Parvovirus B19: diagnosis, distribution and disease associations

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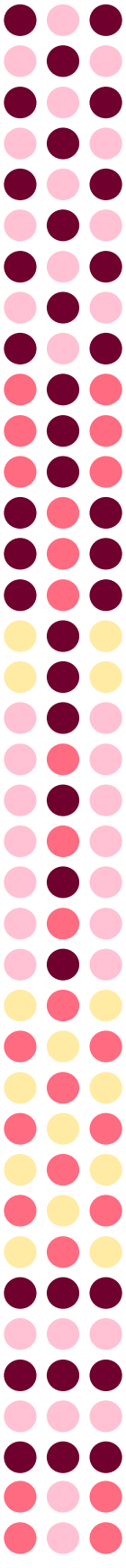

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Transient parvovirus B19 DNAemia after kidney transplantation: a two-sided story

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ABSTRACT

Parvovirus B19 (B19V) DNAemia appears a relatively common finding after kidney transplantation. However, not all DNAemia signifies active infection with replicating virus. This study screened 134 patients post-transplantation for B19V DNAemia and identified two cases in which viral DNA was present after transplantation, with the donor kidney as probable source of the DNA. In both cases intact viral particles could not be detected using an endonuclease method, indicating the presence of non-infectious DNA remnants.

BACKGROUND

Parvovirus B19 (B19V) is a ubiquitous human pathogen with a unique predisposition to infect erythroid progenitor cells (EPC), causing viremia with extremely high viral loads up to 10^{14} IU/ml during acute infection [1]. In immunocompromised patients such as transplant patients, this may lead to severe, and often relapsing, anemia [2-4]. Apart from EPC, B19V DNA appears to enter multiple cell types using mechanisms other than cell-receptor specific entry during acute infection [5, 6]. This results in lifelong detectability of B19V DNA in some tissues, including renal tissue, without apparent disease activity [7-9]. Also in blood, B19V DNA remains detectable for months after infection [10]. Of all transplant patients, attention for B19V infection in kidney transplantation is particularly relevant as kidney transplant patients often suffer from anemia for various reasons, of which B19V infection may be one [11]. Detection of B19V DNA (DNAemia) in blood after kidney transplantation is not uncommon; shortly after transplantation, the incidence is 0.5 to 1.4% but the overall incidence of B19V DNAemia is estimated at 7.6% in the year after kidney transplantation [12, 13]. However, due to the unique characteristic of B19V DNA to persist in tissue lifelong, the clinical relevance of DNAemia after kidney transplantation should be carefully considered. True B19V infection by kidney transplantation appears to be rare, as it supposes (semi)acute infection in the donor combined with a susceptible recipient. Due to an adult seroprevalence of 60-70% many recipients will not be susceptible [13]. Detectable DNA may originate from intact viral particles, causing true infection, or from DNA remnants released from previously infected cells [10, 14-16]. Application of an endonuclease based assay can differentiate between these two scenarios [10]. Endonuclease treatment degrades DNA remnants but not DNA in intact viral particles, which is protected by the viral capsid. This study investigates DNAemia after kidney transplantation and presents two cases where DNAemia after kidney transplantation should not be mistaken for active B19V infection.

METHODS

Patient screening: One hundred ninety-two patients received a living kidney transplantation at the Leiden University Medical Center in the period January 2013 – June 2016. Of these, blood samples were available from 134 patients in the period 30-90 days after transplantation. For each patient, one blood sample in that period was

screened with qualitative B19V PCR for the presence of B19V DNA. If positive, additional donor and longitudinal patient blood samples were tested with quantitative B19V PCR. PCR was also performed on kidney biopsies from the time of transplantation. To determine viral activity, blood samples were also tested using endonuclease pretreatment.

B19V PCR and sequencing: DNA extraction was performed by MagNAPure (Roche Diagnostics) with 200 µl of plasma or serum as input for the PCR, according to the manufacturer's instructions. Subsequently, eluates were tested with an in-house B19V PCR targeting the VP1/VP2 region of all known 3 genotypes as described by Knoester et al [17]. For kidney biopsies, 5 sections of 10 µm were deparaffinized and homogenized with a pre-treatment as used for routine tissue diagnostic procedures in our laboratory. Subsequently, DNA extraction and PCR were performed as described above in duplicate. An in-house PCR for the housekeeping gene beta-globin was performed in duplicate on the kidney biopsies to determine cell count, as described previously, to express DNA load in kidney biopsies as IU per cell [18]. Near whole-genome sequencing on the kidney biopsy and plasma samples of patient 1 was performed as described previously [19].

B19V serology: IgM and IgG serology were performed on blood samples using the Liaison Biotrin Parvovirus IgM/IgG assay (Diasorin) according to the manufacturer's protocol.

