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The impact of alternative donor types on viral infections in pediatric hematopoietic stem cell transplantation

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Abstract

Viral infections remain one of the most important complications following allogeneic HSCT. Few reports compare virus infection between different donor types in pediatric patients. We retrospectively analyzed viral infections and the outcome of one hundred and seventy-one pediatric patients (median 7.38 years) who underwent allogeneic HSCT from matched related donor (MRD, n = 71), 10 of 10 HLA allele-matched unrelated donors (MUD1; n = 29), 9 of 10 HLA allele-matched unrelated donors (MUD2; n = 40), and haploidentical donors (n = 31). PCR screening for BK virus, adenovirus, Epstein-Barr virus, parvovirus B19, human herpesvirus 6, and CMV were performed routinely weekly. Infections between 0-30, 31-100, and 101 days-2 years were identified separately. BK virus and CMV reactivations were significantly low in MRD transplant patients ($P = .046$ and $P < .0001$, respectively), but incidences of all virus infections between MUD1, MUD2, and haplo-HSCT were found statistically not different. The OS was found to be affected by having one or multiple virus infection ($P = .04$ and $P = .0008$). Despite antiviral prophylaxis and treatments, post-transplant viral infections are associated with reduced overall survival. Haplo-HSCT is comparable with MUD transplantation in the setting of viral infections. A larger study group and prospective studies are needed to confirm this observation.

KEYWORDS

outcome, pediatric, stem cell transplantation, virus infection

1 | INTRODUCTION

Delayed immune reconstitution and viral infections remain a significant cause of morbidity and mortality following allogeneic HSCT.[1] The natural history of immune reconstitution is altered in allogeneic transplants by GVHD as well as by an immunosuppressive therapy. Although viral infections are also common after MRD-HSCT, the risk for severe disease is higher in MUD and haplo-HSCT due to more severely depressed T cell-mediated immune responses. The addition of T cell-depleting agents such as ATG to conditioning regimen in MUD transplantation has been associated with a reduced incidence of GVHD but increased risk for delayed immune reconstitution.[2-4] Also, TCR α and β/CD3 depletion for the prophylaxis of GVHD achieved a significant success in haploidentical HSCT, but is associated with increased risk for viral infections.[5] The aim of our study was to compare the incidence of virus infections and outcomes in children who underwent HSCT from MRD, MUD1, MUD2, and haploidentical donors.

Abbreviations: ADV, adenovirus; ATG, anti-thymocyte globulin; CMV, cytomegalovirus; CSA, cyclosporine A; EBV, Epstein-Barr virus; GVHD, graft-vs-host disease; HHV6, human herpesvirus 6; HSCT, hematopoietic stem cell transplantation; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; OS, overall survival; PBSC, peripheral blood stem cells; PTLD, post-transplant lymphoproliferative disease; RIC, reduced-intensity conditioning; TCR, T-cell receptor; TRM, transplant-related mortality.

TABLE 1 Patients' characteristics

Patients	MRD (n = 71)	Matched unrelated donor (10/10—MUD1) (n = 29)	Matched unrelated donor (9/10—MUD2) (n = 40)	HLA-haploidentical donor (n = 31)	*P* value
Age, median (range, y)	5.9 (0.4-17.6)	6 (1.8-17.9)	6.03 (0.7-18)	6.5 (0.4-18)	.84
Male/female	42/29	18/11	22/18	20/11	
Diagnosis, (n)					
Leukemia	22	6	19	15	
MDS/CML/JMML	2	1	2	3	
Hemoglobinopathy	21	8	2	0	
Aplastic anemia	11	9	9	4	
Metabolic disease	5	1	0	3	
Immune deficiency	5	2	2	4	
HLH	5	1	5	1	
NHL	0	1	1	1	
Conditioning regimen					
Myeloablative	65	26	33	0	
Reduced-intensity	6	3	7	31	
Graft type					
Bone marrow	66 (+4 cord)	22	27	0	
PBSC	1	7	13	31	
CD 34 cells $\times 10^6$/kg, median (range)	4.92 (1.45-23)	6.5 (1.16-16.62)	7.01 (1.92-45)	10 (2.49-41.4)	.001
Engraftment, median/mm^3 (range, d)					
Neutrophil	16 (9-50)	16.5 (8-35)	17 (8-35)	14 (8-72)	.13
Platelet	27 (9-75)	25.5 (10-89)	28.5 (10-89)	16 (6-64)	.1
Lymphocyte	21 (7-71)	22 (10-146)	27 (10-130)	18.5 (9-99)	.36
Lymphocyte >1000/mm^3, median (range, d)	35 (9-150)	32 (12-450)	33 (12-450)	40.5 (10-217)	.61
Lymphocyte at day +100, median (range, /mm^3)	1350 (150-6820)	955 (0-6620)	1065 (120-6620)	880 (0-3720)	.08
Acute GVHD					
No	53	16	12	20	
Grade I-II	9	4	12	2	
Grade III-IV	9	9	16	9	
Chronic GVHD					
No	67	27	35	29	
Mild-moderate	2	1	2	2	
Severe	2	1	3	0	
Steroid (>2 mg/kg, >2 wk)					
Yes	20	16	23	14	
No	51	13	17	17	
ATG					
Yes	48	29	40	31	
No	23	0	0	0	
Follow-up median (range, mo)	16 (1-31)	12 (1.5-31)	16 (1.5-31)	12 (1.5-31)	.08

