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Parvovirus B19 in the Context of Hematopoietic Stem Cell Transplantation: Evaluating Cell Donors and Recipients

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Background. Parvovirus B19 (B19V) is a common human pathogen, member of the family Parvoviridae. Typically, B19V has been found to infect erythroid progenitors and cause hematological disorders, such as anemia and aplastic crisis. However, the persistence of genomic deoxyribonucleic acid (DNA) has been demonstrated in tonsils, liver, skin, brain, synovial, and testicular tissues as well as bone marrow, for both symptomatic and asymptomatic subjects. Although the molecular and cellular mechanisms of persistence remain undefined, it raises questions about potential virus transmissibility and its effects in the context of allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients. **Methods.** With this aim, we retrospectively screened allogeneic stem cell donors from 173 patients admitted for allo-HSCT from January 2008 to May 2013 using a seminested polymerase chain reaction approach. **Results.** We found 8 positive donor samples, yielding a 4.6% of parvovirus prevalence (95% confidence interval, 2.36-8.85). Pre- and post-HSCT samples (n = 51) from the 8 recipients of the positive donors were also investigated, and 1 case exhibited B19V DNA in the post-HSCT follow-up (D + 60). Direct DNA sequencing was performed to determine the genotype of isolates and classification, performed by phylogenetic reconstruction, showed a predominance of genotype 1a, whereas the rare genotype 3b was detected in 2 additional patients. By molecular cloning, different B19V 1a substrains polymorphisms were evidenced in the single case in which donor and its recipient were B19V+. **Conclusions.** Our results suggest that HSCT allografts are not a main source for B19V transmission, pointing to potential events of reinfection or endogenous viral reactivation.

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The parvovirus B19 (B19V), a widespread human pathogen, is a member of the family Parvoviridae, genus *Erythrovirus*. It is a nonenveloped virus enclosing a linear single-stranded deoxyribonucleic acid (DNA) genome composed of 1 copy of 5596 nucleotides length. The genome has 2 large open reading frames, codifying for the major structural proteins VP1 and VP2 and for the nonstructural protein NS1.¹ B19V has a pronounced tropism toward erythroid

colony forming units, erythroid burst-forming units and erythroid precursor cells in the bone marrow (BM), which are the natural host cells. The P-blood group antigen serves as its main cellular receptor, being expressed not only on erythroblasts but also in megakaryocytes, endothelium, fetal myocardial, and liver cells.² In immunologically competent individuals, the classic natural history ensues after virus acquisition through respiratory route primarily during childhood. Then, a

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short-lived, high-level viremia appears, declining with the appearance of specific IgM and IgG antibodies.¹ B19V infections usually take an asymptomatic course or present as a self-limited subclinical erythroid aplasia, which may be followed by a cutaneous rash and immune-mediated arthralgia.^{1,3} In contrast, in immunocompromised individuals that are unable to mount an effective immune response, infection may lead to several diseases like chronic pure red cell aplasia, in which suppression of erythroid precursors is permanent.⁴

Viral persistence has also been described in symptomatic as well as asymptomatic immunocompetent individuals.⁵⁻⁷ Remarkably, after primary infection, the viral genomic DNA may remain detectable in several human tissues in the absence of viremia.⁸⁻¹³

Virus DNA can also be persistently detected in the blood,¹⁴ as well as in BM.^{5,7,15,16} In fact, the ability of the virus to persist even in the presence of specific anti-B19V antibodies raise the matter of transmissibility, not so much to the immunocompetent recipients, but to receptors with some degree of immunodeficiency, like immunosuppressed patients for organ or hematopoietic stem cell transplantation (HSCT).¹⁷ It is known that B19V is a frequent contaminant of blood and plasma-derived medicinal products,¹⁸⁻²¹ and there are few reports of clinically relevant B19V infection resulting from transfusion.²² In immunosuppressed subjects, B19V is a rare but significant pathogen, where infection manifests during the posttransplantation period.²³ At least in the kidney transplant setting, B19V was already recognized as an etiological agent transmitted from donor graft,^{24,25} but in the allogeneic HSCT setting, consensus has not been reached, with occasional case reports.²⁶⁻⁴¹