Endonuclease (Benzonase) assay: If B19V DNA positive, a blood sample was retested applying endonuclease treatment as described by Molenaar et al [10]. In short; each plasma sample was split in two 80-100 µl aliquots, the exact amount depending on the availability of sufficient material. To one of the aliquots 250 units of Benzonase (Sigma- Aldrich, the Netherlands) were added. Subsequently both aliquots were incubated for one hour at 37°C in a shaking incubator at 120 rpm. After cooling to room temperature, DNA extraction and quantitative dual target NS-VP2 PCR were performed as described previously [20]. Negative, low-positive and high-positive controls were included in the endonuclease assay.

Histochemical staining of kidney biopsies: Staining of paraffin slides (10 micrometer) was performed using monoclonal anti-Parvovirus B19 antibody, targeted against amino-acid 328-344 of the VP2 capsid protein, clone R9276 (Sigma-Aldrich). A positive control stain was included in the analysis.

Ethics: The investigation was done in accordance with the principles outlined in the declaration of Helsinki. The ethical committee of the LUMC approved the

research protocol. As the study was not subject to the Medical Research Involving Human Subjects Act (WMO) and patient data cannot be traced back to the individual patient, informed consent was waived.

RESULTS

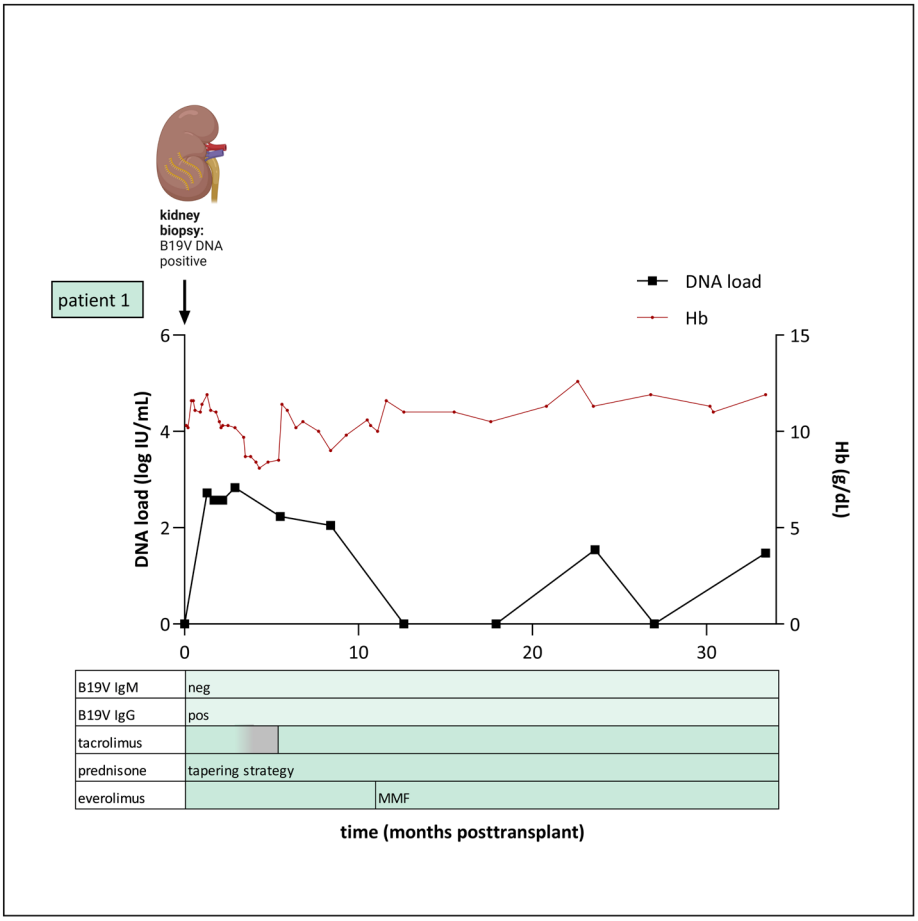
A total of 134 kidney transplant patients were screened for B19V DNAemia 30 to 90 days after kidney transplantation. Clinical characteristics are shown in Table 1.

