Thrombotic Microangiopathy Associated with Parvovirus B19 Infection after Renal Transplantation

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Abstract. Human parvovirus B19 is considered an etiologic agent of aplastic anemia in immunosuppressed patients. Microscopic vasculitis, with or without renal involvement, has recently been attributed to this viral infection in immunocompetent patients. This study describes four cases of thrombotic renal graft microangiopathy presumably secondary to B19 infection. Twelve to 50 days after transplantation, four patients presented a renal graft dysfunction with creatinine rising to 360 to 1088 \( \mu \text{mol/L} \) and requiring hemodialysis in three cases. Renal involvement appeared after a systemic illness characterized by fever, fatigue and arthralgia, aplastic anemia (hemoglobin ranged from 5.3 to 7.8 g/dl), and thrombocytopenia. A thrombotic microangiopathy was observed in the renal biopsies, and the parvovirus B19 genome was isolated by PCR from the specimens. All four patients also became IgM-positive for parvovirus. Three of the four renal biopsies taken at the time of transplantation (T0) from the same patients were found positive for the B19 genome. Graft function recovered, with resolution of the aplastic anemia, within 22 to 110 d. Twenty biopsies performed as routine controls or for suspected acute rejection and nine T0 biopsies of patients with no signs of B19 infection were used. The B19 genome was found in two of 20 posttransplant biopsies and in one of nine T0 biopsies. The temporal association between aplastic anemia and the onset of thrombotic graft microangiopathy, isolation of the viral genome in renal specimens, seroconversion, and endothelial tropism of the virus suggests that B19 could be the etiologic agent of thrombotic microangiopathy in these cases. The development of the disease after infection could depend on other detrimental cofactors, which make the patient more susceptible to microthrombi formation in the renal microvasculature. The renal graft could represent the route of B19 transmission.

In immunocompetent children, parvovirus B19 (a single-stranded DNA virus) is the etiologic agent of infectious erythema, but also of a wide spectrum of diseases such as purpura, chronic arthritis, acute hepatitis, congestive heart failure, gastroenteritis, and encephalopathy (1–5).

Furthermore, B19 is a cause of acute or chronic aplastic anemia in solid-organ transplant recipients (6–11), generally in young people experiencing primary infection. The onset of anemia after transplantation varies from 2 to 34 mo. However, the onset of red blood cell aplasia has been reported to occur a few days after renal transplantation, suggesting different paths of infection, e.g., airway transmission (12,13), blood transfusion, viral reactivation (3), and the transplanted kidney (2,3,13). The diagnosis may be missed, especially in immunosuppressed subjects, when only antibody levels are measured. Direct demonstration of the virus genome by PCR or other assays (6,14) has consequently been preferred.

A recent report (15) describes seven patients with homozygous sickle cell disease and glomerulonephritis with proteinuria, following (1 to 7 wk) aplastic crisis induced by human parvovirus. The histologic renal findings were more suggestive of microscopic vasculitis than of immune complex nephritis, resembling the changes of a polyarteritis nodosa. In this context, systemic necrotizing vasculitis, with or without renal localization, has been associated with chronic B19 infection. Some patients presented a new-onset vasculitis as polyarteritis nodosa and Wegener’s granulomatosis, with serologic evidence of acute B19 infection and with remission after intravenous Ig therapy (16). These findings suggest that parvovirus may cause vasculitis.

We describe four cases of renal allograft dysfunction presumably secondary to acute B19 infection with histologic signs of thrombotic microangiopathy (TMA).

Materials and Methods

Patients

Clinical data on the patients enrolled in the study are reported in Table 1. Only patient 2 had already undergone renal transplantation (Tx), which had failed due to chronic rejection. She also had a high pre-Tx panel reactivity (panel-reactive antibody level, 80%) and received a high-dose course of intravenous Ig (1 g/kg) before transplantation. This patient was the only one with IgG positivity (titer 1:10) for...
B19 at the time of transplantation, but serologic tests were done after intravenous Ig treatment. In addition, the recipients’ serologic titers were negative for HIV1, HIV2, hepatitis A virus, and hepatitis C virus (HCV) and positive for IgG anti-cytomegalovirus (CMV), -herpes simplex virus, -herpes zoster virus, and -Epstein–Barr virus. Two patients were found positive for hepatitis B surface antigen. Primary graft function was satisfactory in three of four patients. Patient 2, with clinical and histologic findings of acute tubular necrosis, required hemodialysis postoperatively. Twenty-one days after transplantation, patient 4 was treated with OKT3 for 10 d because of steroid-resistant clinical and histologic rejection.