To shed some light in the issue of B19V transmissibility in the allogeneic HSCT setting, we retrospectively evaluated the presence of B19V in stem cell donors for allo-HSCT over a 5-year period. Archived DNA samples, obtained from healthy donors were screened, mostly from the same day or close to stem cell infusion. In case of a positive result, all DNA samples obtained from peripheral blood (PB) or BM of its counterpart recipients, pretransplantation and posttransplantation were also evaluated.

MATERIALS AND METHODS

Study Group, Samples, and DNA Isolation

A total of 249 donor-derived DNA samples relative to 173 allo-HSCT from January 2008 to May 2013 (140 related and 33 unrelated allogeneic transplantations) were included in this study. Samples were obtained around the transplantation date and sent to the laboratory for hematopoietic chimerism analysis. Donor genomic DNA was obtained from PB (n = 216) and BM cells (n = 33) by DNAzol reagent (Invitrogen, Life Technologies). A total of 51 existing DNAs from recipient samples were also evaluated. Cells from PB (n = 41) or BM (n = 10) were processed by Ficoll-Paque Plus (GE Healthcare and Life Sciences) from pretransplantation and several posttransplantation time points.

Additionally, B19V+ samples from other 5 regularly monitored cancer patients in pre- and post-allo-HSCT periods, and 2 noncancer patients sent for diagnosis were included in this study to increase B19V genetic variability to be assessed by phylogenetic methods.

This research was approved by the INCA's Ethics Committee (CAAE 53571116.4.0000.5274). The characteristics of the group are shown in Table 1.

B19V Polymerase Chain Reaction Analysis

A seminested polymerase chain reaction (PCR) approach targeting a conserved region of viral NS1 coding sequence was used to investigate the presence of B19V, as described.⁴² Sensitivity of the seminested PCR was estimated by serial

TABLE 1.
Characteristics of patients and transplants per the B19V status of donors

Characteristics	B19V+ donors, n (%)	B19V- donors, n (%)	P ^a
Age: median (range), y	38.5 (9-61)	29.0 (2-67)	0.355
Children (≤18 y)	2 (25.0)	50 (30.3)	0.749
Sex (M:F)	6:2	106:59	0.564
Diseases			
AL	5 (62.5)	85 (51.5)	0.690
SAA	0 (0.0)	24 (14.5)	
CML	1 (12.5)	16 (9.7)	
MDS	1 (12.5)	15 (9.1)	
HL	0 (0.0)	11 (6.7)	
NHL	0 (0.0)	8 (4.8)	
Others ^b	1 (12.5)	14 (8.1)	
Type of transplant			
HLA-related	5/8 (62.5)	136/165 (82.4)	0.156
HLA-unrelated	3/8 (37.5)	29/165 (17.6)	
Source of cells ^c			
BM	7/8 (87.5)	121/158 (75.9)	0.683
PB	1/8 (12.5)	37/158 (24.1)	
GVHD prophylaxis			
CSA + MTX	5 (62.5)	154 (92.8)	0.022 ^a
CSA + MMF	1 (12.5)	1 (0.6)	
CSA-only	2 (25.0)	5 (3.0)	
Other	0 (0.0)	8 (4.8)	
Conditioning regimens			
BuCy	4 (50.0)	76 (45.8)	0.99 ^a
Cy + TBI	0 (0.0)	37 (21.4)	
Cy + ATG	0 (0.0)	12 (6.9)	
Cy + TBI + ATG	1 (12.5)	13 (7.5)	
Bu + Flu/Mel	1 (12.5)	6 (3.5)	
Other	2 (25.0)	22 (13.2)	
Engraftment ^d	7/8 (87.5)	151/159 (95.6)	0.361
Deaths	5/8 (62.5)	86/165 (52.1)	0.566
Cause of death			
TRM	3/5 (60)	55/86 (64.0)	0.858
Relapse/progression	2/5 (40)	31/86 (36.0)	
Early deaths			
Until 30 d	2/5 (40)	10/86 (11.6)	0.068
Until 100 d	3/5 (60)	34/86 (39.5)	0.365

