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Polyomaviruses BK- And JC-DNA quantitation in kidney allograft biopsies

Cristina Costa[a,*,1], Massimiliano Bergallo[a,1], Francesca Sidoti[a], Sara Astegiano[a], Maria Elena Terlizzi[a], Gianna Mazzucco[b], Giuseppe P. Segoloni[c], Rossana Cavallo[a]

[a] Department of Public Health and Microbiology, Virology Unit, Università degli Studi di Torino, Via Santena 9, 10126 Torino, Italy
[b] Biomedical Sciences and Human Oncology, Università degli Studi di Torino, Via Santena 7, 10126 Torino, Italy
[c] Department of Internal Medicine, Renal Transplant Unit, Ospedale Molinette, Università degli Studi di Torino, corso Bramante 88, 10126 Torino, Italy

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ABSTRACT

Background: Polyomavirus-associated nephropathy (PVAN) is one of the most common viral disease affecting renal allograft, with BK being the most frequent causal agent and JCV being considered responsible in <3% of the cases.
Objectives: To quantify polyomaviruses BK and JC load by real-time TaqMan PCR in tissue specimens (renal and ureteral) from kidney transplant recipients.
Study design and methods: One-hundred-thirty-eight specimens (125 kidneys, 13 ureters) obtained from 109 patients were evaluated by quantitative real-time PCR for the detection of BKV- and JCV-DNA. Demographic, virological, and histopathological data were collected.
Results: BKV-DNA was positive in 32 of 109 patients (29.6%) and JCV-DNA in 20 of 109 patients (18.3%). The highest BK viral loads (>10^4 genome equivalents/cell) were found in two renal samples with histopathologically confirmed PVAN; while JC viral load was >10^4 genome equivalents/cell in one ureteral sample.
Conclusions: Although quantitation of viral DNA on renal allograft biopsies could be complementary to histopathological evaluation and the highest viral load are detectable in renal specimens with PVAN, the identification of a diagnostic cut-off should require further studies.

1. Introduction

BKV and JCV are members of the *Polyomaviridae* family with a genome homology of approximately 72%. Human polyomaviruses BK and JC are ubiquitous and following primary infection remain latent in the renourinary tract and B cells[7]. Asymptomatic viruria may occur in both immunocompetent subjects and immunocompromised patients, while in transplanted kidney viral replication may determine polyomavirus-associated nephropathy (PVAN) in 1–10% of the patients, leading to graft failure in 30% up to 80% of the cases[4,5]. Today, PVAN is one of the most common viral disease affecting renal allografts, with BKV being the most frequent causal agent and JCV being responsible in less than 3% of the cases[6,12,2]. However, in a recent study[3] a biopsy-proven PVAN was diagnosed in six renal transplant recipients with exclusive JCV viruria out of 75 patients (8%) with BKV and/or JCV viruria, with an overall incidence during the study period of 0.9%. The definitive diagnosis of PVAN is made on the basis of histopathology, although this presents some drawbacks, including being invasive and limited sensitivity due to (multi)focal involvement. We present here results of quantitation of polyomaviruses BK and JC by real-time TaqMan PCR in renal and ureteral specimens from renal transplant recipients.

2. Materials and methods

All 109 renal transplant recipients who underwent at least one graft biopsy for clinical and/or laboratory suspicion of rejection, PVAN, or other causes, in a 18-month period were evaluated. In 13 patients ureteral specimens, obtained during pyelostomy performed for ureteral stenosis, were studied (for three of these patients a renal sample was also available). Overall, 138 clinical samples were evaluated. Relevant clinical and laboratory data were abstracted from clinical charts and kidney transplant database. Informed consent was obtained from all the patients. BK viral load on concomitant serum and urine samples were available for 55 patients. Extraction from serum and urine samples was performed as previously described.[1] Tissue specimens were formalin-fixed and paraffin-embedded; for DNA extraction, two to four sec-

Abbreviations: Ct, threshold cycle; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; Geq, genome equivalents; IHC, immunohistochemistry; PVAN, polyomavirus-associated nephropathy.

* Corresponding author. Tel.: +39 011 670.5630; fax: +39 011 670.5648.
E-mail address: cristina.costa@unito.it (C. Costa).

[1] Cristina Costa and Massimiliano Bergallo equally contributed to this work and share first authorship.

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Table 1
Technical details on real-time PCR assays employed in this study

Primers	BKV	JCV (modified from McNees et al.[8])	GAPDH
Probe	Q-BKV Real Time PCR kit (Nanogen Adv. Diagnostics)	F 5′-TTCTTCATGGCAAAACAGGTCTT-3′; R 5′-GAATGGGAATCCTGGTGGAA-3′	F 5′-GCCAAAAGGGTCATCATCTC-3′; R 5′-GGGGCCATCCACAGTCTTCT-3′
Cycling conditions	50 °C 2 min; 94 °C 10 min; 95 °C 15 s; 60 °C 1 min	45 cycles	

GAPDH, glyceraldehyde-3-phopshate-dehydrogenase.

tions (thickness, 4 μm) were incubated with 400 μL of lysis buffer (400 mM Tris–HCl 400 pH 7.5; 500 mM NaCl; 50 mM EDTA; 1% SDS) by vortexing for 30 s, boiled for 10 min, vortexed for 30 s, boiled for 10 min and vortexed again for 30 s, then centrifuged for 1 min at 13,000 rpm at room temperature. Three hundred microliters of the supernatant were mixed with 750 μL of absolute ethanol and 30 μL of 3 M sodium acetate pH 5. After centrifugation for 5 min at 13,000 rpm at room temperature, the pellet was washed with 70% ethanol, air-dried for at least 30 min, resuspended in 50 μL of double-distilled H_2O, and stored at −20 °C prior to use.

