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Epidemiology of infections following haploidentical peripheral blood hematopoietic cell transplantation

Michael Slade[1] | Scott Goldsmith[1] | Rizwan Romee[2] | John F. DiPersio[2] | Erik R. Dubberke[3] | Peter Westervelt[2] | Geoffrey L. Uy[2] | Steven J. Lawrence[3]

[1]Department of Medicine, Washington University School of Medicine, Saint Louis, MO, USA

[2]Division of Oncology, Washington University School of Medicine, Saint Louis, MO, USA

[3]Division of Infectious Diseases, Washington University School of Medicine, Saint Louis, MO, USA

Correspondence
Steven J. Lawrence, MD, MSc, Department of Medicine, Washington University School of Medicine, Saint Louis, MO, USA.
Email: slawrenc@dom.wustl.edu

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Abstract

Background: The use of T-cell replete haploidentical hematopoietic cell transplant (haplo-HCT) has increased substantially since the introduction of post-transplant cyclophosphamide (PTCy) regimens. Limited data exist concerning infectious complications of haplo-HCT utilizing mobilized peripheral blood (PB) hematopoietic cells.

Methods: This retrospective cohort study included all adult patients at our institution undergoing PB haplo-HCT with PTCy between June 2009 and June 2015. Infections were microbiologically confirmed. Invasive fungal infections (IFI) classified as "proven" or "probable" by standard definitions were included.

Results: In total, 104 patients were identified. Median follow-up was 218 days (range: 6–1576). A total of 322 episodes of infection were recorded. Eighty-nine percent of patients experienced at least one infection. Median time to first infection was 22 days. Patients experiencing at least one bacterial, viral, and IFI were 62%, 72%, and 6%, respectively. The majority (69%) of bacterial infections were caused by enteric organisms. Seven cases of *Staphylococcus aureus* infection were recorded, with one bacteremia case. Cytomegalovirus (CMV) viremia occurred in 54/71 (76%) at-risk patients at a median time of 24 days. Sixteen (15%) patients developed CMV disease. Nineteen percent (20/104) of patients developed BK polyomavirus-associated cystitis. Six (6%) patients experienced a total of seven IFI. Infection was the primary cause of death for 12% (6/51) of patients and was a secondary cause for 41%.

Conclusion: In PB haplo-HCT patients, a high incidence of CMV viremia and disease was observed. Infections with enteric bacteria were common. Fungal and staphylococcal infections were uncommon. Further studies are needed to compare infectious complications in haplo-HCT with other transplant modalities.

KEYWORDS
haploidentical, hematopoietic cell transplant, peripheral blood graft, stem cell transplant

1 | INTRODUCTION

The combination of unmanipulated haploidentical hematopoietic cell transplant (haplo-HCT) and post-transplant cyclophosphamide (PTCy) for graft-versus-host disease (GVHD) prophylaxis is an emerging HCT strategy that has produced survival outcomes comparable to human leukocyte antigen (HLA)-matched transplantation for the treatment of hematologic malignancies while expanding donor availability.[1–4] While most patients undergoing haplo-HCT receive bone marrow (BM) grafts, peripheral blood stem cells (PBSCs) are

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an alternative graft source that yield higher $CD34^+$ cell counts and obviate the need for donor anesthesia during hematopoietic cell collection.[5] The use of PBSC grafts, which contain up to 10-fold more $CD3^+$ T cells than BM grafts, has been limited by concerns about increased GVHD, although no difference in GVHD has been found in haplo-HCT.[6,7]

The epidemiology and incidence of infectious disease complications associated with haplo-HCT is incompletely understood. The largest study to date consists of 70 patients undergoing BM haplo-HCT with PTCy.[8] They reported a moderate incidence of cytomegalovirus (CMV) reactivation, peaking in the early post-engraftment period. Bacterial infections were highest during the pre-engraftment period and over half of patients had at least one bacterial infection. In matched-related donor (MRD) allogeneic transplants, PBSC grafts have been associated with increased early CMV reactivation[9] and decreased rates of bacterial and fungal infection[10] compared to BM grafts. Meanwhile, in matched-unrelated donor allogeneic transplants, PBSC grafts have been associated with significantly fewer infectious complications.[11] Limited information is available concerning PBSC haplo-HCT.[5,12,13] In this study, we describe the epidemiology of infectious complications associated with haplo-HCT using PTCy and PBSC grafts.

2 | PATIENTS AND METHODS

2.1 | Patients and graft characteristics

This retrospective cohort includes all adult (age ≥18) patients who underwent PBSC haplo-HCT with PTCy at Washington University School of Medicine, in Saint Louis, MO, between June 2009 and June 2015. Patients were included regardless of diagnosis. Data were collected through a follow-up date of September 2015 by manual review of the electronic medical record. The study was approved by the Washington University School of Medicine Institutional Review Board.