^a Age differences were tested with Mann-Whitney test; all others with χ^2 or Fisher exact tests. In the comparisons of GVHD prophylaxis and conditioning regimens, Fisher test was performed comparing the first category vs. the other in 2 × 2 tables.

^b Others include chronic lymphocytic leukemia; juvenile myelomonocytic leukemia; Fanconi anemia; myelofibrosis; multiple myeloma; primary immunodeficiency, and adult T-cell lymphoma.

^c 166 cases with available information.

^d 167 cases with available information.

M:F, male: female; AL, acute leukemias; SAA, severe aplastic anemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphomas; GVHD, graft-versus-host disease; CSA, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil; BuCy, myeloablative combination of busulfan and cyclophosphamide; TBI, total body irradiation; ATG, antithymocyte globulin; Fu, fludarabine; Mel, melphalan.

dilutions between 2×10^{-4} and 1×10^{-3} copies/cells. PCR assays were performed using Platinum *Taq* DNA polymerase and reagents (Invitrogen) in a Veriti 96-well thermal cycler (Life Technologies).

PCR Product Purification and DNA Sequencing

PCR products from the second round of amplification were purified through the Wizard SV Gel and PCR Clean-Up System (Promega). DNA sequencing from forward and reverse strands was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction version 3.1 (Life Technologies). Sequences were read in an ABI PRISM DNA Analyzer 3130xl (Life Technologies).

Phylogenetic Analysis

Forward and reverse DNA sequences were edited by MEGA version 6.0.6 software,⁴³ and aligned using Seaview version 4.5.3.^{44,45} The obtained set of NS1 B19V sequences was analyzed together with worldwide reference sequences from genotypes 1a, 1b, 2, 3a, and 3b, retrieved from GenBank. Simian parvovirus NS1 sequence was used as an outgroup (Table S1, SDC, <http://links.lww.com/TP/B483>).

Bayesian inference using the Markov chain Monte Carlo method was used for phylogenetic analysis using MrBayes 3.1.2 in the web server phylogeny.fr⁴⁶ using a set of 12 B19V worldwide reference sequences. Four chains were run for 10 000 generations, sampling every 10 generations. The first 250 trees sampled were discarded as burn-in. As well, a neighbor-net method implemented on Splitstree4 software⁴⁷ was used to confirm the initial genotyping and to verify hypothetical events of viral recombination through a network analysis using a set of 19 B19V worldwide reference sequences as well as other 41 Brazilian B19V NS1 sequences (Tables S1 and S2, SDC, <http://links.lww.com/TP/B483>).^{42,48-51}

DNA Cloning

B19V+ amplified fragments found in donor and receptor were subjected to molecular cloning in a pCR 2.1 TOPO TA vector (Invitrogen, Life Technologies), per manufacturer's instructions. Plasmidial DNA was isolated and purified using the QIAprep Spin Miniprep Kit (Qiagen) and sequenced using M13F and R regular primers.

Clinical Parameters and Statistical Analysis

Neutrophil recovery was defined as achieving an absolute neutrophil count of 500/ μ L or greater for 3 consecutive days, and date of engraftment, as the first of these 3 days. Deaths during the first 30 and 100 days posttransplantation were considered early deaths. Causes of death were categorized into relapse (patients who never achieved remission or died with relapse at any time after allo-HSCT) or transplant-related mortality (TRM) (deaths from graft-versus-host disease, infection or other causes). Overall survival (OS) was the time elapsed between transplantation and death from any cause; patients who remained alive were censored at the last follow-up. Hematopoietic chimerism was evaluated in PB or BM, by fluorescent STR-PCR as described,⁵² with modifications. Chimerism status was categorized as complete chimerism (CC) (donor pattern), mixed chimerism, and autologous recovery. Kinetics of chimerism was only evaluated when at least 2 sequential samples, with 7 to 15 days apart were available. Statistical analyses were performed using non-parametric tests, with a significance level of *P* less than 0.05.

