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REVIEW

Polyomavirus-associated nephropathy

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Abstract

Polyomaviruses BK and JC are ubiquitous viruses with high seroprevalence rates in general population. Following primary infection, polyomaviruses BK and JC persist latently in different sites, particularly in the reno-urinary tract. Reactivation from latency may occur in normal subjects with asymptomatic viruria, while it can be associated to nephropathy (PVAN) in kidney transplantat recipients. PVAN may occur in 1%-10% of renal transplant patients with loss of the transplanted organ in 30% up to 80% of the cases. Etiology of PVAN is mainly attributable to BK virus, although approximately 5% of the cases may be due to JC. Pathogenesis of PVAN is still unknown, although viral replication and the lack of immune control play a major role. Immunosuppression represents the *condicio sine qua non* for the development of PVAN and the modulation of anti-rejection treatment represents the first line of intervention, given the lack of specific antiviral agents. At moment, an appropriate immunemodulation can only be accomplished by early identification of viral reactivacation by evaluation of polyomavirus load on serum and/or urine specimens, particularly in the first year post-trasplantation. Viro-immunological monitoring of specific cellular immune response could be useful to identify patients unable to recover cellular immunity posttransplantation, that are at higher risk of viral reactivation with development of PVAN. Herein, the main features of polyomaviruses BK and JC, biological properties, clinical characteristics, etiopathogenesis, monitoring and diagnosing of PVAN will be described and discussed, with an extended citation of related relevant literature data.

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Key words: Polyomavirus; Kidney transplantation; Immunosuppressive therapy; Virological monitoring; Cellular immune response

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INTRODUCTION

Polyomavirus-associated nephropathy (PVAN) is one of the most common viral complications in renal transplant recipients and is an increasingly recognized cause of renal transplant dysfunction and graft loss. Since the first description of PVAN in 1995, an increasing prevalence rate from 1% to 10% has been evidenced[1]. The increase in prevalence data could be due to the introduction of new deeply immunosuppressive drugs and/or the relative decline in acute rejection rates. PVAN can lead to kidney graft loss in 10% up to 100% of the cases, determining the return in hemodialysis within 6 to 60 mo, thus significantly and markedly reducing the graft survival.

Viral replication is the single common feature of all patients at risk of nephropathy. Therefore, screening for viral replication is the most useful tool for the identification of patients at risk of developing nephropathy, thus allowing for earlier intervention, in particular a pre-emptive reduction of immunosuppression, with improvement of outcome. Beside virological monitoring, in recent years the role of virus-specific cellular immune response in the control of viral replication has prompted

the development and employment of methods for viro-immunological monitoring.

The complexity regarding diagnosis, monitoring and clinical management of PVAN evidences the need for a multidisciplinary approach, including nephrologists, pathologists, and clinical virologists.

HUMAN POLYOMAVIRUSES AND NEPHROPAHTY

The most characterized polyomaviruses infecting humans and involved in the pathogenesis of PVAN are BK virus (BKV) and JC virus (JCV), named after the initials of the patients in which they were first isolated in 1971[2,3]. In recent years, other polyomaviruses have been described in humans, including WU, KI, Merkel cell virus and others, however their clinical role is still undefined and no association to nephropathy has been evidenced. Moreover, also the non-human primate polyomavirus SV-40 has been detected in human specimens and is associated to nephropathy in these primates in the presence of immunocompromised conditions, such as infection with simian immunodeficiency virus (Table 1). SV-40 was accidentally introduced as a pathogen into the human population as a contaminant of early polio vaccines, both the inactivated one by Salk and the oral one by Sabin, between 1955 and 1964[4]. Apart for polio vaccines, strong serological and molecular evidence suggests that new SV-40 infections may be occurring in the human population, although the route of transmission remains unknown. Pathogenic role of SV-40 in humans is controversial and recent studies evidenced the risk of false positive results because of contamination by common laboratory plasmids containing SV-40 sequences[5-7]. Nevertheless, like other polyomaviruses, SV-40 displays renotropism and is believed to latently persist in the kidney after primary infection.

Polyomaviruses BK and JC are ubiquitous, with seroprevalence rates ranging from 70% to 90% in adult population. Primary infection usually occurs early in the childhood, at a median age of 5 years, and is characterized by low upper respiratory tract morbidity or is asymptomatic. Following primary infection, BKV and JCV remain latent in different sites, including the renourinary tract, as the epidemiologically most relevant latency site, B cells, brain, spleen, and probably other organs. Periodical reactivation may occur in both immunocompetent individuals (in 0% up to 62%) and immunocompromised patients[8] and is evidenced by asymptomatic viruria.

