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Blood Virome After Allogeneic Hematopoietic Stem Cell Transplantation

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Background. Haploidentical allogeneic hematopoietic cell transplant recipients (allo-HCTr) receiving posttransplant cyclophosphamide (haplo-PTCy) are at higher risk for infectious complications, including viral infections.

Methods. We performed a retrospective, single-center, propensity-score matched-pair study including adult haplo-PTCy and allo-HCTr from human leukocyte antigen (HLA)–matched donors, undergoing transplantation in our institution between 2016 and 2022. For each patient, 4 blood samples (day [D] 0, D30, D90, and D180 posttransplantation) were extracted from the biobank and tested with metagenomic next-generation sequencing (mNGS) to describe the blood virome and identify viral RNA/DNA signatures potentially unrecognized by routinely available tests. Routine and symptom-driven polymerase chain reaction (PCR) test results performed during the study period were reviewed.

Results. Twenty-five matched pairs of haplo-PTCy and HLA-matched allo-HCTr were included in the analysis. Plasma mNGS detected a total of 155 and 190 different viral RNA/DNA signatures in haplo-PTCy and HLA-matched allo-HCTr, respectively between D0 and D180. The number of viral signatures was significantly lower in the haplo-PTCy group compared to HLA-matched allo-HCTr at D90 (−1.0 [95% confidence interval {CI}, −1.7 to −.3]; $P = .01$) and during the period between D30 and D180 (−1.9 [95% CI, −3.3 to −.5]; $P = .01$). Certain viral species (Anelloviridae, Epstein-Barr virus) were more prevalent in HLA-matched patients. Symptom-driven PCR tests showed higher infection rates of usual viral pathogens in haplo-PTCy versus HLA-matched allo-HCTr ($P = .02$).

Conclusions. Frequently deployed, targeted PCR tests showed increased viral infection prevalence in haplo-PTCy patients. Conversely, mNGS testing applied at specific timepoints revealed a lower number of commensal viruses in this patient group. More studies on routine use of mNGS are needed to further assess its clinical relevance and value.

Keywords. haploidentical donor; hematopoietic stem cell transplant; viral infection; virome; mNGS.

Allogeneic hematopoietic cell transplantation (HCT) from a haploidentical donor has been increasingly used over the last decade worldwide [1, 2]. In most haploidentical HCTs, graft-versus-host disease (GVHD) prevention is provided by posttransplant cyclophosphamide-containing regimens (haplo-PTCy) [1, 3]. Several studies described an increased number of infectious complications in this patient population followed by higher rates of nonrelapse mortality [4–8]. Notably, haplo-PTCy regimens have been associated with greater risk for infectious complications due to viral pathogens, such as cytomegalovirus (CMV) or Epstein-Barr virus (EBV) as well as BK polyomavirus, human herpesvirus 6 (HHV6), and adenovirus (AdV) infections [6, 7, 9–14]. Most of those data were generated using pathogen-specific polymerase chain reaction (PCR) assays in blood, based on institutional screening practices and/or clinical suspicion. Yet, the clinical signs or symptoms that could trigger the diagnosis of a viral infection in this complex population are very often unspecific or diluted among many other concomitant complications. Therefore, current strategies of targeted molecular screening using PCR assays in plasma for a group of well-defined viruses considered to be clinically relevant (mainly CMV and EBV, also less frequently HHV6 and AdV) are intrinsically limited. However, by using mNGS, a richer picture of the post-HCT blood virome can be depicted [15–17]. mNGS could detect commensal viral

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infections, unrecognized viral reactivation, or rare/unusual viruses. In this propensity-score matched-pair study, we used mNGS to describe the early posttransplant blood virome in haplo-PTCy patients and compared it to the blood virome of human leukocyte antigen (HLA)–matched HCT patients, who had received a GVHD prevention regimen not containing PTCy.

MATERIALS AND METHODS

Study Design, Population, and Objectives

We conducted a retrospective, single-center, propensity-score matched-pair study at the Geneva University Hospitals (HUG). Patients transplanted between November 2016 and January 2022 were eligible for inclusion. Adult (≥18 years of age) allogeneic HCT (allo-HCT) recipients from haploidentical donors receiving PTCy as GVHD prophylaxis were matched to patients who received a graft from HLA-matched donors, either related or unrelated, whose GVHD prophylaxis did not contain PTCy. Participants were matched using propensity score analysis, as described below, in terms of conditioning regimen, CMV donor/recipient (D/R) serostatus, stem cell source, and date of transplant. The primary objective was to describe and compare the blood virome between the 2 patient groups during the early (first 6 months) posttransplant period. Viral identification, thus those constituting the blood virome, was defined as the presence of any viral DNA/RNA detected in plasma by mNGS. Viral detection does not necessarily imply active viral replication. Viral infection was defined as the detection of viral replication by quantitative/qualitative PCR. Secondary objectives included the description and comparison of the number of viral infections detected by routine or symptom-driven PCR during the same posttransplant period.