The lower and upper limits of the 95% confidence interval (95% CI) for the proportion were calculated using Wilson's score interval. Analyses were performed using IBM SPSS Statistics 20.

RESULTS

Frequency of B19V Detection in Donor Samples and Potential Transmissibility to Allo-HSCT Receptors

Eight cases (4.6%; 95% CI, 2.36-8.85) from the evaluated 173 donors were found to carry B19V genomes by PCR analysis (2 BM and 6 PB samples). Remarkably, all except 1 of these 8 samples were collected at the same day of graft infusion. Unfortunately, no serology studies were available for donors and patients. The clinical description of the 8 recipients in which its donor was B19V-positive, as well as the transplant characteristics and specific period of sample collection are displayed in Table S3, SDC, <http://links.lww.com/TP/B483>.

Afterward, all available recipient samples of a B19V+ graft were also retrospectively screened, at both pretransplantation and posttransplantation time points. In 2 cases, DNA samples at 7 to 10 days after HSCT were available, whereas most investigated cases had a D + 30 or engraftment time sample (Table S3, SDC, <http://links.lww.com/TP/B483>). Thus, if B19V transmitted by an allograft resulted in a productive infection in the recipients, we would be able to see it in the early time points investigated.

From the 51 evaluated samples, only 1 BM DNA sample was found positive (patient 8 in Table S3, SDC, <http://links.lww.com/TP/B483>, male, 63 years, with acute myeloid leukemia, subjected to related allogeneic BM transplantation) at D + 60 in the posttransplantation period. Its corresponding positive donor sample was obtained also in the same day of cell infusion. During the posttransplant follow-up, the patient had engraftment at D + 22 and did not exhibit atypical prolonged anemia or cytopenias, or other histopathological or clinical symptoms that could suggest a B19V infection.

Therefore, to test the possibility of B19V transmission through the infused graft, viral DNA sequences from 25 clones from each, donor and recipient, were evaluated. The comparative analysis of donor and recipient DNA sequences showed 4 synonymous mismatches at positions 1521 (C > T), 1656 (A > G), 1710 (A > G), and 1749 (T > A) of the NS1 gene, based on the J35 prototype strain. This indicated no complete sequence similarity between B19V isolates from donor and recipient, strongly suggesting a different viral origin for B19V infection in the receptor. Phylogenetic reconstruction of donor and recipient strains reinforced this result (donor for patient 8 [BRRJ_HSCT_D1] and posttransplantation sample of patient 8 [BRRJ_HSCT_R1]; Figure 1A). Our extensive cloning coverage also ruled out coinfection with 2 or more B19V strains.

As mentioned before, outside the selected period, other 5 hospital patients included in the phylogenetic analysis had also its donor-derived DNA samples tested for B19V, all with negative results. However, 2 of them had 2 occurrences of B19V viremia along their posttransplantation follow-up: patient 9 (R9) had B19V detection 1 week before transplantation and about 1 year and 4 months after transplantation; and patient 10 (R10) had B19V detected 1 month before

