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EXPLORING PARVOVIRUS B19 PATHOGENESIS AND THERAPY AMONG KIDNEY TRANSPLANT RECIPIENTS. CASE REPORT AND A REVIEW OF THE LITERATURE

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Corresponding Author:	Gaetano La Manna IRCCS Azienda Ospedaliero-Universitaria di Bologna Bologna, ITALY
First Author:	Gian Marco Berti, M.D.
Order of Authors:	Gian Marco Berti, M.D.
	Elisabetta Manaresi
	Gisella Vischini
	Michele Provenzano
	Valeria Corradetti
	Maddalena Giannella
	Cecilia Bonazzetti
	Matteo Rinaldi
	Benedetta Fabbrizio
	Giorgio Gallinella
	Gaetano La Manna
	Giorgia Comai
	Matteo Ravaioli
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Dear Professor La Manna,

Thank you for submitting your manuscript to *The Lancet Infectious Diseases*. We will accept this paper but there are a few minor revisions to address from the critical reviewer who has re-reviewed this paper. Please make sure we have author forms, figures submitted according to our guidelines, and all requirements met so we can accept your article.

Your submission has now been assessed by external advisers and discussed by the editorial team. We would like to invite you to **REVISE** your paper in light of the comments below.

When you submit the revised paper, please provide one "clean" copy and one copy where your changes are tracked using Word's tracked change function. In addition, please provide a separate document listing the editorial and referee comments and your replies, point by point. Please also ensure that all elements of the paper and all relevant information has been provided. These documents must be supplied as MS Word files.

It would be helpful if you could let me know whether you can complete the revisions by the suggested deadline.

Yours sincerely,

Dr. Sally Hargreaves
Senior Editor, *The Lancet Infectious Diseases*
Email: sally.hargreaves@lancet.com

Reviewers' comments:

Reviewer #1: The revised manuscript has addressed most of the key issues.
A few additional points to consider:
1. The grammar and language have been improved but there are still some minor errors and awkward phrasings. Another proofread for grammar, typos, and clarity would be beneficial.

Grammar and language have been thoroughly revised, we hope that now it can be acceptable

2. The inclusion of pre-transplant B19V screening results for the donor and recipient adds an interesting angle about potential virus reactivation.

We gladly took into consideration the reviewer's previous suggestion and evaluated the archived recipient and donor pre-transplant peripheral blood stored for immunological cross-match analysis. The reviewer's suggestion and our considerations have been included in the manuscript (p.5 li 19-22).

3. Table 2 provides a good summary of reported cases and outcomes. However, directly comparing the current case findings to those of other reports could further highlight its uniqueness maybe in table or text.

The characteristics of the current case are summarized as a direct mean of comparison to what already reported in the referenced papers (cf. p. 6, li.14-19 to the preceding paragraph).

4. The conclusion emphasizes the importance of the case in elucidating B19V pathogenesis and tissue damage. It calls for more research to improve diagnosis, treatment and achieve complete viral eradication. Mentioning the specific knowledge gaps and priorities for future studies could drive the key points home further.

Thank you for the comment, we agree. Research on B19V infection faces several knowledge gaps, especially in "real life clinical practice", which hampers scientific research to progress in comprehension and developing target treatments to achieve finally a complete virus eradication. With this Grand Rounds we want to bring out the urgency of studies to significantly improve our understanding and management of the intricate landscape of B19V infection to stay abreast and exploit latest therapeutic discoveries. We added a few comments in the conclusion section (p.11, li.7-10).

5. Some of the figures and tables have been updated with additional time points and lab values, which helps show the clinical course and response to treatment. Ensuring all figures and tables are properly formatted and cited in the text would polish the presentation.

Figure 3 has been corrected, by adjusting the x-axis scale. All figures and table have checked.

Manuscript Click here to view linked References

EXPLORING PARVOVIRUS B19 PATHOGENESIS AND THERAPY AMONG KIDNEY TRANSPLANT RECIPIENTS. CASE REPORT AND A REVIEW OF THE LITERATURE

Berti Gian Marco[1] †, Manaresi Elisabetta[5] †, Vischini Gisella[2], Provenzano Michele[2], Corradetti Valeria[2], Giannella Maddalena [3,1], Bonazzetti Cecilia [3,1], Rinaldi Matteo [3,1], Fabbrizio Benedetta [4], Matteo Ravaioli [7,1], Gallinella Giorgio [5,6], La Manna Gaetano [1,2], Comai Giorgia [1,2]

[1]Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum-University of Bologna, Bologna, Italy (GM Berti, gianmarco.berti2@studio.unibo.it; G Vischini, gisella.vischini@aosp.bo.it; M Provenzano, michele.provenzano2@unibo.it¸V Corradetti, valeria.corradetti@aosp.bo.it)

[2]Nephrology, Dialysis and Kidney Transplant Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna (G Comai, giorgia.comai@aosp.bo.it; G La Manna, gaetano.lamanna@unibo.it)

[3]Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria Policlinico di Sant'Orsola, Bologna (M Giannella, maddalena.giannella@unibo.it; C Bonazzetti, cecilia.bonazzetti@aosp.bo.it; M Rinaldi, matteo.rinaldi23@unibo.it)

[4]Pathology Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna (B Fabbrizio, benedetta.fabbrizio@aosp.bo.it)

[5]Department of Pharmacy and Biotechnology, University of Bologna (E Manaresi, elisabetta.manaresi@unibo.it; G Gallinella, giorgio.gallinella@unibo.it)

[6]Microbiology Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna

[7]Hepato-biliary and Transplant Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna (M Ravaioli, matteo.ravaioli6@unibo.it)

† These authors contributed equally to this work.

Correspondence to: Gaetano La Manna, Nephrology, Dialysis and Kidney Transplant Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna, Via Giuseppe Massarenti, 13, Bologna, 40138, Italy, Tel +39-051-2142584, Fax +39-051-2142509, E-mail gaetano.lamanna@unibo.it

Abstract

We describe a case of a 34-year-old male recipient of an ABO-incompatible living donor kidney transplant (LDKT) who experienced repeated hospitalizations for anaemia. Acute kidney injury on a severe and recurrent anaemia related to Parvovirus B19 (B19V) infection was diagnosed through viral and histopathological analysis. In view of the impaired patient's own immune response due to the immunosuppressive regimen, clinical stabilization was achieved by repeated IVIg administration as a maintenance therapy in a prolonged course, although in the absence of viral clearance. The review of literature highlighted a variety of pathological renal lesions associated with B19V infection, although epidemiological data on B19V infection in kidney transplant, standardized diagnostic and therapeutic protocols, and the prospective for specific antiviral therapy are still scanty. Extended awareness of clinical relevance of B19V infection in kidney transplant recipients should direct future efforts toward a better consideration and comprehension of viral-induced pathogenesis, aimed at effective diagnostic and therapeutic appropriateness.

Keywords: parvovirus B19; kidney transplant; anaemia; tubulointerstitial nephritis; glomerular thrombotic microangiopathy; in situ hybridization; immunochemistry, intravenous immunoglobulin.

Introduction

Parvovirus B19 (B19V) is a human pathogenic virus, belonging to the *Parvoviridae* family, *Erythroparvovirus* genus, and is characterized by a pronounced tropism for erythroid progenitor cells in bone marrow. Its single-stranded DNA genome encodes for the structural proteins VP1 and VP2, and for few non-structural proteins, including protein NS1, which play a critical role both in viral life cycle and in B19V induced disease [1-3]. B19V infection is common and worldwide spread. According to a survey in European countries, including Italy, infection is more frequently acquired by age of 25 years, with the highest risk in children aged 7-9 years, but it can occur any time in lifetime, so that at elder ages a high proportion of adult population shows serologic evidence of past infection[4]. The most common route of transmission is through aerosol droplets; other routes include maternal-foetal transmission, with possible severe consequences on the foetus [5], or more rarely through transfusions, administration of blood products [6], or bone marrow or solid organ transplantation [7]. The disease spectrum associated with B19V infection is wide and heterogeneous, depending on individual characteristics (age, hereditary anaemic status) and host's immunologic status (including the use of immunosuppressive drugs). The viral tropism and cytotoxic effect on erythroid progenitor cells in bone marrow accounts for anaemia [8], whose severity and duration depends on underlying clinical conditions and the host ability to mount an effective neutralizing immune response. On the other hand, the pathogenesis and clinical manifestations in other tissues, such as the typical fifth disease in children and arthropathies mainly observed in adults, or atypical and heterogenous manifestations in disparate tissues, depend on activation of inflammatory mechanisms and immune response [9]. Finally, following infection, the virus can persist lifelong in several tissues, posing the issue of a possible reactivation related to immunosuppression [10].

