# Screening for BK virus nephropathy in kidney transplant recipients: comparison of diagnostic tests

## Desempenho de métodos diagnósticos no rastreio de nefropatia pelo vírus BK em pacientes transplantados renais

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## Abstract

Urine cytology and qPCR in blood and urine are commonly used to screen renal transplant recipients for polyomavirus-associated nephropathy (PVAN). Few studies, however, have directly compared these two diagnostic tests, in terms of their performance to predict PVAN. This was a systematic review in which adult (≥ 18 years old) renal transplant recipients were studied. A structured Pubmed search was used to identify studies comparing urine cytology and/or qPCR in urine and plasma samples for detecting PVAN with renal biopsy as the gold standard for diagnosis. From 707 potential papers, there were only twelve articles that matched the inclusion criteria and were analyzed in detail. Among 1694 renal transplant recipients that were included in the review, there were 115 (6.8%) patients with presumptive PVAN and 57 (3.4%) PVAN confirmed. In this systematic review, the qPCR in plasma had better performance for PVAN compared to urine cytopathology.

**Keywords:** biopsy; BK virus; cell biology; DNA; kidney transplantation.

## Resumo

A citologia urinária e a reação da cadeia da polimerase em tempo real (qPCR) em amostras de sangue e/ou urina são comumente utilizados para rastrear nefropatia associada ao polyomavirus (PVAN), em pacientes transplantados renais. Entretanto, poucos estudos comparam diretamente esses testes diagnósticos quanto ao desempenho para predizer esta complicação. Aqui realizamos uma revisão sistemática na qual foram estudados pacientes transplantados renais adultos (≥ 18 anos). Uma pesquisa estruturada Pubmed foi utilizada para identificar estudos comparando citologia urinária e/ou qPCR em amostras de urina e plasma para detectar PVAN, utilizando a biópsia renal como padrão-ouro para o diagnóstico. Dentre os 707 artigos em potencial, apenas 12 atendiam aos critérios de inclusão e foram analisados em maior detalhe. Foram incluídos 1694 pacientes transplantados renais, entre os quais 115 (6,8%) classificados com PVAN presuntivo e 57 (3,4%) PVAN confirmado. Nessa revisão sistemática, o qPCR no plasma tive melhor desempenho para PVAN em comparação com citopatologia urinária.

**Palavras-chave:** biologia celular; biópsia; DNA; transplante de rim; vírus BK.

## Introduction

Kidney transplantation is the treatment of choice for many end-stage renal diseases that would otherwise require dialysis and renal replacement therapy.[1] One of the main threats for graft survival is infection caused by the polyomavirus BK (BKV). The prevalence of clinically significant BKV reactivation after kidney transplantation varies, depending on the study, from 1 to 10% and the incidence of allograft loss due to BKV have ranged from as low as 10% to more than 80% of patients with clinically significant BKV infection.[2] Rapid and sensitive detection of BKV infection, either in urine or plasma, can lead to early management strategy that is critical to prevent irreversible kidney damage and loss.

The diagnosis of BKV nephropathy requires allograft biopsy,[3] however, it may be too late to reverse the damage. Studies have shown that cytological abnormalities ('decoy cells') and polyomavirus DNA are detected in the urine several weeks before kidney damage occurs.[2,4] Decoy cells may be observed in the urinary sediment as a result of renal and urothelial cells infected by BKV.[5]

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Despite being a relatively inexpensive test, the detection of decoy cells requires considerable expertise and these are not specific for BKV infection.[6,7] Detection and quantitation of BKV DNA can be performed using real time real time polymerase chain reaction (qPCR). While it is comparatively more expensive, in comparison to urine cytopathology, the BKV qPCR has the potential for higher test sensitivity, better linearity and independence from personal expertise for accurate results.

In this systematic review, we searched for studies that directly compared the analytical performance of urine cytopathology and qPCR, for predicting the diagnosis of BKV-associated nephropathy, as proven by histopathology.

