs. 1 and 2).

Table 3 Effect of PSCs on the engraftment and aGVHD

	30-day neutrophil engraftment failure		100-day platelet engraftment failure	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Microorganism in stool specimens				
No	1.0	0.361	1.0	0.330
Yes	1.13 (0.86–1.53)		1.16 (0.86–1.57)	
Enterobacteriaceae in stool specimens				
No	1.0	0.201	1.0	0.871
Yes	1.33 (0.86–2.06)		0.96 (0.62–1.50)	
Enterococcus in stool specimens				
No	1.0	0.926	1.0	0.828
Yes	0.97 (0.57–1.67)		1.06 (0.61–1.86)	
Candida in stool specimens				
No	1.0	0.480	1.0	0.041
Yes	1.16 (0.77–1.74)		1.60 (1.02–2.50)	
	100-day grades II to IV aGVHD		100-day grades III to IV aGVHD	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Microorganism in stool specimens				
No	1.0	0.266	1.0	0.629
Yes	0.76 (0.47–1.24)		0.83 (0.39–1.77)	
Enterobacteriaceae in stool specimens				
No	1.0	0.077	1.0	0.279
Yes	0.45 (0.18–1.09)		0.46 (0.11–1.88)	
Enterococcus in stool specimens				
No	1.0	0.253	1.0	0.308
Yes	0.51 (0.16–1.61)		0.05 (0.01–16.98)	
Candida in stool specimens				
No	1.0	0.942	1.0	0.680
Yes	1.02 (0.54–1.96)		0.78 (0.24–2.52)	

PSCs, positive stool cultures; aGVHD, acute graft-versus-host disease; CI, confidence interval.