Case report: a severe case of recurrent anaemia in kidney transplant recipient

A 34-year-old kidney transplanted man was admitted to our ward for acute kidney injury and severe anaemia requiring multiple blood transfusions. His past medical history was remarkable for end-stage IgA nephropathy and ABO-incompatible living donor kidney transplant (LDKT) two months before the hospitalization. Desensitization for ABO-incompatible kidney transplantation was performed, with Rituximab (RTX) at 375 mg/m^2, started at -30 days before transplantation (dbt), tacrolimus (Tac) 0.075 mg/kg and mycophenolic acid

(MMF) 750 mg BD at -14 dbt, Plasma Exchange (PEX) at -6 dbt and Intravenous Immunoglobulins (IVIg) 0·5 g/kg at -1 dbt, followed by induction with Basiliximab, IVIg, oral steroids and MMF, and later maintenance therapy with oral steroids, Tac and MMF. At the time of discharge his renal function was slightly upon normal limits. On admission to the Nephrology Department ultrasonographic medical assessment resulted negative for vascular or surgical complications, laboratory tests manifest severe anaemic (Hb 6·7 g/dL) non-haemolytic condition associated with thrombocytopenia (93 x 10^9/L) and acute kidney injury (**Table1**). Donor-specific antibody (DSA) screening was negative. The patient was initially treated with several blood transfusions. Microbiological tests were carried out and high amount of Parvovirus genome copies (>25,000,000 copies/mL) were detected by qPCR assay (Parvovirus B19 ELITe MGB Assay, EliTech, Italy). Blood cultures and other microbiological tests were negative, including Cytomegalovirus, Epstein-Barr virus and BK Polyomavirus. Considering available literature, immunosuppressive therapy was reduced and IVIg were started resulting in clinical recovery and amelioration of infectious status. Mycophenolate mofetil (MMF) was discontinued as far as B19V active infection was detected. Afterwards, low target levels of serum Tac were settled (range 2-4 ng/mL). As a second step treatment, ten IVIg infusions (0·4 g/kg each) were combined with significantly improvement in B19V infection, clearance of viral load (8,251 copies/mL) and recovery of renal function (serum creatinine 2·08 mg/dL). After resolution of anaemia and thrombocytopenia, renal biopsy was carried out, showing recurrence of primary glomerulopathy (IgAN) and acute tubulointerstitial nephritis (**Figure 1**). A successive hospital admission was recorded few months later because of a comparable episode of anaemia and acute kidney injury. The infection presented again and as much aggressive as the first episode (> 25,000,000 copies/mL) (**Table 1**). Tac was switched to m-TOR inhibitor (everolimus, range 3-8 ng/mL) and nine IVIg infusions were performed again until recovery of renal function and decrease of viral load (180,161 copies/mL at the time of discharge). During this treatment, upper limbs/trunk rash occurred. Given the immunosuppressed status and the consequent inability to mount an efficient immune response, rash was interpreted as a confounding manifestation of immune complexes aggregation (IVIg + B19V antigen) and deposition in skin capillaries (**Figure 2**). The rash disappeared when IVIg treatment was discontinued. Another renal biopsy was performed. The major pathologic findings were acute tubulointerstitial nephritis and features consisting in glomerular thrombotic microangiopathy (TMA) possibly related to viral infection. Given the absence of capillary/glomerular inflammation and negative DSA screening, investigation for antibody

mediated rejection (ABMR) was carried out and C4d peritubular deposition was interpreted as an accommodation phenomenon in ABO-incompatible kidney transplant setting. Therefore, in situ hybridization for B19V was performed on tissue samples, localizing viral genomes in endothelial and tubular cells, a finding highly suggestive for a viral contribution in the renal lesions (**Figure 1**). Furthermore, structural capsid protein (VP) and non-structural protein (NS) immunohistochemistry was performed. All the kidney samples resulted negative for NS and positive for VP immunochemistry in tubular and vascular compartments (**Figure 1**). Desensitization pre-transplant strategy in an ABO-incompatible setting with RTX and the maintenance therapy with Tac contributed to B cell and T cell deficient response to viral infection (PANB CD19+ 0·2%, 1/mmc). Since the longevity of viral clearance is uncertain and given the relapses during the clinical follow-up, we proposed weekly and later monthly administration of IVIg for consolidation and maintenance therapy with a constantly controlled viral load (**Figure 3**). Antibody titres against B19V were measured repeatedly throughout the infection (LIAISON® Biotrin Parvovirus B19 IgG and IgM, DiaSorin, Italy); the detection of IgG correlated with exogenous administration of IVIg, while the constant absence of IgM indicated the lack of a natural immune response capable of clearing the viral infection in the long term. At the last outpatient visit, 19 months after second hospitalization, renal function was stable (serum creatinine 1·86 mg/dL), and persistent low B19V copies were still detectable (8,795 copies/mL). Finally, a retrospective evaluation of archived recipient pre-transplant (-4 days) peripheral blood indicated presence of B19V DNA at low copy number (820 genome copies/mL), while both donor and recipient were B19V DNA negative the year before (-13 months), when samples had been stored for immunological cross-matching analysis. Such low B19V positivity in the pre-transplant period in contrast to past negativity may hint at reactivation of a persistent or latent viral infection due to pre-transplant desensitization therapy, followed by highly active viral replication in the post-transplant period when on immunosuppressive maintenance therapy only.

Discussion, data analysis and literature review

Renal involvement in Human Parvovirus B19

Parvovirus B19 was detected for the first time in human sera in 1975 [11] and the first case of B19V infection in a kidney transplant recipient was reported in 1986 [12]. Since then, several reports have described B19V infection

in kidney transplant recipients, although the extent of this condition is likely underreported and clinical, histological and virologic descriptions are rather heterogenous (**Table 2**).

The most common clinical presentation of B19V infection in immunocompromised patients, including kidney transplant recipients, is anaemia with a low reticulocyte count, due to the direct inhibitory effect on erythropoiesis. In case of aggressive immunosuppressive regimens, viral clearance is delayed, and the acute-phase anaemia is followed by a chronic course, necessitating therapeutic support to avoid persistent red blood cell aplasia (PRCA), until a patient is able to develop an own mature immune response [7]. In addition to anaemia, numerous pathological patterns of renal lesions are associated with B19V infection [13], including acute post-infectious glomerulonephritis (AGN), proliferative glomerulonephritis, collapsing glomerulopathy, focal segmental glomerulosclerosis (FSGS), thrombotic microangiopathy (TMA), and tubulointerstitial nephritis [14]. In kidney transplant recipients, B19V DNA is frequently found in renal tissues and its presence associated with greater allograft injury [15], although acute rejection is rarely seen in transplant population and the long-term outcome does not seem impaired due to B19V infection [16,17].

Compared to reported cases and known pathogenetic mechanisms, our patient strikingly showed a combination of clinical and pathological findings that, taken together, contributed to his atypical and severe clinical presentation. Among these, profound depression of haematopoietic and chronic anaemia, complete anergy of an own adaptive immune response and reliance of a continuous exogenous IVIg administration, complex pathological findings in kidney both in the vascular, glomerular, and tubular components, superimposed on a background of IgA nephropathy and in the context of a complex post-transplant therapeutic management.

Screening and diagnosis of Parvovirus infection in kidney transplant population

Inadequate or delayed antibody response can feasibly occur in an acquired, iatrogenic immunosuppressed setting. Therefore, standard serologic tests for B19V specific antibodies are not diagnostic for discriminating the infection status, that needs to be investigated through direct molecular detection of viral components, mainly viral nucleic acids and/or proteins [18,19]. Viral nucleic acid detection and quantification by qPCR, or in situ tissue hybridization, may provide a rapid and sensitive method of diagnosis in transplant recipients and are recommended for the diagnosis, for differentiating low level persistence viral DNA from acute viral

infection and for localization of infected cells within tissues [20]. After excluding the main common causes of anaemia, bone marrow biopsy/aspiration can be useful to identify the underlying origin of pure red cell aplasia (PRCA). Clinical manifestations are not pathognomonic or can be even cofounding in transplanted patients, such as in the case of anaemia, usually the unique evidence of infection, which is quite common and multifactorial especially in the post-transplant period [21-24]. Few monocentric studies tried to define risk factors of parvovirus infections in kidney transplant population. Deceased donor kidney transplantation, tacrolimus therapy, high HLA mismatches (≥ four mismatches), pancytopenia and haemoglobin levels result positively correlated with high probability of infection in the univariate analysis [16].

Following meta-analysis, the overall reported incidence of positive B19V DNA among kidney transplant patients is 10.3%; in the presence of anaemia, the incidence rate of positive B19V DNA is 27.4% [25]. Prolonged anaemia after kidney transplantation is a relatively common complication. The pathogenesis of anaemia is usually multifactorial including surgical, immunological, infectious and iatrogenic aetiologies. In recent years, viral infections in kidney transplant recipients become increasingly relevant due to new immunosuppressive treatments. It is now generally known that ABO-incompatible kidney transplantation is associated with an increased risk of infectious complications, partly related to the effects of extracorporeal treatments and the strong immunosuppression [26,27]. Renal injury caused by B19V infection could be associated with different histological presentations [13,28]. Moreover, considering nonspecific pathological features on kidney biopsies in transplanted patients such as interstitial nephritis and TMA, the differential diagnosis between B19V and allograft rejection was challenging. In our case, C4d deposition in peritubular capillaries could not have been taken into consideration as a diagnostic element because of graft accommodation in ABO-incompatible kidney transplant setting. In situ DNA hybridization allows identification of infected cells through direct detection of viral DNA in tissue samples. Despite not widely available or standardized in clinical practice, this method could overcome serology limitation in kidney transplant population and clearly demonstrates virus presence with a strong predictive value for disease or active infection. Vascular endothelial and tubular epithelial viral localizations were common features in the two patient's biopsies (**Figure 1; Supplementary Figure 1**). These results were further validated by immunochemistry studies of structural capsid protein (VP) and non-structural protein (NS). In particular, all immunochemistry investigation of NS resulted negative; on the contrary, VP examinations were positive in all kidney samples maintaining tubular and vascular pattern as mentioned above.

