Complications post renal transplantation: literature focus on BK virus nephropathy and diagnostic tools actually available

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Abstract
Clinical diagnosis of kidney transplants related illnesses is not a simple task. Several studies were conducted to define diseases and complications after renal transplantation, but there are no comprehensive guidelines about diagnostic tools for their prevention and detection.

The Authors of this review looked for the medical literature and pertinent publications in particular to understand the role of Human Polyomavirus BK (BKV) in renal failure and to recognize analytical techniques for BK virus associated nephropathy (BKVAN) detection.

Introduction
Clinical diagnosis of kidney transplants related illnesses is not a simple task. Several studies were conducted to define diseases and complications after renal transplantation, but there are no comprehensive guidelines about diagnostic tools for their prevention and detection.

The Authors of this review looked for the medical literature and pertinent publications in particular to understand the role of Human Polyomavirus BK (BKV) in renal failure and to recognize analytical techniques for BK virus associated nephropathy (BKVAN) detection. For reviewing we used Medline and recent pertinent bibliographies.

Kidney pathologies in renal transplants are associated with graft function, immunosuppressive drugs and infections [1]. Moreover cardiovascular, bone and bone marrow diseases, metabolism dysfunctions and cancers could affect these patients [2,3]. Graft function is the most important parameter in evaluation of the allograft status; acute rejection, obstruction, renal artery stenosis could influence renal function resulting in graft dysfunctions and ultimately in chronic renal allograft failure [1,4,5]. Persistent urinary protein excretion and hyperlipidemia are associated with acute rejection, in particular heavy proteinuria has important consequences for extracellular fluid volume regulation and demonstrate the rapid deterioration of renal function associated with pathologic glomerular lesions [6,7]. Serum creatinine levels and urine protein/creatinine ratio (total protein excretion) should be used to screen for changes in renal function. Acute allograft rejection could be also due to interstitial
infiltrates and mild tubulitis that unfortunately are clinically silent and could be detected only by immunohistochemistry (IHC) [1].

**Immunosuppression therapy**

The morbidity and mortality rates associated with renal transplantation and the use of immunosuppressive medications are high. Conventional immunosuppression is based on azathioprine, nevertheless, other immunosuppressive drugs, such as cyclosporine A (CsA), tacrolimus, sirolimus, mycophenolate-mofetil (MMF) and corticosteroids are used [1,8]. To reduce adverse effects of immunosuppressive therapies, it is strongly recommended to monitor routinely blood level of CsA, tacrolimus and sirolimus. The nephrotoxicity associated with azathioprine and MMF is monitored by assessing hemoglobin levels, hematocrit value and white blood cell counts at least weekly for months 1 to 2, every 2 week for months 3 to 4, monthly for months 4 to 12, and then every 3 to 6 months [1,8-12]. Finally toxicity related to corticosteroids is monitored periodically by controlling blood pressure, lipoprotein levels and blood glucose levels [8,11]. Compared with conventional immunosuppression with azathioprine, CsA reduced the incidence of acute rejection and prolonged graft survival but caused chronic tubulointerstitial atrophy and fibrosis that are difficult to distinguish from chronic allograft nephropathy attributable to other causes [1,13]. Instead the role of acute and chronic tacrolimus nephrotoxicity in graft failure is unclear. However the incidence of renal toxicity is roughly proportional to tacrolimus doses and its blood levels [14].

In the other hand sirolimus seems to be efficacious in preventing acute rejection when used in place of, or in combination with, CsA. However very few studies have been conducted to determine the relationship between blood levels of sirolimus and either acute rejection or toxicity [10]. Regarding azathioprine and MMF, hematologic and gastrointestinal toxicities are usually dose-related and respond to dose reductions [12]. Moreover MMF causes leukopenia in renal transplants. Finally clinical signs of corticosteroid toxicity, which are observed relatively soon after the initiation of prednisone treatment, include skin changes, hypertension, peptic ulcer disease and myopathy [8].