All patients received peripherally mobilized hematopoietic cell grafts. Donors were selected by HLA typing, with match grade ranging from 5/10 to 9/10. Optimal donor was determined by, in order, lack of donor-specific antibodies in recipient, CMV serostatus match, and donor health status. Donors were mobilized with granulocyte colony-stimulating factor (G-CSF). Graft cell counts were characterized using flow cytometry. Target $CD34^+$ dose was 5.0×10^6 cells/kg. No T-cell depletion was used.

2.2 | GVHD prophylaxis, opportunistic infection prophylaxis, and surveillance

All patients received 50 mg/kg PTCy on days +3 and +4,[4] mycophenolate mofetil, and either tacrolimus or sirolimus starting on day +5. Unless contraindicated, all patients received herpesvirus, *Pneumocystis*, and fungal prophylaxis. Standard herpesvirus prophylaxis was 400 mg acyclovir three times a day (TID) or 500 mg valacyclovir once daily (QD) until day +180 or cessation of immunosuppression. Patients discharged on ganciclovir (GCV) for CMV treatment were transitioned to standard herpesvirus prophylaxis following completion of treatment. Primary fungal prophylaxis was 400 mg fluconazole QD until day +100. Patients with a history of invasive fungal infections (IFI) or discharged on antifungal treatment received secondary prophylaxis, which was continued until cessation of immunosuppression. *Pneumocystis* prophylaxis was given from day +28 to +180 and consisted of trimethoprim-sulfamethoxazole, dapsone, or atovaquone.

At our institution, patients are actively screened for vancomycin-resistant *Enterococcus* (VRE) colonization. Whole blood CMV polymerase chain reaction (PCR) testing was performed twice weekly during transplant hospitalization and then weekly until day +100. Subsequent monitoring was determined by each patient's physician. Patients received antiviral treatment for CMV disease, consecutive doubling of blood DNA levels on PCR, and for significant viremia defined as >40 000 IU/mL, or an approximate equivalent level when alternative PCR assays were used.

2.3 | Definitions

Periods of infectious risk were defined as transplant to day +30 (pre-engraftment), days +30 to +100 (early post-engraftment), and day >+100 (late post-engraftment).[14] *Neutrophil engraftment* was defined as the first of three consecutive days of absolute neutrophil count >500 cells/mm^3 following post-transplant nadir. *Platelet engraftment* was defined as the first of three measurements within 7 days showing >20 000 platelets/mm^3 without platelet transfusion in the previous 7 days. *Lymphopenia* was defined as an absolute lymphocyte count <200 cells/mm^3. Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were defined per published criteria.[15,16] Cause of death (COD) was determined per algorithm from Copelan et al.[17] Neutropenic fever was defined per established criteria.[18]

All infections were microbiologically confirmed. Bacterial isolates with identical susceptibility patterns were considered to be one infection, if obtained within 7 days of each other, regardless of source. Blood cultures growing bacteria commonly deemed to be skin flora were only considered an infection if verified by a second positive blood culture. CMV infection was defined as detectable CMV DNA by PCR. CMV reactivation, recurrence, and disease were diagnosed according to the criteria proposed by Ljungman et al.[19] BK polyomavirus (BKV) infections were considered clinically significant if BK viruria was accompanied by cystitis without other clinical explanation. Direct fluorescent antibody assays and influenza-specific and/or multiplex PCR assays performed on nasopharyngeal and respiratory specimens were used to diagnose respiratory virus infections. Consecutive positive tests for the same respiratory pathogen were considered a single infection, unless 6 months had passed since previous positive test. IFI were reported according the classification proposed by the EORTC/MSG consensus group.[20] Only "proven" or "probable" infections were included in this analysis. Unless noted above, infections were defined in accordance with the European Society for Bone Marrow Transplant guidelines (EBMT).[21]

2.4 | Statistical analysis

Standard descriptive statistics were used to characterize the study population and evaluate distribution of variables. None of the variables of interest was normally distributed, so median and range were used for central tendency and dispersion of continuous variables. The chi-square test and Fisher's exact test, as appropriate, were used to evaluate distribution of categorical variables. The Mann-Whitney *U*-test was used to compare distribution of continuous variables. Survival analysis was conducted with the log-rank test.