Patient Consent Statement

The study was approved by the local ethics committee (reference number 2022-01094). Only patients who had signed the informed consent for inclusion in the institutional longitudinal cohort and biobank called "The cohort of infectious diseases in allogeneic hematopoietic stem cell transplant patients" were included in this study.

Data Collection

The following data were retrieved from the patient electronic medical records as well as from the dedicated institutional bone marrow transplant database: patient demographics, underlying hematological disease and HCT-related information (conditioning, donor type and HCT source, D/R CMV and EBV serology status, GVHD prophylaxis and posttransplant GVHD grade and time, nonrelapse, and overall posttransplant 1-year mortality). In addition, information on clinically significant viral infectious events, including viral PCR results and drugs administered for prophylaxis or treatment of viral infections, were collected starting at 1 week before transplantation and ending at 6 months posttransplantation from the above-mentioned sources.

Institutional Practices

For all CMV R^+ patients, EBV and CMV DNAemia is monitored in plasma by quantitative PCR weekly during the first 3 months post-HCT and every other week thereafter until 6 months post-HCT [18]. The commercial diagnostic methods used for the identification of EBV and CMV viruses changed during the study period: (1) before December 2019, EBV testing was performed using the artus EBV QS-RGQ kit (Qiagen GmbH, Hilden, Germany) on a Qiasymphony platform; afterward, the Cobas 6800 system (Roche Diagnostics, Indianapolis, Indiana, USA) was put in place; (2) before May 2018, CMV testing was performed via the Real-Time CMV assay (Abbott Molecular Inc., Des Plaines, IL, USA) on the m200 system; afterward, the COBAS CMV for Cobas 6800 test (F. Hoffmann La-Roche Ltd., Basel, Switzerland) was used. Primary CMV prophylaxis by letermovir was introduced on 1 May 2019, to all CMV D^-/R^+ adult patients from day [D] 1 to D100 and to all CMV R^+ patients with grade ≥2 acute GVHD requiring treatment with high-dose corticosteroids up to D180 [19]. Since 1 January 2021, letermovir primary prophylaxis was given to all adult CMV R^+ patients for the first 100 days posttransplantation.

In case of clinical suspicion for viral infection (eg, fever, mental status changes, hematuria), plasma quantitative PCR for different viruses (eg, HHV6, AdV, BK polyomavirus, or parvovirus B19) is performed as clinically indicated. Additional viral investigation by PCR is performed in non-blood samples (eg, BK polyomavirus in urine, AdV and other viral pathogens in respiratory or stool samples) based on clinical suspicion.

Study Procedures: Viral Nucleic Acids Extraction and mNGS

Plasma samples collected at 4 timepoints (days since transplantation) and stored at the above-mentioned institutional biobank were retrieved and analyzed by mNGS: D0 (−7), D30 (±7), D90 (±15), and D180 (±30). Virus nucleic acid extractions and library preparations were done using 2 (RNA and DNA) previously published protocols in parallel [20, 21]. In detail, 220 µL of each plasma sample was centrifuged at 10 000*g* for 10 minutes to remove cells. Then, 200 µL of cell-free supernatant was treated with 40 U of Turbo DNAse (2 U/µL) + 24 µL of 10 × DNAse Buffer (Ambion, Rotkreuz, Switzerland), according to the manufacturer's instructions. A total of 240 µL was recovered to perform the RNA and DNA protocols separately. For the RNA protocol, RNA genome extraction was isolated with TRIzol (Invitrogen, Carlsbad, California, USA). RNA pellet was resuspended in 10 µL, and ribosomal RNA was removed using the Ribo-Zero Gold depletion kit (Illumina, San Diego, California, USA) prior to the preparation

of the libraries (TruSeq total RNA preparation protocol, Illumina). Libraries' concentrations and sizes were analyzed using the Qubit (Life Technologies, Carlsbad, California, USA) and the 2200 TapeStation (Agilent, Santa Clara, California, USA) instruments, respectively. Each library was multiplexed by 24 on the NovaSeq 6000 sequencing system (Illumina) using the 2 × 100-bp protocol. A control submitted to the whole procedure was included to each sequencing lane to assess the presence of potential mNGS contaminants. The mean total number of read pairs obtained per sample was 103 191 027 (range, 16 410 123–395 968 215). For the DNA protocol, DNA genome extraction was isolated with the NucliSens easyMAG magnetic bead system (bioMérieux, Geneva, Switzerland). Double-stranded DNA synthesis was carried out with the DNA polymerase I, large fragment (Klenow) (New England BioLabs, Ipswich, Massachusetts, USA). DNA pellet was resuspended in 5 µL. Libraries were prepared with the Illumina Nextera XT protocol, and libraries' concentrations and sizes were analyzed using the Qubit and the 2200 TapeStation instruments, respectively. Each library was multiplexed by 36 on the NovaSeq 6000 sequencing system using the 2 × 100 bp paired-end protocol. A control submitted to the whole procedure was included to each sequencing lane to assess the presence of potential mNGS contaminants. The mean total number of read pairs obtained per sample was 83 584 934 (range, 2 438 264–674 384 380).

