



Short communication

No correlation between Torque Teno virus viral load and BK virus replication after kidney transplantation



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ABSTRACT

Background: Assessment of the intensity of immunosuppression in transplant recipients to estimate the risk of rejection and infection is not entirely satisfactory at the present time. Determination of Torque teno virus (TTV) viral load appears to be a promising tool in this setting.

Objectives: We evaluated the level of replication and kinetics of TTV during the first three months after kidney transplantation compared to BK virus replication.

Results: In a retrospective cohort of 116 renal transplant recipients, TTV viral load gradually increased during the first three months post-transplantation with no significant difference or discriminatory threshold between patients with and without BK virus replication. However, the level of TTV replication appeared to be indirectly related to the risk of BK virus replication, particularly according to the induction treatment used (antithymocyte globulin: ATG or basiliximab). Among patients receiving ATG, those receiving cyclosporine had significantly lower TTV viral loads ($p < 0.01$) with threefold lower reactivation of BKPyV (13 vs 37%) 3 months post-transplantation. Similarly, among the women in our cohort, TTV viral load was significantly higher in women receiving ATG (6.58 ± 1.57 versus $4.62 \pm 2.0 \log_{10}$ copies/mL for basiliximab: $p < 0.01$), also with threefold higher BKPyV reactivation frequencies (40 vs 13,3%).

Conclusion: The multiparametric variation of TTV viral load does not appear to be individually appropriate for the early detection or monitoring of possible post-transplant BKPyV virus reactivation in renal transplant recipients.

1. Background

The Anelloviridae family, comprising the Alphatorquevirus genus, includes 29 species. Alphatorqueviruses are highly prevalent in humans (90–100%) with low persistent viraemia in immunocompetent subjects [1]. These viruses have not been linked to any human disease at the present time. Data have been recently accumulated concerning the Torque Teno Virus (TTV), the most prevalent of the Alphatorqueviruses and the most abundant virus of the human virome, indicating that TTV viral load could be a useful marker to assess immunosuppression [2]. It has now been clearly demonstrated that an imbalance in immune status has a significant impact on TTV replication. In an individual, the TTV viral load varies between 10^2 and 10^9 copies per milliliter of plasma [3]. Since there is currently no universal marker to assess the intensity of immunosuppression in a transplant recipient treated with an immunosuppressive cocktail, a promising strategy would be to monitor

blood TTV levels.

Various recent studies have evaluated the TTV viral load after heart, lung and kidney transplantation with the primary endpoint being the presence of signs of rejection and/or infection (bacterial, viral, parasitic) in the transplant recipient [2]. Studies evaluating TTV viral load during follow-up after kidney transplantation have been conducted in order to assess the risk of either rejection or Cytomegalovirus disease [4,5]. However, since the early 2000s, an increase in the prevalence of nephropathy caused by human BK polyomavirus (BKPyV) in renal transplant recipients has resulted in a renewed interest in this pathogen. The BKPyV may induce progressive disease, mainly during the first three to six months after transplantation in three successive stages: viraemia, viraemia and if viral replication persists nephropathy [6]. However, it is difficult to predict post-transplant patients who could replicate BKPyV.

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2. Objectives

We therefore wanted to evaluate whether TTV viral load monitoring in the first three months after kidney transplantation could have clinical value to predict an increased risk of BK virus replication.

3. Study design

3.1. Study population and specimen

All 116 consecutive adult renal transplant recipients transplanted between 1st January 2015 and the 31th December 2016 in Amiens hospital, who had completed at least one year of follow-up were enrolled. The exclusion criteria were non-adult patients and those who did not have a minimum one-year follow-up. Induction immunosuppressive therapy included either antithymocyte globulin (ATG) or basiliximab and maintenance therapy included corticosteroids, mycophenolate mofetil and tacrolimus or cyclosporine. This retrospective project was conducted in accordance with the reference methodology (MR-004) and is included in the CHU Amiens - Picardie internal registry of data processing activities, in accordance with Article 30 of the GDPR.

3.2. Quantification of BKPyV and TTV viral load

All samples were stored at -80°C prior to analysis. BKPyV measurements is performed in our centre every month for the first six months and then every three months thereafter. Total nucleic acids were extracted and amplified in a quantitative real-time PCR assay kit (R-gene[®]; Argene, France), as previously described in detail [7]. TTV DNA load was quantified by the TTV R-gene[®] kit (R-gene[®]; Argene, France).

