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Torquetenovirus viremia for early prediction of graft rejection after kidney transplantation

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SUMMARY

Objectives: New biomarkers reflecting the degree of immunosuppression in transplant recipients are needed to provide an optimal personalized balance between rejection and infection risks.
Methods: For this purpose, we investigated TTV viremia dynamics in 66 kidney transplant recipients followed up for two years after transplantation, in relation to BK virus infection and graft rejection.
Results: After transplantation, TTV viremia rose by $\geq$2 $\log_{10}$ copies/mL from baseline to month 3, then declined by $\geq$1 $\log_{10}$ copies/mL thereafter. Higher TTV viremia was associated with recipients of a deceased donor, a lower count of CD8+ T cells and a higher BKV viremia. Importantly, TTV loads were significantly lower in KTR who would later display graft rejection; indeed, patients with TTV viremia lower than 3.4 $\log_{10}$ copies/mL at transplantation or lower than 4.2 $\log_{10}$ copies/mL at month 1 had a higher risk of developing graft rejection in the two following years (hazard ratio (HR) at D0 = 7.30, $p = 0.0007$ and HR at M1 = 6.16, $p = 0.001$).
Conclusions: TTV viremia measurement at early times post transplantation predicts graft rejection and would represent a useful tool to improve kidney transplant monitoring.

Introduction

Long-term graft function and survival after kidney transplantation relies on suitable immunosuppressive treatment to provide an optimal balance between rejection and infection risks. BK virus (BKV)-associated nephropathy (BKVAN) and graft rejection are major causes of graft dysfunction and loss in kidney transplant recipients (KTR).[1] The disruption of the balance between BKV replication and host immune control is a key element of viral pathogenesis.[2] Torquetenovirus (TTV) is a small, non-enveloped, single-stranded DNA virus of the *Anelloviridae* family which has not been linked to any specific human illness but represents a major component of the human virome.[3] Indeed, immunocompetent hosts display low-level viremia while TTV loads are substantially higher in immunocompromised patients.[4] In lung and heart transplant recipients, high TTV loads correlate with high doses of immunosuppressive drugs and are associated with microbial infection occurrence,[5,6] while low levels of TTV viremia are observed in patients with graft rejection episodes.[3] These data suggest that TTV viremia may be linked to BKV replication and/or graft rejection in KTR. In this work, we investigated long-term TTV load kinetics at transplantation and over 24 months in a well-characterized KTR cohort. We analyzed TTV loads according to patient characteristics, immunosuppressive drugs, BKV replication status and rejection episodes, in order to determine if TTV viremia may adequately reflect the degree of immunosuppression and represent a useful predictor of BKV replication and/or graft rejection.

Patients and methods

Patients

Sixty-six adult patients who underwent kidney transplantation between July 2012 and July 2014 in Strasbourg University Hospitals were enrolled in this study. The present study was based on a prospective longitudinal study on anti-BKV neutralizing antibodies (Clinical Trials.gov identifier: NCT02826811). The design of the study has earlier been reported in detail.[7] Briefly, all patients who

Abbreviations: TTV, Torquetenovirus; KTR, kidney transplant recipients; BKV, BK virus; BKVAN, BK virus-associated nephropathy.

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displayed BKV replication in urine in the first two years post-transplantation ($n = 50$) were included. Among them, 28 were also BKV-viremic, including 13 patients with biopsy-confirmed BKVAN. Sixteen KTR who did not display BKV replication during the first two years after transplantation constituted the "BKV-negative" control group. These sixteen KTR had characteristics similar to the whole cohort of BKV-negative patients regarding age, sex, graft rank, donor type and induction/maintenance immunosuppressive treatment. Immunological parameters, including CD4+ and CD8+ T cell counts and gammaglobulin levels, were monitored for all patients.

