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MAJOR ARTICLE

Quantification of Torque Teno Virus Viremia as a Prospective Biomarker for Infectious Disease in Kidney Allograft Recipients

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(See the Editorial Commentary Kotton, on pages 1185–7.)

Background. Drug-induced immunosuppression following kidney transplantation is crucial to prevent allograft rejection, but increases risk for infectious disease. Tailoring of drug dosing to prevent both rejection and infection is greatly desirable. The apathogenic and ubiquitous torque teno virus (TTV) reflects immunocompetence of the host and might be a potential candidate for immunologic monitoring.

Methods. To assess TTV as an infection biomarker, virus load was prospectively quantified in peripheral blood of 169 consecutive renal allograft recipients at the Medical University Vienna.

Results. Patients with infection showed higher TTV levels compared to patients without infection (4.2×10^8 copies/mL [interquartile range, IQR, 2.7×10^7–1.9×10^9] vs 2.9×10^7 [IQR 1.0×10^6–7.2×10^8]; $P = .006$). Differences in TTV load became evident almost 3 months before infection (median 77 days, IQR 19–98). Each log level of TTV copies/mL increased the odds ratio for infection by 23% (95% confidence interval 1.04–1.45; $P = .014$). TTV $>3.1 \times 10^9$ copies/mL corresponded to 90% sensitivity to predict infections. Logistic regression demonstrated independent association between TTV levels and infection.

Conclusions. TTV quantification predicts infection after kidney transplantation and might be a potential tool to tailor immunosuppressive drug therapy.

Keywords. torque teno virus; kidney transplantation; infection; biomarker; immunosuppression; cytomegalovirus; polyomavirus nephropathy.

Organ transplantation is the treatment of choice for patients with end-stage renal disease. In the posttransplant period immunosuppressive drugs are crucial to prevent allograft rejection. Besides this desired effect, reduced immunocompetence of the transplant recipient leads to an increased risk of developing infectious diseases, which are the leading cause of death after kidney transplantation [1]. Currently, monitoring of immunosuppression relies mainly on calcineurin inhibitor drug trough levels in the peripheral blood, which correlate more closely with the risk of drug-related toxicity than the immunosuppressive efficacy [2]. Thus, there is an urgent need for tailored immunosuppressive medication in order to reduce the risk of infection and, at the same time, to prevent allograft rejection.

In this respect, a promising strategy might be the monitoring of peripheral blood levels for the torque teno virus (TTV) [3]. TTV can be detected in up to 90% of healthy individuals [4] and, so far, it has not been linked with any specific human disease [3]. Peripheral blood levels of TTV might mirror the overall strength of innate and specific immunity [5, 6] and thus viral load is closely related to the immunocompetence of the host [7, 8]. The prevalence of TTV in immunocompromised patients after solid-organ transplantation is up to 100% [9] and it is unaffected by conventional antiviral drug therapy used in the posttransplantation setting [10]. TTV levels were shown to be associated with the amount and type of immunosuppressive drugs administered to solid-organ recipients and thus indirectly with allograft rejection and infection [11, 12]. Retrospective cohort studies described associations of low TTV virus levels with graft rejection in lung, liver, kidney, and heart transplantation [10, 13–15] and, conversely, high TTV levels were found to be associated with infection after lung transplantation [11]. Hitherto, the value of TTV for the prediction of infection or organ rejection has not been tested in a prospective study design.

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This prospective study was designed to evaluate TTV as a predictor of infectious disease after kidney transplantation. We hypothesize that a high level of TTV reflects an excessive level of immunosuppression and thus precedes infections. To test this hypothesis, TTV was quantified before and longitudinally after transplantation by means of polymerase chain reaction (PCR) in the peripheral blood of consecutive recipients of a kidney allograft in 2016, performed in one academic centre in Vienna, Austria. Assay results were evaluated in the context of a careful monitoring of viral, bacterial, and fungal infections after transplantation.

MATERIAL AND METHODS

Patients

This prospective study includes all 169 consecutive adult (≥18 years of age) recipients of a kidney allograft transplanted at the Medical University of Vienna, Austria, between 1 January and 31 December 2016. Patients were followed at the outpatient clinic of the Medical University of Vienna and follow-up data were included until 1 March 2017. The present study was approved by the institutional review board of the Medical University of Vienna (approval number: 1785/2016) and registered at the German Clinical Trials Registry (register number DRKS00012335).