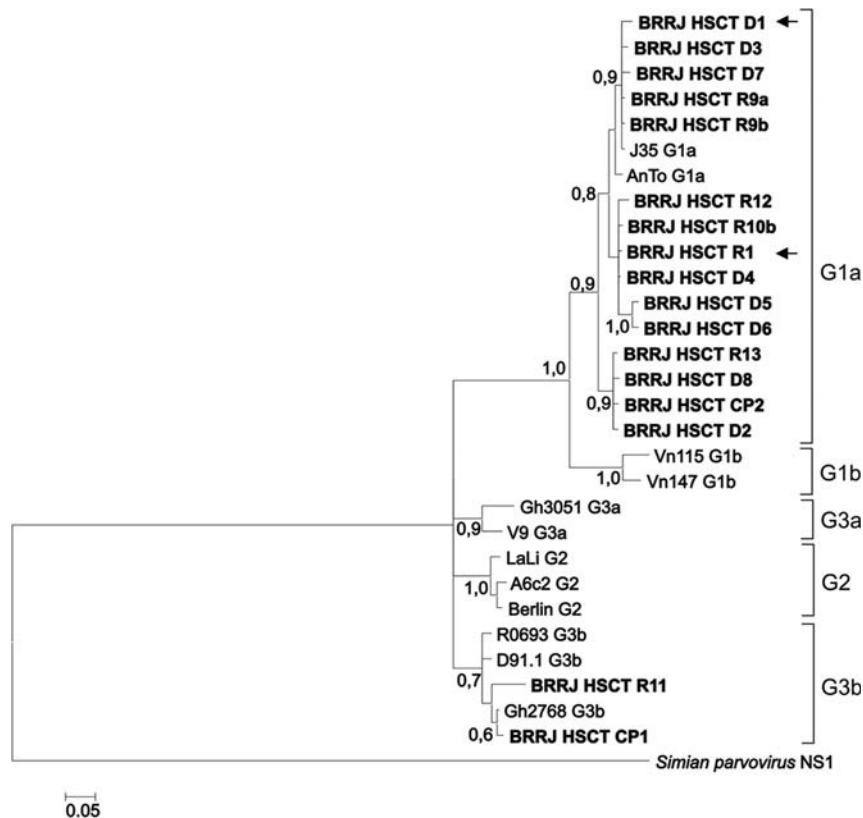


FIGURE 1. Bayesian inferred unrooted phylogenetic analysis from a partial NS1 B19V nucleotide sequence, generated by MrBayes 3.1.2. Bootstrap probabilities values are shown at branches. Bar represents 0.05 nucleotide substitutions per site. BRRJ HSCT, NS1 B19V sequences from positive isolates found in this study; D, donor; R, receptor; CP, positive noncancer samples. The obtained sequences ($n = 17$), corresponding to B19V isolates from 8 donors, 1 receptor and 8 additional sequences from positive external cases) were named as follows: Donor for patient 1 (BRRJ_HSCT_D2), donor for patient 2 (BRRJ_HSCT_D5), donor for patient 3 (BRRJ_HSCT_D4), donor for patient 4 (BRRJ_HSCT_D3), donor for patient 5 (BRRJ_HSCT_D6), donor for patient 6 (BRRJ_HSCT_D7), donor for patient 7 (BRRJ_HSCT_D8), donor for patient 8 (BRRJ_HSCT_D1), posttransplantation sample of patient 8 (BRRJ_HSCT_R1), noncancer patient 1 (BRRJ_HSCT_CP1), noncancer patient 2 (BRRJ_HSCT_CP2), patient 9, first and second detections (a) and (b) (BRRJ_HSCT_R9a and R9b), patient 10 (BRRJ_HSCT_R10b), patient 11 (BRRJ_HSCT_R11), patient 12 (BRRJ_HSCT_R12) and patient 13 (BRRJ_HSCT_R13). Arrows indicate D1 and R1, and refer to the single case where a B19V+ graft and recipient were found (patient 8 in Table S3, SDC, <http://links.lww.com/TP/B483>). G1 refers to genotype 1 (subgenotypes G1a and G1b), G2 refers to genotype 2 and G3 refers to genotype 3 (subgenotypes 3a and 3b).

transplantation and about 3 months post-HSCT. The DNA sequences for R9 pre-(R9a) and R9 posttransplantation (R9b) evidenced a genotype 1a with complete match. Unfortunately, we were not able to successfully determine the nucleotide sequence for the pretransplantation sample (R10a) of recipient 10 due to lack of sample.