2 | PATIENTS AND METHODS

We retrospectively analyzed viral infections and the outcome of one hundred and seventy-one pediatric patients (median 7.38 years) who underwent allogeneic HSCT from January 2014 to September 2016. Patients were divided into four subgroups: patients transplanted from MRD (n = 71), 10 of 10 HLA allele-MUD (MUD1; n = 29), 9 of 10 HLA allele-MUD (MUD2; n = 40), and haploidentical donors (n = 31). Patients' characteristics were summarized in Table 1. In addition to donor types, the whole group was divided into 3 post-transplantation phases. Infections between 0-30, 31-100, and 101 days-2 years were identified separately. Viral infection episodes with different viruses were defined as multiple viral infections. The recurrences of the same pathogen did not count as multiple infections.

The myeloablative conditioning regimen consisted of busulfan (16 mg/kg) plus cyclophosphamide (120-200 mg/kg). RIC regimen consisted of fludarabine (150-180 mg/kg) plus busulfan (8 mg/kg) or cyclophosphamide (40 mg/kg). Serotherapy consisted of ATG (15-20 mg/kg). Haplo-HSCT consisted of a RIC regimen (ATG [days −13 to −9], fludarabine [days −8 to −5], thiotepa [day −4], and melphalan [days −3 and −2]) and TCRαβ/CD3 depletion. To prevent EBV-related PTLD and GVHD, RTX 375 mg/m^2 was added into the conditioning regimen on day-2. MSC was infused at day-1 to suppress alloreactive donor anti-host T-cell responses and to use their ability to promote angiogenesis and support the micro-environment, which facilitate engraftment.

Serology for herpesviruses (HSV, EBV, HHV-6, and CMV), ADV, parvovirus B19, HIV, and hepatitis virus (HAV, HBV, HCV) was assessed in all patients before transplantation. PCR screening tests for CMV, EBV, parvovirus B19, ADV, and HHV6 were performed routinely weekly in transplantation period on inpatients and on outpatients when symptomatic. BK virus was tested in urine and blood routinely in haplo-transplant patients, while MUD and MRD transplant patients were tested only in clinical suspicion of hemorrhagic cystitis. ADV antigen and CMV PCR were tested in stool in patients with diarrhea. PCR test for CMV was carried out routinely every 14 days in post-engraftment period.

ADV and parvovirus B19 DNA PCR were performed by Smart Cycler/Cepheid, Real-Time PCR. EBV and BK virus DNA PCR were performed by VERSANT kPCR Molecular System/Siemens, Real-Time PCR. HHV-6 PCR was performed by Rotor-Gene Q/Qiagen, Real-Time PCR.

CMV reactivation was defined as the positivity of plasma CMV DNA detected by an in-house real-time PCR (VERSANT kPCR Molecular System/Siemens) using TaqMan primers. The cutoff level for anti-CMV treatment was ≥1000 DNA copies/mL.