These considerations lead to the hypothesis that tubular damage may be linked to reabsorption of viral particles rather than direct productive infection of renal tissue. Detection of viral particles in the renal parenchyma is a pivotal result in elucidating B19V pathogenesis, but more studies are needed to understand the mechanisms of B19V infection and the related tissue damage in a non-permissive cell environment such as kidney tissue. Hypotheses about the route of infection in kidney transplant population include an exogenous primary infection in post-transplantation period, reactivation of a latent infection or organ-related transmission. In our patient, detection of low-level viremia in a pre-transplant sample may hind at viral reactivation in the recipient, possibly linked to the immunosuppressive effects of the desensitization therapy with RTX, although conclusive evidence cannot be achieved. Thus far, most reports lack of laboratory data on B19V, including assessment of pre- and post-transplantation serologic and virologic status of the recipient and donor, in order to support towards one hypothesis or the other, an observation prompting for implementation of B19V status analysis in this group of patients.

Prospective for antiviral therapy

The development of preventive or therapeutic measures to contrast B19V infection and its associated diseases suffers from its general appraisal of a mild, self-limited disease and from the inherent difficulties in defining a key target for antivirals. A vaccine to prevent the infection is not available, and no specific antiviral therapy has been approved for B19V, although this would meet clinical needs in case of severe clinical manifestations [18]. Development of targeted antiviral strategies against B19V is now an area of current research [29,30]. A vaccine strategy based on Viral-Like Particles technology (VLP) has been pursued since early after characterization of B19V as a human pathogenic virus, and initial attempts showed both the immunogenicity of VLPs and their capacity to elicit production of neutralizing antibodies [31]. However, first-generation vaccines yielded also relevant side-effects that led to termination of clinical trials [32]. Second generation vaccines, still based on VLP technology but modified to abrogate their associated viral phospholipase activity, also show good immunogenicity [33], but have not been yet evaluated in clinical trials for their efficacy and safety. In the lack of a vaccine, passive immunization by using IVIg has been always considered the only practically available therapeutic option and its use is widely reported in the literature. Given the high seroprevalence of B19V in the general population, IVIG preparations contain high level of anti-B19 IgG [34] and can exert a subsidiary or

replacement neutralizing activity against the virus [35]. Literature is vast but mostly anecdotic, and controlled clinical trials to establish the actual best IVIg administration regimen are not reported. The most structured study reported a good short-term efficacy in controlling B19V viremia and related consequences on hemopoiesis, while the long-term efficacy is more variable and highly depend on the host immune status [36]. In particular, in cases of immunosuppression, repeated IVIg administration might be required, even though viral clearance is generally linked to the capacity of the host to eventually mount an own immune response. In perspective, availability of human monoclonal antibodies might add to the passive immunization options [37]. Recent developments indicated promising strategies in the development of antiviral drugs. Hydroxyurea is a virostatic compound also used for the treatment of sickle cell disease, showing inhibitory activity against B19V and might potentially serve a dual purpose [38]. Broad-range antivirals such as the nucleotide analogues Cidofovir and its derivative Brincidofovir have shown potent antiviral efficacy in in vitro models [39-41], the former also reporting a successful use in a clinical setting [42]. A serendipitous clinical use of Foscarnet, another broad range antiviral, has been reported with good results in a few cases, but its activity has not been demonstrated in controlled in vitro model systems [43]. New directions of antiviral therapy move towards a more selective inhibition of viral and/or cellular systems involved in viral replication. Direct acting antiviral agents can be targeted at the viral NS1 protein, by inhibiting its nuclear translocation [44] or its endonuclease activity, which is essential for promoting replication of the viral genome [45]; a high throughput screening effectively found a few purine derivatives able to inhibit B19V replication in vitro by this mechanism, but their translation to clinical use is still awaiting [46]. Related to kidney transplant population, it should be reminded that there are no established guidelines for managing neither primary nor recurrent disease [18,19], so therapy is mostly empirical (**Table 2**) [16,47-61]. Non-pharmacological measures include supportive therapy with blood transfusions and reduction in immunosuppressive treatment in order to stabilize the acute phase and support the host's production of antibody [13,62-64]. Potent antirejection regimens that involve MMF and Tac should be reconsidered [48,52]. Indeed, Tac seems to be particularly connected with B19V induced anaemia/red cell aplasia [48,50]. In the light of these observations, it could be beneficial switching to another antirejection drug (cyclosporine or m-Tor inhibitor) [48,50,62], however, this too lacks consistent evidence-based studies. Since B19V-specific antibody response is absent or minimal in kidney transplant population, passive immune reconstitution with IVIg can replace the production of neutralizing antibody, thus facilitating viral clearance, resumption of reticulocytotic

and rising of haematocrit. Most common adverse reaction including fever, chills, headache, myalgia, nausea and acute renal failure increase significantly along with rising IVIg dose [19,36].

IVIG administration in our patient was carried out according to guidelines [18] and adjusted over time in order to maintain effective control of viremia and renal function. Initially, we followed the most adopted regimen consisting of repeated high-dose infusions (reported as 400 mg/Kg/day for 5-10 consecutive days [13,16,28,43,50,52,53,62,65]). Then, we opted for a maintenance low-dose regimen able to achieve a low-level viraemic state in a compensated clinical setting. Given its reliance on a single case report, it is essential to acknowledge the limitations of our therapeutic proposal as it cannot be generalized to a broader patient population in the absence of controlled trials. The initial critical presentation, and concerns related to possible toxicity effects on kidney, discouraged the use of antiviral compounds, such as cidofovir or brincidofovir, as an additional therapeutic option. However, given the constant necessity of IVIg therapy and the currently stabilized renal functionality, a pharmacological approach using cidofovir or brincidofovir may be considered to obtain complete viral clearance.

Conclusion

This case report analysed B19V infection-associated kidney disease by clinical and histological findings combined with in situ hybridization and immunohistochemistry techniques in a kidney transplant recipient. In addition to chronic anaemia as a result of persistent infection, results are suggestive of viral tubular and vascular localization in the renal tissue. Tubular viral reabsorption hypothesis appears to be stronger, rather than a direct pathogenetic mechanism driven by productive infection on renal tissue, likely a secondary localization with respect to replication in bone marrow. Further investigations are required to better clarify the pathogenetic mechanisms beyond renal involvement in the course of B19V infection, taking into consideration the contributions of both virus and immune system.

The present case report allowed us to focus attention on B19V infection in kidney transplant recipients and showed the insufficiency of epidemiological studies in existing literature. This could be a valuable starting point for further studies aimed at broadening the knowledge of B19V infection mechanisms and the related tissue damage. B19V is not routinely included in the screening and monitoring of viral infections in kidney

transplant recipients, but its implementation should now be reconsidered also in view of standardisation of diagnostic tools and treatment regimens. In the absence of a standardized diagnostic protocol, clinical relevance of B19V infection may be underestimated. In turn, the lack of a shared standardized therapeutic protocol, might result in various degrees of success or alternatively insufficient long-term responses. Pre-transplant virologic routine screening might help to categorize subgroup of transplanted patients at high risk; post-transplant monitoring can anticipate clinically relevant viral replication and direct therapeutic approaches. Finally, continuing research on antivirals may offer in the future relevant therapeutic options. This study might provide an important basis in the perspective of understanding/clarifying B19V pathogenesis integrating several clinical, histological, laboratory and immunochemistry data, with the ultimate goal of developing more specific treatments to achieve a complete virus eradication.

Search strategy and selection criteria

References were identified through MEDLINE (PubMed) on Dec 21, 2023. For B19V in kidney transplant recipients, we searched with Medical Subject Heading terms including the keywords “Parvovirus B19” AND “Kidney transplant”. Retrieved articles were further scrutinized and selected for completeness of information on clinical, histological and virologic data, and for inclusion of data on follow-up and outcome.

Contributors

GMB, GC, GV, GLM analysed the clinical case. GMB, GC, GV, EM acquired and analysed the data. GG EM contribute processing data and writing manuscript. All coauthors interpreted the results and reviewed the final version of the manuscript.

Patient’s consent for the publication of manuscript materials has been obtained.

Declaration of interests

The author declares no conflict of interest

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Figure legends.

Figure 1. Pathological findings. First biopsy (left). Light microscopy. **a** mesangial proliferation (Periodic-acid Schiff staining, original magnification x200); **b** immunofluorescent staining for IgA, predominant mesangial IgA deposition; **c** acute interstitial nephritis (Periodic-acid Schiff staining, low power x40); **d** accommodation in ABO incompatible kidney transplant setting: positive peritubular capillary staining of C4d; **e** B19V VP capsid protein positivity in tubular cells (immunohistochemical staining of B19V VP capsid antigen, original magnification x100 [66]); **f** B19V in situ hybridization (Digoxigenin-labeled DNA probe [67]): tubular-positive and glomerular-negative staining. Second biopsy (right). Light microscopy. **g** acute interstitial nephritis (hematoxylin and eosin staining, low power x40); **h** glomerular mesangiolysis (Jones' methenamine silver stain, original magnification x200); **i** immunofluorescent staining for IgA, negative; **l** B19V VP capsid protein positivity in tubular cells; **m** B19VB19V in situ hybridization positive in tubular cells and negative in glomerulus.

Figure 2. Skin lesions. Skin rash after administration of Intravenous immunoglobulin (IVIG) due to aggregation in immunocomplexes in an iatrogenic immunosuppressed setting.