Initial Immunosuppression and Rejection Therapy

All patients received triple immunosuppression with tacrolimus, mycophenolic acid, and corticosteroids. Calcineurin inhibitor drug trough levels (tacrolimus and cyclosporine) were monitored at every visit to the outpatient clinic. Our transplant protocol allowed for transplantation across immunologic barriers, including both human leukocyte antigen (HLA) and ABO incompatibilities. HLA incompatibility was defined as the presence of donor-specific anti-HLA antibodies detected by single antigen bead assay with a medium fluorescence intensity of 1000 or higher. Recipient of a HLA-incompatible kidney underwent IgG immunoapheresis according to a local protocol [16]. Recipients of an ABO-incompatible kidney underwent ABO blood group antigen specific immunoapheresis (Glycosorb; Glycorex Transplantation AB, Lund, Sweden) and, in cases of high AB antibody titers (>1:256), received additional induction therapy with an anti-CD20 antibody (Rituximab; Hoffmann-La Roche, Basel, Switzerland). Antibody-mediated and T-cellular rejection were scored according to current Banff classification [17]. T-cellular rejection type I and IIA and borderline rejection were treated with dexamethason and rejection type IIB and III were treated with antithymoglobulin. Antibody-mediated rejection was treated with IgG immunoapheresis.

Infection Prophylaxis and Monitoring

All patients received prophylaxis with trimethoprim and sulfamethoxazole for 6 months after transplantation and valganciclovir for 3 months in cytomegalovirus (CMV) IgG-negative recipients of a CMV IgG-positive organ or after treatment with antithymoglobulin and IgG immunoapheresis. Screening for CMV and BK polyomavirus after transplantation was performed by PCR from peripheral blood once per week until discharge from the ward, on the first visit at the outpatient clinic, on month 3 after transplantation, and every 3 months thereafter. The first visit at the outpatient clinic was within 1 week after discharge from the ward. Epstein Barr virus (EBV) PCR from peripheral blood was performed in EBV IgG-negative recipients on the first visit at the outpatient clinic, 1 month after transplantation, monthly until month 6 after transplantation, and every 3 months thereafter. We did not perform specific screening for other viral infections or bacterial and fungal infections.

Primary End Point

The primary end point was any bacterial, fungal, or viral infection requiring antimicrobial or antiviral treatment or reduction of immunosuppressive drugs in case of BK polyomavirus nephropathy (PVN). Asymptomatic bacteriuria was not treated with antibiotic drugs. Severe infections were defined as infections requiring hospitalization or prolongation of a hospital stay or PVN. PVN was defined according to current Banff classification [18].

TTV Quantification

TTV was quantified prospectively in the peripheral blood at the following time points: before transplantation and after transplantation once per week until discharge from the ward, on the first visit at the outpatient clinic, on month 3 after transplantation, and every 3 months thereafter. Attending physicians were unaware of the TTV results. TTV DNA was extracted from 200 µL of plasma using the NucliSENS easyMAG platform (bioMerieux, France), as recommended by the manufacturer, and eluted in 50 µL of elution buffer. TTV DNA was quantitated by TaqMan real time PCR, as described previously [19]. The quantitative PCR reactions were performed in a volume of 25 µL using 2 × TaqMan Universal PCR Master Mix, containing 5 µL of extracted DNA, 400 nM of each primer, and 80 nM of the probe. Thermal cycling was started for 3 minutes at 50°C, followed by 10 minutes at 95°C, and then by 45 cycles at 95°C for 15 seconds, at 55°C for 30 seconds, and at 72°C for 30 seconds, using the 7300 Real Time PCR System (Applied Biosystems, Foster City, CA). Results were recorded in copies/mL. Details on standards, negative and positive controls, and test performance have been described in detail earlier [13].

Statistical Analysis

Summaries for continuous variables are presented as the median and interquartile range (IQR). The Mann-Whitney U test was used for comparing continuous data. Categorical variables are presented as absolute numbers and percentages, and group comparisons were made using the X^2 test. Logistic regression models were applied to assess whether TTV was independently

associated with infection as the outcome. To allow for the correlation of more than 1 event per patient we used a random-effect model. We added the clinically most relevant covariables into the model, including recipient age, donor age, and estimated glomerular filtration rate (eGFR). We also tested if follow-up time influenced the main effect within the model. The likelihood ratio test was used to test for linearity and interaction with diabetes mellitus and recipient sex. Receiver operating curves were applied for calculation of the area under the curve. Generally, a 2-sided P value < .05 was considered statistically significant. Exact tests were used where applicable. We used MS EXCEL 2010 (Redmond, WA), IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL) and STATA 14.1 (STAT coop., College Station, TX) for data management and analysis.