In contrast to BKV, that is found infrequently in the urine of healthy adults, JC viruria occurs universally, increasing with age. In a study on 400 healthy blood donors, Egli and colleagues evidenced that JC viruria was significantly more frequent and at a higher viral load in comparison to BK viruria (19% *vs* 7%, $P < 0.0001$)[9]. According to the Authors, these data indicate significant differences between BKV- and JCV-infected cells with respect to anatomic location and/or accessibility to T cells in mucosal sites.

Table 1 Polyomaviruses detected in humans and involved in the pathogenesis of polyomavirus-associated nephropathy

Virus	Host	Clinical diseases
BKV	Human	PVAN in renal transplantation Hemorrhagic cystitis in bone marrow transplantation
JCV	Human	Progressive multifocal leukoencephalopathy PVAN in renal transplantation
SV-40	Non-human primate	Unknown; PVAN in renal transplantation?

PVAN: Polyomavirus-associated nephropathy; BKV: BK virus; JCV: JC virus.

At the time of first description of BK in 1971, the pathogenic role of BKV remained elusive and it has been considered an orphan virus for many years afterwards. In 1978, Mackenzie and colleagues first described four features of nephropathy in renal transplantation: (1) the detection of urinary decoy cells; (2) the presence of viral inclusions in uroepithelial cells in graft biopsies; (3) the difficulties in the differential diagnosis with acute rejection; and (4) the role of immunosuppression in the development of these findings[10].

In 1995, Purighalla and colleagues described the first case of PVAN and recognized it as a defined disease entity[11]. Subsequently, several reports with increasing prevalence rates from many transplant centres worldwide followed, evidencing a stepwise increase in incidence of PVAN from approximately 1% in 1995 to 5% or even more in 2001. Subsequently, studies from 2002 to 2004 reported PVAN with prevalence rates of 1% to 10% (mean 5.1%). The majority of the cases occur in the first year posttransplantation, although approximately 25% of cases are diagnosed later. Clinical impact is relevant, as loss of the renal graft ranges from 30% up to > 80% of cases[12-14]; in transplant centres where screening for polyomavirus replication in the urine and plasma is performed, the rate of graft loss is lower[15].

Several studies evidenced that BK viremia is only rarely observed in non-kidney solid organ transplant recipients and biopsy-confirmed cases of nephropathy have been described in few case reports[16-22], despite the similar or even higher level of immunosuppression, thus supporting the role of organ determinants in the pathogenesis of PVAN.

In hematopoietic stem cell transplant recipients, polyomavirus BK replication is commonly associated to hemorrhagic cystitis. Its incidence in this population ranges from 5.7% to 7.7%[23,24]. A case of polyomavirus-induced hemorrhagic cystitis in renal transplantation patient with polyomavirus nephropathy has been reported[25].

Polyomavirus JC was first isolated in brain tissue from a patient with progressive multifocal leukoencephalopathy, a demyelinating disease caused by lytic replication of the virus in oligodendrocytes and observed in the setting of profound cellular immunosuppression, such as ac-

quired immunodeficiency-syndrome. JCV has been also associated to nephropathy in kidney transplant recipients.

In fact, the etiological agent of PVAN is BKV in most of the cases, while JCV has been recognized as the etiologic agent in less than 3% of all reported cases, alone[26,27] or in association to BKV[28]. Nevertheless, it is likely that the role of JCV is more relevant than that reported. In a recent study[29], a biopsy-proven PVAN was diagnosed in six kidney graft recipients with exclusive JCV viruria out of 75 patients (8%) with BKV and/or JCV viruria, thus accounting for an overall incidence during the study period of 0.9%.

A potential role for SV-40 in the etiology of PVAN has been also suggested. Butel and colleagues[30] found that SV-40 seropositivity in children increased with age and was significantly associated to kidney transplantation. A co-infection with BKV and SV-40 has been described in two of six renal transplant patients with PVAN[31].

The pathogenesis of PVAN is still only incompletely understood, although it is now recognized that the interaction of multiple factors concurs to the development of PVAN, including patient, organ, and viral determinants.

In all the cases, the *condicio sine qua non* for the development of PVAN is the presence of intense immunosuppression. Nephropathy has been associated particularly, but not exclusively, to triple immunosuppressive therapy, including tacrolimus, mycophenolate mofetil, and steroids. It has been noted that the emergence of PVAN coincided with the diffuse use of tacrolimus and mycophenolate mofetil, although there is no proved causal relationship, due to the different mechanisms of action of these drugs[1,32]. It seems that the overall level of immunosuppression rather than a specific agent is involved in the pathogenesis of PVAN, although we cannot exclude a drug-specific mechanism. The importance of immunosuppression is also underlined by the fact that the main line of intervention is by modulating immunosuppression, in particular reducing, switching or discontinuing a specific immunosuppressive agent.