**Human Poliomavirus BK and BKVAN**

Viral infections cause several complications in renal transplants that are closely related with the immunosuppressive therapy. On the basis of literature data, viruses implicated in graft failure we could number Varicella zoster, Cytomegalovirus, Influenza A and B, Hepatitis B and C and human Poliomavirus BK and JC [15-18]. In particular BK virus, described for the first time in a transplant recipient, has a remarkable tropism for the genitourinary tract, in fact BKVAN are recognized as an important cause of late allograft failure [19].

BKV is ubiquitous in human populations worldwide. BKV infects young children and the seroprevalence is 70–80% in adults [20,21]. Serologic surveys of populations, using hemagglutination inhibition assay for the detection of antibodies, indicate that seroconversion takes place early in life, at 5–7 years of age [20,21]. Primary infection is usually inapparent and only occasionally may be accompanied by mild respiratory illness or urinary tract disease. During primary infection viremia occurs and the virus spreads to several organs of the infected individual where it remains in a latent state. After the initial infection, the virus disseminates and establishes a persistent infection in the urinary tract and maybe in lymphocytes [20,22,23].

The complete genome of BKV contains 5,153 bp and it is functionally divided into three regions: the early, the late, and the transcriptional control region (TCR). The first region codes for the small and large T-antigens (t-Ag and T-Ag), the second region codes for the viral capsid proteins VP1-VP2-VP3 and agnoprotein, and the last region (TCR) contains the transcriptional control elements for both "early" and "late" gene expression [24] Primary transcripts are required for viral replication, in particular T-Ag promotes unwinding of the double helix and recruitment of cellular proteins required for DNA synthesis whereas in non permisive cells it is involved in neoplastic transformation [24,25] (Fig. 1). Late transcripts encode for viral capsid proteins and agnoprotein, that has a critical role in the regulation of viral gene expression and replication, and in the modulation of certain important host cell functions including cell cycle progression and DNA repair [26]. TCR contains the origin of replication and it is arbitrarily divided into four box alphabetically designated P, Q, R and S. These sequence blocks serve as regulatory regions, or enhancer elements believed to contain several transcription factor binding sites involved in the modulation of viral transcription [24,27,28]. It is not known that genetic alterations are essential for the pathogenesis associated with BKV after kidney transplantation, nevertheless BK-strains with rearranged TCR have been particularly described in subjects under immunosuppressive therapies [24,29,30]. In renal transplants BKV infection may be transmitted via the donor organ, may be acquired in the community or latent BKV could reactivate [31,32]. The incidence of allograft failure has ranged from 15 to 50% in affected individuals [33], but few data are available about BKVAN; it probably due to recent emerging of this disease as an important cause of allograft failure following renal transplantation. BKV urinary shedding of infected urothelial cells occurs in 10 to 60% of renal transplant recipients [34] and literature data suggest that prospective
monitoring of patients at risk for BKVAN may identify those with active infection before renal function deteriorates [35-37]. Recent studies demonstrated that BKVAN develop in as many as 8% of renal allograft recipients, with as many as 50% of patients experiencing graft loss over the next 2 to 3 years of follow-up [34,38,39]. A current study performed by Giraldi et colleagues show that, in a cohort of the 117 patients followed up every three months during a two year period after transplantation, 4 had BKVAN (3.4%) confirmed by quantitative assays on plasma and urine and assessed by allograft biopsy [40].

BKVAN diagnosis
BKVAN diagnosis is very difficult since this disease is often misdiagnosed as acute rejection or drug toxicity. Diagnostic tools available include histopathology by means of renal allograft biopsy, detection of BKV DNA on plasma and urine by polymerase-chain-reaction (PCR) and quantitative PCR (QPCR) and presence of "decoy cells" in the urine sediment. Diagnostic confirmation may be obtained using IHC, in situ hybridization (ISH), and/or electron microscopy (EM) in renal biopsy specimens [34,41-45].