B19V Genotypes and Phylogenetic Reconstruction

Phylogenetic reconstruction by Bayesian Inference (Figure 1) and Network analysis (Figure 2) were consistent in the genotyping of the 17 isolates obtained in this study. Most sequences (15/17) were grouped to genotype 1a, whereas 2 of these sequences (BRRJ-HSCT R11 and BRRJ-HSCT CP1) were grouped to genotype 3b. No evidence of viral recombination was observed by network analysis (Figure 2).

The full set of sequences corresponding to B19V isolates from 8 donors, 1 receptor, and 8 external sequences was deposited on GenBank (accession numbers KM073047 to KM073063).

Clinical Characteristics of the Studied Groups

Patients with a B19V+ donor were not different from the patients with a B19V- donor in respect of age, sex, type of

transplant and cell source. A lower number of patients in the B19V+ donor group received the standard cyclosporine + methotrexate regimen than in the B19V- group. Engraftment data were available for 167 patients; 9 (5.4%) of them did not achieved engraftment (12.5% vs 5.0% of recipients with B19V+ and -ve donors, $P = 0.36$). Median time to neutrophil recovery was 20 days (range, 9-27), and 19 days (range, 6-29) in recipients who had a B19V+ or a B19V- donor, respectively ($P = 0.79$, Mann-Whitney test).

There were 91 deaths reported, which occurred from 3 days to 73.3 months after transplantation. Mortality ratio and frequency of early deaths were not different between recipients who had a B19V+ or a B19V- donor (Table 1). OS of the entire group was 47.4%, with median follow-up of 31 months (95% CI 13-49.3). Having a B19V+ donor had no impact on OS (37.5% vs 47.9%, $P = 0.267$).

In respect of hematopoietic chimerism, 140 patients (4 cases with a B19V+ donor) had at least 1 record. No differences in chimerism status were observed between groups (3/4 with a B19V+ donor and 118/136 with a B19V- donor exhibited CC; $P = 0.498$). In the first 100 days posttransplantation, 118 cases had available samples for kinetics analysis, and again, no differences were observed between groups (75% of