The preferential manifestation in renal allograft as compared to the native kidney of other solid-organ transplant recipients, suggests the role of other factors.

Among patient-related determinants, the following determinants have been identified as possibly contributing to PVAN: age > 50 years, male gender, white ethnicity, pre-transplantation BKV seronegativity in children, interferon (IFN)-γ producing specific T-cells, presence of comorbidities such as diabetes mellitus and cytomegalovirus coinfection.

Among organ determinants, the following factors have been considered as associated to viral replication and PVAN: HLA mismatching, previous episodes of acute rejection (treated with anti-lymphocyte preparations and intravenous steroid boluses), viral load in the renourinary tract, presence of renal injury (including calcineurin-inhibitor toxicity, in fact the reduction of this class of drug represents the very first line of intervention).

Among viral determinants, BKV genotypes (mutations in domain of *VP1* gene) and rearrangements in NCCR with presumably increased viral fitness.

Determinants involved in determining the risk of PVAN are summarized in Table 2.

Table 2 Determinants in the development of polyomavirus-associated nephropathy

Determinants	
Patient-related	Age > 50 yr
	Male gender
	Comorbidities (diabetes mellitus)
	Negative serostatus before transplantation
Organ-related	Degree of HLA mismatching
	Prior rejection episodes
	Renal injury
	Latent infection load
Viral-related	NCCR rearrangements
	Genotype
	Viral fitness
Immunity-related	Intense triple immunosuppression (calcineurin-inhibitor, antiproliferative agent, steroid)
	Rejection and anti-rejection treatment (anti-lymphocyte preparations, iv steroid boluses)
	Positive serostatus of donor
	Low number of BKV-specific T-cells

NCCR: Noncoding control region; BKV: BK virus.

CLINICAL AND HISTOLOGIC FEATURES

PVAN is typically diagnosed within the first year post-transplantation, although approximately 25% of the cases are seen later (range, 1.3-45.1 mo). Clinical presentation may be inconspicuous and lacks of useful features. Varying degrees of renal dysfunction may be seen, although in early stages even normal serum creatinine levels may be detected. PVAN may consist in interstitial nephritis and/or ureteric stenosis with ureteric obstruction, hydronephrosis, and sometimes associated urinary tract infections. Progressive renal failure is reported in approximately 30%-60% of the cases[33].

The stereotypical evolution of PVAN has been thoroughly characterized and is as follows[34]. Most of the cases are preceded by an asymptomatic phase of persistent and significant viruria, as demonstrated by the evidence of a urine viral load > 10^5 copies/mL by polymerase chain reaction or by urine cytology (urinary decoy cells for at least 2 mo). Sustained BK viruria is typically followed within few weeks by viremia. A significant and sustained viremia (identified as > 5000 copies/mL plasma for 3 consecutive weeks) identifies patients with uncontrolled viral replication potentially leading to parenchymal injury. Progression of PVAN leads to eventual deterioration of the kidney graft function. Usually, appearance of viruria and viremia precedes the increase in serum creatinine by weeks or months. Options for clinical interventions vary in relation to the stage of PVAN course. In patients with viremia and viruria, a patient's tailored reduction of immunosuppression before a significant renal injury has

occurred leads to the resolution of the infection in up to 90% of the cases with long term preservation of renal function and a low risk of acute rejection (10%-15%). No intervention is required in the sole presence of viruria. In the presence of viruria and viremia, increased serum creatinine level and renal injury at graft biopsy, the intervention by reducing immunosuppression is mandatory. Late diagnosis and intervention, once that graft dysfunction is evident, decreases significantly the likelihood of viral clearance and is associated with higher probability of graft loss (30% *vs* < 10%). In patients with end-stage PVAN, intervention is usually late and ineffectual; the end-stage PVAN is clinically and histologically similar to end-stage renal disease with progressive obliteration of the renal tubules and decrease in viremia and viruria.