Clinical Presentation

Extrarenal symptoms of illness appeared a mean 18.7 d (range, 6 to 45 d) after transplantation. The patients presented intermittent fever, fatigue, and arthralgia, associated in some cases with diarrhea and pruritic rash. Blood tests showed anemia and reticulocytopenia with no signs of intravascular hemolysis (normal haptoglobin level and absence of schistocytes on blood smear); the hemoglobin level decreased to 5.3 to 7.8 g/dl within a few days. A marked thrombocytopenia was also evident (platelet count, 27,000 to 74,000/mm³) with or without leukopenia. Bone marrow aspiration, performed in patient 4, showed a paucity of erythrocyte precursors with a maturation arrest at pronormoblast stage and the presence of giant pronormoblasts, suggestive of B19 infection.

Renal involvement either appeared simultaneously with the hematologic abnormalities or became evident a few days later, characterized by oliguria, increased body weight, and moderate hypertension. Serum creatinine rose to 360 to 1088 μmol/L, and three of four patients required hemodialysis treatment. Urine analysis showed micro/macromenuria with proteinuria ranging from 1.5 to 3.4 g/d. No urinary signs of tubular toxicity were detected, and the cyclosporin A (CsA) trough level was always in the nontoxic range (200 to 300 ng/L). Graft ultrasound and Doppler showed a regular morphology and a valid perfusion, with a normal vascular resistance index (<0.6). A renal biopsy was performed for diagnostic purposes, and the renal biopsy routinely performed at the time of transplantation (T0) was retrospectively analyzed.

Renal Biopsies

Posttransplantation renal biopsies were performed under ultrasound guidance, using a needle with an outer diameter of 1.2 mm, which yielded a tissue specimen of 0.9 ± 0.2 mm. The T0 biopsies were performed intraoperatively 30 min after revascularization of the graft. Two samples were obtained from each patient for paraffin embedding and freezing. Paraffin-embedded sections were stained using standard methods (hematoxylin and eosin, periodic acid-Schiff, silver-methenamine periodic acid-Schiff, Masson’s trichrome). Frozen tissue sections were processed with antiserum anti-human IgG, IgA, IgM, C3, C4, and C1q (Dako, Glostrup, Denmark), C4, and C1q (Biogenesis, Newfields, United Kingdom).

Controls

Control specimens consisted of the following. Group A: Fourteen graft biopsies performed, for control purposes, at 6 to 12 mo post-Tx in patients with creatinine levels <120 μmol/L and six biopsies taken in patients with suspected acute rejection. Group B: T0 biopsies of 10 transplanted patients who showed no clinical or laboratory signs of B19 infection or TMA during the first year post-Tx.

B19 DNA Detection

The parvovirus genome was detected in plasma and frozen renal tissue by PCR (17), according to the instructions of a commercially available primer kit (direct Parvo B19; DiaTech Srl, Jesi, Italy). Standard precautions were taken to ensure that the PCR assay re-
plasma samples obtained at the time of the biopsy and both genomes. In patients 3 and 4, PCR was also performed on
 proving negative for the CMV, hepatitis B virus, HCV, and HIV in another. B19 DNA was found in all of the biopsies, which