3 | RESULTS

3.1 | Patient demographics

We identified 104 patients for inclusion in the study. As shown in Table 1, our cohort contained a large number of high-risk patients. Acute myeloid leukemia was the most common diagnosis (67%). Twenty-seven percent of patients had received a previous HCT and 33% had active disease at transplantation. The majority (93%) underwent one of three previously described conditioning regimens.[22–24]

3.2 | Overall outcomes

Median follow-up in all patients was 218 days (range: 6–1576) and 292 days (range: 66–1576) in surviving patients. Using microbiological and clinical criteria, 318 infections were diagnosed. Pathogens are summarized in Table 2. Ninety patients (87%) had neutropenic fever, most (94%) within 3 days of transplantation. All patients received anti-pseudomonal coverage, and 59% were either already receiving agents with activity against methicillin-resistant *Staphylococcus aureus* or began this therapy <24 hours after onset of fever. Ninety-three patients (89%) experienced at least one infection, with a median onset to first infection of 19 days (range: 1–315 days). The 15 patients with severe (grade III–IV) aGVHD experienced a higher number of bacterial infections (median 2, range: 0–12) compared to patients without severe aGVHD (median 1, range: 0–11, *P*=.019). Sixty-three (61%), 75 (72%), and 6 (6%) patients experienced at least one bacterial, viral, or fungal infection, respectively (Figure 1). No parasitic infections were observed. Median time to first infection was 23 days for bacterial, 26 days for viral, and 100 days for fungal infection. Incidence of infection per 1000 patient-days was subdivided by standard periods of infectious risk and by neutrophil engraftment (Figure 2A and B).[14] Disease associated with each pathogen class is summarized in Figure 3.

3.3 | Bacterial infections

Overall, 64 patients (62%) experienced a total of 146 bacterial infections during the follow-up period. Mortality was significantly higher for patients experiencing at least one bacterial infection (Figure 4, hazard ratio: 2.32, 95% confidence interval [CI] 1.23–4.36). Enteric pathogens were responsible for 69% of these infections (101/146).

TABLE 1 Demographics and outcomes for 104 consecutive patients undergoing haploidentical peripheral blood stem cell transplant

Characteristics	n (%)[a]
Demographics	
Age (years), median (range)	50 (19-73)
Gender (male)	57 (55)
Race (non-white)	26 (25)
Diagnosis	
AML	70 (67)
ALL	11 (11)
MDS	11 (11)
Other	12 (12)
CMV serostatus	
D^-/R^-	32 (31)
D^+/R^+	31 (30)
D^-/R^+	27 (26)
D^+/R^-	14 (13)
Acute GVHD (grade II-IV)	37 (36)
Chronic GVHD	37 (43)[b]
Active disease at transplant	55 (33)
Conditioning	
Myeloablative[c]	43 (41)
Non-myeloablative[d]	61 (59)
Previous transplant	27 (26)
Neutrophil engraftment (days), median (range)	17.0 (11-78)
Graft composition	
$CD3^+$ (cells × 10^7/kg), median (range)	17.2 (0-68.5)
$CD34^+$ (cells × 10^6/kg), median (range)	5.0 (2.4-14.2)
TNC (cells × 10^8/kg), median (range)	8.1 (0.9-26.3)

[a]Unless otherwise noted.
[b]Assessed in patients surviving ≥day +80.
[c]Most patients received either fludarabine (Flu) and fractionated total body irradiation (TBI) (65%) or Flu, cyclophosphamide (Cy), and 4 days of busulfan (27%).
[d]The majority of patients received Flu, Cy, and a single dose of TBI (93%).
AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; MDS, myeodysplastic syndrome; CMV, cytomegalovirus; D/R, donor/recipient; GVHD, graft-versus-host disease; TNC, total nucleated cells.

Only 26% (26/101) occurred before day 30 and nearly half (46%) occurred after day 100. The most common sites associated with enteric organisms were blood (54%), urinary tract (22%), and gastrointestinal tract (15%). The majority of bloodstream infections (BSIs) prior to day 100 were associated with mucosal barrier injury (19/41, 46%) or central venous catheters (11/41, 27%).

Enterococcus species were responsible for 34% (34/101) of infections by enteric organisms. GVHD of the gut was not associated with either *Enterococcus* (risk ratio [RR]: 0.85, 95% CI 0.38–1.90) or enteric bacterial infection (RR: 1.02, 95% CI 0.67–1.57). Twenty-nine (85%) of the enterococcal infections were caused by VRE organisms. Forty-seven (45%) patients were colonized with VRE. In

TABLE 2 Pathogens associated with 318 infectious episodes

Type	Pathogen	Infections	Patients[a]
Bacterial	**Gram-negative bacilli**	**57**	**30**
	Pseudomonas aeruginosa	9	6
	Enterobacteriaceae species	38	21
	Stenotrophomonas maltophilia	8	6
	Anaerobic species	2	2
	Gram-positive cocci	**63**	**44**
	Staphylococcus aureus	7	4
	MRSA	5	2
	Enterococcus species	34	27
	VRE	29	23
	Coagulase-negative *Staphylococci*	14	14
	Streptococcus pneumoniae	1	1
	Streptococcus species (other)	7	6
	Gram-positive bacilli	**7**	**6**
	Clostridium difficile	**16**	**13**
Viral	**Herpesviruses**	**87**	**58**
	CMV	77	57
	HSV1/2	9	7
	VZV	1	1
	Respiratory viruses	**57**	**33**
	Influenza	14	13
	H1N1	3	3
	H3N2	8	8
	Type B	3	3
	RSV	5	5
	Parainfluenza	12	9
	Type 3	6	5
	Other	6	4
	Other respiratory viruses[b]	26	16
	BKV	21	20
Fungal	***Candida***[c]	**2**	**2**
	Molds	**5**	**4**
	Aspergillus species	3	2
	Zygomycetes	2	2