PVAN displays a (multi)focal distribution in the kidney. Histologic diagnosis of PVAN is based on the detection of typical basophilic nuclear viral inclusion in epithelial cells (renal tubular and/or Bowman's capsular lining urothelium). The virus is identified by immunohistochemical staining for the so-called SV40 Large T antigen, which cross-reacts with polyomaviruses BKV, JCV, and SV40. Histologic patterns of PVAN are based on the identification and extend of inflammatory infiltrates and fibrosis in association with viral infection[34]. The following patterns have been described: pattern A with viral cytopathic changes within a normal renal parenchyma, scant or no tubular atrophy, interstitial fibrosis and inflammation; pattern B with combination of viral cytopathic changes and focal/multifocal areas of tubular atrophy/interstitial fibrosis/inflammation (< 25% for pattern B1; 25%-50% for pattern B2; > 50% for pattern B3); pattern C (end-stage PVAN) with scarce viral cytopatic changes within a diffusely scarred renal tissue and extensive tubular atrophy/interstitial fibrosis/inflammation.

DIAGNOSIS

The definitive diagnosis of PVAN is made by histopathologic evaluation, however this presents some drawbacks, including limited sensitivity due to focal involvement, thus accounting for sampling errors; varying presentations with cytopatic-inflammatory and/or fibrotic/scarring patterns; difficult differential diagnosis from acute rejection, that may coexist with a opposite impact on intervention strategies. As the main line of intervention is by reducing immunosuppression, early diagnosis is fundamental for a pre-emptive treatment, before the instauration of renal injury. Considering the main pathogenic factors of PVAN, i.e., viral replication and failure of immune surveillance, earl diagnosis may be accomplished by virological and viro-immunological monitoring, consisting in monitoring of viral replication and evaluation of virus-specific immune response, respectively.

VIROLOGICAL MONITORING

Viral replication is the single common feature of all renal transplant recipients at risk of PVAN. Therefore, screening for viral replication allows for the identification of patients at risk of developing PVAN, thus permitting earlier intervention consisting in a pre-emptive reduction of immunosuppression[35], with improvement of the outcome. Screening for viral replication presents a high predictive value (100%), as in the absence of viral replication, PVAN is excluded[36]. Moreover, virological monitoring is the most important tool for evaluating the response to the treatment in patients with confirmed PVAN. Different screening assays are available and include: (1) urine cytology, i.e., the detection of decoy cells that are present in 40% to 60% of transplant recipients, although it represents a good screening test with a 100% negative predictive value, positive predictive value is very low (approximately 20%)[33]; (2) quantification of urinary BKV-DNA, with a load 100-fold higher than plasma viral load that is found in 30% to 40% of transplant recipients, with a positive predictive value of approximately 40%[33]; and (3) quantification of plasma BKV-DNA, with a viral load higher than 10^4 copies/mL recommended for a presumed diagnosis of PVAN[32,37,38]; quantification of urinary VP1 mRNA that is likely to mirror active viral replication.

Different screening methods present some drawbacks, for example urine cytology and urinary VP1 mRNA measurement are susceptible to preanalytic hazards due to the type and duration of processing, viruria may differ depending on the type of specimen (supernatant, cell pellet, resuspended urine), micturition intervals and fluctuations of urine content, inhibition of polymerase chain reaction in urine (e.g., depending on urea concentration) for viruria.

As regards VP1 mRNA, this assay was first proposed as a tool for noninvasive diagnosis of PVAN adopting a cut-off of 6.5×10^5 copies/nanogram of total RNA[39] and has been recently described as a complementary assay to investigate viral replication on the basis of the results of a study having found that the mean copy number in patients without PVAN was significantly lower than that in biopsy-proven nephropathy[40], and the assay has been validated in an independent cohort of renal transplant patients[41]. However, the use of VP1 mRNA measurement has been criticized as it is dependent on the purity of the RNA preparation and contamination by the encoding viral genomic VP1 DNA contaminating the VP1 cDNA preparation has been indicated as being potential responsible for falsely high results[42]. We have recently evaluated the role of urine VP1 mRNA quantification in a large cohort of kidney transplant recipients and found that, by analyzing the operating characteristics, VP1 messenger study was not superior to viremia and was otherwise limited by the technical complexity in comparison to DNA detection[43].

According to the Consensus Recommendations of a panel of international experts, screening for polyomavirus replication should be made by urine cytology or urine DNA load evaluation; recommended screening intervals are as follows: every 3 mo up to 2 years posttranplantation

Table 3 Algorythm for the virological monitoring of polyomavirus BK replication in renal transplantation[49]

Assay	Notes	Timing - intervention
Screening Urine cytology (decoy cells) or urine DNA load	Positive screening test (possible PVAN)	Every 3 mo up to 2 yr post-transplantation or in case of allograft dysfunction or when renal biopsy is performed
Adjunctive quantitative tests (threshold) Urine DNA load > 10^7 copies/mL or plasma DNA load > 10^4 copies/mL	Presumptive PVAN	Pre-emptive reduction of immunosuppression
Allograft biopsy	Definitive diagnosis of PVAN	
Monitoring of response to treatment Urine DNA load decreasing or plasma DNA load decreasing Serum creatinine		Every 2-4 wk
	Negative monitoring test (resolved PVAN)	