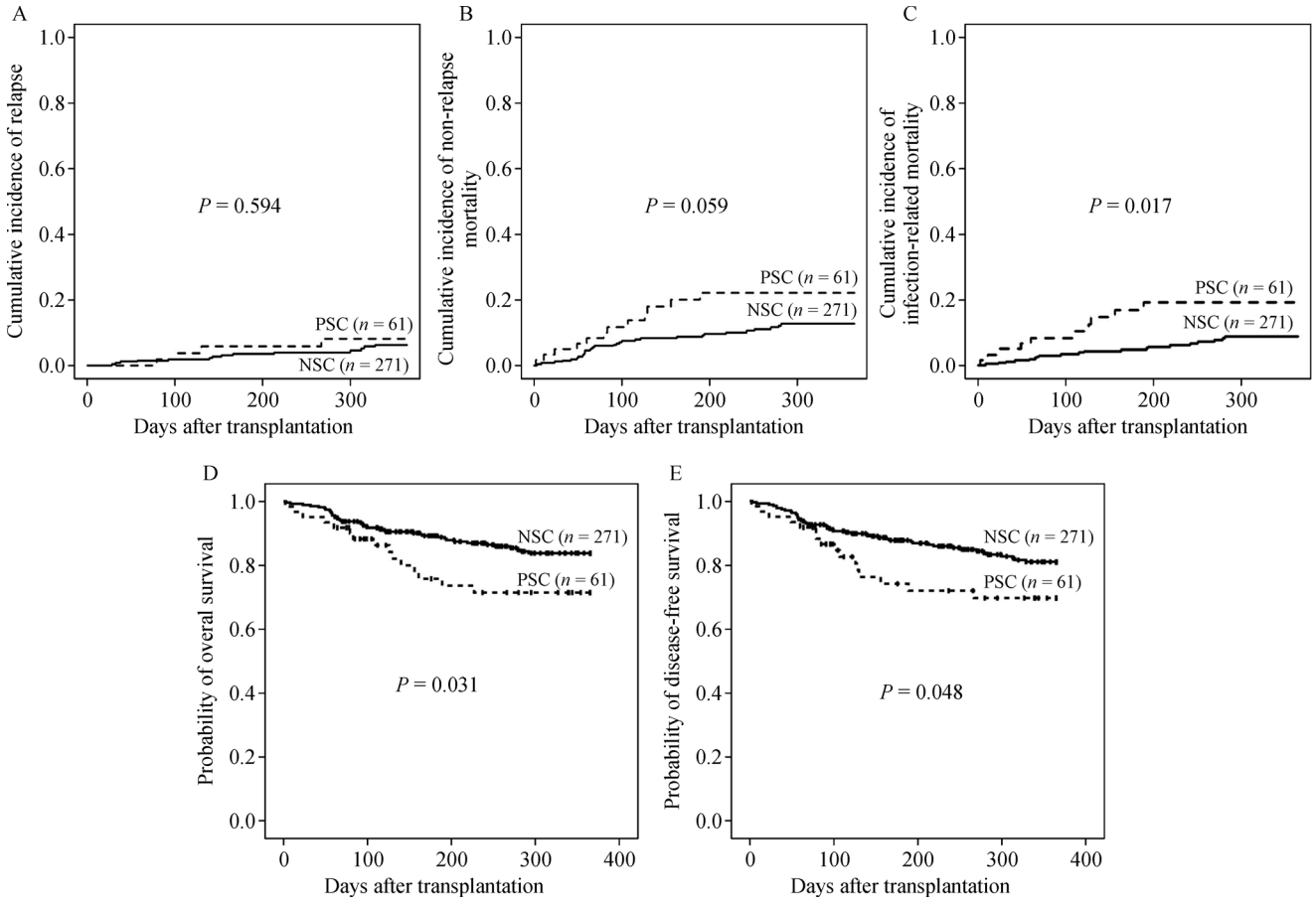

Fig. 1 Clinical outcomes of patients who underwent haplo-HSCT on the basis of the results of stool cultures. (A) relapse, (B) non-relapse mortality, (C) infection-related mortality, (D) overall survival, and (E) disease-free survival. NSC, negative stool cultures; PSC, positive stool cultures.

Discussion

In the present study, we observed that Enterobacteriaceae and *Candida* present in stool specimens were associated with a higher risk of BSI and a higher risk of platelet engraftment failure in patients receiving haplo-HSCT, respectively. PSCs were associated with low survival rates, particularly for those who had *Candida* in their stool specimens. To our knowledge, this study is the first to provide an opportunity for investigating the unexplored role of PSCs in haplo-HSCT recipients.

The presence of *Candida* in stool specimens significantly increased the risk of NRM and was associated with poor OS and DFS possibly because the presence of *Candida* in stools is associated with pulmonary infection after haplo-HSCT is completed, and pulmonary infection after patients receive allo-HSCT negatively influences their survival [18,22–24]. This phenomenon may also be attributed to the negative effect of *Candida* on platelet engraftment. Infection may contribute to the delayed engraftment in allo-HSCT [25,26]. In our study, the NRM of patients with prolonged thrombocytopenia was higher and their survival rates were lower after they received haplo-HSCT compared with patients without prolonged thrombocytopenia [27]. The colonization of the gastrointestinal tract by *Candida* may be a potential risk factor of invasive candidiasis in patients with neutropenia [28,29]. However, invasive candidiasis infection was not identified in the present study. Therefore, stool specimens should be monitored to determine the presence of *Candida* after patients receive haplo-HSCT.

The rate of BSI of patients with Enterobacteriaceae in stool specimens was higher than that of patients who had NSCs. Among patients receiving a HLA identical sibling donor or an unrelated donor HSCT, patients whose gut is colonized by antibiotic-resistant bacteria experience bacteremia [30–32], and the suppression of intestinal microflora can significantly decrease the incidence of bacteremia [33]. A high concentration of Enterobacteriaceae in stool may pose a high risk of the translocation of bacteria in the

Table 4 Effect of PSCs on relapse, nonrelapse mortality, and survival

	1-year relapse		1-year non-relapse mortality		1-year infection-related mortality	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Microorganism in stool specimens						
No	1.0	0.482	1.0	0.057	1.0	0.016
Yes	1.49 (0.49–4.53)		1.91 (0.98–3.72)		2.54 (1.19–5.43)	
Enterobacteriaceae in stool specimens						
No	1.0	0.484	1.0	0.397	1.0	0.368
Yes	0.05 (0.01–263.11)		1.56 (0.56–4.37)		1.73 (0.53–5.72)	
Enterococcus in stool specimens						
No	1.0	0.190	1.0	0.506	1.0	0.799
Yes	2.68 (0.62–11.65)		0.51 (0.07–3.71)		0.77 (0.11–5.67)	
Candida in stool specimens						
No	1.0	0.117	1.0	0.035	1.0	0.002
Yes	2.70 (0.78–9.34)		2.39 (1.06–5.38)		3.87 (1.66–9.03)	

