Monitoring of BK virus replication in the first year following renal transplantation

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Abstract

Background. BK virus-associated nephropathy (BKV AN) is one of the most common viral diseases affecting renal allografts. Screening for viral replication may allow for earlier intervention with reduced allograft loss. A plasma viral load > 10^4 copies/mL of BKV DNA is recommended for a presumed diagnosis of BKVAN.

Methods. We monitored BKV load on serum and urine samples by Real-Time TaqMan PCR in 229 renal transplant recipients in the first year post-transplantation. Overall, 2025 serum and 2025 urine samples were evaluated. A graft biopsy was performed in 47/229 patients to investigate the declining renal function. Operating characteristics [sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV)] and receiver operating characteristic (ROC) curve analysis at different viral load values were calculated.

Results. Serum BKV viral load was > 10^4 in 5/229 patients (2.2%). A histological diagnosis of BKVAN was made in 3/229 patients (1.3%); 3/5 (60.0%) among those with serum viral load > 10^4 and 3/4 (75.0%) in those with > 1.6 x 10^4. Operating characteristics of a serum BK load of 10^4 for the diagnosis of BKVAN were as follows: sensitivity, 100%; specificity, 99.1%; NPV, 100%; PPV, 59.4%. Specificity and PPV rose to 99.6% and 75.0% when using a cut-off level of 1.6 x 10^4 copies/mL.

Conclusions. The recommended level of BK viremia of 10^4 copies/mL is useful to identify patients at risk of BKVAN, although specificity and PPV increase by using a cut-off level of 1.6 x 10^4 copies/mL. BK replication may occur in the first 3 months post-transplantation and subsequently recede. Therefore, the temporal profile of BKV replication has to be accurately evaluated and occasionally elevated values should prompt a closer monitoring.

Keywords: BKV-associated nephropathy; monitoring; polyomavirus BK; renal transplantation

Introduction

Human polyomavirus BK (BKV) is a worldwide-distributed virus the seroprevalence rate of which is ~70–90% in adults. Following primary infection, BK remains latent in the renalurinary tract as the epidemiologically most relevant latency site, and in B cell, brain, spleen and probably other tissues. Reactivation with asymptomatic viruria may occur in both immunocompetent subjects and immuno-compromised patients. In renal transplantation, in the context of intense immunosuppression, viral replication may determine BKV-associated nephropathy (BKVAN) with interstitial nephritis and/or ureteral stenosis in 1–10% of the patients, particularly in the first year following transplantation, and leading to graft failure and return to haemodialysis in 30 to 80% of the cases [1–3]. Today, BKVAN is one of the most common viral diseases affecting renal allografts. Screening for polyomavirus replication may allow for earlier intervention with reduced allograft loss [2]. A presumed diagnosis of BKVAN may be made in the presence of surrogate markers of viral replication, such as plasma viral load > 10^4 copies/mL of BKV DNA [2]. Thus, screening for BKV replication represents the basic strategy to predict early the onset of BKVAN [2,3]. The aim of this study was to investigate the operating characteristics of BKV replication monitoring for the development of BKVAN in renal transplant recipients in the first year post-transplantation.

Materials and methods

Patients and samples

Two hundred twenty-nine renal transplant recipients (140 males, 89 females; mean age ± standard deviation, 53.2 ± 14.3 years) were studied in the first year post-transplantation. BKV replication monitoring was performed on serum and urine samples twice monthly in the first 3 months, and then every 3 months; in the presence of renal function abnormalities or on the basis of clinical judgement, intervals of monitoring were reduced to twice monthly. A complete monitoring was available for 174
patients. Overall, 2025 serum and 2025 urine samples were studied at the Virology Unit of the University of Turin, Italy. The findings of two or more consecutive positive serum or urine samples were defined as sustained viraemia and viruria. Immunosuppressive protocols were as follows: for induction basiliximab (B), mycophenolate mofetil (MMF) and steroid (S) in 75 patients; B, MMF and tacrolimus (FK506) in 10; B, MMF, S and FK506 in 63; B, FK506 and S in 72; B, MMF, S and cyclosporine A (CyA) in 1; B, S and antilymphocyte globulin (ALG) in 3; B, S, MMF and sirolimus in 2; B, S, MMF and CyA in 3; and for maintenance: FK506 and S in 92 patients; FK506, MMF and S in 118; FK506 and sirolimus in 1; CyA and S in 5; S, CyA and MMF in 13. In the presence of BK viraemia, a reduction of immunosuppression level was made.