Bioinformatic Analysis

Paired reads generated by the NovaSeq 6000 platform (RNA and DNA libraries) were processed for virus detection using 2 methods in parallel: (1) the FeVir bioinformatics pipeline designed to detect all vertebrate viruses (mapping against the Virosaurus database version V90v_2023 and the SCANellome database version 2023.1); cross-contamination was removed using a 0.3% threshold and virus reported if a minimal of 300 nucleotides coverage detected) [21]; and (2) by a de novo assembly. Contigs ≥1000 nucleotides were blasted against the RVDB-prot v12.2 viral database (Database U-Rv: https://rvdb-prot.pasteur.fr/). The raw sequencing data were deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject accession number PRJNA1114927.

Statistical Analysis

To make patient groups comparable, propensity score matching was performed with a logistic regression model applied to match patients based on the following variables: type of conditioning regimen (reduced-intensity vs myeloablative conditioning), CMV serostatus (D/$^{-}$R^{-} vs all others), stem cell source (peripheral blood stem cells vs bone marrow), and date of transplant (before and after 1 May 2019, considering the introduction of letermovir as CMV prophylaxis). Of the 25 matched pairs, 23 were fully and 2 partially matched. Data are described by using counts and percentages or means and standard deviations. Results are presented by using frequency measures in both groups. Characteristics were compared using tests taking the matching into account (exact McNemar or *t* test for paired data). In case of missing data leading to incomplete pairs, logistic or linear regression models with mixed effects were used. The prevalence of viral infections is described by group of patients and per type of virus. Exact 95% confidence intervals (CIs) obtained with Clopper-Pearson method are reported. Analyses were conducted at each timepoint (D0, D30, D90, D180) and over the whole study period (D30–D180). The number of detected viruses per patient was described (counts, percentage, mean) by group and per type of virus, at each timepoint and over the whole study period. For prevalence of viral infections, the difference between groups of patients is reported with Tango asymptotic score 95% CI and was tested with the McNemar test. The mean number of viruses detected per patient was compared with a permutation test for paired data. Clinically diagnosed viral infections are described using the same statistical methods. As a sensitivity analysis, comparisons were also carried out removing the pairs of patients with a partial matching. All 95% CIs and statistical tests are 2-sided. The significance level is .05. Analyses were carried out with R software version 4.0.2 (R Core Team [2020]) and the packages MKinfer (Kohl M [2024]) and Ratesci (Laud M [2018]).

RESULTS

Characteristics of Patients and Samples

From 154 adult allo-HCT recipients fulfilling the inclusion criteria, 29 matched pairs of patients were identified. After excluding 4 pairs due to missing peri-transplantation sample(s), the final study group included 25 haplo-PTCy and 25 HLA-matched HCT recipients. The patient baseline characteristics and clinical outcomes during the first 6 months post-transplantation are summarized in Table 1. The groups were comparable in terms of demographics, underlying disease, and HCT characteristics, except for GVHD prophylaxis and cyclophosphamide use. The rate and timing of GVHD were comparable between the 2 patient groups.

Overall, 187 plasma samples were available and analyzed by mNGS for RNA and DNA viral sequences. Samples were available for all patients in both groups at D0 and D30. Concerning D90 and D180, in haplo-PTCy recipients, 22 of 25 (88%) and 19 of 25 (76%) samples were available, respectively. Nine samples were unavailable: 8 due to death (3 patients before D90 and 2 before D180) and 1 due to missing sample from the biobank (D180). For HLA-matched HCT recipients, plasma samples were available for 24 of 25 (96%) at D90, and 22 of 25 (88%) patients at D180. Four samples were not available due to missing

Table 1. Patient Characteristics and Data on Posttransplant Outcomes During the First 6 Months Posttransplantation