Figure 3. Timeline of clinical and therapeutic course.
Haemoglobin serum concentrations (blue line) and serum creatinine trend (grey line) in relation to Log(10)B19V serum genome copy number (orange line) and the IVIG treatment (yellow arrows). Triangles indicate kidney biopsies; star indicates switch of the immunosuppressive therapies from Tac to mTORi. B19V, Parvovirus B19; IVIG, intravenous immunoglobulin; Tac, tacrolimus; mTORi, mammalian target of rapamicyn inhibitor

Supplementary Figure 1.
Digital core biopsy **image and high-resolution zoom into focal areas**. **a**, **b** Parvovirus B19 in situ hybridization with tubular-positivity; **c** Parvovirus B19 in situ hybridization negative in glomerulus

Table 1. Laboratory data of case report

	First hospital admission	Second hospital admission
Days after transplantation	51	171
Haemoglobin, g/dL [13.5-17.2 g/dL]	6·7	6·6
Reticulocytes, % [0.5-2.0]	0·3	0·1
Total white blood cells, 10^9/L [3.6-10.5]	9·37	7·23
Platelets, 10^9/L [160-370]	93	133
Serum creatinine, mg/dL [0.5-1.2]	5·8 (basal 1·7 mg/dL)	4·69 (basal 1·7 mg/dL)
B19V, genome copy number/mL; Log(10)copies/mL	>25000000; >7·39	>25000000; >7·39
LDH, U/L [< 248U/L]	580	325
Haptoglobin, mg/dL [30-200 mg/dL]	53	67
DSA	negative	negative

LDH, lactic dehydrogenase; DSA, donor-specific antibody; [normal range]

Table 2. Parvovirus B19 infection in kidney transplant recipients

Reference	Sample size	PCR	B.M.	K.B.	Treatment*	Outcome**
47	1	+	ND	ND	S IVIG	+
48	1	+	+	+[d]	S IVIG	+
49	4	+(2/4)	+	+[a,c]	NT (3/4) IVIG (1/4)	NT (3/3) + IVIG (1/1) +
50	1	+	+	ND	IVIG (1/1) S (1/1)	+
51	1	+	+	ND	IVIG	+
52	6	+ (4/6)	+(3/6)	ND	R (3/6) IVIG (5/6)	+
53	3	+	+(2/3)	ND	IVIG (3/3) RI (1/3)	+
54	6	+	+(4/6)	+[a]	IVIG	+
55	1	+	+	ND	IVIG	+
56	2	+	ND	ND	IVIG (1/2) RI (1/2)	+
57	1	+	+	ND	IVIG	+
16	39	+	+(13/39)	ND	NT (6/39) RI (4/6) IVIG (29/39)	IVIG group +
58	1	+	+	ND	RI IVIG	+
59	9	+	ND	ND	RI (9/9) S (9/9) IVIG (9/9)	+
60	1	+	+	ND	S IVIG	+
43	50	+	ND	ND	RI (50/50) S (44/50) IVIG (45/50)[b] Foscarnet (5/50)	IVIG group 39/45 + Foscarnet group 10/11 +
61	3	+	ND	ND	RI (1/3) IVIG (2/3)	+

*Treatment undertaken beyond blood transfusions; **resolution of anaemia; PCR, polymerase chain reaction (serum); B.M., bone marrow biopsy/aspiration; K.B., kidney biopsy; NT, no treatment undertaken; RI, reduction of immunosuppression; IVIG, intravenous immunoglobulin; S, switch tacrolimus to cyclosporin/mTOR inhibitor; a, thrombotic microangiopathy evidence in renal tissue sample; b, IVIG (6/45) were converted to foscarnet therapy; c, Parvovirus B19 DNA in situ Hybridization on kidney biopsy; d. interstitial nephritis evidence in renal tissue sample.

Figure 1

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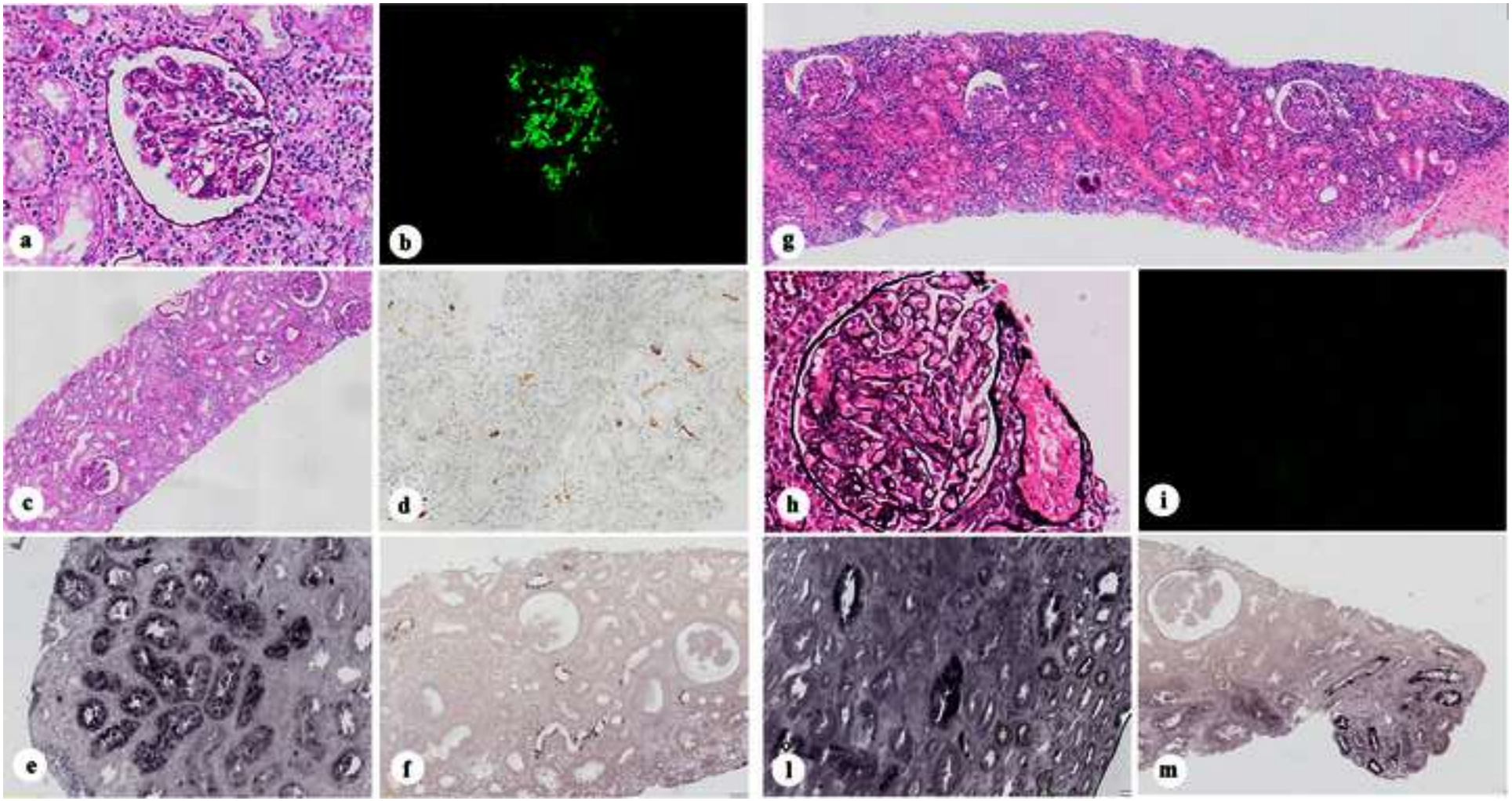

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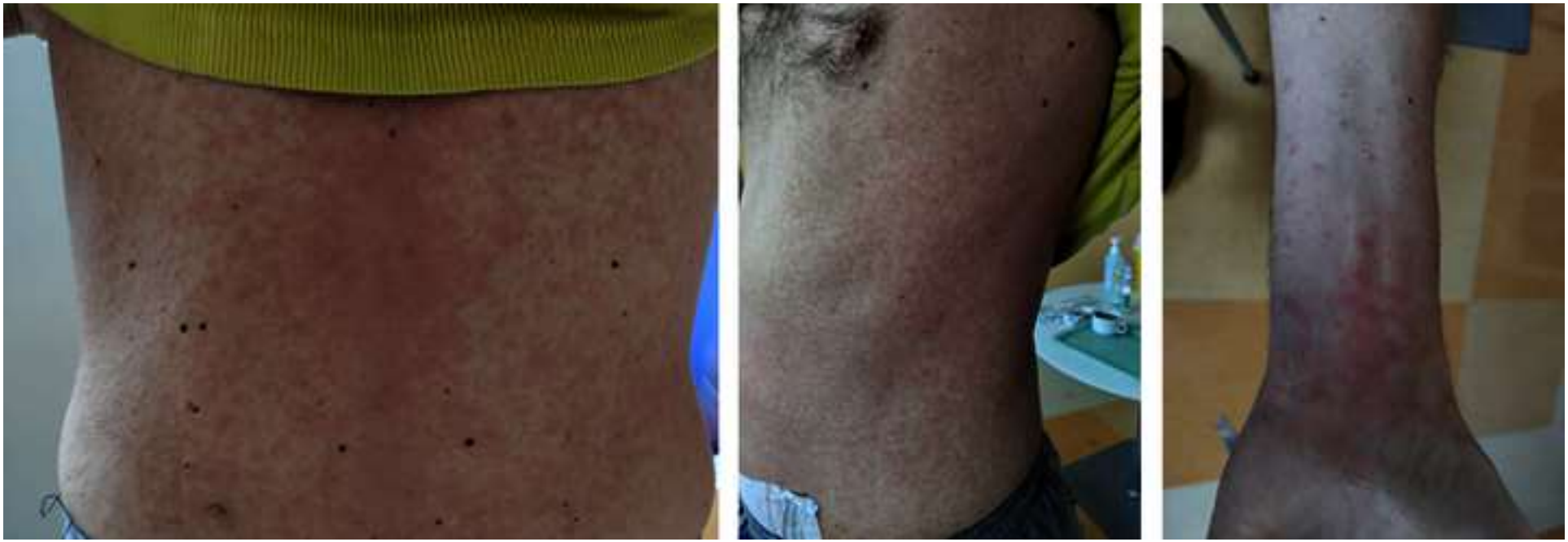