[a]Patients may have multiple infections within a category, so this column is not summative.
[b]Other respiratory viruses include rhinovirus/enterovirus (17), coronavirus (4), adenovirus (3), and metapneumovirus (2).
[c]*Candida* species were *krusei* and *albicans*.
MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; CMV, cytomegalovirus; HSV, herpes simplex virus; RSV, respiratory syncytial virus; BKV, BK polyomavirus.

patients who developed VRE infection, 74% (17/23) had known preceding enteric colonization. This number is significantly higher than patients who did not develop VRE infection (37%, RR: 2.00, 95% CI 1.37–2.90).

A total of 7 *S. aureus* infections were identified from the skin (in 5), lung (in 1), and blood (in 1). The only *S. aureus* BSI occurred in a patient with preceding skin infection. Overall methicillin resistance is summarized in Table 2.

Thirteen patients (13%) developed *Clostridium difficile* infections (CDI), 3 of whom experienced recurrent infection. Of interest, 44% (7/16) of infections were within a week of transplantation. In three patients with CDI, two were found to have pancolitis on computed tomography scan and another had a colonic biopsy consistent with *C. difficile* colitis. However, all three patients had active GVHD of the gut, so the etiology of these findings remained unclear. All but one patient with CDI survived to discharge. The

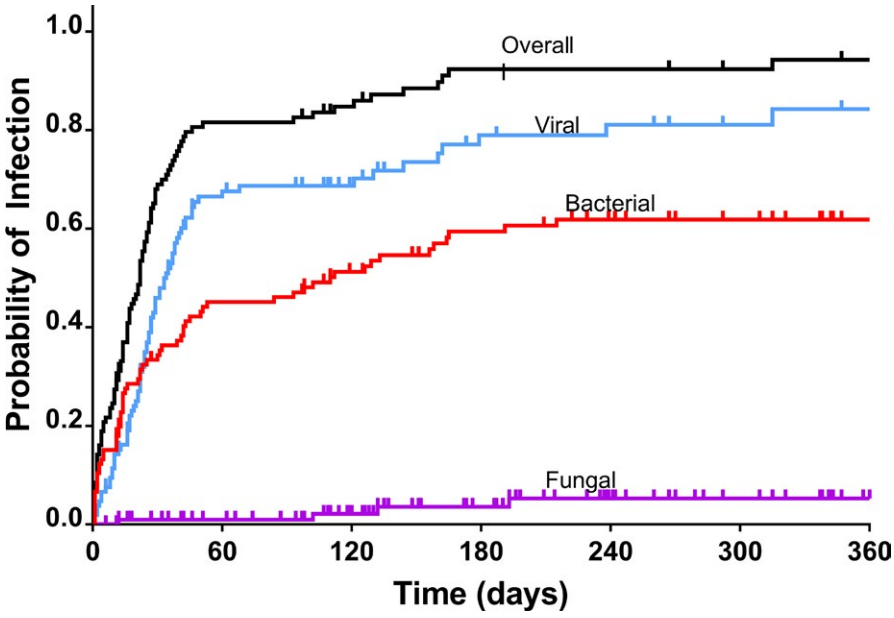

FIGURE 1 Cumulative incidence of infection by pathogen type

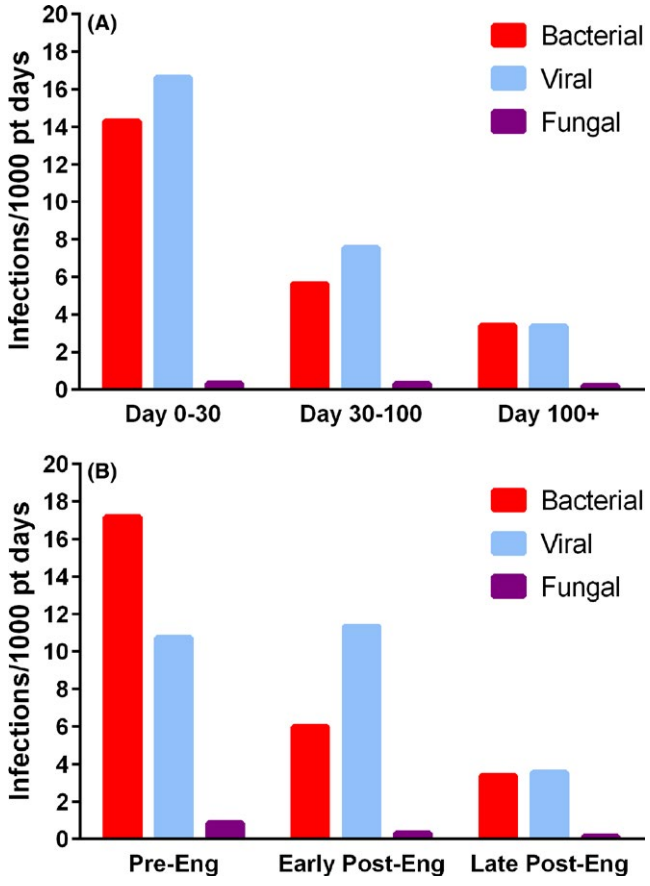

FIGURE 2 (A) Infection rates in periods of risk. (B) Infection rates by neutrophil engraftment. Pre-Eng, Pre-engraftment; Post-Eng, Post-engraftment

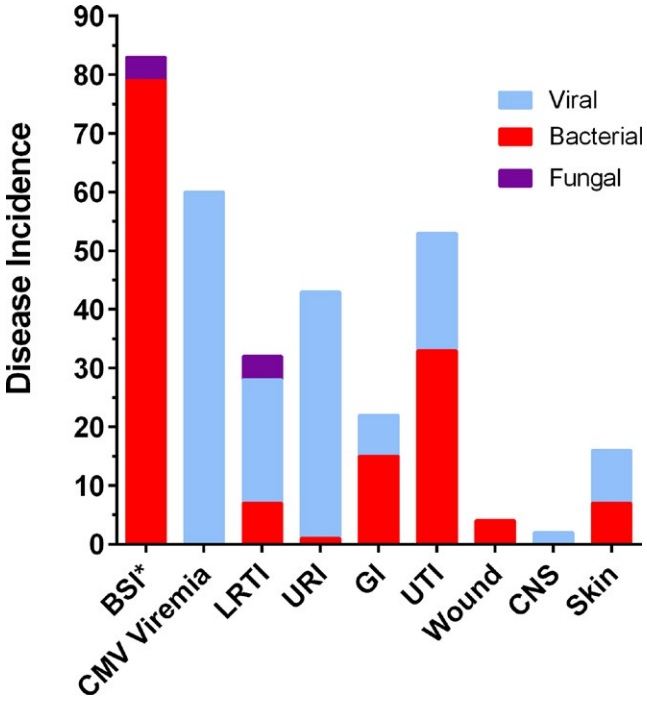

FIGURE 3 Infectious diseases observed in haploidentical peripheral blood stem cell recipients by site and pathogen class. *Significant bloodstream infection (BSI) organisms included *Enterococcus* species (18), *Pseudomonas aeruginosa* (3), other gram-negative bacilli (30), coagulase-negative *Staphylococcus* (13), and *Candida* species (2). LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection; GI, gastrointestinal infection; UTI, urinary tract infection; CNS, central nervous system

other nine infections had median onset of 53 days (range: 21–212). All infections after day +30 occurred in patients diagnosed with aGVHD or cGVHD of the gut. Five episodes were treated with metronidazole alone. Eleven episodes were treated with oral vancomycin, with the standard course of 14 days being extended for one patient with refractory disease. Five patients also received intravenous metronidazole.