4. Results

A total of 139 patients were transplanted at Amiens University Hospital in 2015 and 2016. Longitudinal plasma TTV viral load was measured in these patients at month 0 (M0), M1, M2 and M3 post-transplantation in order to compare these results with the risk of post-transplant BKPyV replication. Samples were available for 116 patients, whose characteristics are listed in Table 1 with no significant differences between plasma BKPyV-positive and plasma BKPyV-negative patients and patients not included (data not shown). Twenty-four of these 116 patients (20.7%) had a positive plasma BKPyV viral load during the first year after transplantation and 17 (14.7%) had a positive BKPyV viral load during the first three months. Longitudinal monitoring of TTV viral load is shown in Fig. 1A for BKPyV-positive (17 patients) and BKPyV negative patients. A gradual increase in TTV viral load was observed over the first three months, with no significant

Table 1
Patient characteristics.

Characteristics	BKPyV negative (n = 99)	BKPyV positive (n = 17)	P value
Female gender	29	4	NS
Age mean (± SD)	48.1 (± 13.5)	47 (± 16)	NS
Positive Donor CMV serology	38	6	NS
Transfusion	10	1	NS
ATG induction	39	10	NS
Cyclosporine	33	4	NS
Tacrolimus	66	13	
Mean TTV viral load (log ₁₀ copies/ml (± SD)			
J0	1.85 ± 1.45	1.60 ± 1.43	NS
M1	2.98 ± 1.72	2.68 ± 1.86	NS
M2	4.71 ± 1.58	4.23 ± 0.60	NS
M3	5.16 ± 2.07	4.73 ± 2.20	NS

NS: not significant.

difference between both groups ($p > 0.05$). Similarly, we were unable to determine a TTV viral load threshold to distinguish between BKPyV-positive patients and BKPyV-negative patients at any time point. In addition, for 13/17 patients for whom matched plasma BKPyV and TTV viral loads were available at 3 months, we failed to demonstrate any correlation between these two parameters (Fig. 1B). Determination of TTV viral load during the first three months after kidney transplantation therefore does not appear to be a useful parameter to predict possible BKPyV replication. In order to highlight a potential indirect role of TTV viral load on BKPyV replication, we studied the influence of known BKPyV replication risk factors on TTV viral load during the first three months after transplantation [8]. As shown in Fig. 1C, among these factors, immunosuppressive induction appears to play a role in TTV viral load, as a significantly different viral load was observed 2 and 3 months post-transplant between patients who received ATG or basiliximab with tacrolimus as calcineurin inhibitor. The mean M3 TTV viral loads were 6.03 and 4.41 log₁₀ copies/mL in the ATG/tacrolimus and basiliximab/tacrolimus groups, respectively ($p < 0.01$). Furthermore, 37% and 17.3% of transplant recipients were tested positive for BKPyV during the first three months after transplantation for these same groups respectively. This difference was not observed for cyclosporine, which generally induced a lower level of BKPyV reactivation. The second known risk factor for BKPyV reactivation associated with differences in TTV viral load was the gender of transplant recipients associated with ATG induction therapy (Fig. 1D). It has now been demonstrated that the rate of BKPyV reactivation is much lower in women than in men, but the proportion of women of our cohort treated with basiliximab, who tested positive for BKPyV was 13.3% versus more than 40% of women treated with ATG. These differences between both groups could be explained by mean differences of TTV viral load of 0.5 log₁₀ at month 2 and 1.96 log₁₀ at M3 (4.62 ± 2.0 versus 6.58 ± 1.57 log₁₀ copies/mL; $p < 0.01$) (Fig. 1D). Other factors known to be associated with TTV replication, such as age, donor CMV serology [2,9] and granulocyte count, were compared with BKPyV data in transplant recipients, but no obvious link was demonstrated (data not shown).

5. Discussion

This study, which was designed to analyse a possible link between the level of TTV, the most abundant component of the virome, and post-renal transplantation development of BKPyV replication clearly illustrates the difficulty of using this popular and promising tool in the kidney transplant community in this setting. As already demonstrated by other teams, this test appears to have a higher positive predictive capacity for the risk of developing episodes of rejection. As in this study, most other studies have failed to demonstrate a clinically predictive TTV viral load threshold.