Figure 3

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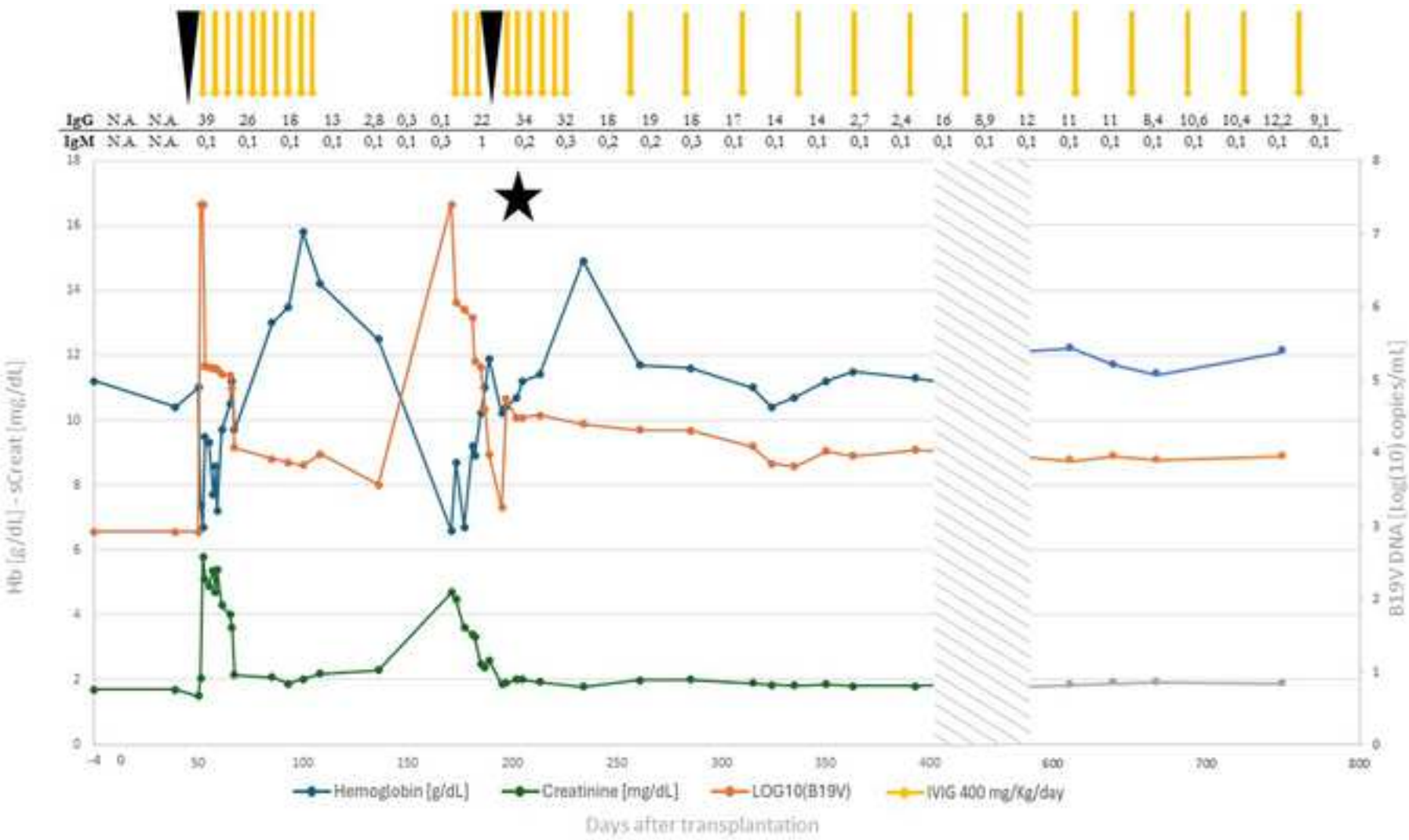

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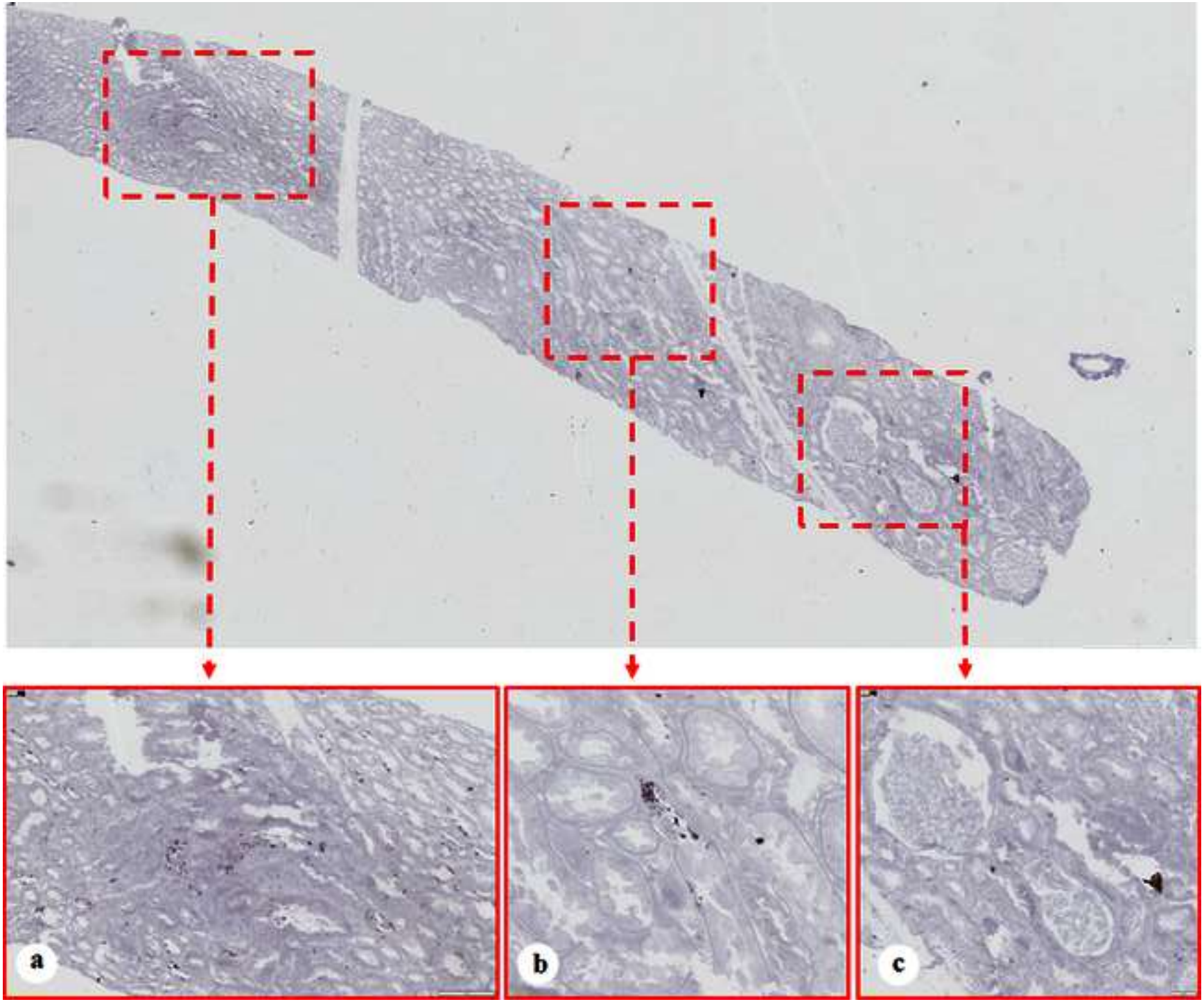

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EXPLORING PARVOVIRUS B19 PATHOGENESIS AND THERAPY AMONG KIDNEY TRANSPLANT RECIPIENTS. CASE REPORT AND A REVIEW OF THE LITERATURE

Berti Gian Marco[1] †, Manaresi Elisabetta[5] †, Vischini Gisella[2], Provenzano Michele[2], Corradetti Valeria[2] , Giannella Maddalena [3,1], Bonazzetti Cecilia [3,1], Rinaldi Matteo [3,1], Fabbrizio Benedetta [4], Matteo Ravaioli [7,1] , Gallinella Giorgio [5,6], La Manna Gaetano [1,2], Comai Giorgia [1,2]

[1]Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum-University of Bologna, Bologna, Italy (GM Berti, gianmarco.berti2@studio.unibo.it; G Vischini, gisella.vischini@aosp.bo.it; M Provenzano, michele.provenzano2@unibo.it, V Corradetti, valeria.corradetti@aosp.bo.it)

[2]Nephrology, Dialysis and Kidney Transplant Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna (G Comai, giorgia.comai@aosp.bo.it; G La Manna, gaetano.lamanna@unibo.it)

[3]Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria Policlinico di Sant'Orsola, Bologna (M Giannella, maddalena.giannella@unibo.it; C Bonazzetti, cecilia.bonazzetti@aosp.bo.it; M Rinaldi, matteo.rinaldi23@unibo.it)

[4]Pathology Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna (B Fabbrizio, benedetta.fabbrizio@aosp.bo.it)

[5]Department of Pharmacy and Biotechnology, University of Bologna (E Manaresi, elisabetta.manaresi@unibo.it; G Gallinella, giorgio.gallinella@unibo.it)

[6]Microbiology Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna

[7]Hepato-biliary and Transplant Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna (M Ravaioli, matteo.ravaioli6@unibo.it)

† These authors contributed equally to this work.