Clinical charts of all the patients and the database of the Regional Reference Transplant Centre were reviewed. During the study period, 47 of 229 patients underwent at least one kidney allograft biopsy (overall 63 procedures) to investigate the declining renal function. The occurrence of BKVAN was diagnosed by histopathological evaluation on formalin-fixed and paraffin-embedded 4-µm sections, using periodic acid-Schiff, Masson's trichrome, phosphotungstic acid haematoxylin and acid fucsin-orange G stains. Moreover, immunohistochemistry with immunoperoxidase staining was performed on fixed material using polyclonal anti-SV40 antibody (dilution 1:20 000; Lee Biomolecular Research Labs, San Diego CA, USA).

Thirty healthy subjects, including health care workers in the Nephrology Ward (8 subjects) or in the Virology Unit (4 subjects), and 50 non-transplant patients with lupus nephritis (14 not treated and 36 treated with steroids or immunosuppressive agents, as described elsewhere [4]) were also evaluated.

**PCR for BKV-DNA quantification in serum and urine samples**

Extraction procedure from serum and urine samples was performed as previously described [5]. BKV-DNA quantification was performed by Real-Time TaqMan PCR using a commercial kit (BKV Q-PCR Alert Kit; Nanogen Advanced Diagnostic, Milano, Italy) for the detection of the target viral gene encoding for the large T-antigen of BKV with the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). According to the manufacturer’s instructions, PCR amplifications were set up in a reaction volume of 25 µL that contained 5 µL of extracted sample or negative control (sterile double-distilled H2O) or plasmid. Thermal cycling was as follows: 50°C for 2 min, an initial denaturation step at 95°C for 10 min that was followed by 45 cycles at 95°C for 15 s (denaturation) and 60°C for 1 min (reannealing and extension). Standard curves for the quantification of DNA were constructed by plotting the threshold cycle against the logarithm of serial 10-fold dilutions, ranging from 10² to 10⁵, of the corresponding plasmid. Amplification data were analysed by the Sequence Detection System software (Applied Biosystems). Each sample was subjected to simultaneous TaqMan PCR for the housekeeping gene human β-globin; results were considered acceptable only in the presence of β-globin positivity. The assay was linear in the range 10²–10⁵ copies per reaction, corresponding to an absolute quantification of 10³ copies/mL.

Different values for serum viral load (ranging from 3 × 10² to 1.6 × 10⁴ copies/mL) were investigated as markers for possible evolution towards BKVAN or presumed BKVAN, as appropriate.

**Statistical analysis**

Operating characteristics, including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and receiver operating characteristic (ROC) curve analysis, were evaluated by using commercially available software (MedCalc; version 9.2.1.0).

**Results**

Results are summarized in Table 1. Real-time PCR for BKV DNA was positive (i.e. >10⁴ copies/mL) in 93 of 2025 (4.6%) serum samples, obtained from 21 of 229 patients (9.2%), and in 228 of 2025 (11.3%) urine samples, obtained from 42 of 229 patients (18.3%). Sustained viraemia and viruria were found in 11 and 19 patients, respectively, with a mean time to becoming negative of 34.7 ± 23.0 days (mean ± SD). Considering serum samples, BKV viral load was >10⁴ in 36/2025 specimens (1.8%), from 5/229 patients (2.2%). A histologically confirmed diagnosis of BKVAN was made in three of these patients, giving an overall BKVAN prevalence of 1.3% in the 229 kidney transplant recipients and 60% (3/5) among patients with serum viral load >10⁴. Graft loss subsequently occurred in one of these three patients (33.3%). Considering the 42 remaining patients in which a renal biopsy was performed, 2 presented a serum viral load <3 × 10⁵ and the others were negative. None of them presented histopathological findings associated with BKVAN. Two patients presented a serum viral load >10⁴ in the first month following transplantation; however, with reduction of immunosuppression, the viral loads subsequently reduced at the following determinations 1 week later until they returned negative.