PVAN: Polyomavirus-associated nephropathy.

or when allograft dysfunction occurs or when allograft biopsy is performed. In the presence of a positive screening tests (possible PVAN), adjunctive quantitative assays with identification of cut-off levels are recommended, including a urine DNA load > 10^7 copies/mL or plasma DNA load > 10^4 copies/mL. In the presence of a positive adjunctive test above the threshold (presumptive PVAN), allograft biopsy is recommended in order to make a definitive diagnosis of PVAN and prompt intervention. Response to the treatment is monitored every 2-4 wk by evidencing a reduction of urine and plasma DNA load until resolution of PVAN.

The performance characteristics of tests for screening of polyomavirus replication have been evaluated in recent studies. Based on a study by Viscount and colleagues[44] on a cohort of 114 kidney transplant recipients with four cases of confirmed PVAN, BKV-PCR may prove superior to urine cytology, particularly in terms of sensitivity and positive predictive value. A plasma DNA load > 1.6 × 10^4 copies/mL evidenced an improved specificity to 96% (*vs* 91% for plasma load of 10^4 copies/mL, 92% for urine load > 2 × 10^7 copies/mL, and 84% for urine cytology) without reducing sensitivity (100% for all the molecular assays, as all the PVAN cases presented this viral load, *vs* 25% for urine cytology), however positive predictive value was only 50% (*vs* 29% for plasma load of 10^4 copies/mL, 312% for urine load > 2 × 10^7 copies/mL, and 5% for urine cytology). Nevertheless, the important clinical value of a negative polymerase chain reaction test is well established. In a similar study performed at the Renal Transplant Centre of Turin, Italy, on 229 patients with three cases of confirmed PVAN (accounting for an overall prevalence of 1.3%), a viremia level > 1.6 × 10^4 copies/mL was found in four cases, three of which with PVAN. The following operating characteristics for the diagnosis of PVAN were achieved: sensitivity 100%, specificity 99.6%, positive predictive value 75%, and negative predictive value 100%; of course, the low number of PVAN cases represents a limitation of this study[15].

Virological monitoring for PVAN at the Renal Transplant Centre of Turin, Italy, includes screening of viruria and viremia twice monthly in the first 3 mo posttransplantation, thereafter every 3 mo during the first 2 years and then yearly until the 5th year. Due to the possibility of self-limiting (transient) replication, positive screening assays are confirmed within 2-4 wk.

Renal biopsy is performed in case of suspected rejection, PVAN or declining renal function; in fact, also considering the potential for sampling errors due to focal involvement, renal biopsy is necessary to exclude other pathologic processes, such as acute rejection that may coexist[45]. At our centre, considering that in early stages of PVAN viral inclusions may be absent, as well as inflammation may be scarce[46], beside histopathologic evaluation, also quantification of polyomavirus DNA on renal graft biopsies and/or ureteric specimens is performed. Polyomavirus-DNA quantitation could be useful in the presence of little evidence of viral cytopathy[46]. In a study on quantitation of polyomaviruses BK and JC in human kidneys, the highest tissue viral loads (e.g., > 10^3 copies/cell) were found in active PVAN, while it was significantly lower in pre-PVAN biopsies and in specimens from patients with asymptomatic viruria[47]. Similar results were obtained by our group in a study on 109 renal transplant patients with two cases of histologically confirmed PVAN, with tissue BKV load > 10^4 copies/cell in both PVAN cases and < 10^2 copies/cell in patients with asymptomatic viruria or pre-PVAN[48]. Overall, these studies evidenced that viral load are significantly higher in active PVAN and underline the low sensitivity of tissue polymerase chain reaction in early stages of infection, possibly reflecting the low sensitivity due to focal involvement, as already described for histopathology.

Recommended algorithm for virological monitoring of polyomavirus BK replication in renal transplant recipients[49] is summarized in Table 3.

VIRO-IMMUNOLOGICAL MONITORING

Among determinants potentially involved in the pathogenesis of PVAN, there are immunity-related risk factors. These include immunosuppressive therapy, use of steroids, HLA-mismatching, rejection and anti-rejection treatment, and factors related to polyomavirus-specific

immune response[50]. Both humoral and cellular response may be associated to BKV replication and PVAN.