	1-year disease-free survival		1-year overall survival	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Microorganism in stool specimens				
No	1.0	0.046	1.0	0.030
Yes	1.79 (1.01–3.17)		1.93 (1.07–3.51)	
Enterobacteriaceae in stool specimens				
No	1.0	0.893	1.0	0.658
Yes	1.07 (0.39–2.96)		1.26 (0.45–3.49)	
Enterococcus in stool specimens				
No	1.0	0.832	1.0	0.699
Yes	1.13 (0.36–3.62)		1.26 (0.39–4.03)	
Candida in stool specimens				
No	1.0	0.008	1.0	0.013
Yes	2.50 (1.27–4.92)		2.48 (1.21–5.07)	

PSCs, positive stool cultures; aGVHD, acute graft-versus-host disease; CI, confidence interval.

blood stream. An early BSI can increase the risk of the subsequent development of grade II or higher of aGVHD [34], and the mortality of BSI in patients with neutropenia can be as high as 20% [35,36]. Thus, decreasing gut colonization by Enterobacteriaceae is likely a good approach to improve the clinical outcomes of haplo-HSCT recipients.

The role of microbiota following allo-HSCT has been investigated, and aGVHD significantly decreases in mice transferred in a germ-free environment [37,38]. Several clinical studies on intestinal decontamination in patients receiving allo-HSCT have shown promising results. Beelen *et al*. [39] demonstrated that the median concentrations of anaerobic bacteria are high in the post-transplant period in patients who have contracted grades II to IV of aGVHD, and an antimicrobial therapy targeted toward intestinal anaerobic bacteria significantly reduces the severity of aGVHD. However, these results have not been reproduced in other studies [40,41], and administering a broad-spectrum antibiotic for patients who receive allo-HSCT increases GVHD-related mortality in mice and humans [42,43]. In the present study, the microorganisms in the stool specimens were not associated with the occurrence of aGVHD. Conflicting results have been obtained likely because these studies have been conducted in a nonsterile clinical setting instead of a microbiologically isolated environment, and decontaminating regimens have been successful to varying degrees [44,45].

Diversity in the intestinal flora is lost after patients receive allo-HSCT, and an intestinal microbiome exhibiting restricted diversity is an independent risk factor of the increased mortality of patients who receive allo-HSCT [46,47]. In the present study, one or two kinds of microorganisms were observed in the stool specimens of most patients in the PSC group, suggesting a restricted diversity of the intestinal microbiome. This finding might also contribute to the poor survival of the PSC group. However, we did not use pyrosequencing to screen the

Table 5 Multivariate analysis of risk factors of clinical outcomes

Outcome	HR (95% CI)	*P* value
Treatment failure as defined by overall survival		
Candida in stool specimens		
No	1	
Yes	2.53 (1.22–5.24)	0.012
Disease risk index		
Low risk	1	
Intermediate risk	1.41 (0.59–3.38)	0.441
High risk	3.18 (1.19–8.47)	0.021
Very high risk	25.44 (6.91–93.73)	<0.001
Treatment failure as defined by disease-free survival		
Candida in stool specimens		
No	1	
Yes	2.60 (1.31–5.18)	0.006
Disease risk index		
Low risk	1	
Intermediate risk	1.64 (0.69–3.89)	0.263
High risk	3.98 (1.53–10.37)	0.005
Very high risk	41.05 (11.02–152.95)	<0.001
Non-relapse mortality		
Candida in stool specimens		
No	1	
Yes	2.39 (1.06–5.38)	0.035
Infection-related mortality		
Candida in stool specimens		
No	1	
Yes	3.87 (1.66–9.03)	0.002
Relapse		
Disease risk index		
Low risk	1	
Intermediate risk	2.63 (0.34–20.37)	0.355
High risk	11.08 (1.33–92.05)	0.026
Very high risk	470.00 (37.69–5860.29)	<0.001

CI, confidence interval; HR, hazard ratio.