Correspondence to: Gaetano La Manna, Nephrology, Dialysis and Kidney Transplant Unit, IRCCS-Azienda Ospedaliero-Universitaria di Bologna, Via Giuseppe Massarenti, 13, Bologna, 40138, Italy, Tel +39-051-2142584, Fax +39-051-2142509, E-mail gaetano.lamanna@unibo.it

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Abstract

WeIn this Ground Round, we describe a case of a 34-year-old male recipient of an ABO-incompatible man with living donor kidney transplant (LDKT) LRKT) ABO incompatible who experienced repeated hospitalizations for unexplained anaemia. Acute kidney injury on a severe and recurrent anaemia related to Parvovirus B19 (B19V) infection was diagnosed throughbased on viral and histopathological analysis. In viewthe absence of the impaired patient'spatient own immune response due to the immunosuppressive regimen, clinical stabilization was achieved by repeated IVIgIVIG administration as a maintenance therapy in a prolonged course, although in the absence of viral clearance. TheA review of literature highlighted a variety of is provided, highlighting the various pathological patterns of renal lesions reported as associated with B19V infection, althoughthe insufficiency of epidemiological data on B19V infection in kidney transplant, the lack of standardized diagnostic and therapeutic protocols, and the prospective for specific antiviral therapy are still scanty. Extended awareness of clinical relevance of B19V infection in kidney transplant recipients should direct future efforts toward a better consideration and comprehension of viral-induced pathogenesis, aimed at effective diagnostic and therapeutic appropriateness.

Keywords: parvovirus B19; kidney transplant; anaemia; tubulointerstitial nephritis; glomerular thrombotic microangiopathy; in situ hybridization; immunochemistry, intravenous immunoglobulin.

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Introduction

Parvovirus B19 (B19V) is a human pathogenic virus, belonging to the *Parvoviridae* family, *Erythroparvovirus* genus, and is characterized by a pronounced tropism for erythroid progenitor cells in bone marrow. Its single-stranded DNA genome encodes for the structural proteins VP1 and VP2, and for few non-structural proteins, including protein NS1, which play a critical role both in viral life cycle and in B19V induced disease [1-3]. B19V infection is common and worldwide spread. According to a survey in European countries, including Italy, infection is more frequently acquired by age of 25 years, with the highest risk in children aged 7-9 years, but it can occur any time in lifetime~~lifelong~~, so that at elder ages a high proportion of adult population shows serologic evidence of past infection[4]. The most~~infection [4]. Most~~ common route of transmission is through aerosol droplets; other routes include maternal-foetal transmission, with possible severe consequences on the foetus [5], or more rarely through transfusions~~transfusion~~, administration of blood products [6], or bone marrow or solid organ transplantation [7]. The disease spectrum associated with B19V infection is wide and heterogeneous, depending on individual characteristics (age, hereditary anaemic status) and host's immunologic status (including the ~~included~~ use of immunosuppressive drugs).~~) of the host.~~ The viral tropism and cytotoxic effect on erythroid progenitor cells in bone marrow accounts for anaemia [8], whose severity and duration depends on underlying clinical conditions and the host ability to mount an effective neutralizing immune response. On the other hand, the pathogenesis and clinical manifestations in other tissues, such as the typical fifth disease in children and arthropathies mainly observed in adults, or atypical and heterogenous manifestations in disparate tissues, depend on activation of inflammatory mechanisms and immune response [9]. Finally, following infection, the virus can persist lifelong in several tissues, posing the issue of a possible reactivation related to immunosuppression [10].

Case report: a severe case of recurrent anaemia~~Anaemia~~ in kidney transplant~~Kidney Transplant~~ recipient

A 34-year-old kidney transplanted man was admitted to our ward for acute kidney injury and severe anaemia requiring multiple blood transfusions. His past medical history was remarkable for end-stage IgA nephropathy and ABO-incompatible living donor kidney transplant (LDKT) ~~LRKT) ABO incompatible~~ two months before

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the hospitalization. Desensitization for ABO-incompatible kidney transplantation~~therapy,~~ was performed, with Rituximab (RTX) at 375 mg/m^2, started at -30 days before transplantation (dbt), tacrolimus (Tac) 0.075 mg/kg and mycophenolic acid (MMF) 750 mg BD at -14 dbt, Plasma Exchange (PEX) at -6 dbt and Intravenous Immunoglobulins (IVIg~~Immunoglobulin (IVIG,~~) 0·5 g/kg at -1 dbt, followed by induction with Basiliximab, IVIg~~IVIG,~~ oral steroids and MMF, and later maintenance therapy with oral steroids, Tac and MMF. At the time of discharge his renal function was slightly upon normal limits. On admission to the Nephrology Department ultrasonographic medical assessment resulted negative for vascular or surgical complications, laboratory tests manifest severe anaemic (Hb 6·7 g/dL) non-haemolytic condition associated with thrombocytopenia (93 x 10^9/L) and acute kidney injury (**Table1**). Donor-specific antibody (DSA) screening was negative. The patient was initially treated with several blood transfusions. Microbiological tests were carried out and high amount of Parvovirus genome copies (>25,000,000~~25000000~~ copies/mL~~ml~~) were detected by qPCR assay (Parvovirus B19 ELITe MGB Assay, EliTech, Italy). Blood cultures and other microbiological tests were negative, including Cytomegalovirus, Epstein-Barr virus and BK Polyomavirus. Considering available literature, immunosuppressive therapy was ~~medications were~~ reduced and IVIg were~~IVIG,~~ started resulting in~~with,~~ clinical recovery and amelioration of infectious status. Mycophenolate mofetil (MMF) was discontinued as far as B19V active infection was detected. Afterwards, low target levels of serum Tac were settled (range 2-4 ng/mL). As a second step treatment, ten IVIg~~IVIG,~~ infusions (0·4 g/kg each) were combined with significantly improvement in B19V infection, clearance of viral load (8,251~~8251,~~ copies/mL) and recovery of renal function (serum creatinine 2·08 mg/dL). After resolution of anaemia and thrombocytopenia, renal biopsy was carried out, showing recurrence of primary~~baseline~~ glomerulopathy (IgAN) and acute tubulointerstitial nephritis (**Figure 1**). A successive~~Similar,~~ hospital admission was recorded few months later because of a comparable episode of anaemia and acute kidney injury. The infection presented again and as much aggressive as the first episode (> 25,000,000~~25000000~~ copies/mL~~ml~~) (**Table 1**). Tac was switched to m-TOR inhibitor (everolimus, range 3-8 ng/mL) and nine IVIg infusions were ~~IVIG infusion therapy was~~ performed again until~~with,~~ recovery of renal function and decrease of viral load (180,161~~180161,~~ copies/mL at the time of discharge). During this treatment, upper limbs/trunk rash occurred. Given~~Since,~~ the immunosuppressed status and the consequent inability to mount an efficient immune response, rash~~it,~~ was interpreted as a confounding manifestation of immune complexes aggregation (IVIg~~IVIG,~~ + B19V antigen)

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and deposition in skin capillaries (**Figure 2**). The rash disappeared when IVIgIVIG, treatment was discontinued. Another renal biopsy was performed. The majorMajor, pathologic findings were acute tubulointerstitial nephritis and features consisting in glomerular thrombotic microangiopathy (TMA) possibly related to the viral infection. GivenSince the absence of capillary/glomerular inflammation and negative DSA screening, investigation for antibody mediated rejection (ABMR) was carried out and C4d peritubular deposition was interpreted as an accommodation phenomenon in ABO- incompatible kidney transplant setting. Therefore, in situ hybridization for B19V was performed on tissue samples, localizing viral genomes in endothelial and tubular cells, a finding highly suggestive for a viral contribution in the renal lesions (**Figure 1**). Furthermore, structural capsid protein (VP) and non-structural protein (NS) immunohistochemistry was performedcarried out. All the kidney samples resulted negative for NS and positive for VP immunochemistry in tubular and vascular compartments (**Figure 1**). Desensitization pre-transplant strategy in an ABO-incompatibleABOi setting with RTX and the maintenance therapy with Tactacrolimus contributed to B cell and T cell deficient response to viral infection (PANB CD19+ 0·2%, 1/mmc). Since the longevity of viral clearance is uncertain and given the relapses during the clinical follow- up, we proposed weekly and later monthly administration of IVIgIVIG for consolidation and maintenance therapy with a constantly controlled viral load (**Figure 3**). Antibody titrestiters against B19V were measured repeatedly throughout the infection (LIAISON® Biotrin Parvovirus B19 IgG and IgM, DiaSorin, Italy); the detection of IgG correlatedcorrelates with exogenous administration of IVIgIVIG, while the constant absence of IgM indicateddemonstrates the lack of a natural immune response capable of clearing the viral infection in the long term. At the last outpatient visit, 19 months after second hospitalization, renal function wasis stable (serum creatinine 1·86 mg/dL), and persistent low B19V copies werecan still detectable (8,795be measured (8795 copies/mLml). Finally, a retrospective evaluation of archived recipient pre-transplant (-4 days) peripheral blood indicated presence of B19V DNA at low copy number (820 genome copies/mLml), while both donor and recipient were B19V DNA negative the year before (-13 months), when samples had been stored for immunological cross-matching analysis. Such low B19V positivity in the pre-transplant period in contrast to past negativity may hint at reactivation of a persistent or latent viral infection due to pre-transplant desensitization therapy, followed by highly active viral replication in the post-transplant period when on immunosuppressive maintenance therapy only.