Characteristic	Haploidentical (n = 25)	HLA-Matched (n = 25)	*P* Value
Demographics			
Female sex	8 (32)	10 (40)	.77
Age at transplantation, y, mean (SD)	56.7 (14.7)	57.8 (13.5)	.68
Underlying disease			
AML/MDS	15 (60)	14 (56)	>.99
Other[a]	10 (40)	11 (44)	
HCT characteristics			
Conditioning, RIC	21 (84)	21 (84)	>.99
HCT source, PBSCs	25 (100)	25 (100)	>.99
EBV serostatus			
D^+/R^+	23 (92)	20/24 (83.3)	.23
D^-/R^+	1 (4)	4 (16.7)	.25
CMV serostatus			
D^+/R^+	8 (32)	12 (48)	.39
D^-/R^+	6 (24)	2 (8)	.29
GVHD prophylaxis			
Tacrolimus	25 (100)	15 (60)	.002
Cyclosporine	0 (0)	10 (40)	.002
Mycophenolate mofetil	25 (100)	0 (0)	<.0001
Methotrexate	0 (0)	25 (100)	<.0001
Cyclophosphamide	25 (100)	0 (0)	<.0001
Letermovir prophylaxis	4 (16)	4 (16)	>.99
Engraftment			
Engraftment day, mean (SD)	19.2 (4.2)	18.7 (4.1)	.67
Engraftment on or before day 30 post-HCT	24 (96)	25 (100)	>.99
GVHD			
Any (up to 180 days post-HCT)	17 (68)	13 (52)	.39
Day, mean (SD)	47.3 (34.7)	50.0 (33.0)	.80
Days 0–30 post-HCT	5 (20)	5 (20)	
Days 31–90 post-HCT	10 (40)	7 (28)	
Days 91–180 post-HCT	2 (8)	1 (4)	
Grade ≥2	13 (52)	10 (40)	.58
Day, mean (SD)	50.7 (39.4)	38.7 (24.5)	.08
Days 0–30 post-HCT	5 (20)	5 (20)	
Days 31–90 post-HCT	6 (24)	5 (20)	
Days 91–180 post-HCT	2 (8)	0 (0)	
Death			
Days 0–180 post-HCT	5 (20)	0 (0)	.06
Days 31–90 post-HCT	3 (12)	0 (0)	
Days 91–180 post-HCT	2 (8)	0 (0)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: AML/MDS, acute myelogenous leukemia/myelodysplastic syndrome; CMV, cytomegalovirus; D, donor; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; HCT, hematopoietic cell transplantation; PBSCs, peripheral blood stem cells; R, recipient; RIC, reduced-intensity conditioning.

[a]Other includes acute lymphoblastic leukemia (7), chronic lymphocytic leukemia (2), chronic myelomonocytic leukemia (5), lymphoma (3), and primary/secondary myelofibrosis (4).

samples from the biobank (1 patient at D90 and D180 and 2 other patients at D180).

Blood Virome by mNGS

Overall, 155 and 190 viruses were detected during the first 6 months posttransplantation in haplo-PTCy and HLA-matched patients, respectively (Supplementary Table 1). The composition of the blood virome between the 2 groups did not significantly differ at baseline (D0), neither in terms of prevalence of viral detection nor in the mean number of viruses detected per patient (Figure 1, Table 2). The overall prevalence of viral detection stayed comparable during the whole study period, but the respective composition differed over time. By D90, all patients in both groups had 1 or more viruses detected, while at D180 all but 2 patients (in the haplo-PTCy group) had at least 1 virus identified. This increase in the prevalence of virus detection in both groups at D90 and D180 was predominately attributed to anelloviruses: 87.8% and 75.4% of the viruses detected at D90 and 66.7% and 85.7% of the viruses detected at D180 in the haploidentical and HLA-matched groups, respectively, were from the Anelloviridae family. The mean number of viral detections per patient was similar between the 2 groups at all prespecified timepoints, except for D90, when it was lower in the haplo-PTCy group (−1.0 [95% CI, −1.7 to −.3]; $P = .01$). Similarly, when we looked at all timepoints posttransplant together (data between D30 and D180) the mean number of viral detections per patient was lower in the haplo-PTCy group (−1.9 [95% CI, −3.3 to −.5]; $P = .01$).

Although both groups were comparable at baseline, the respective distribution of viral families/species composing the blood virome varied posttransplantation. Notably, viral detection was lower in the haplo-PTCy compared to the HLA-matched patients for human Papillomaviridae at D90 (−23.8% [95% CI, −45.1% to −4.7%]; $P = .03$) and for torque teno mini virus (TTMV) and torque teno midi virus (TTMDV) from the Anelloviridae family: for TTMV at D30 (−20.0% [95% CI, −40.8% to .9%]; $P = .06$), D90 (−33.3% [95% CI, −58.0% to −2.6]; $P = .03$), D180 (−41.2% [95% CI, −64.0% to −15.2]; $P = .01$), and during the whole study period (−36.0% [95% CI, −56.8% to −11.8]; $P = .01$), and for TTMDV during the whole study period (−32.0% [95% CI, −55.7% to −2.9%]; $P = .03$). A trend for more frequent detection of EBV in HLA-matched patients between D30 and D180 (−20.0% [95% CI, −40.8% to .9%]; $P = .06$) was observed, as shown in Table 3 and Supplementary Table 1. The diversity of viral families and species detected posttransplantation did not appear to differ between the 2 groups (Figure 2).