CMV disease was defined as clinical symptoms associated with detection of CMV in the fluid, lavage fluid, or biopsy specimen obtained from the affected organ, except CMV retinitis, which can be diagnosed during retinal examination by an experienced ophthalmologist.[6]

All patients received acyclovir prophylaxis against CMV infection until discontinuation of immunosuppression. IVIG was given weekly during inpatient treatment and thereafter according to immunoglobulin G (IgG) level <6 g/L.

The lymphocyte count >1000/mm^3 and lymphocyte count at day 100 were analyzed in all patients. Analysis on immune reconstitution (CD3, CD4, CD8, CD19, CD16/56) to quantify T, B, and NK cells was performed post-transplant at days 30, 60, 90, 120, and 180 in haplo-HSCT patients.

Neutrophil engraftment day was defined as the first day of three consecutive days with absolute neutrophil count >0.5 × 10^9/L. Platelet engraftment day was defined as the first of three consecutive days without transfusion support for at least 7 days. Lymphocyte engraftment was defined as the first day of three consecutive days with absolute lymphocyte count >0.5 × 10^9/L.

Acute and chronic GVHD were defined and graded according to the EBMT Guideline.[7] GVHD prophylaxis consisted mostly of CSA alone or a CSA and short-term MTX combination in MRD and MUD transplantations, whereas MMF was used in haploidentical donor transplantations. MMF was added routinely to patients with ABO mismatched donors for GVHD prophylaxis for up to 60 days.

All data were analyzed using the SPSS, version 18.0 for Windows (SPSS, Chicago, IL, USA) statistical package. Categorical data are presented as frequency and percentage (%). Continuous variables are presented as median, minimum, and maximum. The comparisons among groups were made by Kruskal-Wallis for continuous variables with non-normal distribution and a chi-square test for categorical variables. Paired-sample *t* test was used for the comparison of dependent variables. A two-tailed *P* value <.05 was considered statistically significant. Kaplan-Meier method was used for survival analysis.

3 | RESULTS

One hundred and seventy-one pediatric patients undergoing HSCT were enrolled to study. The median duration of follow-up was 14 months (range 1-31 months). The main diagnosis, HLA disparity, donor type, conditioning regimen, the use of corticosteroids and ATG, the median CD34+ cells of the grafts, engraftment days of neutrophils, platelets, and lymphocytes, lymphocyte count at day 100 and the day lymphocyte count >1000/mm^3, and the existence of acute and chronic GVHD according to donor type are summarized in Table 1.

The median engraftment days of thrombocytes and lymphocytes were achieved about 5 to 10 days earlier in patients receiving haplo-HSCT than in patients receiving MRD or MUD HSCT, but these differences were statistically not different (Table 1). The lymphocyte count at day 100 and the day when the lymphocyte count was greater than 1000/mm^3 did not significantly differ between the four groups (Table 1). Immunophenotypic analysis reveals a progressive but slow increase in lymphocyte subset counts from day +30 through later post-transplantation time points in all patients with haplo-HSCT. Our data at day +30 post-transplantation showed delayed immune reconstitution for CD3+ T cells, CD4+ T helper cells, CD8+ T cytotoxic cells, and CD19+ B cells. Only CD16/56 NK cell recovery was found fast and reached a normal value within 30 days post-transplantation with median 230 cells/mm^3 (range 0-1281). CD3+ and CD8+ T cells began to recover at day +90 with median 770.35 cells/mm^3 (range

TABLE 2 Immune reconstitution at days 30, 60, 90, 120, and 180 in haplo-transplant patients

	Day 30	Day 60	Day 90	Day 120	Day 180
CD3+ T cells (cells/mm^3, range)	121.12 (9.9-2009.20)	347.13 (9.9-17071.0)	770.35 (17.70-1931.80)	728.20 (127.45-3097.70)	1474.50 (26.4-10339.20)
CD4+ T helper cells (cells/mm^3, range)	40.50 (5.7-547.1)	86.7 (3-489.10)	137.20 (8.4-765.70)	165.90 (47.50-1294.70)	384.10 (2.9-4315)
CD8+ T cytotoxic cells (cells/mm^3, range)	75.50 (3.5-1602)	279.15 (1.4-1574.50)	589.80 (6-1628.20)	562.30 (104-1797.60)	1138.80 (23.50-6025.60)
CD19+ B cells (cells/mm^3, range)	0.65 (0-14.10)	1.7 (0-578.64)	1.77 (0.10-1800.70)	270.46 (0.39-1353.60)	47.10 (0.6-701.90)
CD16/56 NK cells (cells/mm^3, range)	230 (0-1281)	298.02 (0-1047.70)	248.20 (1.30-1012.20)	174.40 (58-850.70)	189.6 (18-2709.50)