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Discussion, data analysis and literature review

Renal involvement in Human Parvovirus B19

Parvovirus B19 was detected for the first time in human sera in 1975 [11] and the first case of B19V infection in a kidney transplant recipient was reported in 1986 [12]. Since then, several reports have described B19V infection in kidney transplant recipients, although the extent of this condition is likely underreported and clinical, histological and virologic descriptions are rather heterogenous (**Table 2**).

The most common clinical presentation of B19V infection in immunocompromised patients, including~~included~~ kidney transplant recipients, is anaemia with a low reticulocyte count, due to the direct inhibitory effect on erythropoiesis. In case of aggressive immunosuppressive regimens, viral clearance is delayed, and the acute-phase anaemia~~anemia~~ is followed by a chronic course, necessitating therapeutic support to avoid persistent red blood cell aplasia (PRCA), until a patient is able to develop an own mature immune response [7]. In addition to anaemia, numerous pathological patterns of renal lesions are associated with B19V infection [13], including acute post-~~-~~infectious glomerulonephritis (AGN), proliferative glomerulonephritis, collapsing glomerulopathy, focal segmental glomerulosclerosis (FSGS), thrombotic microangiopathy (TMA), and tubulointerstitial nephritis [14]. In kidney transplant recipients~~transplanted patients~~, B19V DNA is frequently found in renal tissues and its presence associated with greater~~higher~~ allograft injury [15], although acute rejection is rarely seen in transplant population and the long-term outcome does not seem impaired due to B19V infection [16,17].

Compared to reported cases and known pathogenetic mechanisms, our patient strikingly showed a combination of clinical and pathological findings that, taken together, contributed to his atypical and severe clinical presentation. Among these, profound depression of haematopoietic and chronic anaemia, complete anergy of an own adaptive immune response and reliance of a continuous exogenous IVIg administration, complex pathological findings in kidney both in the vascular, glomerular, and tubular components, superimposed on a background of IgA nephropathy and in the context of a complex post-transplant therapeutic management.

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Screening and diagnosis of Parvovirus infection in kidney~~Kidney~~ transplant population

Inadequate or delayed antibody response can feasibly occur~~may be mounted~~ in an acquired, iatrogenic immunosuppressed setting. Therefore, standard serologic tests for B19V specific antibodies are not diagnostic for discriminating the infection status, that needs to be investigated through direct molecular detection of viral components, mainly viral nucleic acids and/or proteins [18,19]. Viral nucleic acid detection and quantification by qPCR, or in situ tissue hybridization, may provide a rapid and sensitive method of diagnosis in transplant recipients and are recommended for the diagnosis, for differentiating low level persistence viral DNA from acute viral infection and for localization of infected cells within tissues [20]. After excluding the main common causes of anaemia, bone marrow biopsy/aspiration can be useful to identify the underlying origin~~cause~~ of pure red cell aplasia (PRCA). Clinical manifestations are not pathognomonic or can be even cofounding in transplanted patients, such as in the case of~~like~~ anaemia, usually the unique evidence of infection, which is quite common and multifactorial especially in the post-transplant period [21-24]. Few monocentric studies tried to define risk factors of parvovirus infections in kidney transplant ~~(KT)~~ population. Deceased donor kidney transplantation, tacrolimus therapy, high HLA mismatches (≥ four mismatches), pancytopenia and haemoglobin levels result positively correlated with high probability of infection in the univariate analysis [16].

Following meta-analysis, the overall reported incidence of positive B19V DNA among kidney transplant~~Kidney Transplant (KT)~~ patients is 10.3%; in the presence of~~among KT patients with~~ anaemia, the incidence rate of positive B19V DNA is 27.4% [25]. Prolonged anaemia after kidney transplantation is a relatively common complication. The pathogenesis of anaemia is usually multifactorial including surgical, immunological, infectious and iatrogenic aetiologies. In recent years, viral infections in kidney transplant recipients become increasingly relevant due to new immunosuppressive treatments. It is now generally known that ABO-incompatible~~ABOi~~ kidney transplantation is associated with an increased risk of infectious complications, partly related to the effects of extracorporeal treatments and the strong immunosuppression [26,27]. Renal injury caused by B19V infection could be associated with different histological presentations [13,28]. Moreover, considering nonspecific pathological features on kidney biopsies in transplanted patients such as interstitial nephritis and TMA, the differential diagnosis between B19V and allograft rejection was~~has been~~ challenging. In our case, C4d deposition in peritubular capillaries could not have been taken into consideration as a diagnostic element because of graft accommodation in ABO-incompatible~~ABOi~~ kidney transplant setting.

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In situ DNA hybridization allows identification of infected cells through direct detection of viral DNA in tissue samples. Despite not widely available or standardized in clinical practice, this method could overcome serology limitation in kidney transplantKT, population and clearly demonstratesdemonstrate, virus presence with a strong predictive value for disease or active infection. Vascular endothelial and tubular epithelial viral localizationslocalization, were common features in the two patient's biopsies (**Figure 1; Supplementary Figure 1**). These results were further validated byfrom, immunochemistry studies of structural capsid protein (VP) and non-structural protein (NS). In particular, all immunochemistry investigation of NS resulted negative; on the contrary, VP examinations were positive in all kidney samples maintaining tubular and vascular pattern as mentioned above. These considerations lead to the hypothesis that tubular damage may be linked to reabsorption of viral particles rather than direct productive infection of renal tissue. Detection of viral particles in the renal parenchyma is a pivotal result in elucidating B19V pathogenesis, but more studies are needed to understand the mechanisms of B19V infection and the related tissue damage in a non-permissive cell environment such as kidney tissue. HypothesesHypothesis, about the route of infection in kidney transplantKT, population include an exogenous primary infection in post-transplantation period, reactivation of a latent infection or organ-related transmission. In our patient, detection of low-level viremia in a pre-transplant sample may hind at viral reactivation in the recipient, possibly linked to the immunosuppressive effects of the desensitization therapy with RTXRituximab, although conclusive evidence cannot be achieved. Thus far, most reports lack of laboratory data on B19V, including assessment of pre-, and post-transplantation serologic and virologic status of the recipient and donor, in order to lend, support towards one hypothesis or the other, an observation prompting for implementation of B19V status analysis in this group of patients.

Prospective for antiviral therapy

The development of preventive or therapeutic measures to contrast B19V infection and its associated diseases suffers from its general appraisal of a mild, self-limited disease and from the inherent difficulties in defining a key target for antivirals. A vaccine to prevent the infection is not available, and no specific antiviral therapy has been approved for B19V, although this would meet clinical needs in case of severe clinical manifestations [18]. Development of targeted antiviral strategies against B19V is now an area of currentactive research [29,30]. A vaccine strategy based on Viral-Like Particles technology (VLP) has been pursued since early after

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characterization of B19V as a human pathogenic virus, and initial attempts showed both the immunogenicity of VLPs and their capacity to elicit production of neutralizing antibodies [31]. However, first-generation vaccines yielded also relevant side-effects that led to termination of clinical trials [32]. Second generation vaccines, still based on VLP technology but modified to abrogate their associated viral phospholipase activity, also show good immunogenicity [33], but have not been yet evaluated in clinical trials for their efficacy and safety. In the lack of a vaccine, passive immunization by using IVIg~~IVIG~~ has been always considered the only practically available therapeutic option and its use is widely reported in the literature. Given the high seroprevalence of B19V in the general population, IVIG preparations contain high level of anti-B19 IgG [34] and can exert a subsidiary or replacement neutralizing activity against the virus [35]. Literature is vast but mostly anecdotic, and controlled clinical trials to establish the actual best IVIg~~IVIG~~ administration regimen are not reported. The most structured study reported a good short-term efficacy in controlling B19V viremia and related consequences on hemopoiesis, while the long-term efficacy is more variable and highly depend on the host immune status [36]. In particular, in cases of immunosuppression, repeated IVIg~~IVIG~~ administration might be required, even though viral clearance is generally linked to the capacity of the host to eventually mount an own immune response. In perspective, availability of human monoclonal antibodies might add to the passive immunization options [37]. Recent developments indicated promising strategies in the development of antiviral drugs. Hydroxyurea is a virostatic compound also used for the treatment of sickle cell disease, showing inhibitory activity against B19V and might potentially serve a dual purpose [38]. Broad-range antivirals such as the nucleotide analogues Cidofovir and its derivative Brincidofovir have shown potent antiviral efficacy in in vitro models [39-41], the former also reporting a successful use in a clinical setting [42]. A serendipitous clinical use of Foscarnet, another broad range antiviral, has been reported with good results in a few cases, but its activity has not been demonstrated in controlled in vitro model systems [43]. New directions of antiviral therapy move towards a more selective inhibition of viral and/or cellular systems involved in viral replication. Direct acting antiviral agents can be targeted at the viral NS1 protein, by inhibiting its nuclear translocation [44] or its endonuclease activity, which is essential for promoting replication of the viral genome [45]; a high throughput screening effectively found a few purine derivatives able to inhibit B19V replication in vitro by this mechanism, but their translation to clinical use is still awaiting [46]. Related to kidney transplant~~KT~~ population, it should be reminded that there are no established guidelines for managing neither primary nor recurrent

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disease [18,19], so therapy is mostly empirical (**Table 2**) [16,47-61]. Non-pharmacological measures include supportive therapy with blood transfusions and reduction in immunosuppressive treatment in order to stabilize the acute phase and support the host's production of antibody [13,62-64]. Potent antirejection regimens that involve ~~mychophenolate mofetil (~~MMF~~)~~ and ~~tacrolimus (~~Tac~~)~~ should be reconsidered [48,52]. Indeed, Tac seems to be particularly connected with B19V induced anaemia/red cell aplasia [48,50]. In the light of~~Considering~~ these observations, it could be beneficial switching to another antirejection drug (cyclosporine or m-Tor inhibitor) [48,50,62], however, this too lacks consistent evidence-based studies. Since B19V-specific antibody response is absent or minimal in kidney transplant~~KT~~ population, passive immune reconstitution with IVIg can~~(IVIG)~~ replace the production of neutralizing antibody, thus facilitating~~ helping in~~ viral clearance, resumption of reticulocytotic and ~~a~~ rising of~~in~~ haematocrit. Most common adverse reaction including fever, chills, headache, myalgia, nausea and acute renal failure increase significantly along with rising IVIg~~the IVIG~~ dose [19,36].

