BKV-infection in kidney graft dysfunction

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ABSTRACT

Introduction: BKV nephropathy (BKN) causes kidney graft loss, whose specific diagnosis is invasive and might be predicted by the early detection of active viral infection. **Objective:** Determine the BKV-infection prevalence in late kidney graft dysfunction by urinary decoy cell (DC) and viral DNA detection in urine (viruria) and blood (viremia; active infection). **Methods:** Kidney recipients with >1 month follow-up and creatinine >1.5 mg/dL and/or recent increasing >20% (n = 120) had their urine and blood tested for BKV by semi-nested PCR, DC searching, and graft biopsy. PCR-positive patients were classified as 1+, 2+, 3+. DC, viruria and viremia prevalence, sensitivity, specificity, and likelihood ratio (LR) were determined (Table 2x2). Diagnosis efficacy of DC and viruria were compared to viremia. **Results:** DC prevalence was 25%, viruria 61.7%, and viremia 42.5%. Positive and negative patients in each test had similar clinical, immunossupressive, and histopathological characteristics. There was no case of viremia with chronic allograft nephropathy and, under treatment with sirolimus, patients had a lower viruria prevalence (p = 0.043). Intense viruria was the single predictive test for active infection (3+; LR = 2.8).[1,6-4,9] **Conclusion:** DC, BKV-viruria and -viremia are commun findings under late kidney graft dysfunction. Viremia could only be predicted by intense viruria. These results should be considered under the context of BKN confirmation.

Keywords: BK virus, decoy cells, kidney transplantation, PCR, viremia, viruria.
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INTRODUCTION

Opportunistic infections are a major cause of morbidity after transplantation. Such fact has stimulated studies toward virus epidemiology, biology, and pathogenic effects among this high risk population.[1] BK virus (BKV), a member of the Polyomavirus family, is a common viral infection, as cytomegalovirus, which is associated with high risk of renal disorder after kidney transplantation.[2]

The primary infection occurs during early childhood, and becomes latent in the kidneys. As soon as the immunosuppression starts at the time of transplantation, the virus can reactivate and develop a clinically relevant disease, which is manifested as tubulointersticial nephritis (known as BKV nephropathy [BKN] and/or ureteral stenosis).[3-6]

BKN has been associated with graft dysfunction in 3% to 14% of kidney recipients, leading to graft failure in up to 67% of the cases.[2,7,8] Specific diagnosis of BKN depends on the pathological tissue patterns on the graft biopsy specimen, which can be confounded with cellular rejection mainly when both pathologies coexist. Thus, BKN diagnosis, based exclusively on the pathological criteria, might be difficult.[4-11]

This study was designed to investigate the prevalence of BKV infection among patients under late kidney graft dysfunction. It combines three non-invasive methodologies: urinary cytology (looking for decoy cells; DC), viruria (urinary viral DNA), and viremia (active infection; blood viral DNA), and takes into consideration the importance of an early diagnosis and the need of graft biopsy invasiveness to define a suspicion of BKN.

PATIENTS AND METHODS

Patients

Kidney transplant recipients (n = 120), from living or deceased donors, with serum creatinine levels above 1.5 mg/dL or recent increase of > 20%[2,12] were allocated during 1 year of

outpatient follow-up. After signing an informed consent, previously approved by the local Ethic Committee, patients performed a graft biopsy, and urine and blood samples were collected. Patients who refuse to participate, those younger than 18 years old or less than 1 month of post-transplant follow-up, and patients with a known and reversible cause for graft dysfunction were excluded from the study.

Urinary cytology

Papanicolau standard staining was performed in urinary sediment. DC were recognized for their typical round-glass intranuclear inclusions with a peripheral of hyperchromatic chromatin.[13-15]

Molecular biology

DNA extraction

Pellet urinary sediment was used for DNA extraction following the protocol previously published by Takayama *et al*.[4] Blood samples collected in EDTA anticoagulant were submitted to DNA extraction using a commercial kit (PureLinkGenomic, Invitrogen, USA).

Polymerase Chain Reaction (PCR)

Semi-nested PCR was performed in order to detect the BKV DNA, as validated by Nickeleit *et al*.[13] The outer primer pair, 5' AAGTCTTTAGGGTCTTCTAC 3' and 5' GTGCCAACCTATGGAACAGA 3', was used to generate a 176-bp, a common amplicon among *Polyomaviridae* memberships. It was used the primer 5' AAGTCTTTAGGGTCTTCTAC 3' with 5' GAGTCCTGGTGGAGTTCC 3' in order to obtain a 149-bp fragment, a specific BKV-region.[16]

A spectrophotometer, using a 260 nm length wave (Bio Photometer, Eppendorf, Germany), was used to standardize the total DNA content at 0.5 and 0.1 μg in blood and urine samples, respectively, for PCR reaction. Amplification steps were performed from a 25 μl reaction-mixture containing 1.5 mM $MgCl_2$, 0.5 μM of each primer, dNTPs 200 μM and 2.5 U of Taq polymerase (Invitrogen, Brazil).