Early identification provides the opportunity for intervention with reduction of the immunosuppression in an effort to control BKV replication and prevent BKVAN. The risk factors predisposing to BKVAN appear to be multiple, with immunosuppressive regimens containing tacrolimus and MMF representing recognized associations [41,46]. Several investigators have begun to define risk factors for BKV disease among renal transplant recipients. The serologic status of the donor and the recipient appears to be a predictor of BKV infection, but it is not currently clear whether it influences the development of BKV nephritis. Tubular injury could be a factor promoting viral replication in an immunocompromised state induced by tacrolimus or MMF. The load of dormant BKV in the grafted organ is likely to be another important risk factor: no dormant virus, no re-activation and most likely, no BKVAN [47]. On these basis, since no specific anti-viral therapy is available, reduction in immunosuppression remains the mainstay of treatment with an increased risk of subsequent rejection. Therefore an accurate diagnosis is important, as it allows for early intervention and possible recovery of renal function.

Urine cytology is based on decoy cells recovery. Decoy cells are epithelial cells with enlarged nuclei and large basophilic ground-glass intranuclear viral inclusions, screening for their presence provides a simple and an inexpensive tool for the diagnosis of BKV nephropathy, nevertheless, Papanicolaou-stained urine sediment is not to be considered a specific morphological marker of BKV disease [48,49].

Electron microscopy is very sensitive for detection of BK virions, but the finding of viral particles is not diagnostic of BKVAN, since the ultrastructural appearance of BK virus is poorly typical. Virions are arranged in paracrystalline arrays of naked, round, electron-dense structures that measure 45 nm in diameter. It is important to emphasize that electron microscopy cannot distinguish BKV from JC virus [41] (Fig. 2).

The histological diagnosis of BKVAN requires evaluation of a renal biopsy with demonstration and confirmation of the polyomavirus cytopathic changes by IHC and ISH [41]. BKVAN is characterized by the presence of polyomavirus cytopathic changes in the epithelium of the renal tubules and urothelial lining. The infected cells have an enlarged nucleus with a gelatinous basophilic inclusion resulting from the accumulation of the newly formed virions [50]. Confirmation of the polyomavirus infection is usually performed with immunohistochemical stains for the simian virus 40 (SV40) large T antigen (AgT), which identifies all polyomavirus infections due to cross-reactivity between SV40 and both BKV and JCV. Distinction between the different types of polyomavirus requires the use of species-specific antibodies, ISH or in situ PCR. Systematic studies comparing the clinical utility of each method have not been performed [50]. The sections are
stained with hematoxylin-eosin and examined by means of light microscopy in order to evaluate the integrity of the tissue before proceeding to molecular analysis, to identify possible pathologic changes, and in particular to search for the presence of morphologic equivalents of cellular polyomavirus infection. In situ hybridization and immunohistochemistry are carried out to define the viral status of the infected tissues. The reactions are detected by means of the streptavidin-biotin method and are revealed using diaminobenzidine as a chromogen. In situ hybridization is performed to localize the nucleic acid sequences of BKV and JCV at the subcellular level using commercially available biotinylated DNA probes [51].