RESULTS

Patients and Transplant Outcome

This prospective study includes 169 consecutive adult recipients of a kidney allograft transplanted in 2016 at the Medical University of Vienna. Median follow-up was 218 days (IQR 133–316). Baseline characteristics are listed in Table 1. Median recipient age at transplantation was 55 years (IQR 43–64 years) and 51 (30%) patients were female. Thirty-six (21%) of the patients had a previous kidney transplant and 28 (17%) were recipients of a living donor kidney. Nine patients (5%) were transplanted across ABO and 17 (10%) across HLA antibody barriers. One hundred fifty-three patients (90%) received induction therapy with interleukin-2 receptor antagonist and 16 (10%) with antithymoglobulin.

Patient survival was 94% and death censored graft survival was 92% at the end of the follow-up period (median 7 month, maximum 14 months). The main cause of death was infection (n = 6; 60%; Supplementary Table 1). Acute rejection was detected in 23 (14%) of the allograft recipients. All rejection episodes were documented within 3 months after transplantation (Supplementary Table 2).

Torque Teno Virus Kinetics

In total, 978 samples for TTV assessment were taken with a mean of 6 measurements per patient (Supplementary Figure 1).

Table 1. Baseline Characteristics of the Total Study Cohort and the Event Cohort Stratified According to Patients With and Without Infections

Characteristics	Total Cohort (n = 169)	Event Cohort (n = 71)	P Value	Infection[a] (n = 22)	No Infection[a] (n = 49)	P value
Recipient characteristics						
Recipient age, years (IQR)	55 (43–64)	56 (43–64)	.885	59 (46–64)	56 (43–63)	.442
Recipient female, n (%)	51 (30)	19 (27)	.761	5 (23)	16 (33)	.421
Recipient BMI, kg/m^2 (IQR)	26 (23–29)	26 (24–29)	.993	25 (23–28)	26 (24–29)	.558
Renal replacement therapy,[b] years (IQR)	2.7 (1.6–4.4)	2.6 (1.7–4.2)	.977	2.4 (1.4–4.5)	2.7 (2.3–4.3)	.654
Diabetes mellitus, n (%)	24 (14)	9 (13)	.839	2 (9)	7 (14)	.711
Major comorbidity,[c] n (%)	84 (50)	33 (47)	.673	12 (55)	21 (43)	.443
CMV IgG positive, n (%)	120 (71)	48 (68)	.877	15 (68)	35 (71)	>.99
EBV IgG positive, n (%)	165 (97)	68 (96)	>.99	20 (91)	48 (98)	.225
HCV antibody positive, n (%)	3 (2)	0	>.99	0	0	NA
HBc antibody positive, n (%)	19 (11)	8 (11)	>.99	3 (14)	5 (10)	.696
HIV antibody positive, n (%)	4 (2)	1 (1)	>.99	1 (5)	0	.310
Donor characteristics						
Living donor, n (%)	28 (17)	7 (10)	.329	2 (9)	5 (10)	>.99
Donor after circulatory death, n (%)	13 (8)	9 (13)	.327	1 (5)	8 (16)	.257
Donor age, years (IQR)	55 (43–67)	56 (45–67)	.542	57 (52–68)	57 (45–68)	.817
Donor female, n (%)	82 (49)	33 (46)	>.99	9 (41)	24 (49)	.611
Donor CMV IgG positive, n (%)	117 (69)	47 (66)	.648	16 (72)	31 (63)	.589
Transplant characteristics						
Retransplantation, n (%)	36 (21)	12 (17)	.484	4 (18)	8 (16)	>.99
ABO incompatible transplantation, n (%)	9 (5)	2 (3)	.515	0	2 (4)	>.99
Donor specific antibody, n (%)	17 (10)	4 (6)	.325	1 (5)	3 (6)	>.99
HLA A, B, DR mismatch, n (IQR)	3 (2–4)	3 (3–4)	.878	3 (2–4)	3 (3–4)	.745
ATG induction, n (%)	16 (10)	5 (7)	.625	1 (5)	4 (8)	>.99
CMV prophylaxis, n (%)	58 (34)	22 (31)	.882	8 (36)	14 (29)	.583

Mann-Whitney U test was used for comparing continuous data and group comparisons were made using the X^2 test. Exact tests were used where applicable.

Abbreviations: ATG, antithymoglobulin; BMI, body mass index; CMV, cytomegalovirus; EBV, Epstein Barr virus; HBc, hepatitis B-core antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IgG immunoglobulin G; IQR, inter quartile range; NA, not applicable.

[a]For comparison of the clinical baseline data within the event cohort we stratified patients according to their first documented event (infection or no infection).

[b]Time between the start of renal replacement therapy and transplantation.

[c]Any history of major cardiovascular, gastrointestinal, pulmonary, or malignant disease.