Immunosuppressive treatment

After transplantation, immunosuppression consisted of induction therapy with either basiliximab or thymoglobulin, cyclosporine or tacrolimus, mycophenolate mofetil and steroids. Steroids were initially given at a dose of 1 mg/kg/day and were then progressively tapered off during the first four months post-transplant. In immunologically high-risk patients or patients with a history of acute rejection, steroids were continued at a dose of 0.1 mg/kg/day. Target trough levels of tacrolimus were 10–12 ng/ml in the first three months, 8–10 ng/ml from four to six months, and 6–8 ng/ml thereafter. Target trough levels of cyclosporine were 150–200 ng/ml in the first six months, 125–150 ng/ml from six to 12 months, and 75–125 ng/ml thereafter. The target for the mycophenolic acid area under the concentration time curve (AUC) from 0 to 12 h at month 1 and month 3 was 30–60 mg.h/l.

Viral load quantification

TTV DNA load quantification was measured on the day of transplantation (D0), and at 1, 3, 6, 12 and 24 months after transplantation using the TTV R-GENE® kit (bioMérieux, Marcy l'Etoile, France).[8] A total of 385 samples were collected, only 5 patients having more than one missing time point.

BKV replication was monitored using BKV quantitative real-time PCR (BK Virus R-GENE® kit, bioMérieux, Marcy l'Etoile, France) in urine and blood samples harvested at the time of transplantation (day 0), monthly for the first six months and then every three months over two years after transplantation, according to KDIGO and European guidelines.[9,10] In case of a BKV load >8 $\log_{10}$ copies/ml in urine, mycophenolate mofetil dose was reduced by 50%. Detection of BKV viremia at ≥ 4 $\log_{10}$ copies/ml prompted adjustment of the immunosuppressive regimen with a 50% reduction of calcineurin inhibitors, a 50% reduction or cessation of mycophenolate mofetil and/or introduction of leflunomide.

Graft rejection diagnosis and management

All patients displaying graft dysfunction – based on biological findings (increased serum creatinine, onset of proteinuria)- or upon the first detection of donor-specific antibodies – underwent kidney biopsy, excepted patients for whom biopsy was contraindicated. Biopsy specimens were classified as acute cellular rejection, antibody-mediated rejection or rejection-free according to the 2013 Banff classification.[11] In cases of cellular rejection, patients received 3 doses of 500 mg of methylprednisolone. In the event of antibody mediated or mixed rejection, patients were treated with steroid pulses, intravenous immunoglobulin (IVIg; 1 g/kg for 2 days repeated every three weeks for 3 cycles), plasmapheresis and rituximab (375 mg per square meter of body surface area) in the absence of advanced chronic lesions.

Table 1
Characteristics of kidney transplant recipients included in the study ($n = 66$).

	Patient cohort ($n = 66$)
Age, years, median (range)	54.4 (18–71.1)
Male, *n* (%)	40 (60.6)
1st transplantation, *n* (%)	45 (68.2)
Living donor, *n* (%)	13 (19.7)
HLA mismatches, median (range)	4.0 (0.0–6.0)
ATG, *n* (%)	34 (51.5)
Tacrolimus, *n* (%)	37 (56.1)
Rituximab, *n* (%)	5 (7.6)

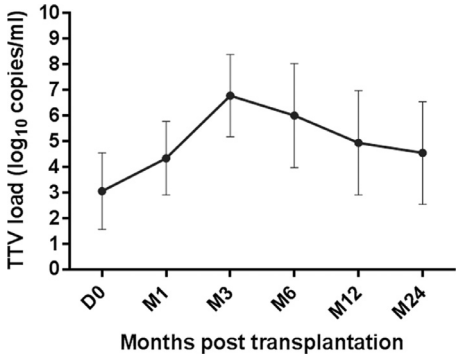

Fig. 1. TTV loads dynamics after kidney transplantation. TTV loads were measured at transplantation and at month (M) 1, 3, 6, 12 and 24 after transplantation. At each timepoint, geometric mean and standard deviation values are presented. TTV loads increase after transplantation up until M3 then decrease from M3 to M24.