We observed high variation in viral load levels with high variation between populations. Many factors seem to influence the level of TTV replication, such as individual factors (age, sex, ...) although many of these factors probably remain unidentified, factors related to the immunosuppressive regimen and probably also factors specific to the virus given its great diversity and the existence of many subtypes [10]. We also tried to identify variables that could be directly correlated during the first three months following transplantation with TTV replication intensity and indirectly correlated with BKPyV replication. For example, we evaluated calcineurin inhibitor concentrations, calculated their areas under the curve, lymphocyte and granulocyte counts at each time-point, pre-graft serology, a transfusion episode during transplantation, but failed to demonstrate any solid correlation.

Although there is no gold standard for monitoring the level of immunosuppression, the measurement drug through levels of calcineurin inhibitors should not be supplanted in the monitoring of post-renal transplantation.

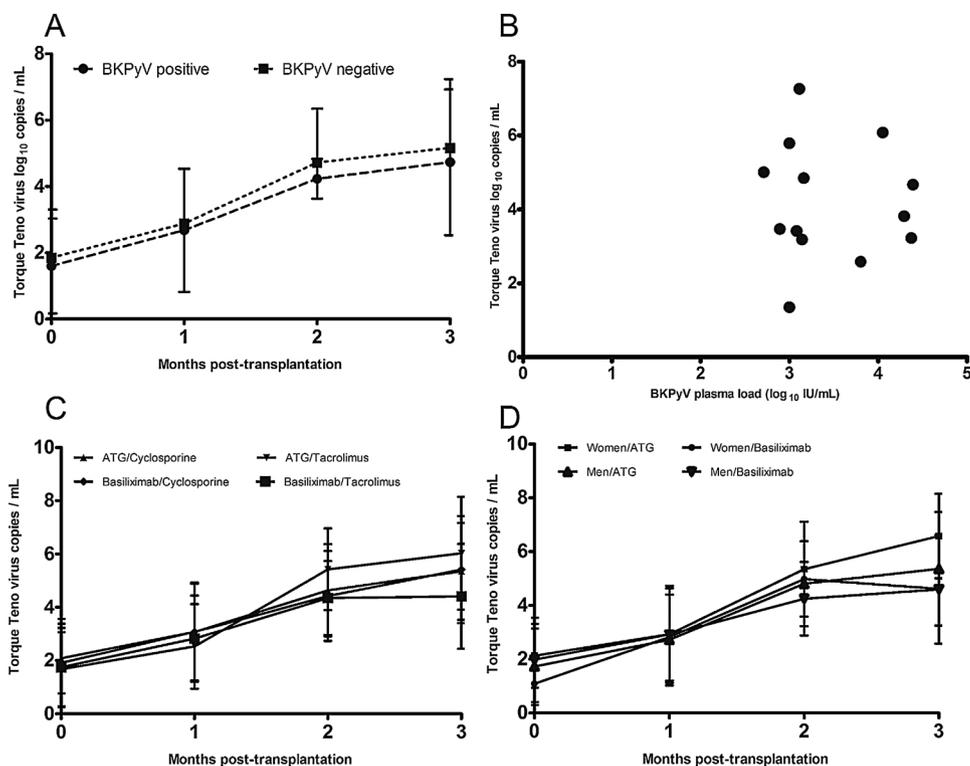


Fig. 1. TTV viral load 3 months post-renal transplantation and BKPvV replication.

A Longitudinal monitoring of the level of TTV replication according to the presence or absence of BKPvV.

B Month 3 matched plasma TTV and BKPvV viral loads.

C Longitudinal follow-up of TTV during the first three months after transplantation according to immunosuppressive treatment

D Longitudinal follow-up of TTV during the first three months post-transplantation according to sex and induction immunosuppressive treatment

Conflict of interest

All authors have no conflict of interest to declare.

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Author contribution statement

LH, VD and EB conceived of the presented idea.

LH, FH, GD and EB wrote the manuscript with support from.

LH, VM, CF and FH performed the analytic calculations and performed statistical analysis.

SC supervised the project.

All authors discussed the results and contributed to the final manuscript.

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