Analysis of Targeted PCR Tests

In addition to mNGS, we examined the number and results of routine or symptom-driven viral PCR performed on blood and other samples in both groups. Symptom-driven PCR tests were performed in case of a clinically significant infectious event. Overall, 84% and 100% of haplo-PTCy and HLA-matched patients had routine CMV and EBV tests performed in blood during the 6 months posttransplant period, respectively. Routine

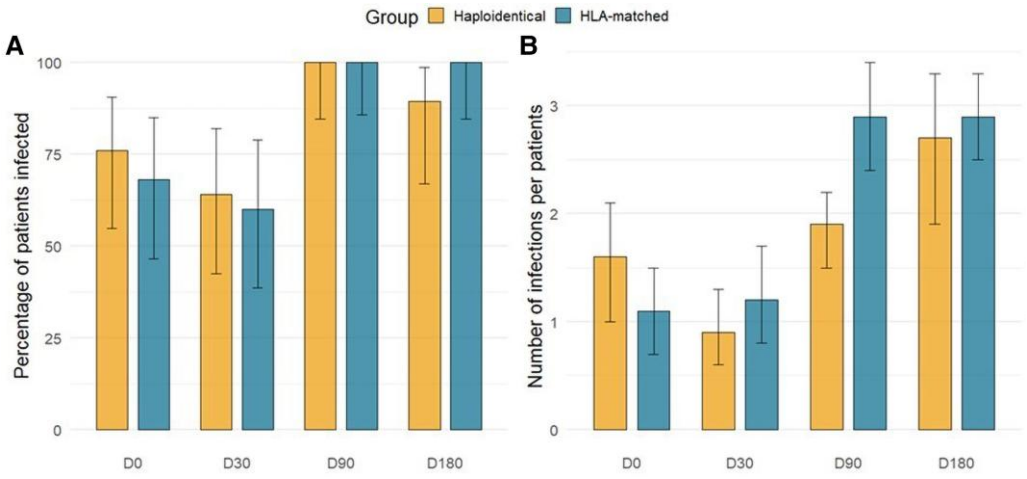

Figure 1. Prevalence (*A*) and mean number (*B*) of viruses detected by metagenomic next-generation sequencing in blood per patient in the 2 groups at baseline (day 0) and at the prespecified timepoints (days 30, 90, and 180) during the first 6 months posttransplantation. Abbreviations: D, day; HLA, human leukocyte antigen.

Table 2. Prevalence and Mean Number of Detected Viruses in the 2 Patient Groups at Baseline (Day 0) and at the Prespecified Timepoints (Days 30, 90, and 180) During the First 6 Months Posttransplantation Assessed by Metagenomic Next-Generation Sequencing in Blood

	Day 0		Day 30		Day 90		Day 180		Days 30–180	
Comparisons	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched
Patients with a sample, No.	25	25	25	25	22	24	19	22	25	25
Cases with detected viral RNA/DNA										
No. of patients	19	17	16	15	22	24	17	22	25	24
% (95% CI) of infected patients	76 (54.9–90.6)	68 (46.5–85.1)	64 (42.5–82)	60 (38.7–78.9)	100 (84.6–100)	100 (85.8–100)	89.5 (66.9–98.7)	100 (84.6–100)	100 (86.3–100)	96 (79.6–99.9)
No. of infections	40	28	23	30	41	69	51	63	115	162
Mean (95% CI) per patient	1.6 (1.0–2.1)	1.1 (.7–1.5)	0.9 (.6–1.3)	1.2 (.8–1.7)	1.9 (1.5–2.2)	2.9 (2.4–3.4)	2.7 (1.9–3.3)	2.9 (2.5–3.3)	4.6 (3.6–5.6)	6.5 (5.4–7.3)
Mean (95% CI) per infected patient	2.1 (1.6–2.6)	1.6 (1.3–1.9)	1.4 (1.1–1.8)	2.0 (1.5–2.5)	1.9 (1.5–2.2)	2.9 (2.4–3.4)	3.0 (2.4–3.6)	2.9 (2.5–3.3)	4.6 (3.6–5.6)	6.8 (6.0–7.5)
Difference Haplo vs HLA-matched[a]										
No. of matched pairs analyzable	25		25		21		17		25	
% (95% CI) of infected patients	8 (−13.1 to 29.1)		4 (−24.2 to 31.5)		0 (−15.5 to 15.5)		−11.8 (−34.3 to 8.8)		4 (−9.9 to 19.5)	
P value	.41		.78		>.99		.16		.32	
Mean No. (95% CI) of infections per patient	0.5 (−.2 to 1.2)		−0.3 (−.9 to .4)		−1 (−1.7 to −.3)		−0.4 (−1.1 to .4)		−1.9 (−3.3 to −.5)	
P value	.16		.37		.01		.36		.01	

Abbreviations: CI, confidence interval; Haplo, haploidentical; HLA, human leukocyte antigen.