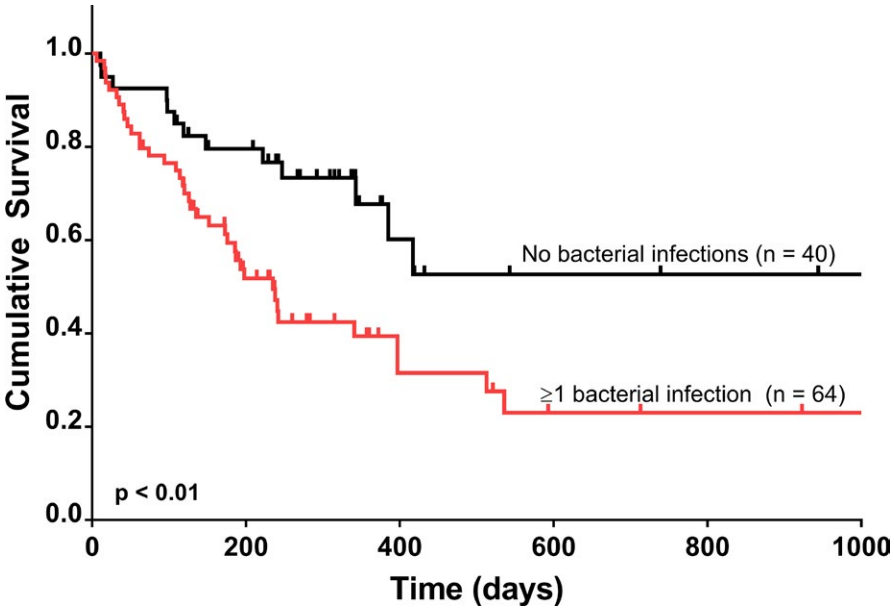

FIGURE 4 Association of bacterial infection with overall survival

3.4 | Viral infections

Fifty-seven patients (55%) experienced a total of 76 episodes of CMV viremia. The median onset of CMV was 24 days for initial infection (range: 3–240 days) and 32 days overall (range: 3–1217 days). Recipient CMV serostatus and aGVHD were not associated with time to infection. Fifty-one episodes were considered reactivation and 6 were primary infection. Nineteen episodes were recurrent infection, none of which followed primary infection. Four of 14 (29%) donor positive–recipient negative (D^+/R^-) patients experienced primary infection, compared to 2 of 32 (6%) D^-/R^- patients. The median follow-up in these groups was 132 and 241 days, respectively. The first episode of CMV viremia was, when compared to recurrent viremia, more likely to require treatment (79% vs 21% of episodes, RR: 3.75, 95% CI 1.55–9.05). Initial treatment for viremia was maintenance-dose GCV or valGCV for 38 episodes and induction-dose GCV or valGCV for 11 episodes. Twenty-seven episodes resolved without anti-CMV treatment. Thirty-one episodes (41%) were treated with multiple medications or dosages, including foscarnet (12%) and CMV-specific immunoglobulin (9%). Four patients were tested for CMV resistance because of treatment failure; one was positive for an M460V mutation of the UL97 gene.

Sixteen patients (15%) experienced CMV-associated end-organ disease, affecting the gut in 7 cases, the lungs in 6 cases, and both in 2 cases. One case involved the retina, meninges, and lungs. The first episode of CMV viremia was significantly more likely to be associated with CMV disease (28% vs 0%, RR: 27.31, 95% CI 1.68–443.50). CMV viremia before day +30 was significantly associated with CMV disease (29.7% vs 7.5%, RR: 3.98, 95% CI 1.50–10.59), as was positive recipient CMV serostatus (RR: 5.77, 95% CI 1.38–24.13). Primary infection (RR: 1.40, 95% CI 0.39–4.97), receiving steroids at time of viremia onset (RR: 0.84, 95% CI 0.35–2.01), and receiving a maintenance dose of GCV as initial therapy (RR: 0.83, 95% CI 0.33–2.04) were not significantly associated with CMV disease.

On pre-transplant screening, 78 (75%) and 27 (26%) patients were positive for herpes simplex virus (HSV)-1 and HSV-2 antibodies, respectively. HSV reactivation occurred in seven patients (7%). Six patients (86%) were receiving prophylactic doses of acyclovir or valacyclovir at time of reactivation. Three patients presented with oral lesions (attack rate: 3.8%) on days +8, +15, and +93. Three patients presented with genital lesions on days +1, +160, and +170. One patient presented with HSV-2 meningitis on day +92 (overall attack rate: 14.8%) and later developed leg and genital lesions. All patients with HSV reactivation were successfully treated and survived to discharge.

We observed 14 Influenza infections in 13 patients (13%). All infections occurred between November and April. Seven patients were hospitalized for influenza infection, five were treated as outpatients, and two were nosocomially acquired. Patients generally receive influenza vaccine starting at day +120 or at the beginning of flu season, whichever is later at our institution. Median onset of infection was day +153 (range: 1–991), with only three infections occurring before day +120. Four infections in unvaccinated patients occurred before day +150. Of the remaining seven patients, three became infected despite vaccination. Most patients were treated with 75 mg of oseltamivir twice daily (BID) for 5 days. However, four hospitalized patients received higher doses or longer treatment based on acuity of illness. All patients hospitalized for influenza survived to discharge. Two patients had co-infections with other respiratory viruses, one with parainfluenza and one with respiratory syncytial virus (RSV). No testing for viral clearance was performed. However, none of the nine patients who received subsequent respiratory virus testing (median day after initial infection: 35, range: 14–149) were still shedding influenza virus. Two of five patients with RSV infection presented with lower respiratory tract involvement, defined as a new oxygen requirement and radiological

findings consistent with atypical pneumonia. These two patients, along with one patient with only upper respiratory symptoms, were treated with nebulized ribavirin (2 g TID for 5 days) and survived to discharge. The other patients' RSV infections resolved without treatment.