17.70-1931.80) for CD3+ T cells and 589.80 cells/mm^3 (range 6-1628.20) for CD8+ T cytotoxic cells. CD4+ T helper cells reached normal values at day +180 post-transplantation (median 384.10 cells/mm^3 (range 2.9-4315). CD19+ B cells were found still low after 6 months of transplantation. Lymphocyte subsets are shown in Table 2.

Cumulative incidence of acute grade I-II and grade III-IV GVHD was 15.8% and 25.1% in whole group. Forty percent of MUD2 patients (16/40) had severe acute GVHD (≥grade III). Of the children with severe acute GVHD, 88.4% developed at least one viral infection and 60.5% developed multiple viral infections. Mild-moderate chronic GVHD was observed in seven patients, six of them had one viral infection, and two of them had multiple viral infections. Severe chronic GVHD was observed in six patients (Table 1). Among these patients, five of them had at least one viral infection and four of them had multiple infections.

One hundred and sixty-nine viral episodes were documented within the median follow-up of 14 months (range 1-31 months). Sixty-six patients (38.6%) had no evidence of viral infections post-transplant, 63 of 105 (60%) had one viral reactivation/infection, 27 of 105 (25.71%) had two, 14 of 105 (13.33%) had three, and two of 105 (1.9%) had four viral reactivations/infections with different viruses. Most of the viral reactivations/clinical infections (80.6%) occurred during the first 3 months post-HSCT (Table 3). Recipients of MUD1, MUD2 grafts, and recipients of haploidentical donor grafts seemed to have a higher incidence of multiple viral infections than those of MRD grafts (16/40 [42.5%]—11/29 [37.9%] and 10/31 [32.3%] vs 6/71 [8.45%], $P < .0001$). We found an increased risk of having multiple viral infection among those patients who have a total lymphocyte count<1000/mm^3 at day +100 (32% vs 18%, $P = .035$).

CMV was the most prevalent infection (n = 84) followed by BK virus (n = 48) among all transplant groups. CMV was detected in 49.12% (84/171) of the patients, of which 89% (75/84) of these patients were diagnosed as having CMV reactivation. CMV appeared more often among the recipients of MUD1, MUD2, and haplo-HSCT than those of MRD grafts ($P < .0001$); 67.7% of patients with haplo-HSCT, 62.1% of patients with MUD1 HSCT, and 62.5% of patients with MUD2 HSCT were positive for CMV reactivation/infection, whereas only 28.2% of patients with MRD-HSCT were positive for CMV reactivation/infection (Table 3). Fifteen cases were associated with symptoms of grade I-II acute GVHD, and 35 cases were associated with symptoms of grade III-IV acute GVHD at the time of virus detection. Among our patients with CMV disease, four patients (one of them was associated with CMV hepatitis) developed CMV pneumonia with a fatal outcome. Two patients with CMV retinitis and three patients with CMV enteritis were treated with intravenous ganciclovir successfully.

BK virus was the second most seen infection. BK virus-associated hemorrhagic cystitis was diagnosed in 48 HSCT patients (12 haplo/12 MRD/9 MUD1/15 MUD2). Twenty-six patients were positive for BK virus at 0-30 days post-HSCT. Twenty patients were positive at 31-100 days, and two patients were positive at >100 days post-HSCT. BK virus was detected more often among the recipients of MUD1, MUD2, and haplo-HSCT than those of MRD grafts ($P = .046$); 38.7% of patients with haplo-HSCT, 37.5% of patients with MUD2 HSCT, and

