BK polyomavirus-associated nephropathy (BKPyVAN) has emerged as a significant cause of allograft failure after kidney transplantation, affecting up to 10% of kidney transplant (KTx) recipients. BKPyV is acquired in childhood and persists latent in the urinary tract until its reactivation in the immunosuppression state. The nephropathy is a consequence of BKPyV reactivation in the urinary tract of the donor kidney/ureter with subsequent viremia and invasion of the virus in the graft causing viral cytopathic changes, inflammatory response and functional deterioration.

BKPyV replication in urine precedes BKPyV viremia by a median of 4 weeks, and histologically documented nephropathy by a median of 12 weeks. Renal allograft loss secondary to BKPyVAN ranges from 10% to 100% of cases, and prognosis depends on the intensity of inflammatory infiltrates and active tubulitis at the time of diagnosis.

Since there is no effective antiviral therapy for BKPyV, early identification of patients under BKPyV replication is imperative because it allows immunosuppression reduction strategy to stop or slow the progression of inflammation caused by the virus.

There are several studies evaluating the impact of early detection of BKPyV replication in the graft loss progression using surrogate markers in urine or blood. Therefore, post-transplant routine screening for BKPyV replication is currently recommended for all KTR. International guidelines recommend to screen for BKPyV replication by testing urine or plasma at least every 3 months during the first 2 years post-transplant, then annually thereafter for 5 years, and if there is an unexplained serum creatinine rise of after treatment for acute rejection.

Currently, available screening tests include: urine cytology to identify virally loaded epithelial cells (also named “decoy cells”), quantitative nucleic acid amplification testing (NAT) by polymerase chain reaction (PCR) to identify BKPyV in urine or plasma or blood samples, or urine electronic microscopy to detect three-dimensional viral aggregates (Haufen). However, screening methods for BKPyVAN have only limited predictive value to predict nephropathy development, since definitive diagnosis requires histological confirmation based on cytopathic changes and immunohistochemistry (cross-reaction antibodies raised against simian virus 40 clone) or in situ hybridization. Kidney biopsy is invasive and impractical to be used widely as a screening test to allow early detection of nephropathy. On the other hand, BKPyV replication has a negative predictive value (NPV) higher than 99%. Therefore, the goal is a perfect screening test as a surrogate of the accurate diagnosis of BKPyV replication and its clinical correlation with risk of development of nephropathy.

Few studies have directly compared the performance of the available screening methods to predict BKPyVAN. In this edition of the Brazilian Journal of Nephrology, the study of Pinto et al. presented a systematic review of studies.
that directly compared the analytical performance of screening methods to predict the diagnosis of BKPyVAN proven by histopathology. From 707 potential articles initially identified, only 12 met inclusion criteria and were included in the final analysis, representing the paucity of data in the literature regarding this issue and the lack of quality data. The authors have demonstrated a better diagnostic performance of quantitative NAT than urine cytopathology for the detection of BKPyVAN.

Urine cytology to detect the presence of “decoy cells” is an acceptable unspecific alternative screening method. These cells have also been described in adenovirus and cytomegalovirus infections. The testing is inexpensive and has a high NPV but a very low positive predictive value (PPV) for BKPyVAN. If the test is used, an additional confirmatory testing is required before therapy is changed in such patients based on the presence of urine “decoy cells” alone. Quantitative NAT provide superior PPV and NPV compared to urine cytology, and in the absence of BKPyV viruria or viremia by PCR, the diagnosis of PVAN is highly unlikely.

Whether to screen KTx with NAT of plasma or urine has been controversial. Negative urine NAT for BKPyV has almost 100% NPV but patients with BKPyV viruria exceeding established thresholds should be tested additionally by NAT on the blood. NAT in urine, in the absence of an elevated BKPyV plasma load is not associated with an increased risk for BKPyVAN. Pinto et al. concluded that positive BKPyV in two or more urine samples was helpful to predict BKPyV viremia with 100% sensitivity, 94% specificity and a PPV of 50% and NPV of 100%. Plasma NAT has the best PPV for BKPyVAN and has been used for most transplant centers for BKPyV screening; the sensitivity to predict BKPyVAN was 60-100%, specificity was 33-100%, and PPV was 72-100%.

Although the threshold of BKPyV viruria and viremia that is associated with PVAN has not been defined, in patients with sustained plasma BKPyV DNA load of > 4 log 10 GEq/mL or equivalent, the diagnosis of presumptive BKPyVAN can be made.

Haufen technique confirmed BKPyVAN with a 99% concordance rate; PPV was 97% and NPV was 100%. Although it seems promising, electronic microscopy is not widely available and other reproducible studies are required.

There are many limitations to validate the accuracy of a screening test to detect BKPyV replication. First of all, the validity of a screening test can only be determine if the accuracy of the screening test can be compared to some “gold standard” that establishes the true disease diagnosis. The “gold standard” diagnosis of BKPyVAN (renal biopsy), however, is ineffective in at least 10 to 36.5% of cases in which a negative biopsy cannot exclude BKPyVAN diagnosis due to the focal nature of the disease and a second biopsy should be performed later on in suspected cases. Therefore, the accuracy assessment of a determinate screening test is hampered by the absence of a really “gold standard” diagnosis.

Second, there are quite intra laboratory variations and many laboratories use in-house PCR-methods. Variations in sample type, DNA extraction, techniques, primers and probe sequences can yield significant differences in the amount of virus quantified and limits assay detection. As reported by Pinto et al., the different techniques limit the comparison between quantitative NATs and there is a need for standardization for BKPyV-related tests. PCRs with primers and probes targeting the variable regions of the genome, like the NCCR or VP1 regions, may give false negative results or incorrect viral loads when samples contain rare genotypes. In addition, urine “decoy cells” detection presents a considerable pre-analytical logistics and an analytical laboratory expertise; the exam should be performed in fresh urine, and inconclusive results are frequent during the first months post-transplant due the high urinary sediment.

Screening tests are widely used in Medicine to assess the likelihood of a defined population to have a particular disease, and the ideal surrogate should fulfill four criteria: 1 - the measurement of the surrogate needs to be clearly defined, reproducible and easier to access than the corresponding endpoint; 2 - a strong biological rationale has to exist between the clinical endpoint and the surrogate; 3 - the relationship between the surrogate and the clinical endpoint should be well established qualitatively and quantitatively through relevant epidemiological studies and 4 - an estimate of the expected clinical benefit should be derived from the estimate reduction of surrogate incidence in clinical randomized trials. At this moment, none of the available tests comprise all requirements above and plasma NT is still the best available option.
Future research include multicenter clinical trials to validate new screening testing as predictors of BKPyVAN development, such as BKPyV-specific antibody titers and cellular immune response, and the presence of viral variants bearing rearranged noncoding control regions (rr-NCCRs) associated with an increase replication capacity and disease in KTx.

REFERENCES