During the study period, 33 patients (32%) tested positive for BKV in the urine. Of these, 21 symptomatic infections in 20 patients (19%) were recorded. Of these, 65% (13/20) experienced hemorrhagic cystitis of grade II (n=6), grade III (n=6), and grade IV (n=1). Sixty-five percent of symptomatic patients (13/20) received treatment with 500 mg of ciprofloxacin BID. Treatment was deferred in four cases because of patient preference, whereas two patients received cidofovir and one patient received continuous bladder irrigation.

In surviving patients, the median absolute lymphocyte count at day +30 (n=97) and day +100 (n=80) was 200 cells/mm^3 (range: 0–2794) and 539 cells/mm^3 (range: 20–4176), respectively. The 1-year cumulative incidence of CMV viremia was not significantly different in patients who were lymphopenic at day +30 (57% vs 60%, *P*=.38) and +100 (63% vs 64%, *P*=.93). Similarly, the cumulative incidence of respiratory virus infection was not significantly different in patients who were lymphopenic at day +30 (41% vs 25%, *P*=.10) and +100 (39% vs 32%, *P*=.50). In contrast, lymphopenia at day +30 and at day +100 was both associated with a lower 1-year cumulative incidence of BK cystitis (10% vs 29%, *P*=.03; and 15% vs 44%, *P*=.03, respectively).

3.5 | Fungal infections

Seven fungal infections were diagnosed in the follow-up period. Six were classified as "proven" according to EORTC/MSG criteria[20] via blood culture (n=3), biopsy (n=2), and autopsy (n=1). The positive blood cultures were for *Candida* (n=2) and Zygomycetes (n=1) species. Disseminated mucormycosis was found in kidneys and lungs of one patient who died following a stroke. Both patients who developed a fungal infection during their transplant hospitalization died before discharge. One infection was classified as "probable" by galactomannan assay and cavitary nodules on chest computed tomography. No *Pneumocystis* infections were observed. Nineteen patients (18%) were discharged from their initial hospitalization while receiving either secondary prophylaxis with voriconazole (n=15) or therapeutic antifungal treatment (n=4). Only one of these patients later developed a fungal infection.

3.6 | Mortality and readmissions

During the follow-up period, 51 patients (49%) died. Per published criteria,[17] the most common primary COD was relapse (54%), followed by GVHD (17%). Primary and contributing COD in periods of infectious risk are summarized in Table 3. Infection was the primary COD in 12% (6/52). Infection was a contributing COD in an additional 41%. Fifteen patients were microbiologically diagnosed with 35 new infections within 7 days of death. Of these patients, 33% had moderate aGVHD, 27% had severe aGVHD, and 47% had cGVHD. Sixty-nine percent of the infections were bacterial, 26% were viral, and 6% were fungal. The most common bacterial pathogens were *Enterococcus* species (42%) and gram-negative bacilli (46%). The 30-day readmission rate in patients who survived to discharge was 56% (50/90). Of the 50 readmissions in this time period, infection was the primary cause of 16 (32%).

4 | DISCUSSION

In this analysis, we describe infectious complications in 104 patients receiving PB haplo-HCT with PTCy. Overall, infections were a primary or contributing COD in more than half of the patients who died in the follow-up period. At present, the largest published report of infectious complications in patients undergoing haplo-HCT was by Crocchiolo et al.[8], who analyzed a cohort of 70 patients. The majority (94%) received BM grafts. They observed the established progression of infectious agents during traditional periods of risk. Bacterial infections peaked in the pre-engraftment period at a rate of approximately 11 per 1000 patient-days. Viral infections were most prominent in the early post-engraftment period, occurring at a rate of 15 per 1000

TABLE 3 Cause of death among 51 haploidentical peripheral blood stem cell transplant recipients who died during follow-up

	Days 0–30		Days 30–100		Days 100+		Overall	
Cause of death	Primary	Contrib	Primary	Contrib	Primary	Contrib	Primary	Contrib
Relapse	1	0	3	0	24	0	28	0
Non-engraftment	1	1	1	0	0	0	2	1
aGVHD	1	0	4	0	4	0	9	0
cGVHD	0	0	0	0	0	1	0	1
Infection	3[a]	4	3[b]	3	2[c]	11	8	18
Organ failure	2	2	0	1	0	2	2	5
Other	0	0	1	1	1	0	2	1
Total	8	–	12	–	31	–	51	–