TABLE 3 Etiologies of the documented viral infections

Virus	MRD (n = 71)	Matched unrelated donor (10/10–MUD1) (n = 29)	Matched unrelated donor (9/10–MUD2) (n = 40)	HLA-haploidentical donor (n = 31)	*P* value
CMV (d)	20 (28.2%)	18 (62.1%)	25 (62.5%)	21 (67.7%)	
0-30	16	10	15	14	**<.0001**
31-100	4	6	9	6	
>100	0	2	1	1	
BK virus (d)	12 (16.9%)	9 (31%)	15 (37.5%)	12 (38.7%)	
0-30	6	2	11	7	**.046**
31-100	6	6	3	5	
>100	0	1	1	0	
EBV (d)	1 (1.4%)	3 (10.3%)	3 (7.5%)	2 (6.5%)	
0-30	0	0	1	0	.25
31-100	1	2	1	1	
>100	0	1	1	1	
ADV (d)	2 (2.8%)	2 (6.9%)	6 (15%)	5 (16.1%)	
0-30	1	1	2	1	.06
31-100	1	1	4	3	
>100	0	0	0	1	
HHV-6 (d)	1 (1.4%)	0	0	0	
0-30	0	0	0	0	.70
31-100	1	0	0	0	
>100	0	0	0	0	
Parvovirus B19 (d)	3 (4.2%)	1 (3.4%)	3 (7.5%)	5 (16.1%)	.15
0-30	1	0	2	4	
31-100	1	1	1	1	
>100	1	0	0	0	

31% of patients with MUD1 HSCT were positive for BK virus (Table 3). A majority of patients with detection of BK virus in urine had no or mild clinical symptoms. Three patients were diagnosed with BK virus nephropathy and treated with cidofovir. One of them had viral clearance with cidofovir treatment. The second one had viral clearance with leflunomide and quinolone treatment, but the third one is still on leflunomide and quinolone treatment.

EBV was detected by PCR in the blood of nine of 171 (5.2%) patients. None of them developed post-transplant lymphoproliferative disorder. The rest of them were associated with asymptomatic viremia (Table 3). EBV infection was found statistically different between MRD and MUD1 HSCT ($P = .039$). Treatment of these children showing EBV replication consisted of reduction in immunosuppression and rituximab treatment in three children.

VZV infection was not seen in all groups under acyclovir prophylaxis. HHV-6 infection was detected in only one patient. This patient developed HHV-6-associated pneumonia, rash, fever, lymphadenopathy, and parotitis. He has no sign of HHV-6 encephalitis, and he was treated with ganciclovir and IVIG successfully.

Altogether 12 patients were found positive for parvovirus B19 PCR in the follow-up period; seven patients at 0-30 days post-HSCT (pre-HSCT 4), four patients at 31-100 days, and one patient at >100 days. All of these patients received IVIG treatment. Most of them (5/12) cleared the viremia in 1 month. Two patients, who were already positive pre-HSCT, are still positive for parvovirus B19 after 3 months in one patient (viral load 218455 copy/mL) and 6 months in another patient (viral load 14410 copy/mL) post-HSCT, but they do not have any symptoms or anemia. The duration of cytopenias during the first 100 days post-HSCT did not differ between those with or without parvovirus B19 viremia. Parvovirus B19 infections were found statistically different between MRD and haplo-HSCT patients (4.2% vs 16.1%, $P = .04$). Parvovirus B19 infections did not differ between patients with haplo and MUD1-MUD2 HSCT.

Respiratory viruses manifested as upper respiratory tract infection in four patients: RSV in two patients, parainfluenza in two patients, coronavirus in one patient, and none of them had pneumonia.

Among the 171 patients, 15 patients developed ADV infection. ADV infection was seen in nine patients at 31-100 days post-HSCT (Table 3). Ten patients had localized ADV disease (gastrointestinal tract) without viremia, and four patients had ADV disease with viremia. Patients with ADV viremia were treated with cidofovir successfully. ADV infection seemed to have a significantly higher incidence in patients with MUD2 and haplo-HSCT than those with MRD-HSCT ($P = .017$, $P = .014$). The incidence of ADV infection was not statistically different between MUD1-MUD2 and haplo-HSCT.