For efficient early diagnosis of BKVAN, various molecular approaches are recommended. Quantitative PCR is a non-invasive method clinically useful since it is high sensitive and specific and it supplies quantitative data that allow pharmacological therapy management by clinicians because specific antiviral therapy for BKVAN does not currently exist and the reduction in immunosuppression depend on viral loads in urine and plasma specimens of kidney transplants [32,33,36,52]. Nevertheless it is important to underline that the relationship between BKV viruria and viremia, the cut-offs and predictive values of BKV viruria and viremia for the occurrence of BKVAN, are still largely undefined [33]. In fact some literature studies from 2004 to nowadays showed that measurements of BKV viruria and BKV viremia have a different prognostic value for patient's therapeutic response and duration of therapy. In accordance with Drachenberg et colleagues BKV viruria precedes BKV viremia and it is a prerequisite for histologically proven BKVAN because the viral replication within the graft finally leads from viruria to viremia [53]. This hypothesis is also sustained by other Authors that maintained that viremia is not present in patients with low-level/limited viral replication in the urinary tract [34,43,44,52,54]. Moreover, in relation to these Authors, viremia is not useful for screening because of blood inhibitors present in plasma sample. Finally, although analytical and physiological variations may be significant when comparing viral urine load in patients with BKVAN, there is general agreement that repeated values above 10^7 BKV copies per milliliter are associated with BKVAN [32,53]. On the other hand, a recent study performed by Basse et collaborators suggested that BKV viremia is a rare event after renal transplantation but it has emerged as the most specific test for BKV associated nephropathy [55]. Some Authors retain BKV viremia as the standard for BKVAN diagnosis since the presence of the virus in the blood represents a significant tissue damage and confirm the renal parenchymal involvement [37,56]. Therefore serial determinations of BK viremia are the best tool to demonstrate resolution of the disease after immunosuppression has been decreased [37,55-58]. Nevertheless, a study carried out by Hymes et colleagues from June 2003 to January 2006 on 20 renal transplant children showed that most patients remained PCR-positive despite reduction of immunosuppression. Moreover they did not identify any one drug as more prevalent among patients with BK viremia [59].

**Conclusion**

In conclusion, there are several aspects of BKVAN pathology in kidney transplant patients requiring evaluation; it includes BKV transmissibility within kidneys transplanted, target organ effects, risk factors, time frame of reactivation and the best treatment options. Therefore it is essential to understand and to monitor the delicate balance between viral infection, immune regulation in the transplant population and immunosuppressive therapy in order to minimize viral injury and rejection risk to patients with BKV infection. Measuring of BKV DNA in urine and serum is an useful and non invasive tool for early detection and monitoring, nevertheless a combined approach of molecular techniques must be utilized to identify BK virus-associated nephropathy at an early phase facilitating well timed clinical intervention.
References

1. Kasieke BL, Vazquez MA, Harmon WE, Brown RS, Danovitch GM, Gaston RS, Roth D, Scadding JD, Singer GG, for the American Society of Transplantation: Recommendations for the outpatient surveillance of renal transplant recipients. J Am Soc Nephrol 2000, 11:S1-S86.

2. Jelola TK, Ross H, Smith R, Huang M, Fenton S, Catran D, Schiff J, Cardella C, Cole E: Renal transplant outcome in high-cardiovascular risk recipients. Clin Transplant 2007, 21:609-614.

3. Matignon M, Dan K, Fruchaud C, Audard V, Grimbert P, Lang P: Kidney transplantation: indications, results, limitations, and perspectives. Presse Med 2007, 36:1829-1834.

4. Foster CE, Weng RR, Smith CV, Imagawa DK: The influence of organ acceptance criteria on long-term graft survival: outcomes of a kidney transplant program. Am J Surg 2007, 195:149-152.

5. Johnston O, O’Kelly P, Spencer S, John Donohoe, Walshes J, Little DM, Hickey D, Connor J: Reduced graft function (with or without dialysis) vs immediate graft function—a comparison of renal allograft recipients. Nephrol Dial Transplant 2006, 21:2270-2274.

6. Sancho A, Gavela E, Avila A, Morales A, Fernández-Nájera JE, Crespo JF: Ultrastructural evidence for virus infection in man. Adv Exp Med Biol 1968, 20:323-330.

7. Hirsch HH, Steiger J: Polyomavirus BK. Lancet Infect Dis 2003, 3:611-623.

8. Kim HS, Benson JW, Frisque RJ: Transcription and replication in the human polyomaviruses. In Human Polyomaviruses Edited by: Wiley-Liss. INC. New York; 2001:73-126.