As regards humoral response to BKV and PVAN risk, it has been evidenced that BKV-seropositive recipients are not protected from viral replication and PVAN[12]. For example, in a study on 78 renal transplant patients, PVAN occurred in four of the 59 (76% of the whole study population) seropositive individuals and in one of the 19 (24%) seronegative patients[12]. On the other hand, BKV-seronegative recipients are at higher risk of viral replication and nephropathy[51-53]. For example, BK viruria was significantly more frequent in seronegative recipients in comparison to seropositive patients (58% *vs* 21% in a study population of 24 and 56 pediatric kidney transplant patients, respectively; $P < 0.005$)[51]. Nevertheless, although specific antibodies may accelerate the clearance of primary infection, they seem to play only a minimal role in the containment of PVAN. In fact, although a significant increase in immunoglobulins G titer is seen in patients with decreasing viremia values and after the resolution of PVAN[52-54], individuals with elevated immunoglobulins G levels can still develop PVAN, thus suggesting a role of defective cellular immune response in the onset of nephropathy[55].

Until recently, studies on immune response to BKV have been limited, due to the little pathologic potential in immunocompetent individuals. It has been evidence that BK-seropositive healthy subjects present CD4+ and CD8+ cells specific for BKV Large T antigen and the capsid protein VP1; in particular, CD4+ T cells with cytotoxic potential seem to play a role in maintaining memory responses to BKV and contribute to the immune control of viral replication[56].

In renal transplant patients, it has been evidenced that control of BKV replication and PVAN is correlated with the development or reconstitution of BKV-specific cellular immune response; whereas the lack of a protective immunity may favour the occurrence of active infection and progression to PVAN[54,57]. The fundamental role of T-cell immune response in the control of BKV replication was first observed by the indirect evidence of increased incidence of viral reactivation and development of disease in relation to the degree of immune compromise[8,32,49,58].

Evaluation of BKV-specific cellular immune response could be accomplished by lymphocyte stimulation with inactivated cultured virus or specific epitopes/overlapping peptide pools derived from VP1 and Large T antigens (i.e., virus-specific stimulation step) and detection of cellular immunity by labelled major histocompatibility complex class I tetramers, by intracellular staining and flow cytometry analysis, or by the enzyme-linked immunosorbent spot (ELISPOT) assay for IFN-γ.

By using one of these assays, several studies have evidenced that kidney transplant recipients with with BK viremia or nephropathy failed to mount or expand a virus-specific cellular immune response, in comparison to seropositive healthy individuals or renal transplant patients with no evidence of infection (negativity of viruria and viremia) or with evidence of infection in the presence of good renal function (evidence of viruria, but no increase in serum creatinine)[54]. In particular, in patients with PVAN, no IFN-γ-secreting cell was detectable; whereas, in patients with PVAN, after reduction of immunosuppression, an increase in the number of IFN-γ-secreting cells to levels similar to those of healthy subjects was evidenced, in concomitance with a reduction of viremia and viruria. In seronegative healthy individuals no cellular immunity is detectable[54].

A defined association between viremia dynamics and BKV-specific cellular immunity has been evidenced. In a study on renal transplant recipients, cellular response to both Large T antigen and VP1 resulted significantly lower in patients with increasing or persistent viral load (n = 22 patients) in comparison to those with decreasing (al least 2 $\log_{10}$ copies/mL) viral load or past PVAN (n = 20 patients)[57]. An example of the course of BK viral load and virus-specific cellular immune response in a kidney transplant recipient with polyomavirus reactivation treated with pre-emptive reduction of maintenance immunosuppression has been reported by Comoli and colleagues[55]: the emergence of BKV-specific T-cells coincides with the reduction of viral load; the concomitant reduction of serum creatinine indicates stabilization of graft function. This last finding seems to argue against the hypothesis that mounting of cellular immunity is a major determinant of tissue damage, as previously proposed for hemorrhagic cystitis in bone marrow transplantation[54,57,59]. Nevertheless, an inflammatory reaction mediated by different effectors may contribute to graft damage in case of prolonged viral cytopathic damage[60,61]. Overall, these observations represent the basis by which it seems reasonable to manage therapeutic modulation by complementing quantification of viral load (virological monitoring) with measurement of specific cellular immunity (viro-immunological monitoring).