TABLE 4 Overall survival and TRM between four groups

	MRD	MUD1 (10/10)	MUD2 (9/10)	Haplo	*P* value
OS at day 100 (%)	95.7	82.7	82.5	90.32	
OS at the end of follow-up (%)	90.6	49	68.8	71.7	**<.0001**
TRM at day 100 (%)	2.8	6.9	17.5	9.7	.058
TRM at the end of follow-up (%)	5.6	44.8	22.5	19.4	**<.0001**

TABLE 5 Overall survival according to viral infection

	OS at day 100 (%)	OS at the end of follow-up (%)	*P* value
Infection (−)	93.75	83.4	**.04**
Infection (+)	88.7	66	
Multiple infection (−)	91.3	81.6	**.0008**
Multiple infection (+)	84.1	48.28	

Fourteen recipients of the allogeneic grafts passed away during the first 100 days post-HSCT. The cumulative incidences of TRM were 8.2% at day 100 (14/171 patients). Eleven of these 14 patients (78.57%) meanwhile had viral infection. Seven of them had multiple viral infections during their follow-up (7/11 patients, 63.63%). Forty-one patients passed away at the end of follow-up. Nineteen of them had multiple viral infections. Four patients (one of them was associated with CMV hepatitis) developed CMV pneumonia with a fatal outcome. The cumulative incidences of TRM were 18.7% at the end of follow-up (32/171 patients). Twenty-four of these 32 patients (75%) meanwhile had viral infection (15/32 patients—multiple viral infection, 62.5%). The Kaplan-Meier estimates of overall survival for all of the patients were 89.4% at day 100 and 72.8% at the end of follow-up. The overall survival and the cumulative incidences of TRM were summarized in Tables 4 and 5. Overall survival was influenced with having one or multiple viral infection ($P = .04$ and $P = .0008$). TRM at day 100 was not statistically significant between four groups, but TRM at the end of follow-up was found statistically significant ($P < .0001$). This significance was especially prominent between MRD and MUD1/MUD2/haplo-transplantations ($P < .0001$, $P = .008$, and $P = .03$). Comparison of overall survival at the end of follow-up between the four groups was associated with significantly better survival in the MRD group (log rank test, $P < .0001$).

4 | DISCUSSION

Viral infections due to impaired immune reconstitution remain a cause of morbidity and mortality in HSCT patients. The incidence of viral infections can be the best indicator of immune recovery. This study, with 171 pediatric patients, is the second largest study, which reports the incidence of virus infections in children following allogeneic HSCT. We found that viral infections are especially common in the first 100 days post-HSCT, suggesting a non-optimal immune reconstitution after allogeneic HSCT. Children with severe acute GVHD are more likely to develop multiple viral infections probably due to prolonged and intensive immunosuppressive treatment, especially the use of corticosteroids. The largest study by Hiwarkar et al[8] consisted of 291 pediatric patients with 34% MRD, 31% MUD, and 35% ≥1 ag HLA-mismatched donors. They reported CMV (16%), ADV (15%), and EBV (11%) frequently during period of CD4 T-cell lymphopenia and found that ADV and EBV reactivations had additional associations with the use of HLA-mismatched grafts. Rustia et al's[9] study was the third largest study of 140 pediatric patients with 40.7% MRD and 49.3% MUD. They found similar incidence results for CMV (21.4%), ADV (11.4%), and EBV (10%) like Hiwarkar et al. In our study, we found that BK virus and CMV reactivations were significantly low in MRD transplant patients compared with MUD1/MUD2 and haplo-HSCT. To the best of our knowledge, our study is the only study that compared the viral incidence between haplo-HSCT and MRD/MUD HSCT. We detected the incidence of CMV reactivation in 28.2% ofMRD patients but more than 60% of haplo- and MUD1/MUD2 HSCT recipients were positive for CMV reactivation. This difference can be explained with ethnic and geographical differences in CMV seroprevalence in Turkish population.[10,11] In our country, the seroprevalence rates in the age groups of 1-6, 7-14, and 14-49 years were detected as 82%, 92%, and 97.8%, respectively.[11]

BK virus was the second most seen virus in our study. BK virus is more frequent in the presence of MUD, haplo-HSCT, cord blood transplantation, GVHD, advanced age, and persistent thrombocytopenia.[12-14] We also found BK virus frequently among the recipients of MUD1, MUD2, and haplo-HSCT than those of MRD grafts.

ADV is increasingly recognized as an important pathogen in the recipients of allogeneic HSCT.[15] The reported mortality of patients with sustained high ADV DNA level in plasma is remarkably high, varying from 20% to 80% in different studies.[16,17] In our study, 16.1% of haplo-HSCT patients, 15% of MUD2 HSCT patients, and 6.9% of MUD1 HSCT patients developed ADV infection.