polymerase (750 mM Tris-HCl, pH 8.8, 200 mM (NH₄)₂SO₄, Taq
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PCR. Three sections of frozen renal tissue 5 µm thick were added to 200 µl of the proteinase K/lysis buffer preparation and incubated overnight at 56°C. After denaturation, 10 µl of the preparation was used in the PCR. A first amplification was performed in a 100-µl preparation containing: 10 µl of the digested sample; 0.5 µl of licensed Taq polymerase (5 U/µl) (Advanced Biotechnologies, Surrey, United Kingdom); 10 µl of the reaction buffer supplied with the Taq polymerase (750 mM Tris-HCl, pH 8.8, 200 mM (NH₄)₂SO₄; 0.1% (vol/vol) Tween, 1.5 mM MgCl₂); 5 µl of solution with 50 pmol of two primers included in the “direct Parvo B19” kit amplifying a sequence of 1112 bp of the parvovirus B19 “orf2” region; and 74.5 µl of sterile water. Two liters of the first amplification was used in a second amplification with a set of primers that amplify a sequence of 104 bp contained in the 1112-bp amplification product. Thirty-five cycles of both first- and second-round amplification were performed at 95°C for 1 min, 55°C for 1.5 min, and 72°C for 1 min in a Perkin-Elmer 2400 automated thermal cycler. Each sample was tested at least twice. Each amplification performed included a negative control (sterile water instead of the sample preparation) and a positive control, which was purified parvovirus B19 DNA. Amplification products were detected by ethidium bromide staining after agarose (1%) or polyacrylamide (10%) gel electrophoresis.

Other Virologic Tests
Serum samples of the patients were analyzed for specific IgG and IgM antibodies to B19 by enzyme immunoassay (Biotrin, Dublin, Ireland) with baculovirus B19 capsid protein as the antigen. Serologic and cultural analysis for other opportunistic infectious agents was also performed. The graft biopsies were also tested for hepatitis B virus, HCV, CMV, and HIV genomes, using commercially available PCR and reverse transcription-PCR kits (DiaTech Srl). CMV antigenemia, using an anti-pp65 monoclonal antibody (Biosoft, Paris, France), and blood and urine cultures for CMV were routinely tested.

Results
The histologic findings were suggestive of a TMA (Figures 1 and 2) with prevalent glomerular lesions in three of four cases (patients 1, 2, and 4). The glomeruli presented a thickening of the basement membrane, endothelial swelling and the presence of fluffy material in the subendothelial space, thrombosis of capillary lumina, congested and dilated capillaries (glomerular “paralysis”) with the presence of intracapillary red cell fragments, and focal and parcellar necrosis and/or sclerosis. In addition, a vascular involvement with a striking narrowing of the lumen and luminal thrombosis of the small arteries was evident in case 3. Moderate interstitial edema and focal tubular atrophy and dilation were observed. No lesions of acute rejection or intimal proliferation of media arteriae were observed. Immunofluorescence showed focal staining of glomerular tufts with fibrinogen, C3 (+) in one case and granular IgM in another. B19 DNA was found in all of the biopsies, which proved negative for the CMV, hepatitis B virus, HCV, and HIV genomes. In patients 3 and 4, PCR was also performed on plasma samples obtained at the time of the biopsy and both

proved positive for B19. All of the patients became serologically positive for anti-B19 IgM. All microbiologic tests for other infectious agents, and particularly for CMV and HCV, were negative for active infection. T0 biopsies of the patients showed no histologic abnormalities, and B19 DNA was isolated by PCR in three of four cases.

Follow-Up
A rapid and progressive resolution of thrombocytopenia and leukopenia was observed, with a simultaneous normalization of the red blood cell count and hemoglobin level 22 to 56 d after the onset of anemia in two of four cases. Patients 2 and 3 remained anemic for 12 and 9 mo post-Tx, respectively.

The patients’ renal graft function recovered and serum creatinine returned to premorbidity levels (range, 108 to 175 µmol/L) 22 to 110 d after the onset of the disease. At 12 mo
post-Tx, renal function was still stable (range, 96 to 201 μmol/L) and urinalysis had normalized. A routine control biopsy of the graft was performed 6 mo after transplantation in three of four cases. The glomerular lesions observed ranged from a moderate focal segmental sclerosis to severe sclero-hyalinosis (patients 2 and 3), associated with mild to moderate tubular atrophy and interstitial fibrosis. Intimal hyperplasia with luminal thrombosis of the arterioles and small arteries was observed. The B19 genome was still present in the renal tissue of patient 3, but had disappeared from all patients’ plasma samples obtained at the sixth month of follow-up.