## Material and Methods

### Criteria for considering studies for this review

We selected for inclusion in this review studies involving patients who had undergone kidney transplantation not combined with receipt of other transplanted organs.

### Types of studies

Cross section, prevalence and cohort studies were included. Studies involving 10 or less patients were not included.

### Types of participants

Adult (≥ 18 years old) renal transplant recipients were considered for study, regardless of sex, race, or nationality.

### Types of interventions

Since biopsy is gold standard test for BKV nephropathy, we included only studies that compared biopsies with urine cytology and/or qPCR.

### Types of outcome measures

The outcome measure was nephropathy caused by BKV, as confirmed by renal biopsy. Additional information such as BKV viral load in plasma and urine; presence of 'decoy cells' on urine cytopathology; use of SV40 antibody staining on biopsied tissue was investigated and associated with the outcome.

### Search strategy

We searched PubMed electronic database using the strategy demonstrated in Table 1. The search was conducted on 14th February 2014 and included all papers retrieved in the database.

### Exclusion criteria

Papers that were not written in English and/or not conducted in humans were excluded. Since this study aimed for a comparison of diagnostic tests, we excluded review articles, case reports, studies involving patients younger than 18 years old, studies of patients submitted to transplant procedures other than renal transplantation (even when combined), drug intervention studies, studies in which biopsies were not performed to confirm nephropathy and studies that did not compare biopsies with at least one of the tests under study. Attempts were made to contact corresponding authors when articles were not available on Pubmed or when additional information was required. In the situations when a response was not received, the respective articles were excluded.

### Studies included in the review and data synthesis

The flow-chart diagram in Figure 1 shows the total number of papers screened and number of manuscripts that met the inclusion criteria. Additional data were extracted from these studies.

### Ethical aspects

The study was approved by the Institutional Review Board (protocol numbers 3531/11 and 915/12).

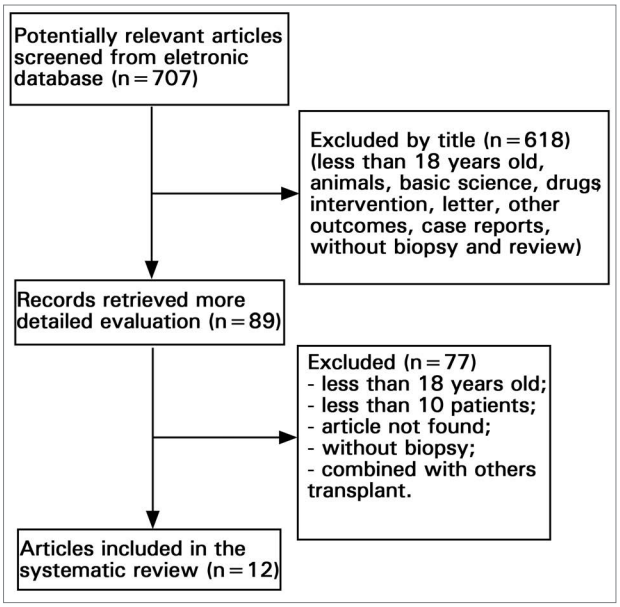
## Results

The systematic search initially identified 707 potential articles. However, a total of 12 articles were included in the final analyses. A total of 1694 renal transplant recipients were included in this review (Table 2). Using biopsy as gold standard there were 115 cases (6.8%) of presumptive nephropathy without observation of BKV and 57 cases (3.4%) of polyomavirus-associated nephropathy (PVAN). The range of sensitivity, specificity, PPV (positive predictive (PPV) value) and NPV (negative predictive (NPV) value) using qPCR as non-invasive test to detect and predict PVAN in plasma was 60-100%, 33-100%, 7-65% and 72-100% respectively (Table 3). The range of plasma viral load at the time of diagnosis was 2.7 - 7 *log*. The threshold of ≥ 3.7 *log* for PVAN provide specificity of 91% and positive predicted value (PPV) of 29%, whereas > 4.2 *log*