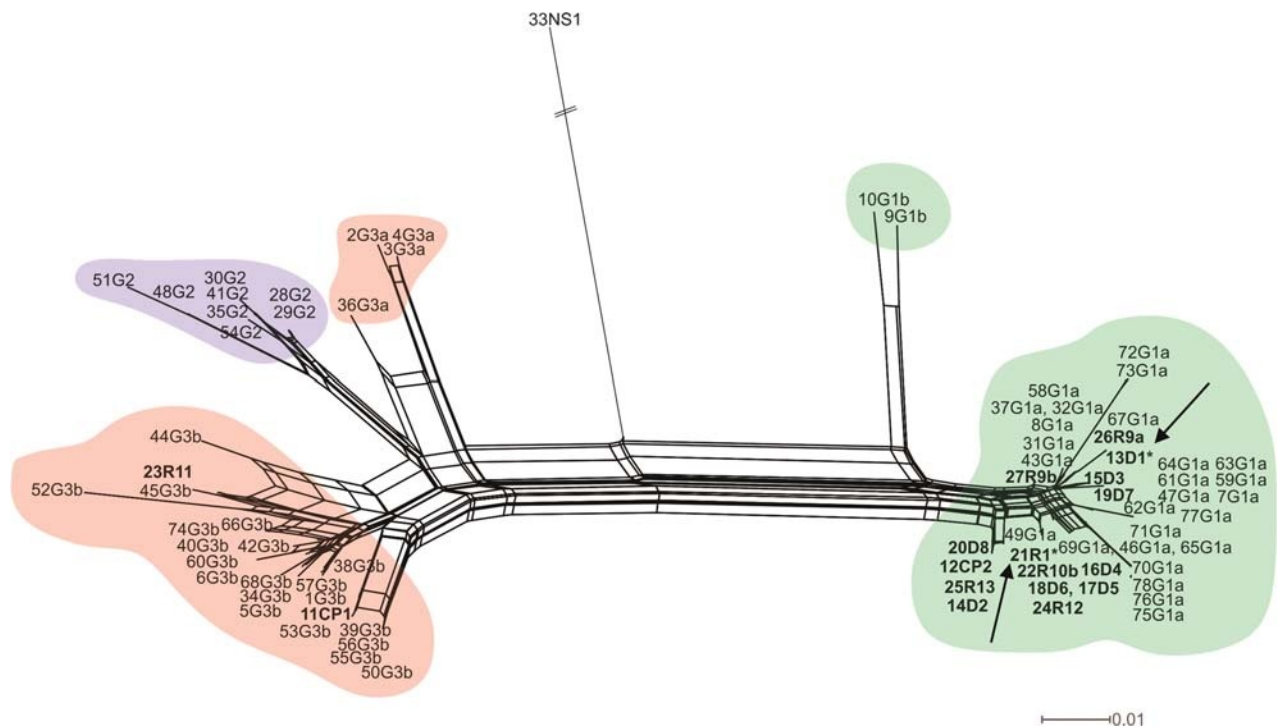


FIGURE 2. Unrooted phylogenetic analysis from a partial NS1 B19V nucleotide sequence, generated by Splitstree4 analysis. Bar represents 0.01 nucleotide substitutions per site. G1 refers to genotype 1 (subgenotypes G1a and G1b), G2 refers to genotype 2 and G3 refers to genotype 3 (subgenotypes 3a and 3b). Colored circles indicate distinct clades, G1a and G1b to the right and G3a, G2 and G3b to the left. Codes for isolates are found in Tables S1 and S2, SDC, <http://links.lww.com/TP/B483>.

cases with a BV19+ donor exhibiting CC vs. 85% in cases with a B19V- donor; $P = 0.194$).

DISCUSSION

In this work, we intended to exploit an existing DNA repository bank in which DNA samples collected from donors close or at the day of cell infusion, and their respective recipients, pre- and post-allo-HSCT collected at several timepoints, were available to evaluate the possibility of B19V transmission by allogeneic stem cell infusion. We aimed also to infer the potential effects of infusing a B19+ allograft in HSCT recipients. The group of HSCT evaluated in this study is characterized by a predominant use of BM as cell source, which reflects the clinical practice in Latin American countries, such as Brazil.^{53,54} Economic constraints, as well as a high number of transplants for marrow failure syndromes, where the use of BM is chosen over other cell sources, are responsible for the disparity with developed countries, where PB predominates as cell source. In fact, in our work, BM failure syndromes (severe aplastic anemias and myelodysplastic syndromes) account for 22.5% of diagnoses. To the best of our knowledge, no similar evaluation was performed so far, either in developed or underdeveloped settings.

In our study, a 4.6% of prevalence of B19V DNA was detected in asymptomatic stem cell donors (95% CI, 2.36-8.85). In normal blood donors, B19V DNA detection is lower (95% CI, 0.075%-1%)⁵⁵ than that observed in this work. However, the positivity ratio obtained in this study is in accordance with a related study that evaluated HSCT plasma donor samples.³¹

Nevertheless, due to the newly recognized phenomenon of B19V persistence, there are issues regarding whether a qualitative positive PCR, or even the detection of genomes by next-generation sequencing technologies could be interpreted as a distinctive sign of active B19V replication producing infectious virions or could simply reflect the viral decay from persistently infected cells without active replication.⁵⁵⁻⁵⁷ Follow-up studies with acutely infected immunocompetent patients monitored for over 100 weeks have shown that, after a short peak of extremely high viremia, viral loads fall during the first few weeks of infection; then viral DNA persisted at low levels for long periods.^{5,58-60} Such low-level B19V viral loads appear to be safe in respect of transmissibility via blood component transfusions.⁶¹