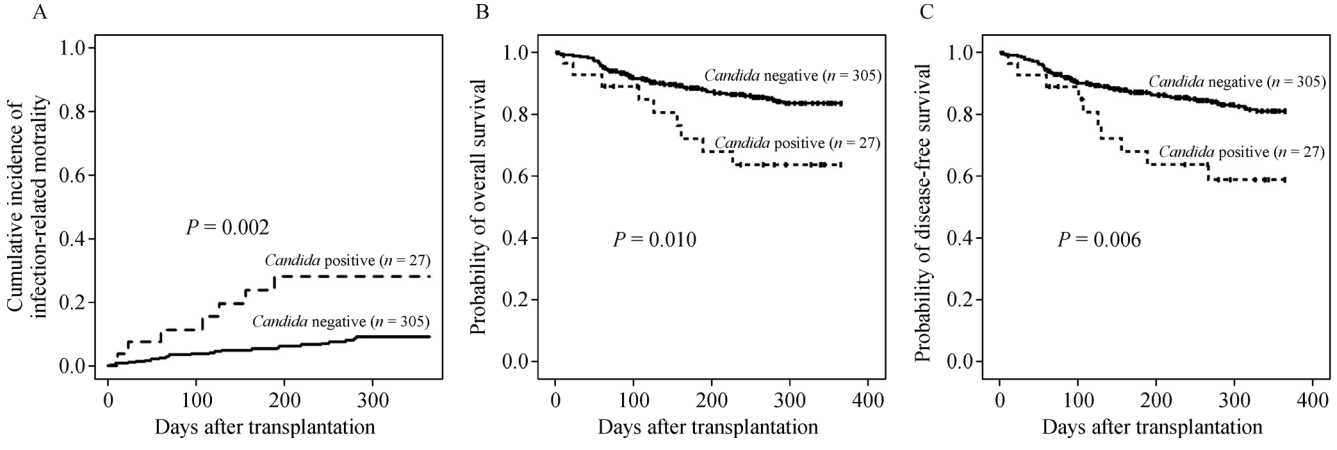

Fig. 2 Survival of haplo-HSCT recipients with and without *Candida* in stool specimens. (A) Infection-related mortality, (B) overall survival, and (C) disease-free survival.

microorganisms in the stool specimens, and we could not further estimate the intestinal flora diversity in haplo-HSCT recipients.

Several factors, such as previous broad-spectrum antibiotics, corticosteroids, and transplantation, may negatively affect the microbiota in the gut of patients after they undergo halo-HSCT. However, these factors were all comparable between PSC and NSC groups. This similarity might be due to the widespread use of spectrum antibiotics in the neutropenia phase of patients with a hematologic disorder. Moreover, corticosteroids are widely used to prevent anaphylactic reactions and serum sickness because ATG is one of the most important components of the conditioning regimen for haplo-HSCT recipients. These findings might partially support the finding that exposure to previous broad-spectrum antibiotics and corticosteroids was similar between PSC and NSC groups. Only three patients received autologous stem cell transplantation, and we could not investigate any further correlation between previous transplantation and microbiota after patients underwent haplo-HSCT.

A major limitation of this study is its retrospective design and the relatively small specimen size of the PSC group. Our cohort did not include other haplo-HSCT protocols, such as haplo-HSCT with depleted T cells or with post-transplant CY. As such, the role of microorganisms in the stool specimens of haplo-HSCT recipients should be further examined. Second, the prevalence of bacteria might vary, possibly influencing the association between microorganisms in stool specimens and clinical outcomes. Third, oral decontamination differed between adults (fluoroquinolone, which shows anti-coccus and anti-bacillus activities) and children (gentamicin, which induces anti-bacillus activity only). However, the age of patients and the proportion of children were comparable between PSC and NSC groups. In addition, age did not influence the clinical outcomes in multivariate analysis. Therefore, age might not affect the clinical outcomes in the present study. Lastly, oral decontamination might vary between patients because some patients might not have taken oral decontamination because of severe vomiting, nausea, or oral mucositis. However, the rates of severe oral mucositis and gastrointestinal tract during preconditioning regimens were comparable between PSC and NSC groups, indirectly suggesting that the oral decontamination status was comparable between the groups.

In summary, our results showed that PSCs considerably influenced the clinical outcomes of patients who received haplo-HSCT, particularly those who had *Candida* in their stool specimens. Additional prospective studies involving pyrosequencing to screen microorganisms in stool specimens might help further identify the effect of intestinal microbiota on the clinical outcomes of haplo-HSCT.

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Compliance with ethics guidelines

Lijuan Hu, Qi Wang, Xiaohui Zhang, Lanping Xu, Yu Wang, Chenhua Yan, Huan Chen, Yuhong Chen, Kaiyan Liu, Hui Wang, Xiaojun Huang, and Xiaodong Mo declare that they have no potential financial conflict of interests related to this manuscript. Informed consent was obtained from all patients or their guardians, and the study was conducted in accordance with the *Declaration of Helsinki*. The study protocol was approved by the Ethics Committee of Peking University People's Hospital.

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