**Table 1** Search strategy used in the study (PubMed)

| |
|---|
| (humans) |
| AND |
| (((transplant) OR (graft survival[mh]) OR ("graft survival") OR (graft rejection[mh]) OR ("graft rejection")) |
| AND |
| ((kidney[mh]) OR (kidney) OR ("allograft loss") OR (kidney disease[mh]) OR ("kidney disease"))) |
| AND |
| ((molecular diagnostic techniques[mh]) OR ("molecular diagnostic techniques") OR (molecular biology[mh]) OR ("molecular biology") OR ("molecular biology") OR (PCR) OR ("polymerase chain reaction") OR ("polymerase chain reaction") OR (polymerase chain reaction[mh]) OR (cytological techniques[mh]) OR ("cytological techniques") OR ("decoy cells") OR (papanicolaou) OR (biopsy) OR (viremia) OR (viruria) OR ("viral load")) |
| AND |
| (("BK virus") OR (polyomavirus infection[mh]) OR ("polyomavirus infection") OR (polyomavirus) OR ("BK nephropathy")) |

**Figure 1.** Flow-chart of article screenned.




enhanced the specificity to 96% and PPV to 50%. Sensitivity and NPV were 100% in both cases.[8] In those studies where cytology test were performed (n = 506 patients), decoy cells were found in 30.6% (n = 155) of the patients. In comparison with qPCR, decoy cells showed better range on NPV (97-100%), while sensitivity, specificity and PPV were diminished (Table 4). In one study, the BKV replication indicated by decoy cell shedding in urine, BKV viremia (qPCR), and PVAN (histopathology) occurred in 29%, 13%, and 6% respectively, and the median time for detection was 3.7 months, 5.4 months and 6.5 months after transplant, respectively.[2] In all studies range time for the detection of viruria, decoy cell and viremia were 0.03-12 month, 0.5-16.1 month and 0.9-25 month after transplant respectively. The early (day 5) detection of BKV viruria may predict the occurrence of both BKV viremia and nephropathy.[9] Also, the finding of two or more consecutively positive urine samples was shown to be a helpful tool to predict BKV viremia (sensitivity 100%; specificity 94%; positive and negative predictive values of 50% and 100%, respectively).[10] It was demonstrated that 20% patients became viremic when BKV copies in the urine achieved 7 *log*/mL - a percentage that increased to 33%, 50% and 100% at 8 *log*, 9 *log* and ≥ 10 *log*, respectively.[11] Such an association has not been demonstrated for decoy cells.

## Discussion

This study shows the paucity of data in the literature regarding the comparison of the performance of qPCR (either blood or urine) and urine cytopathology for the diagnosis of PVAN. It seems clear that viruria (defined as detection of BKV DNA in the urine) precedes the detection of decoy cells on urinary cytology, which antecedes viremia and PVAN.[2] Detection of decoy cells and BKV viruria are important markers of BKV replication but poor predictors of PVAN.

The cut-off to determine the clinical relevance of BKV viremia remains controversial. The American Society of Transplantation (AST) recommends that in the presence of plasma loads > 4 *log* for three or more weeks the diagnosis PVAN should be presumed and biopsy should be considered for definitive diagnosis.[12] While the American Society of Transplantation and the Kidney Disease Improving Global Outcomes Group suggest a BK viral load of 4 *log* copies (10.000 copies) as a cut-off value for PVAN, there is no US Food and Drug Administration approved or standardizes methods for BK viral load evaluation. The diagnosis of BKV is currently based on different