The most important clinical manifestation of EBV infection is the PTLD, which occurs in <1% of the patients after HSCT.[18] We found nine patients who showed EBV reactivation. Interestingly, we detected the lack of PTLD, as reported also by Kanakry et al[19] and Crocchiolo et al.[20]

Parvovirus B19 can cause rare but significant infectious complications after transplantation.[21] The predominant clinical manifestation of parvovirus B19 disease is anemia; chronic or recurrent anemia, as well as pure red cell aplasia, may be seen post-transplant. Organ

invasive manifestations such as hepatitis, myocarditis, and pneumonitis can also be observed. Rahiala et al[22] reported in their study that the incidence of parvovirus B19 was 30%. We detected the incidence of parvovirus B19 only 7%; the majority of them were patients transplanted from haploidentical donors.

The other end-point of this study was the outcome of these patients with viral infections. Yan et al[23] reported in their study that haploidentical HSCT correlated with a higher incidence of overall mortality and TRM compared with HLA-identical sibling donor HSCT. They found the incidence of infection-related mortality was higher after haplo-HSCT than after HLA-identical sibling donor HSCT (21.2% vs 13.4%, $P = .002$). Rustia et al[9] found in their study similarly that the overall survival for patients with viremia and viral disease was significantly lower compared with those without viremia (58% vs 74.2%, $P = .03$) and viral disease (48.2% vs 71.2%, $P = .024$). In our study, overall survival was comparable among MUD1/MUD2 and haplo-HSCT patients, whereas overall survival was superior for patients with MRD-HSCT ($P < .0001$). Similar to other studies, in the presence of one or multiple viral infection, the overall survival was found significantly lower compared with those without viral infection ($P = .04$ and $P = .0008$). MUD2 HSCT led to the highest incidence of TRM at day 100 compared with MRD, haplo, and MUD1 HSCT (17.5% vs 2.8%, 9.7%, and 6.9%). This significance was statistically borderline ($P = .058$). Haplo-HSCT was associated with superior TRM than MUD1 HSCT at the end of follow-up ($P = .03$). Based on these observations, the haploidentical donor option could become the second choice when there are no suitable MRD in terms of viral infection.

The main limitations of this study are its retrospective design and absence of routine surveillance in outpatients. Except CMV, surveillance for viruses was performed only in clinical suspicion, so the frequency of these infections might have been underestimated. In conclusion, CMV and BK virus infections were found to be significantly low in MRD-HSCT in this study. MUD and haplo-HSCT are comparable with each other in the setting of all viral infections, which is not stated in previous studies. Despite antiviral prophylaxis and treatments, post-transplant viral infections are associated with reduced overall survival and increased hospitalization, but these complications are significantly preventable with good follow-up and preemptive treatment without any clinical disease. For this reason, especially in countries where it takes a long time to find suitable donors, viral diseases should not interfere with the decision of haplo-HSCT considering the time spent on the patient. A larger study group and prospective studies are needed to confirm this observation. In these patients with post-transplant viral disease, the potential widespread application of virus-specific T lymphocyte therapy may likely reduce long-term morbidity and mortality.

AUTHORS' CONTRIBUTIONS

D.A. and G.O.: Concept/design; D.A. and A.A.: Data analysis/interpretation; D.A.: Data collection and drafting and manuscript writing; A.A.: Statistics; F.E.: Data review and interpretation; and G.O.: Critical revision of article and approval of the article.