The reaction program consisted of 5 minute-denaturation at 94° C and were followed by 35 cycles of denaturation at 94° C during 1 minute, annealing at 56° C for 1 minute and extension at 72° C for 5 minutes. A final extension at 72° C for 10 minutes was added.

Free DNA samples were analyzed as negative controls, and DNA obtained from a known BKV-positive patient was used as positive control in each test. All negative tests had their content and quality DNA certified by amplification using a primer for β-globine detection.

Semi-quantitative PCR analysis

The amplified regions were detected in agarose gel 2.5% stained with ethidium bromide. The amplified urine and blood products were qualified as 1+, 2+, 3+, according to that observed at the transilluminator, corresponding, respectively, to weak, intermediate, and strong intensity of amplification.

Histology

All biopsies were classified according to Banff criteria 1997 for kidney graft pathologies definition.[17]

Statistical analysis

Data were expressed as percentage, mean and standard deviation or median and range. Comparisons of age and creatinine levels were performed by Student's t-test. Other characteristics and risk factors of patients were analyzed by Chi-squared or Fisher's exact test, as indicated. Sensitivity and specificity were described as percentage and likelihood ratio (LR), and were calculated by using Table 2x2. For study purposes, DC and viruria values were compared with the presence of BKV DNA in peripheral blood. The software *Statistical Package for Social Sciences 14.0* was used for all statistical analysis, being significant p values ≤ 0.05 and confidence intervals 95%.

RESULTS

Among the 120 kidney recipients under late graft dysfunction, the prevalence of DC was 25.0% (95% CI: 17.5-33.7), viruria 61.7% (95% CI: 52.4-70.4), and viremia 42.5% (95% CI: 33.5-51.9). Serum creatinine 2.3 mg/dL (0.8-8.5 mg/dL), and median follow-up was 1.6 years.

There was predominance of male gender 60.8% (n = 73/120) and 56.7% were recipients from deceased donors (n = 68/120). Patients receiving a second or third allograft represented 10.0% of the sample (12/120), and there was 16.7% (17/102) of anti-HLA class I pre-sensitized patients. Induction with immunosuppressants had been used by 15.0% (18/120) of them at the time of transplantation, and current prophylatic immunosuppressive scheme was a combination of the following drugs: prednisone (n = 103; 94.2%), mofetil mycofenolate (n = 80; 66.7%), tacrolimus (n = 60; 50.0%), cyclosporine (n = 55; 45.8%), azatioprine (n = 24; 20.0%), and sirolimus (n = 14; 11.7%). General characteristics are presented in Table 1.

Clinical characteristics, pre-transplant immunological risk, current immunosuppressive protocol, and kidney histopathology findings were similar among negative and positive patients, taking into consideration each laboratory test performed. An exception was the higher rate of patients treated with sirolimus, showing a lower prevalence of positive urine BKV-DNA detection (p = 0.043). Another difference was observed among patients with current diagnosis of chronic allograft nephropathy (CAN). In this group, no case of BKV-DNA in peripheral blood p = 0.020) was detected.

Table 2 describes the comparison of diagnostic efficacy of urinary cytopathic effect (DC) and BKV-viruria with

Table 1. Clinical characteristics of kidney transplant recipients under late graft dysfunction studied for BKV infection

Recipient's Characteristics*	n (%)	
Male gender	73	60.8
Re-transplant	12	10.0
Pre-sensitization**	17	16.7
Deceased donor	68	56.7
Induction therapy***	18	15.0
Previous CMV disease	34	28.3
Previous AR	19	15.2
Previous CAN	14	11.7
CyA-Aza-Pred	17	14.2
CyA-MMF-Pred	31	25.8
FK-MMF-Pred	44	36.7
FK-Sirol-Pred	10	8.3

* n = 120
** patients evaluated for anti-HLA antibodies before transplantation (n = 102)
*** basiliximab
CMV: cytomegalovirus
AR: acute rejection defined by biopsy
CAN: chronic allograft nephropathy
CyA: cyclosporine
Aza: azatioprine
Pred: prednisone
MMF: mofetil mycofenolate
FK: tacrolimus
Sirol: sirolimus.