9. Agha I, Brennan DC: BK virus and immunosuppressive agents. Adv Exp Med Biol 2006, 577:174-184.

10. Randhawa P, Zygmont D, Shapiro R, Vats A, Weck K, Swalsky P, Finkelstein S: Viral regulatory region sequence variations in kidney tissue obliterates with BK virus nephropathy. Kidney Int 2003, 64:743-747.

11. Harihara S: BK virus nephritis after renal transplantation. Kidney Int 2006, 69:655-662.

12. Hirsch HH: BK virus: opportunity makes a pathogen. Clin Infect Dis 2005, 41:354-360.

13. Djamali A, Samaniego M, Much B: Medical care of kidney transplant recipients after the first posttransplant year. Clin J Am Soc Nephrol 2006, 1:623-640.

14. Pang XL, Doucette K, LeBlanc B, Godfried SM, Preiksaitis JK: Monitoring of polyomavirus BK virus viruria and viremia in renal allograft recipients by use of a quantitative real-time PCR assay: one-year prospective study. J Clin Microbiol 2007, 45:3568-3573.

15. Drachenberg CB, Beskow CO, Cangro CB, Bourquin PM, Simsir A, Fink J, Weir MR, Klassen D, Bartlett ST, Papadimitriou JC: Human polyomavirus in renal allograft biopsies: morphological findings and correlation with urine cytology. Hum Pathol 1999, 30:970-977.

16. Hirsch HH, Knowles W, Dickenmann M, Passweg J, Klimkait T, Mihatsch MJ, Steiger J: Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. N Engl J Med 2002, 347:488-496.

17. Nickelet V, Klimkait T, Binet IF, Dalquen P, Del Zenero V, Thiel G, Mihatsch MJ, Hirsch HH: Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. N Engl J Med 2000, 342:1309-1315.

18. Alagandani GJ, Thyagarajan R, Gruber SA, Morawski K, Garlick J, El-Amm JM, West MS, Sillix DH, Chandrasekar PH, Harihara A: Infections complications after kidney transplantation: current epidemiology and associated risk factors. Clin Transplant 2006, 20:401-409.

19. Beimler J, Sommerer C, Zeier M: The influence of immunosuppression on the development of BK virus nephropathy—does it matter? Nephrol Dial Transplant 2007, 22:66-71.

20. Giraldi C, Noto A, Tenuta R, Greco F, Perugini D, Dodaro S, Spadafora M, Lo Bianco AM, Savino O, Papalia T, Greco R, Bonfiglio R: Prospective study of BKV nephropathy in 117 renal transplant recipients. New Microbiol 2007, 30:127-130.

21. Lau F, Zaman F, Veeramachaneni R, Jones L, Uribe-Uribe N, Turbat-Herrera EA, Herrera GA: BK polyomavirus in renal transplants: role of electronic microscopy and immunostaining in detecting early infection. Ultrastruct Pathol 2007, 31:199-207.

22. Mannion RB, Hoffmann SC, Kampen RL, Cheng OC, Kleiner DE, Ryschkewitsch C, Amm JM, West MS, Sillix DH, Chandrasekar PH, Harihara A: Evaluation of BK polyomavirus nephropathy. N Engl J Med 2000, 342:1309-1315.

23. Mitterhofer AP, Mitterhofer AM, Tinti F, Barile M, Dal Maso M, Chiarini F, Pietropaolo V: BK virus receptors and tropism. J Am Soc Nephrol 2004, 15:1571-1575.

24. Randhawa P, Vats A, Shapiro R: The pathobiology of polyomavirus infection in man. Adv Exp Med Biol 2006, 577:148-159.

25. Knowles WA: Discovery and epidemiology of the human polyomaviruses BK virus (BKV) and JC virus (JCv). Adv Exp Med Biol 2007, 577:197-204.