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REFERENCES

1. Ljungman P. Prevention and treatment of viral infections in stem cell transplant recipients. *Br J Haematol*. 2002;118:44-57.
2. Ballen K, Woo Ahn K, Chen M, et al. Infection rates among acute leukemia patients receiving alternative donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2016;22:1636-1645.
3. Srinivasan A, Wang C, Srivastava DK, et al. Timeline, epidemiology, and risk factors for bacterial, fungal, and viral infections in children and adolescents after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2013;19:94-101.
4. Verdeguer A, de Heredia CD, González M, et al. Observational prospective study of viral infections in children undergoing allogeneic hematopoietic cell transplantation: a 3-year GETMON experience. *Bone Marrow Transplant*. 2011;46:119-124.
5. Lang P, Handgretinger R. Haploidentical SCT in children: an update and future perspectives. *Bone Marrow Transplant*. 2008;42(Suppl. 2):S54-S59.
6. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis*. 2002;34:1094-1097.
7. Apperley J, Masszi T. Graft vs. host disease. In: Apperley J, Carreras E, Gluckman E, Masszi T, eds. *Haematopoietic Stem Cell Transplantation, The EBMT Handbook*, 6th edn. European School of Haematology: Paris, France; 2012:217-234.
8. Hiwarkar P, Gaspar HB, Gilmour K, et al. Impact of viral reactivations in the era of pre-emptive antiviral drug therapy following allogeneic haematopoietic SCT in paediatric recipients. *Bone Marrow Transplant*. 2013;48:803-808.
9. Rustia E, Violago L, Jin Z, et al. Risk factors and utility of a risk-based algorithm for monitoring cytomegalovirus, Epstein-Barr virus, and adenovirus infections in pediatric recipients after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2016;22:1646-1653.
10. Jansen MA, van den Heuvel D, Bouthoorn SH, et al. Determinants of ethnic differences in cytomegalovirus, Epstein-Barr virus, and herpes simplex virus type 1 Seroprevalence in childhood. *J Pediatr*. 2016;170:126-34.e1-6.
11. Ataman S, Colak D, Günseren F, et al. Investigation of cytomegalovirus seroepidemiology in Antalya with a population-based cross-sectional study and review of related data in Turkey. *Mikrobiyol Bul*. 2007;41:545-555.
12. Carreras E. Early complications after HSCT. In: Apperley J, Carreras E, Gluckman E, Masszi T, eds. Haematopoietic Stem Cell Transplantation, *The EBMT Handbook*, 6th edn. Paris, France: European School of Haematology; 2012:177.
13. Silva Lde P, Patah PA, Saliba RM, et al. Hemorrhagic cystitis after allogeneic hematopoietic stem cell transplants is the complex result of BK virus infection, preparative regimen intensity and donor type. *Haematologica*. 2010;95:1183-1190.
14. Ruggeri A, Roth-Guepin G, Battipaglia G, et al. Incidence and risk factors for hemorrhagic cystitis in unmanipulated haploidentical transplant recipients. *Transpl Infect Dis*. 2015;17:822-830.
15. Robin M, Marque-Juillet S, Scieux C, et al. Disseminated adenovirus infections after allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcome. *Haematologica*. 2007;92:1254-1257.
16. Schilham MW, Claas EC, van Zaane W, et al. High levels of adenovirus DNA in serum correlate with fatal outcome of adenovirus infection

in children after allogeneic stem-cell transplantation. *Clin Infect Dis*. 2002;35:526-532.
17. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. *Blood*. 2010;115:3861-3868.
18. Styczynski J, Reusser P, Einsele H, et al.; Second European Conference on Infections in Leukemia. Management of HSV, VZV and EBV infections in patients with haematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. *Bone Marrow Transplant*. 2009;43:757-770.
19. Kanakry JA, Kasamon YL, Bolaños-Meade J, et al. Absence of post-transplantation lymphoproliferative disorder after allogeneic blood or marrow transplantation using post-transplantation cyclophosphamide as graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant*. 2013;19:1514-1517.
20. Crocchiolo R, Bramanti S, Vai A, et al. Infections after T-replete haploidentical transplantation and high-dose cyclophosphamide as graft-versus-host disease prophylaxis. *Transpl Infect Dis*. 2015;17:242-249.
21. Eid AJ, Brown RA, Patel R, Razonable RR. Parvovirus B19 infection after transplantation: a review of 98 cases. *Clin Infect Dis*. 2006;43:40-48.
22. Rahiala J, Koskenvuo M, Norja P, et al. Human parvoviruses B19, PARV4 and bocavirus in pediatric patients with allogeneic hematopoietic SCT. *Bone Marrow Transplant*. 2013;48:1308-1312.
23. Yan CH, Xu LP, Wang FR, et al. Causes of mortality after haploidentical hematopoietic stem cell transplantation and the comparison with HLA-identical sibling hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2016;51:391-397.

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