Tissue samples were tested for BKV- and JCV-DNA by real-time quantitative TaqMan PCRs targeting viral genes encoding for the large T-antigens of each virus with the 7300 Real Time PCR System (Applied Biosystems, Monza, Italy). For BKV-DNA a commercial kit was used (Q-BKV, Nanogen Advanced Diagnostics, Milano, Italy), following the manufacturer's instruction, while JCV-DNA was tested as previously described.[8] Details are reported in Table 1. Amplifications were set up in a reaction volume of 25 μL, including 5 μL of extracted sample or plasmid dilutions. No template control (sterile double-distilled H_2O) was included in each PCR run. Standard curves for BKV- and JCV-DNA quantification were constructed by plotting the threshold cycle (Ct) against the logarithm of serial 10-fold dilutions (ranging from 10^2 to 10^5) of pBKV provided with the Real-Time kit and a plasmid containing full-length JCV-DNA (courtesy of T. Musso), respectively. The plasmid DNA concentration was determined by measuring the optical density at 260 nm. The DNA content in micrograms was converted to genomic copies using Avogadro's number (6.023×10^{23}) and the number of nucleotide pairs in the plasmid, and the average molecular weight of a nucleotide pair was assumed to be 660 μg. All measurements were performed in duplicate. To correct for the variable amount of DNA in different tissue samples, each sample was subjected to simultaneous TaqMan PCR for the housekeeping gene Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH, Accession No. J04038), targeting the region between exons 6 and 8; primers and probe were designed using the Primer Express software (Version 3.0, Applied Biosystems; Table 1). Results were considered acceptable only in the presence of GAPDH-positivity. Standard curves for the GAPDH gene quantitation were constructed by plotting the Ct against the logarithm of serial dilutions of DNA extracted from peripheral blood leukocytes. The Ct values and number of BKV-DNA copies, JCV-DNA copies, and diploid sets of GAPDH-gene were calculated from the standard curve. The assay was linear in the range 10^2–10^5 copies per reaction. In case of results above the linear range serial 10-fold dilutions of extracted samples were made and quantification was obtained considering the mean of the results within the linear range. A "nucleotide–nucleotide blast" search for short nucleotide sequences performed at the National Center for Biotechnology Information and the National Library of Medicine web site confirmed that the primer pairs used for JCV amplify only 92 clinical isolates of JCV and should not amplify other viruses pathogenic to humans. Amplification data were analyzed by the Sequence Detection System software (Applied Biosystems).

For histopathologic evaluation periodic acid-Schiff, Masson's trichrome, phosphotungstic acid hemotoxylin, and acid fucsin-orange G stains were performed. In case of suspected PVAN, immunohistochemistry (IHC) with immunoperoxidase staining was performed on fixed material using polyclonal anti-SV40 antibody (dilution 1:20,000; Lee Biomolecular Research Labs. San Diego CA), cross-reacting with BKV and JCV.

Statistical analysis was performed using the chi square test with a commercially available software (MedCalc; Version 9.2.1.0). A p value <0.05 was considered significant.

3. Results

Results are summarized in Table 2. Real-time PCR for BKV-DNA was positive in 37/138 transplant biopsies (26.8%) from 32/109 patients (29.6%): 30/124 (24.2%) renal and 7/14 (50%) ureteral samples, from 26/96 (27.9%) and 6/13 (46.2%) patients, respectively (for two patients BKV-positivity was concordant on renal and ureteral specimens). The positivity rate did not significantly differ between renal and ureteral samples. Concordant negative and positive results were found in all the cases, thus the highest load was considered in each patient. Median BKV viral load was 125 Geq/10^4 cells (range 40–1,023,067,263; 25th percentile 40, 75th percentile 361); tissue viral load was >75th percentile in 10 samples and >90th percentile (i.e. 66668.8) in five: 137,126 in a renal specimen with post-transplantation glomerulopathy, 36,448 and 601,057 in two ureteral samples from a patient in whom renal specimen resulted negative to BKV and JCV, 493,047,821 and 1,023,067,263

Table 2
Results of polyomaviruses BK- and JC-DNA detection in renal and ureteral specimens from renal transplant recipients

	Samples *N* (%)	Pts *N* (%)	Viral load (Geq/10^4 cells)
BKV-DNA	37 (26.8%)	32 (29.6%)	Median 125 (range 40–1,023,067,263; 25th percentile 40; 75th percentile 361). Two PVAN[a], 4.93×10^8 and 1.02×10^9
	30/124 (24.2%) R	26/96 (27.9%) R	Median 40, mean ± S.D. 4907.0 ± 25494.4
	7/14 (50.0%) U	6/13 (46.2%) U	Median 1059, mean ± S.D. 106,626 ± 242646.4
JCV-DNA	24 (17.4%)	20 (18.3%)	Median 40 (range 36–23,060,245; 25th percentile 40; 75th percentile 265)
	21/124 (16.9%) R	17/96 (17.7%) R	Median 40, mean ± S.D. 216.4 ± 401.2
	3/14 (21.4%) U	3/13 (23.1%) U	Median 1020, mean ± S.D. 1020.0 ± 1385.9
Total (*N*)	138	109	

Pts, patients; R, renal samples; U, ureteral samples; S.D., standard deviation.
[a] Patients with histologically confirmed PVAN.