Table 2. Comparison of diagnostic efficacy of urinary cytopatic effect (DC) and BKV-viruria with BKV-viremia in kidney transplant recipients under late graft dysfunction

	BKV viremia + n (%)	BKV viremia - n (%)	LR	95% CI	Post test probability (%)
Urine BKV PCR (1)					
Negative	11 (21.6)	35(50.7)	0.43	0.24–0.75	24.1
1+ (2)	5 (9.8)	14 (20.3)	0.48	0.19–1.26	26.2
2+ (2)	8 (15.7)	7 (10.1)	1.55	0.60–3.99	53.4
3+ (2)	27 (52.9)	13 (18.8)	2.81	1.61–4.89	67.5
Decoy Cells (DC) (3)					
Negative	35 (68.6)	55 (79.7)	0.86	0.69–1.07	38.9
Positive	16 (31.4)	14 (20.3	1.55	0.83–2.87	53.3

Viruria: urine BKV PCR
Viremia: peripheral blood BKV PCR
Pb: peripheral blood
LR: likelihood ratio
CI: confidence interval
PCR: polymerase chain reaction
(1) Sensitivity: 78.4% (95% CI: 64.7-88.7), Specificity: 50.7% (95% CI: 38.4-63.0)
(2) Intensity of amplification: 1 (weak); 2 (intermediate); 3 (strong)
(3) Sensitivity: 31.4% (95% CI: 19.1-45.9), Specificity: 79.7% (95% CI: 68.3-88.4)
Prevalence of BKV in peripheral blood: 42.5% (95% CI: 33.5-51.9).

BKV-viremia among the studied patients. In this study, strong urine BKV amplification (3+) achieved a LR of 2.8 predicting viremia, indicating a post test probability (PTP) of 67.5% (95% CI: 1.61-4.89). The efficacy of DC for viremia prediction was not confirmed when taking into account CI achieved and its ability to improve the pre-test odds.

DISCUSSION

Early BKN diagnosis and reduction of the immunosuppressive therapy are the major strategies to improve graft survival and stabilize serum creatinine after kidney transplantation.[18-20]

The most important tool to establish this diagnosis depends on the graft pathological pattern, but the multifocal appearance in the parenchyma, the heterogeneous nature of viral inclusions, and the immunohistochemical findings compromise the accuracy of the anatomopathological BKN diagnosis.[2,21,22] As a consequence, complementary and non-invasive tests, such as DC into the urinary sediment, viruria and viremia detection, and renal function follow up,[9,19,22] can be important to early predict and manage nephropathy.

Among the population studied, the prevalence of DC, viruria, and viremia using these complementary tests were, respectively, 25%, 62%, and 42%. Besides these high frequencies of BKV infection signals,[5,21,23-26] this study also confirms the previous evidence of no clinical or histopathological characteristics associated with BKV infection.[2,24,26,27,28]

In agreement with others,[26-28] there was no association with acute rejection or with its treatment. On the other hand, reinforcing the importance of an early diagnosis, no case of viremia among patients with current CAN highlights their probable severe renal damage hindering the viral replication. Another interesting finding was the lower prevalence of viruria among patients under treatment with sirolimus. This drug acts as an anti-proliferative agent and has been suggested as an alternative treatment for viral replication control in established BKV disease.[30,31]

Molecular detection of polioviruses has been considered as a gold-standard, and its efficacy has been confirmed by a variety of studies.[2,13,24,32] Nowadays, regarding the countless different molecular techniques available for Polyomavirus detection,[16,32] quantitative PCR with viral load definition is the most sensitive and specific approach. Urinary DC, considered as having the higher negative predictive value up to now,[5,24] has been substituted by urine PCR. DC has a significant sensitivity for BKN detection, but PCR is up to 4 times more sensitive for viruria monitoring in asymptomatic patients, and also has the stability advantage upon the DC for that.[32,33] However, not taking viruria into account, as suggested by some authors,[34,35] can be contested.[36] Despite the controversy, the uncommon occurrence of viruria or viremia among health people are due to JCV, a less uropathogenic agent.[24,28,35,37-41] Hence, BKV viruria itself probably have clinical relevance.

The present study showed the urinary high BKV amplification intensity as the single test able to predict viremia occurrence (3+; LR = 2.8; 95% CI: 1.6-4.9). In this group, the presence of DC did not achieve clinical significance for viremia prediction (LR = 1.55; 95% CI: 0.8-2.9). Such finding becomes even more relevant due to the fact that viremia only occurs under active replication, not being observed during latent periods.[15,42] BKV-PCR can be clinically used as a non-invasive test in order to identify kidney transplant recipients under risk or suspicion of BKN. Nickeleit and coworkers demonstrated that the viremia detection by semi-nested PCR can achieve up to 100% sensitivity and 88% specificity for nephropathy diagnosis.[13] Quantitative-PCR has already defined some viral load cut-offs correlated with BKN risk: > 1.6E+04 copies/mL in plasma; > 2.5E+07 copies/mL in urine; > 7700 copies/mL in total blood.[24,43]

There is a direct correlation between viremia load, graft dysfunction degree, severity of histological BKN tissue patterns, and number of infected renal cells in histological slide.[37] Such correlation suggests that viremia occurs mainly, if not entirely, from the viral replication started in the kidney. This is the reason why the amount of viremia is more predictive of BKN than the viruria.[16,24,44,45]

Finally, viremia is a late BKV-infection event, associated with graft dysfunction and works as a marker for BKN development.[28] It occurs 16 to 33 weeks previously to the establishment of BKN,[13] and invariably appears later than viruria.[36] Thus, an intense BKV-viruria predicts viremia, and probably BKN.

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