**Table 2** Prospective studies that compared qPCR, urine citology and kidney biopsy in the diagnosis of PVAN in kidney transplant receptors

| Author | n | Decoy cells [a] | Viremia (n) | Viral Load (plasma) | Presumptive PVAN(n)[b] | PVAN + (n) |
|---|---|---|---|---|---|---|
| Hirsch *et al.*[2] | 78 | 23 | 10 | 4.4 - 7 *log* | 5 | 5 |
| Pang *et al.*[11] | 183 | NA | 44 | Median 2.84 (0-5.86) | 0 | 8 |
| Thamboo *et al.*[28] | 97 | 15 | 4 | 3.3 - 5.4 *log* | 7 | 3 |
| Viscount *et al.*[8] | 204 | 26 | 16 | > 3.7 *log* | 12 | 4 |
| Almerás *et al.*[24] | 123 | NA | 13 | 2.7 - 5.6 *log* | 11 | 3 |
| Babel *et al.*[10] | 233 | NA | 16 | Mean 5.9 (range 4.3-7.5)[c] | 10 | 6 |
| Helanterä *et al.*[22] | 68 | NA | 0 | NA | 5 | 0 |
| Girmanova *et al.*[29] | 120 | NA | 6 | > 4.5 *log* | 3 | 3 |
| Pollara *et al.*[16] | 75 | 39 | 26 | 2.8 - 6.5 *log* | 19 | 7 |
| Saundh *et al.*[9] | 112 | NA | 12 | Mean 5.5 *log* (range, 3.6 - 6.5) | 10 | 2 |
| Knight *et al.*[21] | 349 | NA | 57 | 5.7 *log* (SD ± 5.9) | 17 | 15 |
| Menter *et al.*[23] | 52 | 52 | 17 | > 7 *log* | 16 | 1 |

[a] Number of patients diagnosed with decoy cells on cytopathology; [b] Number of patients with diagnosis of nephropathy but with no visualization of BKV by SV40 or viral alterations characteristics; [c] Mean peak of viral load; NA: Not applicable; PVAN: Polyomavirus-associated nephropathy; sd: Standard deviation.

**Table 3** Performance of BKV viremia detected by qPCR in the prediction of PVAN

| Author | Molecular target | Primer or probe | Sequence (5'-3') | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|---|
| Hirsch *et al.*[2] | NI | Primer 1, | AGCAGGCAAGGG TTCTATTACTAAAT | 100 | 88 | 50 | 100 |
| | | Primer 2, | GAAGCAACAGCA GATTCTCAACA | | | | |
| | | Probe | AAGACCCTAAAGACTTT CCCTCTGATCTACA CCAGTTT labeled with 6-carboxyfluorescein at the 5` end and 6-carboxytetramethylrhodamine at the 3` end | | | | |
| Pang *et al.*[11] | VP1 gene | BKpangF | ATGTGACCA ACACAGC | 60 | 76 | 65 | 72 |
| | | BKpangR | CTG TGCCATCAAACACC | | | | |
| | | BKpangP1 | AGGAGAACCCAGA GAGTGGA-fluorescein | | | | |
| | | BKpangP2 | LC-Red 640-GGCAGCCTATGT ATGGTATGGAA-phosphate | | | | |
| Thamboo *et al.*[28] | VP1 gene | NI | (5`-AGG TAG AAG AGG TTA GGG TGT TTG ATG GCA CAG-3`) dual-labeled at the 5` end with 6-carboxyfluorsecein (FAM) and the 3` end with 6-carboxytetramethylrhodamine (TAMRA) | 67 | 33 | 20 | 80 |
| Viscount *et al.*[8] | VP2 gene | Primer PoL1s, | CACTTTTGGGGGACCTAGT | 100 | 96 | 50 | 100 |
| | | Primer PoL2as, | CTCTACAGTAGCA AGGGATGC | | | | |
| | | Probe 1, PoLP1, | TCTGAGGCTGCTGCT GCCACAGGATTTT-fluorescein | | | | |
| | | Probe 2, PoLP2, | LC-Red 640-AGTAG CTGAAATTGCTG CTGGAGAGGCTGCT-phosphate | | | | |