Statistics

The distributions of continuous data were compared using non-parametric Mann–Whitney and Kruskal–Wallis tests when comparing different groups of patients and Wilcoxon signed-rank tests for paired comparisons; mean values indicated in the text correspond to the geometric mean. The distribution of categorical variables was compared using chi-square or Fisher's exact tests. Bayesian methods were used to estimate TTV viremia distribution during follow-up using a mixed effects linear model. Kaplan-Meier analyses were used to estimate the association of TTV viremia with acute rejection occurrence.

Results

TTV load kinetics

Patient characteristics are presented in Table 1. Positive TTV viremia was detected in 86% of KTR on the day of transplantation (D0) with a mean load of 3.06 $\log_{10}$ copies/mL, and in 96% of patients during follow-up. Only TTV-positive patients were subsequently considered for the analysis of TTV loads ($n = 63$). TTV kinetics showed an increasing phase up until M (month) 3 (M1 mean TTV load: 4.35 $\log_{10}$ copies/mL, M3 mean TTV load: 6.79 $\log_{10}$ copies/mL) with a 98% probability for TTV viremia to rise by ≥ 2 $\log_{10}$ copies/mL from baseline to M3 (Fig. 1). This was followed by a decreasing phase (M6 mean TTV load: 6.01 $\log_{10}$ copies/mL, M12 mean TTV load: 4.95 $\log_{10}$ copies/mL and M24 mean TTV load: 4.55 $\log_{10}$ copies/mL) with an 81% probability for TTV viremia to decline by ≥ 1 $\log_{10}$ copies/mL from M3 to M24.

TTV loads and patient characteristics

No difference in TTV loads was observed depending on recipient age (Fig. 2(A)), graft rank or HLA mismatches. Interestingly, TTV loads were lower in recipients of living donors compared to those

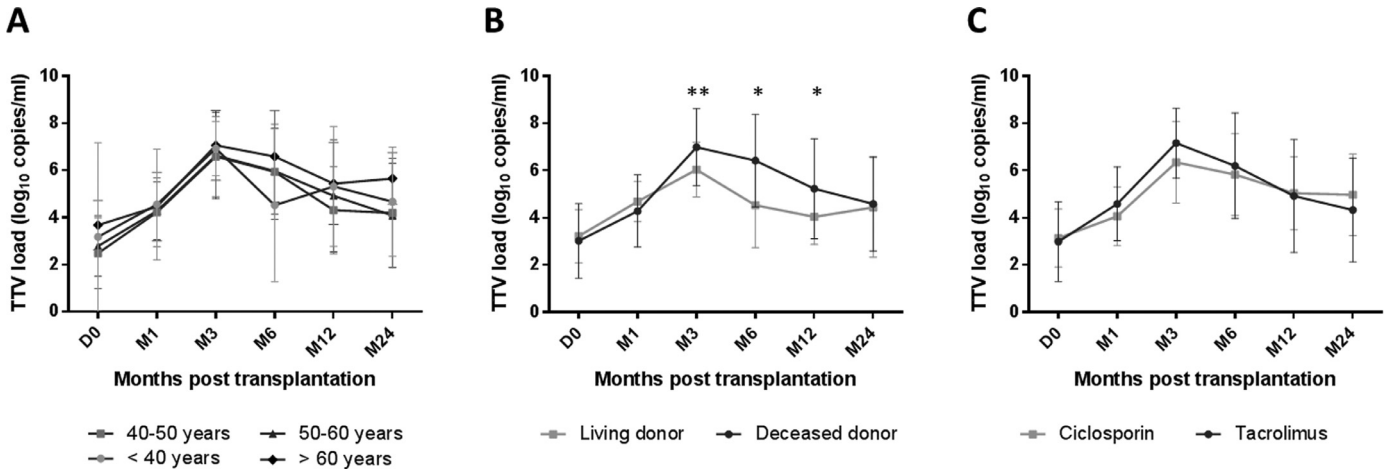

Fig. 2. TTV loads and patients characteristics. TTV loads are presented according to age (A), donor type (B) or calcineurin inhibitor (C). At each timepoint, geometric mean and standard deviation values are presented. One asterisk indicates a *p*-value lower than 0.05 and two asterisks indicate a *p*-value lower than 0.01.