[a]*Candida* bloodstream infection (BSI), *Enterococcus faecium* BSI, *Stenotrophomonas maltophilia* BSI.
[b]*E. faecium* BSI (2), disseminated *Mucor*.
[c]Multifocal pneumonia (no organism identified), *Bacteroides uniformis* BSI.
Contrib, contributory; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

patient-days.[8] We observed a slightly higher rate of early bacterial infection and a markedly higher rate of early viral infections (Figure 2A). Of note, patients reported by Crocchiolo et al.[8] received routine bacterial prophylaxis with levofloxacin, whereas ours did not. Our median time to neutrophil engraftment was shorter (17 vs 20 days). This difference may have skewed the attribution of infections into the "pre-engraftment" period, despite neutrophil recovery. In particular, a large number of viral infections occurred after neutrophil engraftment but before day 30 (Figure 2A vs B). Our observed rate of IFI and increased early CMV reactivation is consistent with previous literature comparing PBSC and BM grafts.[9,10] We also observed a high incidence of early neutropenic fever in our cohort. The association of PB haplo-HCT with post-infusion fever is increasingly recognized in the literature.[25–27]

The high incidence of enterococcal infections in this population was notable. Our finding that colonization with VRE is associated with later infections is consistent with previously published work, as is the association between enterococcal infection and early mortality.[28] In contrast, we observed few *S. aureus* infections, similar to previous reports in allogeneic HCT.[29]

The observed rate of CDI was comparable to the previously reported rates of 9%–25%, as was our rate of recurrent infection.[30–32] The high proportion of early-onset CDI has been reported in the literature.[30] An association between CDI and gastrointestinal GVHD has also been noted, which was not observed in our population.[31] CDI had no discernible impact on survival.

The role of graft source on CMV infection is still a matter of debate. In the MRD setting, Young et al.[11] found no difference in CMV infection within a large retrospective cohort of patients undergoing HCT with either BM or PBSC. However, early CMV reactivation and higher rates of reactivation and disease in patients receiving PBSC grafts were reported by Guerrero et al.[9], who analyzed a cohort of MRD patients randomized to PBSC or BM grafts. They found altered prevalence and proliferation of CMV-specific $CD4^+$ cells following G-CSF mobilization. Compared to previously published data in BM haplo-HCT, the rate of CMV viremia associated with previous donor and/or recipient CMV seropositivity and CMV disease were considerably higher (76% vs 54%, 12% vs 4%).[8] The rate of CMV viremia among seropositive recipients was similar to another cohort of PBSC haplo-HCT patients (90% vs 79%), although the incidence of CMV disease was considerably higher (12% vs 0%).[12] The association of early viremia and recipient CMV pre-transplant seropositivity with CMV disease is consistent with altered CMV immunity in this setting and may indicate the need for an alternative approach to treatment of CMV viremia in this population. We also observed a relatively high rate of HSV prophylaxis failure, especially among patients seropositive for HSV-2.

We observed a low rate of incident IFI in our cohort, similar to previously reported rates in haplo-HCT with BM grafts.[8] This low rate may reflect the efficacy of receiving extended primary antifungal prophylaxis until day +100, or the relatively large proportion (18%) of patients receiving secondary fungal prophylaxis.[5,12] Our inclusion of only "probable" and "proven" fungal infections is consistent with the literature, but likely results in underestimating the true incidence of fungal infection in our population. Follow-up time in our cohort is also limited and may also contribute to an underestimation of fungal infections, which often occur later in patients with cGVHD.

Our study has several limitations. First, by excluding suspected infections that were not microbiologically confirmed, we underestimate the true incidence of infection in our population. Febrile episodes with no identified organism are a common occurrence in this population.[33] Furthermore, many patients with suspected pneumonia cannot undergo bronchoscopy owing to thrombocytopenia. However, by employing strict criteria, our results are more specific than relying on retrospective clinical judgment. Second, our study did not include a comparison group. This makes contextualizing the reported rates of infection more difficult, especially given regional variations in some pathogens. However, it also allows us to provide an extremely broad and comprehensive description of the infectious experience within this novel cohort. Pathogen-specific comparative studies are a direction of future research and may help illuminate differences in immune reconstitution following haplo-HCT when compared to more traditional donors. Third, we were limited to collecting data from our own medical records system. Generally, patients receive all transplant-related care at our institution. In the case of acute illness, some patients likely received treatment closer to home and these episodes would not be captured in this study. However, these patients are usually transferred to our institution when hospitalized and most data should be captured. Overall, our data represent a conservative estimate of the infectious experience of this population.

To our knowledge, our cohort represents the largest analysis of infectious complications following haplo-HCT with PTCy presently in the literature. It is also the first detailed analysis of infectious complications in patients transplanted under this protocol receiving PBSC grafts. Further studies are needed to compare the infectious experience in patients undergoing haplo-HCT to patients undergoing other HCT protocols.

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DISCLAIMER

The content is solely the responsibility of the authors and does not necessarily represent the official view of the NIH.

AUTHOR CONTRIBUTIONS

M.S., S.G., R.R., and S.J.L. designed the study. M.S., S.G., R.R., J.F.D., E.R.D., P.W., G.L.U., and S.J.L. collected the data. M.S. and S.J.L. analyzed the data and drafted the article. All authors edited and approved the final report prior to submission.

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