Continued Table 3.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Almerás *et al.*[24] | VP2 gene | Primer PoL1s,<br>Primer PoL2as,<br>Probe 1, PoLP1,<br>Probe 2, PoLP2, | CACTTTTGGGGGACCTAGT<br>CTCTACAGTAGCAAGGGATGC<br>TCTGAGGCTGC TGCTGCCA CAGGATTTT-fluorescein<br>LC-Red 640-AGTAGCTG AAATTGCTGC TGGAGAGGCTGCT-phosphate | 100 | 91 | 15 | 100 |
| Babel *et al.*[10] | VP1 gene | NI | NI | 100 | 96 | 43 | 100 |
| Girmanova *et al.*[29] | Gene that encode large T Ag | Commercial kit | BKV Q-PCR Detection Alert Kit (Chemagen) | 100 | 68 | 7 | 100 |
| Pollara *et al.*[16] | Gene that encode large T Ag | Commercial kit | BKV Q.Alert Kit (Nanogen Advanced Diagnostics, Italy) | 95 | 100 | NI | NI |
| Saundh *et al.*[9] | Gene that encode large T Ag | BKV Forward<br>BKV Reverse<br>BKV Probe | TGA CTA AGA AAC TGG TGT AGA TCA<br>YTCC TT TAAT GA AAA ATG GGA<br>FAM AGT GTT GAG AAT CTG CTG TTG CTT C BHQ-1 | 100 | 91 | 17 | 100 |
| Knight *et al.*[21] | NI | NI | NI | 100 | 87 | 26 | 100 |
| Menter *et al.*[23] | NI | Primer 1,<br>Primer 2,<br>Probe | AGCAGGCAAGGGTTCTATTACTAAAT<br>GAAGCAACAGCAGATTCTCAACA<br>AAGACCCTAAAGACTTTCCCTCTGAT CTACACCAGTTT labeled with 6-carboxyfluorescein at the 5` end and 6-carboxytetramethylrhodamine at the 3` end | 100 | 57 | 41 | 100 |

Ag: Antigen; BKV: BK vírus; NI: Not informed; NPV: Negative Predictive Value; PPV: Positive Predictive Value; PVAN: Polyomavirus-associated nephropathy.

**Table 4** Performance of urine cytopathology in the prediction of PVAN

| Author | Decoy cell (n) | PVAN (n) | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| Hirsch *et al.*[2] | 23 | 5 | 100 | 71 | 29 | 100 |
| Thamboo *et al.*[28] | 15 | 3 | 67 | 85 | 20 | 98 |
| Viscount *et al.*[8] | 26 | 4 | 25 | 85 | 5 | 97 |
| Pollara *et al.*[16] | 39 | 7 | 100 | 53 | 18 | 100 |

NPV: Negative Predictive Value; PPV: Positive Predictive Value; PVAN: Polyomavirus-associated nephropathy.

qPCR approaches, but since there is no standard method for BKV viral load assessment, it is essential that institutions implement clinical validation studies certifying their own methodology to be used as a guide for clinical treatment.[2,13-19]

The definitive PVAN diagnosis is made histopathologically[20] in a context in which the viral infection may be difficult to differentiate from organ rejection. In our review, only four articles reported the use of SV40 staining in the histopathological test.[21-24] Therefore, the absence of a confirmatory test may underestimate the actual frequency of PVAN. The SV40 should be performed when clinicians suspect of BKV infection, despite the absence of visible alterations on the examined tissue.[25] The AST recommends a minimum of two core biopsies with medullary tissue preferable in an intention to decrease the false negative diagnosis of PVAN, which can be as high as 20-30% (12, 26). Therefore, a negative biopsy does not rule out PVAN.[26]

## Conclusion

This study demonstrates the paucity of data in the literature on the comparison of diagnostic tests for the prediction of PVAN. qPCR has an overall better diagnostic performance than urine cytopathology for the detection of PVAN. However, the cut-off for qPCR tests remain poorly defined. In contrast to cytomegalovirus (CMV), for which the World Health Organization has produced international standards,[27] there is a need for standardization for BKV-related tests. Additional prospective studies are ultimately required in order to elucidate the ideal cut-off for viral load in the plasma and urine, for the early diagnosis of PVAN, as well as the moment for occurrence of viremia, and co-factors associated with the transplant recipient.

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The different techniques limit the comparison between qPCR assays included, once there is no international standard for BKV quantification.

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