<table>
<thead>
<tr>
<th>Serum BKV DNA</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;3000 copies/mL</td>
<td>100</td>
<td>95.1</td>
<td>21.1</td>
<td>100</td>
</tr>
<tr>
<td>&gt;5000 copies/mL</td>
<td>100</td>
<td>96.5</td>
<td>27.3</td>
<td>100</td>
</tr>
<tr>
<td>&gt;7000 copies/mL</td>
<td>100</td>
<td>99.7</td>
<td>50.3</td>
<td>100</td>
</tr>
<tr>
<td>&gt;10 000 copies/mL</td>
<td>100</td>
<td>99.7</td>
<td>59.4</td>
<td>100</td>
</tr>
<tr>
<td>&gt;13 000 copies/mL</td>
<td>100</td>
<td>99.1</td>
<td>60.0</td>
<td>100</td>
</tr>
<tr>
<td>&gt;16 000 copies/mL</td>
<td>100</td>
<td>99.6</td>
<td>75.0</td>
<td>100</td>
</tr>
<tr>
<td>&gt;10¹⁰ copies/mL</td>
<td>100</td>
<td>95.5</td>
<td>27.3</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 1. Operating characteristics of BKV-DNA quantification in serum and urine samples in relation to a histologically confirmed diagnosis of BKVAN in 229 renal transplant patients.**
The aim of this study was to investigate the operating characteristics of BKV replication monitoring for the development of BKVAN in renal transplant recipients in the first year post-transplantation.

By 1 year post-transplantation, 21 patients (9.2%) developed viraemia and 42 (18.3%) viruria. While cumulative incidence of BK viraemia was similar to that reported in other studies, ranging from 9 to 40% [6,7,3,8], viruria occurred at a lower frequency in comparison to that of other studies, ranging from 24 to 40% [7,3,8]. This could be attributable to different follow-up sampling timings (e.g. twice monthly in the first 3 months in our study versus weekly in others [7]), thus missing episodes of transient viruria, micturition intervals and fluctuations of urine content [2], although it is to note that the difference between our data on viruria and those of others [8] is not statistically significant. Considering the prevalence of BK viruria and viraemia in healthy controls, our results seem to be in contrast with those obtained from other studies [10]. We could speculate that these data are related to a major susceptibility to BK contact in our control group, including mainly Nephrology and Virology operators, as previously demonstrated for Blood Transfusion Centre operators [11]; while considering the occurrence of BK viraemia and viruria in non-transplant patients with lupus nephritis, this has been already discussed elsewhere [4].

The identification of a relevant threshold of BK viraemia or viruria for a presumed diagnosis of BKVAN represents an important issue in clinical management of renal transplant recipients [12]. A multidisciplinary international panel of experts recommends screening for BK replication on urine every 3 months in the first 2 years post-transplantation and yearly thereafter [2]. Viruria can be studied either through urine cytology (decoy cells) or quantification of viral load. Although decoy cell evaluation has a high NPV, as their absence virtually excludes BKVAN, it is to note that sensitivity and PPV are low and cannot differentiate between human polyomavirus BK and JC [13]. Regarding urine BK load, there is general agreement that repeated values >10^7 copies/mL are associated with BKVAN, while the detection of BK viraemia >10^4 copies/mL is considered the most reliable surrogate marker of presumed BKVAN in renal transplant recipients. The results obtained in this study concur with the internationally recommended cut-off levels. It is to note that the adoption of a cut-off level of 1.6×10^4, as recently suggested in a study by Viscount et al. [8], ameliorates operating characteristics, particularly in terms of PPV (Table 1).

This also has to be interpreted considering the time from transplantation, as our study focused on the first year post-transplantation, while in the study by Viscount et al., samples were collected at a median of 2 years after transplantation. Moreover, like most studies investigating BKVAN, our study was limited by the low number of affected patients.

A definitive diagnosis of BKVAN is made by the demonstration of typical viral cytopathic findings at histopathology. However, given the focal involvement of early BKVAN and the possibility of tissue-sampling errors, clinical management is often based on the surrogate markers of viral replication. On the other hand, the requirement of tissue evaluation in patients who are suspected to have BKVAN...
remains, also taking into account that a renal biopsy is necessary to exclude other pathologic processes, such as acute rejection that may coexist [14].

Quantification of viraemia and viruria is mandatory in the follow-up of kidney graft recipients, as evidenced by the analysis of operating characteristics on a large group of patients [2]. It has to be taken into consideration that BK replication may occur in the first 3 months following transplantation and may subsequently recede, probably due to the early development of a specific cellular immune response [15,16], as in immunocompetent subjects. Therefore, the temporal profile of BKV replication has to be accurately evaluated and occasionally elevated values should prompt a closer monitoring in order to be confirmed, as well as the modulation of immunosuppressive therapy.

Conflict of interest statement. None declared.

References


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