Controls

**Group A.** The 14 graft biopsies performed during routine follow-up showed a normal renal histology or a mild chronic allograft nephropathy, whereas the other six confirmed the clinical suspicion of acute rejection (moderate or severe). The B19 genome was absent in 18 of 20 biopsies of this group. One positive graft specimen was obtained from a patient who presented 5 mo post-Tx (and 1 mo before the biopsies) with a raised plasma creatinine (20%) with the appearance of moderate proteinuria, followed by B19 seroconversion with IgM positivity at the time of the biopsy. The second patient never developed clinical or serologic evidence of B19 disease post-Tx.

**Group B.** All T0 biopsies analyzed showed no relevant histologic lesions. Nine of 10 biopsies were B19 DNA-negative. The only positive T0 specimen proving positive was obtained from the graft that was persistently positive 6 mo post-Tx in the patient with no evidence of B19 infection.

**Discussion**

This article describes four renal allograft recipients who had a renal TMA associated with an aplastic anemia due to B19 infection. In solid-organ transplant recipients, a parvovirus B19 infection should be suspected in the presence of aplastic anemia following aspecific symptoms of viral infection (fever, fatigue, cutaneous rash), or even if no such symptoms are detected. The infection is confirmed by the seroconversion and/or by isolation of the viral genome from the blood, because B19 IgM antibodies, B19 DNA, or both are rarely found in patients with an outbreak and without an illness suggesting parvovirus infection (8–10,18). In our patients, B19 infection was suspected because of the onset of acute anemia in the presence of a low reticulocyte count, normal erythrocyte indices, and no signs of hemolysis in association with clinical and laboratory signs of viral infection. In one patient (patient 4), a bone marrow biopsy confirmed the erythroid hypoplasiasis suggestive of B19 infection (19). All of our patients became IgM positive for B19, and the viral genome was isolated from the blood of the two patients tested. No other virologic or pharmacologic causes of aplastic anemia were documented.

In our patients, acute aplastic anemia was associated with a severe rise in serum creatinine; renal biopsies showed a TMA with predominant glomerular or vascular involvement. TMA is a complication of renal transplantation reported in 0.5 to 5% of kidney transplant patients, usually during the first 3 mo post-Tx. Direct toxicity of CsA on endothelial cells, CMV infection, and previous TMA have been implicated in the pathogenesis of this condition (20–22). A renal TMA occurring in HCV-positive renal allograft recipients with positive anti-cardiolipin antibody test has been recently reported (23,24). All of our patients received CsA from the time of transplantation; none presented signs of CsA nephrotoxicity or high trough levels of CsA; all patients obtained graft function recovery without CsA withdrawal. The four patients were HCV serologically negative pre- and posttransplantation, and none seroconverted post-transplant: HCV and CMV genome, in both plasma and graft specimens taken pre- and posttransplant, were absent. The hypothesis that B19 could be an etiopathogenic agent rather than an opportunistic infectious agent in TMA after kidney transplantation is supported by the following observations. (1) The serologic status for B19 pretransplantation was known for three of four of our cases and all were negative for IgM; all patients became B19 positive and the viral genome was detected by PCR in diseased grafts and *a posteriori* in three of four of the patient biopsies performed at the time of transplantation. Nine of 10 of the T0 biopsies used as controls (group 2) were negative for B19 DNA, and none of the recipients developed a symptomatic B19 infection or became IgM positive within 1 yr of transplantation. (2) The B19 genome disappeared from all plasma samples and from three of four biopsies performed 6 mo after the onset of the disease. In group A, 18 of 20 biopsies performed either for routine follow-up or because acute rejection was suspected were negative for the B19 genome and for histologic features of TMA. A positive biopsy was found in a patient whose plasma creatinine level was increased by 20% and who had persistent proteinuria followed by IgM seroconversion 5 mo after transplantation and 1 mo before the biopsy. The other patient never showed hematologic or serologic signs of B19 infection.