Table 1. Patient characteristics

Patients screened for B19V DNA	n = 134
Age, years, mean (SD)	51 (14)
Male sex, n (%)	83 (61.9)
B19V IgG-positive at baseline, n (%)	93 (69.4)*
Time to screening sample, days after transplantation, mean (SD)	65 (14)
Positive B19V PCR-result, n	2

* 1 IgG status not available

Two of the 134 patients tested positive for B19V DNA in the screening, after which additional donor kidney and recipient blood samples were tested. The longitudinal B19V DNAemia profiles of both patients are shown in Figure 1, accompanied by clinical information and the course of hemoglobulin (Hb) levels.


kidney biopsy: B19V DNA positive
patient 1
DNA load
Hb
DNA load (log IU/mL)
Hb (g/dL)
0
2
4
6
0
5
10
15
0
10
20
30
B19V IgM
neg
B19V IgG
pos
tacrolimus
prednisone
tapering strategy
everolimus
MMF
time (months posttransplant)

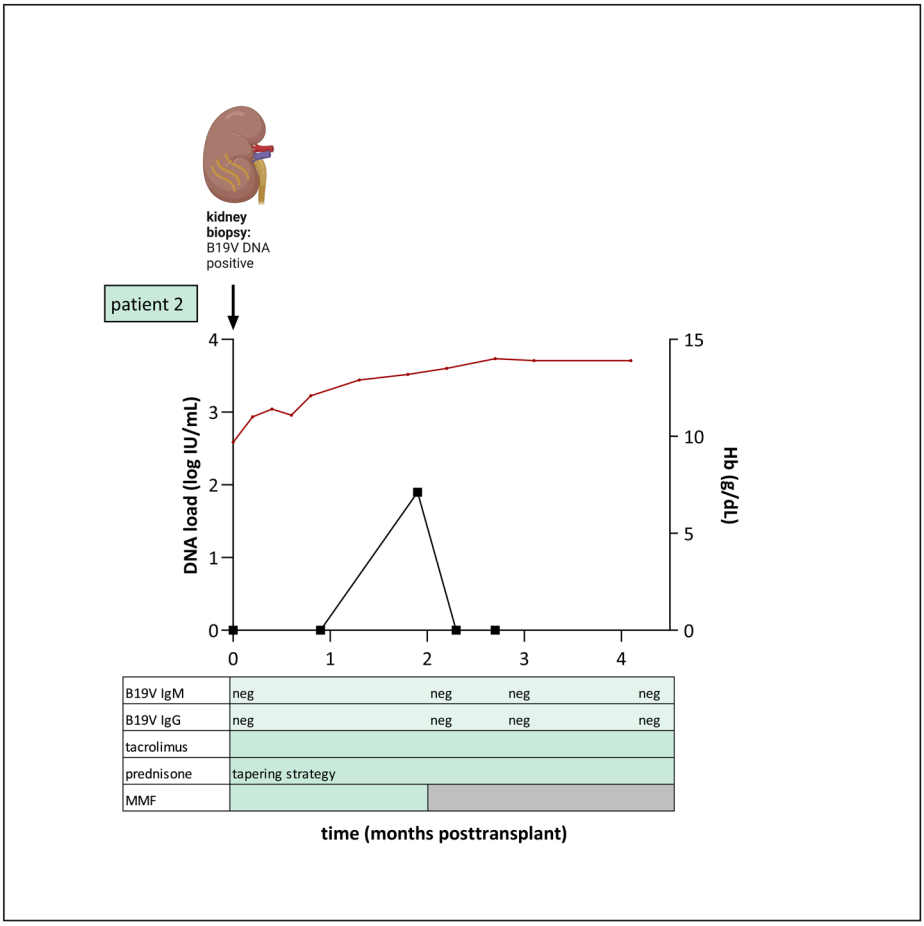


Figure 1: *Longitudinal course of B19V DNAemia in 2 kidney transplant recipients, in relation to hemoglobin levels and information regarding seroconversion and immunosuppressive treatment. MMF, mycophenolate mofetil. Dark green bars represent active treatment; gray bars represent no treatment. (Figure partly created with Biorender.com)*

Patient 1, a 49-year-old male, was B19V IgG-seropositive before transplantation and B19V DNA-negative in blood. He received a living-related kidney transplant. The kidney biopsy at the time of transplantation tested positive for B19V DNA (200 IU/cell). After transplantation, patient 1 tested consistently positive for B19V DNA in blood for more than 10 months, with a gradual decrease in DNA load. Endonuclease testing of all DNA-positive blood samples showed 100% degradability of the detected DNA. Patient 1 showed no obvious symptoms of active B19V infection after transplantation. The patient suffered from anemia 4 months post-transplantation with a nadir hemoglobulin (Hb) level of 8.0 g/dL (see figure), which recovered in the following months. The presence of B19V DNAemia was unknown to clinicians in the years after transplantation and he received no specific B19V treatment such as IVIG. His immunosuppressive regimen was moderated by a temporary stop of tacrolimus due to a concomitant BK-nephropathy (see figure), but this did not lead to clearance of B19V DNA, whereas his Hb-levels did improve.