[a]A positive or negative difference means that the percentage or the mean is higher or lower, respectively, in haploidentical patients than in HLA-matched patients.

testing viral PCR positivity did not differ significantly between the 2 groups (+9.5% [95% CI, −15.2% to 33.6%]; $P = .41$). A significantly higher number of symptom-driven viral PCR assays were performed in haplo-PTCy compared to HLA-matched patients in blood and other samples during the 6 months of follow-up (+36 [95% CI, 6.1–59.5]; $P = .02$); all of them were

Table 3. Blood Detection of Viral Families by Metagenomic Next-Generation Sequencing Between Haploidentical and Human Leukocyte Antigen–Matched Allogeneic Hematopoietic Cell Transplant Recipients

	Day 0		Day 30		Day 90		Day 180		Days 30–180	
Viral Family[a]	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched
Anelloviridae	16 (64)	12 (48)	8 (32)	9 (36)	21 (95.5)	24 (100)	17 (89.5)	22 (100)	22 (88)	24 (96)
Flaviviridae	5 (20)	4 (16)	4 (16)	4 (16)	2 (9.1)	5 (20.8)	4 (21.1)	4 (18.2)	5 (20)	5 (20)
Herpesviridae	1 (4)	1 (4)	4 (16)	6 (24)	1 (4.5)	3 (12.5)	1 (5.3)	2 (9.1)	6 (24)	9 (36)
Human Papillomaviridae	3 (12)	4 (16)	3 (12)	3 (12)	0	6 (25)	3 (15.8)	0	6 (24)	8 (32)
Human Polyomaviridae	2 (8)	0	1 (4)	1 (4)	0	3 (12.5)	4 (21.1)	0	5 (20)	4 (16)
Other[b]	0	0	1 (4)	1 (4)	2 (9.1)	0	3 (15.8)	2 (9.1)	5 (20)	3 (12)
Any viruses	19 (76)	17 (68)	16 (64)	15 (60)	22 (100)	24 (100)	17 (89.5)	22 (100)	25 (100)	24 (96)

Values represent the No. (%) of patients positive by metagenomic next-generation sequencing for at least 1 virus.

Abbreviations: Haplo, haploidentical; HLA, human leukocyte antigen.

[a]Viral families are presented in alphabetical order.

[b]Other includes adeno-associated virus 2, human-associated circovirus 2, human-associated gemykibivirus 1, human adenovirus, and Norwalk virus.