of deceased donors, particularly at M3 (−0.95 $\log_{10}$ copies/mL, $p = 0.003$), M6 (−1.90 $\log_{10}$ copies/mL, $p = 0.020$) and M12 (−1.19 $\log_{10}$ copies/mL, $p = 0.013$) (Fig. 2(B)). Bayesian analysis showed that a higher TTV viremia was associated with a lower count of CD8+ T cells: each CD8+ T cell count decrease of 100 cells/mm^3 led to a 0.80 $\log_{10}$ TTV load increase (probability of 90%). No association was found between TTV loads and other immunological parameters such as CD4+ T cell count and gamma globulin levels.

TTV loads and immunosuppressive treatment

No difference in TTV loads at D0 or during follow-up was observed depending on thymoglobulin or basiliximab induction treatment (data not shown), and tacrolimus or cyclosporine maintenance treatment (Fig. 2(C)). Likewise, no association was found between TTV loads and cyclosporine or tacrolimus trough levels at any time, nor with mycophenolic acid AUC at M1, M3 and M12 (data not shown).

TTV loads and BKV reactivation

TTV loads at transplantation were not significantly higher in patients displaying BKV replication compared to BKV-negative KTR. Regarding patients who displayed BKV replication in blood, higher TTV loads were associated with a higher BKV viremia: each 0.2 $\log_{10}$ TTV load increase led to a BKV viremia increase of 1.50 $\log_{10}$ copies/ml (probability of 90%). BKV-viremic patients also displayed sustained high TTV loads at M6 compared to non-BKV-viremic KTR (mean TTV load 6.93 vs 5.47 $\log_{10}$ copies/ml, respectively, $p = 0.015$) (Fig. 3).

TTV loads predict graft rejection occurrence

Six patients displayed acute cellular rejection, two patients exhibited antibody-mediated rejection, and six patients developed mixed rejection during follow-up. TTV loads were lower in patients who subsequently developed graft rejection than in graft rejection-free KTR, notably at D0 (−1.50 $\log_{10}$ copies/mL, $p = 0.009$). Additionally, a viral load threshold of 3.4 $\log_{10}$ copies/ml at transplantation allowed prediction of graft rejection: 39% (11/28) of KTR with a D0 TTV load lower than 3.4 $\log_{10}$ copies/ml displayed graft rejection versus only 3% (1/31) of KTR with higher TTV loads ($p = 0.007$; hazard ratio (HR) = 7.30; 95% confidence interval (CI) = 2.32–22.9; negative predictive value (NPV) = 0.92; positive predictive value (PPV) = 0.63) (Fig. 4(A)). At M1, a viral load threshold of 4.2 $\log_{10}$ copies/ml also allowed prediction of graft rejection: 48% (11/23) of KTR with a M1 TTV load lower than 4.2 $\log_{10}$ copies/ml displayed graft rejection versus only 9% (3/33) of KTR with higher TTV loads ($p = 0.001$; HR = 6.16; 95% CI = 2.07-18.3; NPV = 0.92; PPV = 0.48) (Fig. 4(B)). The same observations were made when dividing the cohort into BKV-viremic and non BKV-viremic groups (Supplementary Figure 1).