The hypothesis that human parvovirus causes TMA is supported both by the pathogenic mechanisms of TMA and by the tropism of B19. TMA on native or transplanted kidney is characterized by microthrombi formation in the renal microvasculature, and could be determined by various factors acting on endothelial cells, platelets, red cells, or on the coagulation pathway (25). Parvoviruses could cause endothelial damage by two mechanisms: production of circulating immune complexes, with subsequent deposition in the vascular endothelium, or direct invasion of the endothelium. In our patients, no circulating immunocomplexes or significant immunofluorescent deposits on renal biopsies were detected. A direct infection and injury of endothelial cells has been suggested by the observation that the receptor for B19 on erythrocytes, the P antigen, is also present on endothelial cells (26,27). This hypothesis would be strengthened by the demonstration of viral DNA with high sensitive *in situ* PCR. Furthermore, B19 infection has recently been related to histologic evidence of vasculitis in infected fetuses (28) and to systemic necrotizing vasculitis and panarteritis nodosa in humans (16,29).

B19 could cause TMA on the graft kidney, depending on recipient susceptibility (immunosuppression, serologic protection, etc.) and on the association with other endothelium-
damaging conditions. All of our patients developed TMA within the first month post-Tx. Patient 2 had high panel-reactive antibody levels pretransplant, which could mediate an additional endothelial injury (30) and increase the capability of B19 to trigger microthrombi on damaged endothelium at the renal microvasculature level. Patient 3 showed a delayed primary graft function recovery, and the hypoxic-ischemic insult could have increased the endothelial damage (31). Finally, patient 4, 20 d before the onset of TMA related to B19 infection, had received a cycle of OKT3, which could allow viral replication and also induces tumor necrosis factor-α release, increasing the procoagulant activity of endothelial cells (32). A parvovirus infection after the first months post-Tx or in the absence of any other endothelium-damaging agents could determine a lesser or absent clinical and histologic graft involvement.

The donor kidney could be the route of transmission of the infection in our affected cases, as reported by other investigators (7). Our patient 4 seems to be paradigmatic (Figure 3). Before transplantation, the patient was serologically negative for B19 and received a donor kidney found to be B19 positive by PCR. At post-Tx day 25, he presented histologically confirmed acute rejection that was steroid-resistant and responded to a 10-d course of OKT3 treatment, without significant changes in platelet or erythrocyte count. At day 50, the patient presented signs and symptoms of viral infection and developed an acute aplastic anemia. The bone marrow biopsy was suggestive of erythroid hypoplasia with rare giant pronormoblast and numerous eosinophilic intranuclear inclusions suggestive of viral infection. In addition, a rapid deterioration of graft function was observed and the renal biopsy showed mild features of TMA. B19 DNA was found in plasma, bone marrow, and renal tissue, disappearing from the plasma within 30 d of the onset of the disease with progressive resolution of anemia and improvement in graft function.

Concerning the outcome of the disease, in 22 to 110 d after the onset of TMA, all of our patients recovered to premorbidity serum creatinine levels, which remained stable after 6 and 12 mo of follow-up, although the control biopsies showed various degrees of glomerular and vascular damage. None of the patients was withdrawn from CsA therapy, and only one patient (patient 4) was treated with a 5-d course of intravenous immunoglobulins. Ig therapy has been proposed in immunosuppressed patients with chronic parvovirus infection anemia (11,33,34) and in some patients with polyarteritis nodosa, Wegener’s granulomatosis, and systemic necrotizing vasculitis associated with B19 infection (27,35). In the absence of a controlled study evaluating the efficacy of intravenous Ig for B19 in transplanted patients, and considering the spontaneous recovery observed in our untreated patients, the usefulness of Ig administration should be further evaluated.

In conclusion, B19 parvovirus infection should be taken into consideration as a possible cause of de novo TMA of renal grafts. The temporal association between B19-related hematologic abnormalities and the onset of graft TMA, the isolation of the viral genome from renal specimens followed by seroconversion, and the endothelial tropism of the virus suggests that a cause-and-effect relationship could exist between B19 infection and posttransplant TMA. Screening donors and performing a thorough follow-up of recipients could provide information on the route and risk of transmission of B19 infection with the transplanted kidney, and on the risk of TMA onset after renal transplantation.
References


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