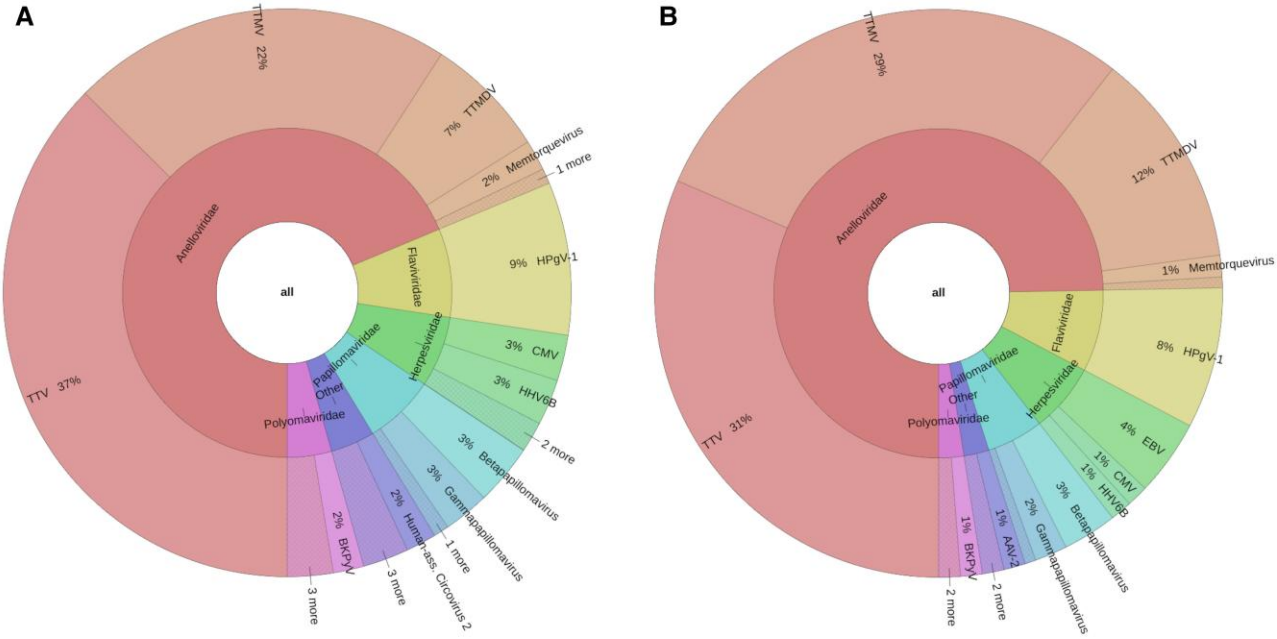

Figure 2. Diversity of viral families and species detected by metagenomic next-generation sequencing between days 30 and 180 posttransplantation in blood. The diversity of viruses represents the proportion of infected patients by a specific virus and viral family between day 30 and day 180. *A*, Haploidentical allogeneic hematopoietic cell transplantation recipients receiving posttransplant cyclophosphamide. *B*, Human leukocyte antigen–matched patients. Abbreviations: AAV-2, adeno-associated virus 2; BKPyV, BK polyomavirus; CMV, cytomegalovirus; HHV6B, human herpesvirus 6B; HPgV-1, human pegivirus type1; TTMDV, torque teno midi virus; TTMV, torque teno mini virus; TTV, torque teno virus.

positive (Table 4, Supplementary Tables 2 and 3). BK polyomavirus (+32.0 [95% CI, 8.6–53.0]; $P = .01$) and HHV6 (+20.0 [95% CI, 4.0–39.1]; $P = .03$) infections were more frequently detected in haplo-PTCy versus HLA-matched patients (Table 4, Supplementary Table 3).

DISCUSSION

Several studies have highlighted that allo-HCT recipients with haploidentical donors and PTCy-containing GVHD prevention regimens have a higher risk for posttransplant infectious complications, including bacterial, fungal, and viral [9–14, 22]. In most studies, viral infections were predominately diagnosed based on traditional and targeted molecular testing by PCR, therefore limiting diagnosis to a panel of viruses that

Table 4. Proportion of Patients With Virus Detected by Routine and Symptom-Driven Polymerase Chain Reaction by Timepoint in Blood and in Nonblood Samples

	Day 0		Day 30		Day 90		Day 180		Days 30–180	
Type of PCR and Virus	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched	Haplo	HLA-Matched
Routine PCR	24	18	19	24	17	21	12	20	21	25
CMV	2 (8.3)	0	7 (36.8)	8 (33.3)	9 (52.9)	8 (38.1)	7 (58.3)	7 (35.0)	10 (47.6)	11 (44)
EBV	1 (4.2)	0	1 (5.3)	5 (20.3)	3 (17.6)	8 (38.1)	4 (33.3)	2 (10.0)	6 (28.6)	10 (44)
Any virus	3 (12.5)	0	8 (42.1)	11 (45.8)	12 (70.6)	14 (66.7)	9 (75)	9 (45.0)	14 (67.7)	16 (64.0)
Symptom-driven PCR	1	2	12	3	9	7	12	6	20	11
CMV	0	0	0	0	0	0	0	0	0	0
EBV	0	0	0	0	0	2 (28.6)	1 (8.3)	0	1 (5)	2 (18.2)
HHV6	0	0	4 (33.3)	2 (66.7)	2 (22.2)	0	1 (8.3)	0	7 (35)	2 (18.2)
Adenovirus	0	1 (50)	1 (8.3)	1 (33.3)	3 (33.3)	1 (14.3)	2 (16.7)	2 (33.3)	5 (25)	3 (27.3)
BK polyomavirus	0	0	7 (58.3)	0	4 (44.4)	2 (33.3)	3 (25)	0	10 (50)	2 (18.2)
Other[a]	1 (100)	1 (50)	2 (16.7)	0	2 (22.2)	3 (42.9)	6 (50)	4 (66.7)	8 (40)	6 (54.5)
Any virus	1 (100)	2 (100)	12 (100)	3 (100)	9 (100)	7 (100)	12 (100)	6 (100)	20 (100)	11 (100)

Data are presented as No. (%).

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; Haplo, haploidentical; HHV6, human herpesvirus 6; HLA, human leukocyte antigen; PCR, polymerase chain reaction.