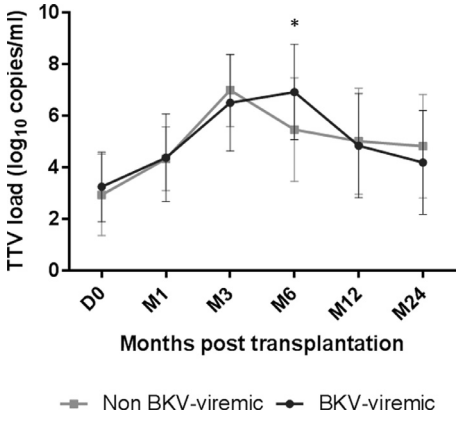

Fig. 3. TTV loads and BKV viremia. TTV loads are presented according to BKV status (viremic or non-viremic). At each timepoint, geometric mean and standard deviation values are presented. One asterisk indicates a *p*-value lower than 0.05.

Discussion

In this long-term longitudinal study on KTR, for the first time, we (i) describe a relationship between TTV and BKV viremia and (ii) determine cutoff values of TTV viremia allowing to identify, as early as in the first month after transplantation, the patients who are at high risk of graft rejection.

Longitudinal TTV load dynamics in KTR are characterized by a first increasing phase from baseline to M3, and a decreasing phase from M3 to M24. Some shorter-term studies in KTR have been published recently and show similar kinetics for the first months.[8,12,13] Long-term TTV loads have previously been reported in heart and lung transplant recipients, who display similar kinetics.[3,5] However, TTV loads in lung transplant recipients are approximately 100-fold higher, paralleling the higher intensity of immunosuppressive therapy in these patients compared to KTR. Interestingly, we found lower longitudinal TTV loads for recipients