Unfortunately, due to the lack of sample availability, no accurate quantitative data could be generated in the present study, thus at present, we are not able to affirm that the detected B19V genomes reflect a real source of infectious virions. In fact, in the series herein investigated, we were not able to associate the presence of B19V DNA in PB or BM of donors close to the time of HSCT, with transmissibility via transplanted graft. Nevertheless, with our assay, which is able to detect 1 viral gEq in 5000 cells, we estimate that we would have been able to detect medium to high viral loads, and therefore, clinically relevant infectious episodes, either in BM or PB.

One molecularly confirmed case of B19V transmission by BM transplantation from a B19V viremic donor at the time of BM harvest was previously reported.³³ Results from another case report also suggested that the occurrence of chronic pure red cell aplasia in a 19-year-old man was due to B19V transmitted through HSCT.³⁵ In the case described here, in

which B19V was detected in donor and posttransplant recipient samples, despite extensive molecular investigation we were not able to prove transmission by allo-HSCT, because the obtained DNA sequences from patient 8 displayed a similar genotype 1a than its donor-derived sequences, but harbored different substrain polymorphisms. Molecular cloning also helped to rule out polyclonal infections and/or intergenotypic recombinant viruses, phenomena already described for B19V infections.^{40,48,50} Considering the age (63 years), underlying disease (acute myeloid leukemia), and the immunosuppressed state of the recipient at the time of detection (D + 60), the most plausible explanation would be reinfection or, alternatively, endogenous viral reactivation from reservoir tissues (not available for PCR evaluation). Additional evidence came from 2 other cases in the present study (R9 and R10), where an endogenous reactivation or even reinfection was also suggested.

In general, B19V infection is a rare event in HSCT as well as in solid organ transplantation.²³ In a group of 201 patients after BM transplantation, the incidence of B19V infection was low (1.5%), and anemia was the main clinical manifestation observed.⁶² Other few cases were also reported,^{34,36-41} some of them linked to transmission by platelet transfusion, erythrocytes concentrate infusion or to acute infection. Therefore, although B19V appears to be transmitted by blood-derived products, hematopoietic allografts do not seem to be a source of frequent virus transmission. It is likely that the low incidence of documented parvovirus B19 infection may be attributed to virus prophylaxis and to the common current practice of IVIg infusions to stem cell recipients.^{33,38}

We also asked whether receiving a graft from a donor with a potential B19V infection could have an indirect effect on early transplantation parameters, such as engraftment, hematopoietic chimerism, or TRM. No significant differences were observed between the patients receiving a B19V-positive or -negative graft, suggesting that detected DNA is not coming from an active B19V infection. Nevertheless, we acknowledge that the low sample number in this work and the few numbers of recipients with a positive donor strongly limit the power of this study. Moreover, the well-known competing risks in the post-HSCT setting (ie, early TRM in 4/8 cases receiving a B19V+ allograft) may have prevented an accurate characterization of B19V infectious events. Therefore, the issue of B19V transmissibility via allo-HSCT warrants further more powerful prospective studies.

In respect of B19V circulating virus strains, our study corroborates a recent work that stated that genotype 1 is the most worldwide spread type, being followed by the rare genotype 3b.^{63,64} In Brazil, other previous studies had demonstrated the existence of genotypes 1, 2, and 3,^{42,49} whereas others detected only 1 and 3 genotypes^{48,65} or solely genotype 1.^{50,66}

Our study has some limitations: it did not extensively evaluate different types of donor and recipient paired samples, including an evaluation of viral loads in different sample types, did not include serological evaluation of donor and recipients and did not quantify the virus load to infer virus activity.

However, considering the paucity of reports in this context, its main importance is to raise the attention to a virus for which several aspects of basic virus biology still need to be disclosed in the context of allo-HSCT and to contribute to the issue of virus transmission by HSCT grafts.

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