Prolonged and deep immunosuppression is considered as the most important determinant in the development of PVAN. In particular, although viral replication and PVAN has been associated to the overall level of immunosuppression rather than a specific drug, triple therapy including tacrolimus, mycophenolate mofetil and prednisone[62-64] seems to be associated with a higher risk than cyclosporine. The mechanism of action of anti-rejection drugs interferes with the T cell activity. Calcineurin inhibitors (i.e., cyclosporine and tacrolimus) interfere with T cell activation (signal-1); whereas mammalian target of rapamycin (mTOR) inhibitors (i.e., sirolimus and everolimus) and anti-proliferative agents (i.e., azathioprine and mycophenolate mofetil) interfere with T cell proliferation downstream of the interleukin-2-receptor activation (signal-3)[35,63,65].

Tacrolimus trogh levels > 8 ng/mL, and higher doses of tacrolimus or mycophenolate mofetil, have been associated to polyomavirus replication and the development of PVAN[66]. Conversely, the reduction of tacrolimus trough levels to 6 ng/mL and of the daily dose of myco-

Table 4 Recommended treatment of polyomavirus-associated nephropathy by reduction or switching of immunosuppression[49]

Switching	Decreasing
Tacrolimus → Cyclosporine A (trough levels 100-150 ng/mL)	Tacrolimus (trough levels < 6 ng/mL)
Mycophenolate mofetil → Azathioprine (dose ≤ 100 mg/d)	Cyclosporine A (trough levels 100-150 ng/mL)
Tacrolimus → sirolimus (trough levels < 6 ng/mL)	Mycophenolate mofetil dose ≤ 1 g/d
Mycophenolate mofetil → sirolimus (trough levels < 6 ng/mL)	Cyclosporine A (trough levels 100-150 ng/mL)
Mycophenolate mofetil → leflunomide	

phenolate mofetil to ≤ 1 g, determined an improvement or stabilization of PVAN in most of the cases[67]. According to current consensus recommendations[49], tacrolimus trough levels should be targeted to 6 ng/mL, cyclosporine A to 100-150 ng/mL, and mycophenolate mofetil should be reduced to a daily dose ≤ 1 g or discontinued.

As no effective antiviral therapy is available, the milestone of treatment is represented by the pre-emptive reduction of immunosuppression on the basis of virological monitoring, although no protocol has been defined.

Based on the knowledge of the mechanisms of action, it can be hypothesized that the frequency of BKV-specific IFN-γ producing T cells is impacted by the immunosuppressive treatment. A recent study, using IFN-γ ELISPOT assays, investigated immunosuppressive drug levels and BKV Large T antigen-specific T cell responses in kidney transplant recipients *in vivo* and in healthy donors after titrating immunosuppression *in vitro*[68]. Based on their results, in kidney transplant patients *in vivo* ($n = 16$), responses resulted inversely correlated with tacrolimus trough levels ($P < 0.002$), but not with mycophenolate mofetil, prednisone or the overall immunosuppressive dosing. *In vitro* tacrolimus concentrations > 6 ng/mL resulyed in inhibition of BKV-specific T cell responses more than 50%, while inhibition was less than 30% with tacrolimus concentration < 3 ng/mL. Cyclosporine A resulted in > 50% inhibition of BKV-specific cellular responses at concentrations of 1920 ng/mL and less than 30% at concentrations below 960 ng/mL (corresponding to clinical C_0 trough levels of 200 and 100 ng/nL, respectively). No inhibition of BKV-specific T cell responses was observed for mycophenolate mofetil levels up to 8 μg/mL, leflunomide 50 g/mL, or sirolimus concentrations of 64 ng/mL. Overall, the results obtained by Egli and colleagues[68] suggested that calcinuerin-inhibitor concentrations are crucial for the development and/or recovery of BKV-specific T cell responses. Therefore, reduction of calcineurin inhibitors should be considered as the first line of intervention in the presence of a presumptive diagnosis of PVAN, whereas switching to mTOR inhibitors may represent an alternative or the second line of intervention. These data should be confirmed in clinical trials.

TREATMENT

There is no approved and defined treatment for PVAN. The main line of intervention is by reducing immmunosuppression. Antiviral agents have been used, but no defined treatment is recommended and results are conteoversial. More recently, the use of the immunomodulant agent leflunomide, together with the reduction of immunosuppression has been proposed.

As the majority of cases of PVAN have been associated to triple immunosuppressive therapy including combinations of calcineurin inhibitors (tacrolimus, cyclosporine A), antiproliferative agents (mycophenolate mofetil, azathioprine) and corticosteroids, most of recommended strategies includes decreasing, switching or stopping the ongoing treatment.

Immunosuppressive reduction

The recommended treatment of PVAN by modification of maintenance immunosuppression is summarized in Table 4[49]. Discontinuation of mycophenolate mofetil or azathioprine and reduction of immunosuppression by 25%-50% were commonly used strategies[69]. Graft failure after immunosuppression reduction alone can be observed in approximately 8% of the patients[69]. Following reduction of immunosuppression, biopsy-proven acute rejection has been observed in approximately 25% of patients[49]. These episodes of rejection may be treated by steroid without recurrence of PVAN[12,63].