[a]Other includes adeno-associated virus 2, human-associated circovirus 2, human-associated gemykibivirus 1, human adenovirus, Norwalk virus.

are known to be associated with clinical complications. In contrast, data on viruses that are part of the blood virome in the early posttransplant period in high-risk allo-HCT recipients remain limited [23]. By using the unbiased mNGS method, we compared viral detection in haplo-PTCy and HLA-matched allo-HCT recipients, based on the hypothesis that stronger immunosuppression due to PTCy may be associated with higher rates of viral reactivation or promote unexpected viral infections. Following a careful patient selection via propensity score analysis, at baseline both groups were comparable in terms of baseline characteristics and mNGS viral detection rates. In contrast to prior reports, our data showed that detection of viral sequences was more frequent in HLA-matched patients on D90 and during the whole study period between D30 and D180. This may, in part, be attributed to the small number of patients included and to the limited testing on prespecified timepoints in our study, leading to lack of data on viral replication between the tested timepoints. Indeed, when looking at our PCR data, symptom-driven viral PCR tests were more frequently requested and found positive in haplo-PTCy patients, suggesting that clinicians considered an infectious complication more often in this patient group. The above could suggest that if mNGS was applied more frequently or on a symptom-driven approach, the distribution of positive mNGS results could be different. This question could be addressed in future studies, with routine use of mNGS in case of suspicious clinical presentations, rather than depending on predefined timepoint testing. We further hypothesized that the higher rates of viral detection by mNGS in the HLA-matched patient group could be related to selection biases, in case of increased rates of GVHD and consequent immunosuppressive treatment in those patients. However, there were no differences in the timing or rates of any and grade ≥2 GVHD between the 2 groups.

Finally, partly explaining the above difference is the increased prevalence of certain Anelloviridae in the HLA-matched group posttransplantation: TTMV at D90 and D180 timepoints and during the whole study period, and TTMDV during the whole study period. Anelloviridae presence and replication possibly depends on the balance between T-cell number and function after allo-HCT [24]. Torque teno virus (TTV) replication has been described in T-lymphocytes and therefore it is plausible that T-cell depletion can lead to lower TTV detection in the PTCy group [25]. Studies have suggested a slower T-cell immune reconstitution during the first 3 months after HCT in patients receiving haplo-PTCy with the number of T cells meeting the level observed in HLA-matched patients at 6–12 months posttransplantation [26–29]. Although this interaction may be more complex, it is likely that this may, in part, explain the relatively higher numbers of Anelloviridae in the HLA-matched versus haploidentical groups, during the first 6 months posttransplantation in this study. On the other hand, it is known that anellovirus detection increases with higher immunosuppression levels [30]. As detailed above, there were no significant differences in terms of GVHD prevalence and treatment between the 2 patient groups. Additional studies are required to further describe the prevalence of Anelloviridae reactivation posttransplantation, in patients with and without GVHD, and how this could be associated with other direct and indirect measurements of the patient's overall immune status.

Our data suggest that all allo-HCT recipients will reactivate 1 or more viruses during the first 6 months posttransplantation. The fact that those high-risk patients are viremic for at least 1 virus during the fragile early posttransplant period cannot be ignored. The clinical significance of this finding remains thus to be defined and should be further assessed in future studies to rule out potential complex role and associations with immune reconstitution, GVHD, disease relapse, and mortality.

mNGS testing in blood can depict a much broader picture of the blood virome than traditional molecular testing. Members of the Polyomaviridae family (human polyomavirus types 6 and 7, Merkel cell polyomavirus) were frequently detected in both groups, some (BK polyomavirus, JC virus, Merkel virus) already known to be associated with specific syndromes, but other species without an identified clear clinical impact for the time being [16, 31]. Some other rarely observed viruses were also identified in both patient groups, however without statistically significant differences considering the very low numbers: human-associated circovirus 2, adeno-associated virus 2 and 5, human-associated gemykibivirus 1, trichodysplasia spinuloza virus, and human herpesvirus 7. After a careful chart review of those patients, we were not able to identify potential associations between those viruses and clinical syndromes.

This study has limitations, including the relatively small number of patients and the fact that we tested only blood samples and from only 3 specific timepoints posttransplantation without additional symptom guided sampling. Moreover, combining HLA-matched related and unrelated donors could have also affected outcomes and our observations, but due to limited number of patients we were not able to narrow our selection to HLA-matched related donors. Furthermore, the use of propensity score matching to make the 2 patient groups more comparable led to a reduction in the number of inclusions, which in turn might have as well altered the representativeness of the study population.

In conclusion, this study reveals a higher number of viral sequences in HLA-matched HCT recipients compared to recipients of haploidentical grafts treated with PTCy when performed at 3 specific timepoints, partially due to a higher prevalence of Anelloviridae detection. Nevertheless, PCR tests performed routinely or upon clinical suspicion documented more frequent infectious events in the haplo-PTCy patient population. Viral replication is a frequent event in the early posttransplant period, with all patients replicating at least 1 viral pathogen, albeit with clinical implications that have not yet been identified. More studies are required to assess the performance and clinical value of symptom-driven mNGS testing on HCT outcomes.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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