IVIG administration in our patient was carried out according to guidelines [18] and adjusted over time in order to maintain effective control of viremia and renal function. Initially, we followed the most adopted regimen consisting of repeated high-dose infusions (reported as 400 mg/Kg/day for 5-10 consecutive days [13,16,28,43,50,52,53,62,65]). Then, we opted for a maintenance low-dose regimen able to achieve a low-level viraemic~~viremic~~ state in a compensated clinical setting. Given its reliance on a single case report, it is essential to acknowledge the limitations of our therapeutic proposal as it cannot be generalized to a broader patient population in the absence of controlled trials. The initial critical presentation, and concerns related to possible toxicity effects on kidney, discouraged the use of antiviral compounds, such as cidofovir or brincidofovir, as an additional therapeutic option. However, given the constant necessity of IVIg~~IVIG~~ therapy and the currently~~now~~ stabilized renal functionality, a pharmacological approach using cidofovir or brincidofovir may be considered to obtain complete viral clearance.

Conclusion

This case report analysed~~In this Ground Round we analyse~~ B19V infection-associated kidney disease by clinical and histological findings combined with in situ hybridization and immunohistochemistry techniques in a kidney transplant recipient. In addition to chronic anaemia as a result of persistent infection, results are suggestive of~~stand for~~ viral tubular and vascular localization in the renal tissue. Tubular viral reabsorption

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hypothesis appears to be~~was~~ stronger, ~~supported~~ rather than a direct pathogenetic mechanism driven by productive infection on renal tissue, likely a secondary localization with respect to replication in bone marrow. Further investigations are required to better clarify the pathogenetic mechanisms beyond renal involvement in the course of B19V infection, taking into consideration the contributions of both virus and immune system.

The present case report allowed us to focus attention on B19V infection in kidney transplant recipients and showed the insufficiency of epidemiological studies in ~~the~~ existing literature. This could be a valuable~~an excellent~~ starting point for further studies aimed at broadening the ~~investigation which should improve~~ knowledge of~~in~~ B19V infection mechanisms and the related tissue damage. B19V is not routinely included in the screening and monitoring of viral infections in kidney transplant recipients, but its implementation should now be reconsidered also in view of standardisation of diagnostic tools and treatment regimens.~~recipient patients.~~ In the absence of a standardized diagnostic protocol, clinical relevance of B19V infection may be underestimated. In turn,~~, and in~~ the lack~~absence~~ of a shared standardized therapeutic protocol, might result in various degrees of success or alternatively~~and~~ insufficient long-term responses~~ are achieved~~. Pre-transplant virologic routine screening might help to categorize subgroup of transplanted patients at high risk; post-transplant monitoring can anticipate clinically relevant viral replication and direct therapeutic approaches. Finally, continuing research on antivirals may offer in the future relevant therapeutic options. This study might provide an important basis in the perspective~~This study is of paramount importance for the purposes~~ of understanding/clarifying B19V pathogenesis integrating several clinical, histological, laboratory and immunochemistry data, with the ultimate goal of developing~~. The goal of the study is to raise awareness of scientific research and direct future efforts toward updating clinical comprehension and therapeutic mindset in order to develop~~ more specific treatments to achieve a complete virus eradication.

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Search strategy and selection criteria

References were identified through MEDLINE (PubMed) on Dec 21, 2023. For B19V in kidney transplant recipients, we searched with Medical Subject Heading terms including the keywords “Parvovirus B19” AND “Kidney transplant”. Retrieved articles were further scrutinized and selected for completeness of information on clinical, histological and virologic data, and for inclusion of data on follow-up and outcome.

Contributors

GMB, GC, GV, GLM analysed the clinical case. GMB, GC, GV, EM acquired and analysed the data. GG EM contribute processing data and writing manuscript. All coauthors interpreted the results and reviewed the final version of the manuscript.

Patient’s consent for the publication of manuscript materials has been obtained.

Declaration of interests

The author declares no conflict of interest

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Figure legends.

Figure 1. Pathological findings. First biopsy (left). Light microscopy. **a** mesangial proliferation (Periodic-acid Schiff staining, original magnification x200); **b** immunofluorescent staining for IgA, predominant mesangial IgA deposition; **c** acute interstitial nephritis (Periodic-acid Schiff staining, low power x40); **d** accommodation in ABO incompatible kidney transplant setting: positive peritubular capillary staining of C4d; **e** B19V VP capsid protein positivity in tubular cells (immunohistochemical staining of B19V VP capsid antigen, original magnification x100 [66]); **f** B19V in situ hybridization (Digoxigenin-labeled DNA probe [67]): tubular-positive and glomerular-negative staining. Second biopsy (right). Light microscopy. **g** acute interstitial nephritis (hematoxylin and eosin staining, low power x40); **h** glomerular mesangiolysis (Jones' methenamine silver stain, original magnification x200); **i** immunofluorescent staining for IgA, negative; **l** B19V VP capsid protein positivity in tubular cells; **m** B19VB19V in situ hybridization positive in tubular cells and negative in glomerulus.

Figure 2. Skin lesions. Skin rash after administration of Intravenous immunoglobulin (IVIG) due to aggregation in immunocomplexes in an iatrogenic immunosuppressed setting.

Figure 3. Timeline of clinical and therapeutic course.
Haemoglobin serum concentrations (blue line) and serum creatinine trend (grey line) in relation to Log(10)B19V serum genome copy number (orange line) and the IVIG treatment (yellow arrows). Triangles indicate kidney biopsies; star indicates switch of the immunosuppressive therapies from Tac to mTORi. B19V, Parvovirus B19; IVIG, intravenous immunoglobulin; Tac, tacrolimus; mTORi, mammalian target of rapamicyn inhibitor

Supplementary Figure 1.
Digital core biopsy **image and high-resolution zoom into focal areas**. **a**, **b** Parvovirus B19 in situ hybridization with tubular-positivity; **c** Parvovirus B19 in situ hybridization negative in glomerulus

Table 1. Laboratory data of case report

	First hospital admission	Second hospital admission
Days after transplantation	51	171
Haemoglobin, g/dL [13.5-17.2 g/dL]	6·7	6·6
Reticulocytes, % [0.5-2.0]	0·3	0·1
Total white blood cells, 10^9/L [3.6-10.5]	9·37	7·23
Platelets, 10^9/L [160-370]	93	133
Serum creatinine, mg/dL [0.5-1.2]	5·8 (basal 1·7 mg/dL)	4·69 (basal 1·7 mg/dL)
B19V, genome copy number/mL; Log(10)copies/mL	>25000000; >7·39	>25000000; >7·39
LDH, U/L [< 248U/L]	580	325
Haptoglobin, mg/dL [30-200 mg/dL]	53	67
DSA	negative	negative

LDH, lactic dehydrogenase; DSA, donor-specific antibody; [normal range]

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Table 2. Parvovirus B19 infection in kidney transplant recipients

Reference	Sample size	PCR	B.M.	K.B.	Treatment*	Outcome**
47	1	+	ND	ND	S IVIG	+
48	1	+	+	+[d]	S IVIG	+
49	4	+(2/4)	+	+[a,c]	NT (3/4) IVIG (1/4)	NT (3/3) + IVIG (1/1) +
50	1	+	+	ND	IVIG (1/1) S (1/1)	+
51	1	+	+	ND	IVIG	+
52	6	+ (4/6)	+(3/6)	ND	R (3/6) IVIG (5/6)	+
53	3	+	+(2/3)	ND	IVIG (3/3) RI (1/3)	+
54	6	+	+(4/6)	+[a]	IVIG	+
55	1	+	+	ND	IVIG	+
56	2	+	ND	ND	IVIG (1/2) RI (1/2)	+
57	1	+	+	ND	IVIG	+
16	39	+	+(13/39)	ND	NT (6/39) RI (4/6) IVIG (29/39)	IVIG group +
58	1	+	+	ND	RI IVIG	+
59	9	+	ND	ND	RI (9/9) S (9/9) IVIG (9/9)	+
60	1	+	+	ND	S IVIG	+
43	50	+	ND	ND	RI (50/50) S (44/50) IVIG (45/50)[b] Foscarnet (5/50)	IVIG group 39/45 + Foscarnet group 10/11 +
61	3	+	ND	ND	RI (1/3) IVIG (2/3)	+

*Treatment undertaken beyond blood transfusions; **resolution of anaemia; PCR, polymerase chain reaction (serum); B.M., bone marrow biopsy/aspiration; K.B., kidney biopsy; NT, no treatment undertaken; RI, reduction of immunosuppression; IVIG, intravenous immunoglobulin; S, switch tacrolimus to cyclosporin/mTOR inhibitor; a, thrombotic microangiopathy evidence in renal tissue sample; b, IVIG (6/45) were converted to foscarnet therapy; c, Parvovirus B19 DNA in situ Hybridization on kidney biopsy; d. interstitial nephritis evidence in renal tissue sample.

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