Patient 2, a 54-year-old male, was B19V IgG-seronegative and B19V DNA-negative before transplantation. The patient received a living-related kidney transplant from a B19V-seropositive donor. The kidney biopsy at the time of transplantation tested weak-positive in 1 of 2 duplicates for B19V DNA, indicating a DNA load around the limit of detection. After transplantation, patient 2 tested positive at one timepoint with a low B19V DNA-load in blood of 79 IU/mL. A false-positive finding was ruled out by confirmation with a different dual-target PCR-method in a different laboratory. The sample was 100% degradable in the endonuclease assay. Clinically, the patient showed no signs of a B19V infection with a stable Hb-level of around 13 g/dL in the months post-transplantation. His immunosuppressive regimen was also moderated due to a concomitant BK-nephropathy, but in the months following the positive sample, the patient remained B19V IgM and IgG-negative.

DISCUSSION

This study describes 2 patients testing positive for B19V DNA in blood after kidney transplantation, which was found to be unrelated to B19V replication, and could be attributed to 'passive' B19V DNA remnants, most likely originating from the donor kidney. This interpretation was supported by the degradation of the B19V DNA in an endonuclease test; by the fact that the B19V load did not decrease with tapering of immunosuppression; by the relatively low DNA loads in blood as opposed to the high viral loads commonly seen in active infection; by the absence of

seroconversion; and by the lack of symptoms consistent with B19V infection. Patient 1 did suffer from anemia, but anemia occurs in 20-51% of kidney transplant patients at various time points after transplantation and the improving course of anemia does not correspond to the persistent detection of B19V DNA [11].

In previous studies, it was already observed that low-level B19V DNAemia after transplantation does not correspond to symptomatic infection. Barzon et al describe a pediatric cohort of 75 transplantations in which 24 of kidney biopsies test positive for B19V DNA. Twenty recipients become DNA-positive in the year after transplantation, of which 10 are B19V-seronegative before transplantation. However, only 4 patients are reported to develop symptoms of B19V infection after transplantation. Data regarding seroconversion are not mentioned [21]. A similar observation was made recently by Rezahosseini et al [22]. In their study, 6 previously B19V IgG-negative SOT recipients were identified with B19V DNAemia after transplantation. Seroconversion was documented in 4 patients but in the other 2 patients, DNAemia was low-level and transient and IgG was negative one year after transplantation. This illustrates that post-transplant B19V DNAemia can be caused by two mechanisms: either true B19V infection with viral replication; or clinically irrelevant presence of inactive B19V DNA fragments (i.e. remnants from earlier infection). Although the above-mentioned studies by Barzon et al and Rezahosseini et al report on patients in which passive transfer of clinically irrelevant DNA seems probable, they do not distinguish between these two manifestations by additional laboratory testing and do not comment on the possible different interpretations of DNAemia. The relative proportions of true infection and clinically irrelevant DNAemia can still be considered unknown. The endonuclease assay used in this study appears to be a useful and reliable tool to distinguish between active B19V infection and presence of inactive B19V DNA remnants, which can be applied in research settings, for example, to determine proportions of true infection vs irrelevant DNAemia, and in clinical practice to guide patient management.

This study also showed that B19V DNAemia can arise from the graft after kidney transplantation. Patient 1 was B19V IgG-seropositive before transplantation, so B19V DNA may have been derived from a previous infection in the recipient. However, the course and properties of DNAemia in patient 1 strongly suggested that DNAemia originated from the donor kidney, which was retrospectively found to be positive with for B19V DNA. In patient 2, it is also likely that the B19V DNA-

positive kidney is the source of the B19V DNA transmission, as the patient had no evidence of a previous B19V infection. Unfortunately, it was not possible to investigate the genetic relationship between donor and recipient B19V DNA, because the viral loads proved too low for reliable sequencing (data not shown).

It is remarkable that B19V DNAemia is reported in many patients with evident kidney damage, e.g. kidneys from deceased donors or concomitant BK-reactivation [23]. Our 2 patients also showed BK-reactivation. It can be hypothesized that B19V DNA present in tissue, will be released into the bloodstream during tissue damage, for example, induced by BK-reactivation. This mechanism has been described for donor-derived cell-free DNA, which is used as a biomarker for tissue injury [24]. This mechanism could also explain clinically irrelevant B19V DNAemia after transplantation. Immunohistochemical staining for B19V capsid antigen in kidney biopsies was negative (data not shown), which is in line with persistence of DNA in kidney tissue but not persistence of intact viral particles.

In summary, this study showed that B19V DNAemia after kidney transplantation is not necessarily associated with active infection. The nature and origin of B19V DNAemia should be carefully considered before it is assumed to have clinical relevance, to avoid erroneous treatment decisions.

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