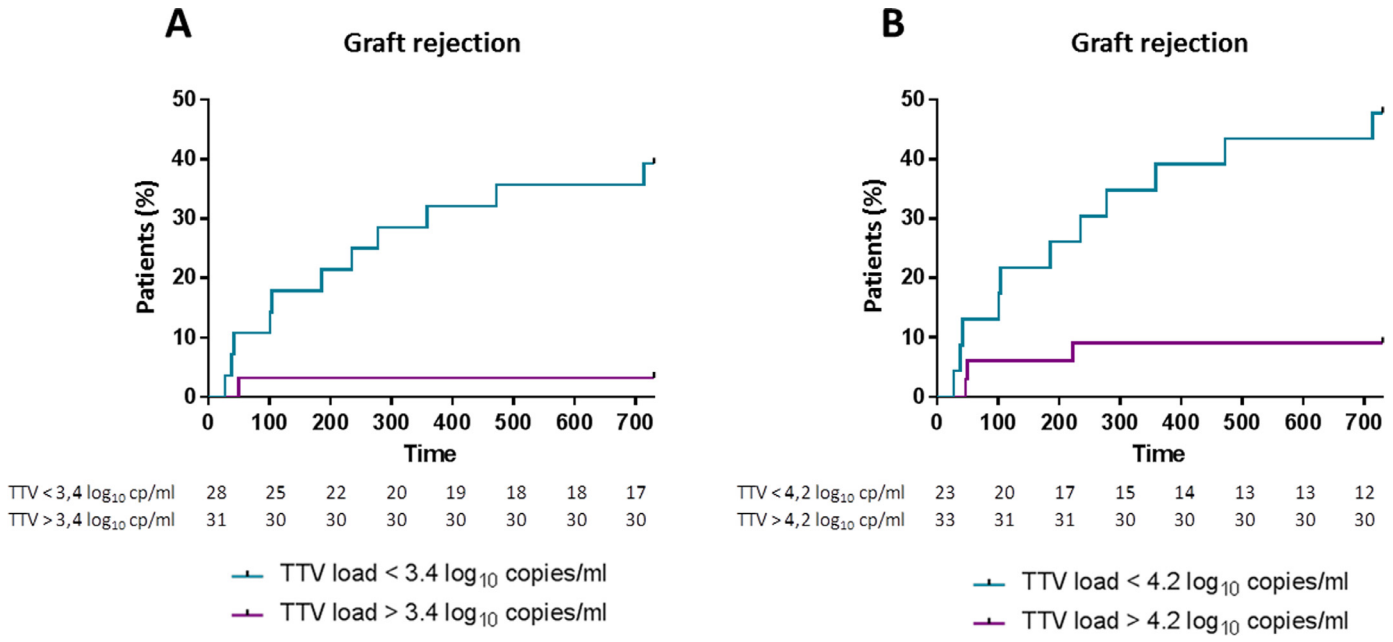

Fig. 4. Prediction of graft rejection according to TTV load. Kaplan–Meier curves represent graft rejection cumulative incidence according to TTV viral load (log_{10} copies/ml) at the time of transplantation (panel A) and at M1 post transplantation (panel B). Green curves indicate TTV loads lower than the cutoff while purple curves indicate TTV loads higher than the cutoff value (3.4 log_{10} copies/ml at D0, 4.2 log_{10} copies/ml at M1). Numbers at risk are indicated at each timepoint.

from living donors, who usually receive less intense immunosuppressive therapy, confirming the link between TTV load and overall immune function.

Since TTV loads mirror the intensity of immunosuppression,[4,14] we sought to investigate its relationship with the immunological parameters and immunosuppressive therapy. We found an inverse correlation of TTV loads with CD8+ T cell count, but not with CD4+ T cell count nor with gamma globulin levels. Regarding immunosuppressive drugs, we did not find significantly different TTV loads between KTR receiving thymoglobulin or basiliximab induction therapy, and tacrolimus or cyclosporine maintenance therapy. We also found no correlation between TTV loads and cyclosporine, tacrolimus or MPA blood levels. Correlation between TTV loads and tacrolimus blood levels have been reported in lung transplant recipients,[5] suggesting that the impact of this drug on the immune response compared to that of cyclosporine is significantly different only when high doses are given.

TTV and BKV can both be found in the urinary virome,[15] but the relationship between TTV loads and BKV viremia has not been hitherto studied in KTR. In this work, we show that in BKV-viremic recipients, sustained high TTV loads were associated with high BKV loads in blood. While the development of BKV replication – essentially from donor origin in KTR[7,16] – may rather depend on donor/recipient strain mismatch and serostatus,[7,17] TTV loads might mirror more sustained and/or deeper immunosuppression leading to more intense BKV replication following transplantation.

Several longitudinal studies on lung, heart or liver transplantation[3,5] reported low TTV loads in patients displaying graft rejection. In KTR, one cross-sectional analysis showed lower TTV loads in patients displaying antibody-mediated rejection.[18] In our longitudinal analysis, we not only show that TTV loads are lower in patients who subsequently develop graft rejection, but also define cutoff values of TTV load that allow prediction of graft rejection. Hence, patients who display TTV loads lower than 3.4 log_{10} copies/ml at transplantation, and/or lower than 4.2 log_{10} copies/ml one month after transplantation, have a 4–5-fold higher risk of graft rejection during the two years following transplantation. This should guide clinicians to better adapt immunosuppression level in order to prevent rejection episodes or DSA development. Indeed, TTV could be used as a predictive biomarker for various events such as microbial infections, cancer or graft rejection in solid organ transplantation, as summarized by Focosi and Maggi.[19]

A limitation of our study is the small number of recipients investigated. Large multicenter prospective trials investigating TTV loads with standardized methods would be needed to employ TTV as a biomarker reflecting the degree of immunosuppression in routine surveillance.[19] Nevertheless, our findings point to a close relationship between TTV load kinetics, BKV viremia and graft rejection, and strongly suggest that it could be possible for patients to be further assigned to lower and higher risk groups for high BKV viremia and graft rejection early after transplantation. This early stratification could be useful for transplant physicians, potentially allowing adaptation of the immunosuppressive strategy and optimal patient monitoring.

Acknowledgments

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Declarations of interest

None

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.05.010.

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