26. Lundst A, Dillner J: Serological diagnosis of human polyomavirus infection. Adv Exp Med Biol 2006, 577:96-101.

27. Ashok A, Axwood WJ: Virus receptors and tropism. Adv Exp Med Biol 2007, 577:60-72.

28. Doerries K: Human polyomavirus JC and BK persistent infection. Adv Exp Med Biol 2006, 577:102-116.
46. Binet I, Nickeleit V, Hirsch HH, Prince O, Dalquen P, Gudat F, Miehatsch M, Thiel G: Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. Transplantation 1999, 67:918-923.

47. Van Gorder MA, Della Pelle P, Henson JW, Sachs DH, Cosimi AB, Colvin RB: Cynomolgus polyoma virus infection: a new member of the polyoma virus family causes interstitial nephritis, ureteritis, and enteritis in immunosuppressed cynomolgus monkeys. Am J Pathol 1999, 154:1273-1284.

48. Kapila K, Nampoory MR, Johny KV, Pacsa AS, Ali-Ayadh B, Mathew JR, Nair MP, Halim MA, George SS, Francis IM: Role of urinary cytology in detecting human polyoma bk virus in kidney transplant recipients. A preliminary report. Med Princ Pract 2007, 16:237-239.

49. Kipp BR, Sebo TJ, Griffin MT, Ihrler JM, Halling KC: Analysis of Polyomavirus-Infected Renal Transplant Recipient’s Urine Specimens. Am J Clin Pathol 2005, 124:854-861.

50. Drachenberg CB, Papadimitriou JC: Polyomavirus-associated nephropathy: update in diagnosis. Transpl Infect Dis 2006, 8:68-75.

51. Boldorini R, Veggiani C, Barco D, Monga G: Kidney and urinary tract polyomavirus infection and distribution: molecular biology investigation of 10 consecutive autopsies. Arch Pathol Lab Med 2005, 129:69-73.

52. Mannon RB: Polyomavirus nephropathy: what have we learned? Transplantation 2004, 77:1313-1318.

53. Drachenberg CB, Hirsch HH, Ramos E, Papadimitriou JC: Polyomavirus disease in renal transplantation: review of pathological findings and diagnostic methods. Hum Pathol 2005, 36:1245-1255.

54. Vera-Sempere FJ, Rubio L, Felipe-Ponce V, Garcia A, Mayordomo F, Sanchez-Plumed J, Beneyto I, Ramos D, Zamora I, Simon J: PCR assays for the early detection of BKV infection in 125 Spanish kidney transplant patients. Clin Transplant 2006, 20:706-711.

55. Basse G, Mengelle C, Kamar N, Guitard J, Ribes D, Esposito L, Rostaging L: Prospective evaluation of BK virus DNAemia in renal transplant patients and their transplant outcome. Transplant Proc 2007, 39:84-87.

56. Randhawa P, Ho A, Shapiro R, Vats A, Swalsky P, Finkelstein S, Uhrmacher J, Weck K: Correlates of quantitative measurement of BK polyomavirus (BKV) DNA with clinical course of BKV infection in renal transplant patients. J Clin Microbiol 2004, 42:1176-1180.

57. Leung AY, Chan M, Tang SC, Liang R, Kwong YL: Real-time quantitative analysis of polyoma BK viremia and viruria in renal allograft recipients. J Virol Methods 2002, 101:51-56.

58. Limaye AP, Jerome KR, Kuhner CS, Ferrenberg J, Huang ML, Davis CL, Corey L, Marsh CL: Quantitation of BK virus load in serum for the diagnosis of BK virus-associated nephropathy in renal transplant recipients. J Infect Dis 2001, 183:1669-1672.

59. Hymes LC, Warshaw BL: Polyomavirus (BK) in pediatric renal transplants: evaluation of viremic patients with and without BK associated nephritis. Pediatr Transplant 2006, 10:920-922.