Acute rejection and PVAN may coexist. In cases of concurrent biopsy-confirmed acute rejection and PVAN, a two-step approach of anti-rejection treatment followed by the reduction of immunosuppressive treatment has been adopted by several studies, that obtained the stabilization or improvement of allograft function[12,31,70,71].

The response to the immunosuppression reduction should be monitored by viruria and viremia evaluation every 2-4 wk[49]. Some studies evidenced clearance of viruria and viremia in most of the patients, with clearance rates ranging from 7% to 80% for viruria and from 40% to 96% for viremia. However, based on creatininemia evaluation, renal function did not always improve with the reduction of immunosuppression[72-74].

Immunosuppression reduction with antivirals

Antiviral agents, in particular cidofovir, have been investigated in addition to the reduction of immunosuppression for the management of PVAN. However, results evidenced a scarce, if any, significant effect in clearing viruria or viremia[75-78]. Other studies reported clearance of viremia in 50% to 100% of the cases[75,79-82]. The effect of cidofovir on renal function was variable, with some studies evidencing stabilization of creatinine[79-81] and others

showed no effect on renal function[75,77]. The pronounced nephrotoxicity limits the use of cidofovir particularly in renal transplantation and an adequate hydratation is required. Vidarabine is used in the treatment of BKV-associated cystitis in bone marrow transplant recipients; its efficacy in nephritis is unknown[33].

Immunosuppression reduction with leflunomide

More recently, the use of the immunomodulant agent leflunomide has been proposed for the treatment of patients with PVAN, in addition to the reduction of immunosuppression. Leflunomide is an immunosuppressive agent, however its metabolite A77 1726 exhibits antiviral activity *in vitro*. Among the few studies that have evaluated the role of leflunomide in treating PVAN, three reported results of viral clearance with treatment[83-85] with significant decreases in viremia and viruria with leflunomide alone or leflunomide plus cidofovir. Moreover, treatment with leflunomide stabilized or improved renal function in most of the cases.

Other interventions and retransplantation

Among drugs having evidenced a polyomavirus-inhibitory activity *in vitro*, beside cidofovir and leflunomide, there are certain quinolone antibiotics. Based on the results of a recent study, a 1-mo fluroquinolone course after transplantation is associated with significantly lower rates of BK viremia at 1 year in comparison to patients with no fluoroquinolone, therefore suggesting the usefulness of these antibiotics at preventing viremia in kidney graft recipients[86]. However, these results should be further confirmed by other studies, given the lack of clinical trials.

Amantadine has been used in the treatment of PVAN with poor effect[33]. γ globulin has been used in order to augment the immune response, however the real efficacy is unknown[33].

Consideration of retransplantation in the context of PVAN has become an increasingly relevant issue, with some unsolved questions regarding timing of retransplantation, i.e., preemptive *vs* after progression to renal failure. There is only limited experience about this in patients who have lost a previous graft due to PVAN. Retransplantation has been reported in a total of 21 cases[87-90], four of which were performed pre-emptively. All preemptive cases were performed with concomitant graft nephrectomy because of the risk of possible reinfection. Nevertheless, Cooper and colleagues[90] reported for the first time a successful preemptive retransplantation for PVAN in a patient without simultaneous graft nephrectomy. This cases evidenced that retransplantation could be pursued without nephrectomy for patients with PVAN provided the absence of viral replication and an active surveillance protocol. The need for close monitoring of viral replication both in the immediate posttransplantation setting (to minimize the risk of development of PVAN) and in the context of graft failure (to indicate proper management and retransplant option) remains critical.

CONCLUSION

In the past decade, PVAN has emerged as one of the most relevant viral diseases occurring in renal transplantation. Its increasing incidence has underlined the role played by immunosuppression in its pathogenesis. The major determining factors are now recognized as the occurrence of uncontrolled viral replication and the failure of immune surveillance. Therefore, since a preemptive reduction of immunosuppression represents the mile stone for the treatment of PVAN, virological and viro-immunological monitoring are necessary and should be performed according to current recommendations. At moment, the reduction of immunosuppression represents the first line of intervention, however clinical controlled trials are required to identify the best therapeutic strategies. A multidisciplinary approach is fundamental to optimize the clinical management of renal transplant recipients and, despite of the relevance of consensus recommendations, these cannot substitute for clinical judgement and individualized care taking into account the different points of view.

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