Before transplantation, 80% of the patients were TTV positive and all patients except 1 turned TTV positive within month 3 after transplantation. Before transplantation, a median of 1.9×10^4 TTV copies/mL (IQR 2.4×10^3–8.0×10^4) was detected (Figure 1). After transplantation, TTV load increased steeply up to a peak level of 4.3×10^8 TTV copies/mL (IQR 3.6×10^7–2.9×10^9; $P < .001$ compared to pretransplant levels) at month 3 posttransplantation (median day 92, IQR 87–170). Thereafter, TTV levels decreased modestly to a median of 4.2×10^6 TTV copies/mL (IQR 3.2×10^5–1.7×10^8) at the last available visit (day 269, IQR 183–318; $P < .001$ compared to TTV at peak level).

Infections After Transplantation

To test the value of TTV quantification to predict infectious disease posttransplantation we included all TTV measurements taken after stabilization of TTV viremia (>day 92 posttransplantation) and analyzed the corresponding follow-up periods for the presence of infectious disease events (event cohort). Infectious disease occurred in 31 patients. We allowed for multiple events per patient. In total, 41 TTV measurements followed by infections and 83 TTV measurements with a subsequent follow-up period without infection were registered. Details on the involved pathogens and organ systems of the infections are displayed in Table 2. The majority of the infections were caused by bacteria (n = 26) or viruses (n = 12) and involved the urinary tract (n = 14) or the transplant kidney (n = 11).

Baseline characteristics of patients with and without infections at the time of transplantation are displayed in Table 1. No differences concerning risk factors for infections, including recipient age, frequency of diabetes mellitus or other major comorbidities, intensity of induction therapy, or CMV prophylaxis, were found in patients developing infectious diseases compared to patients without infections (Table 1).

Analyzing clinical data after transplantation, at the time of TTV assessment, we found higher C-reactive protein levels and more infections preceding an infection event compared to follow-up periods without infection (Table 3). No differences in allograft rejections or antirejection therapy, CMV viremia, prophylactic virostatic treatment, or type and amount of immunosuppressive drugs and calcineurin inhibitor trough level were noted in patients developing infections compared to patients without infection (Table 3).

Follow-up periods without infection occurred at a later time point posttransplantation compared to infections (264 days posttransplantation [IQR 189–292] vs 192 [IQR 139–267]; $P = .003$), but there was no difference in the time point of TTV assessment preceding infections and follow-up periods without infections (day 164 posttransplantation [IQR 105–195] vs 174 [IQR 104–189]; $P = .292$).

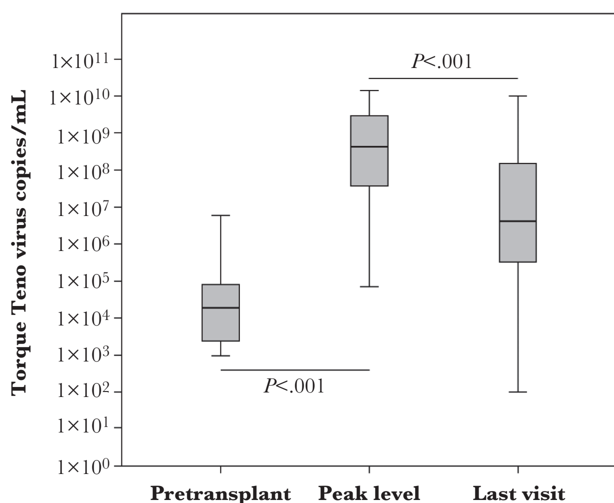

Figure 1. Torque teno virus (TTV) copies/mL in peripheral blood of kidney transplant recipients are displayed in relation to detection time. Individual levels were combined in box plots. TTV load increased steeply after transplantation up to month 3 posttransplantation and showed a modest decrease at the last documented visit. The Mann-Whitney U test was performed for statistical comparison of TTV levels between time points in patients with full dataset.

Torque Teno Virus Quantification in the Context of Infections After Transplantation

TTV levels detected before the occurrence of an infection were higher compared to levels preceding a follow-up period without infection (4.2×10^8 [IQR 2.7×10^7–1.9×10^9] vs 2.9×10^7 [IQR 1.0×10^6–7.2×10^8]; $P = .006$). Median time between TTV assessment and the event was 77 days (IQR 19–98). The odds ratio (OR) for an infection increased by 23% per log level increase of TTV copies/mL (95% confidence interval [CI], 1.04–1.45; $P = .014$) and showed a linear effect. Applying receiver operating statistics an area under the curve of 0.65 (95% CI, 0.55–0.75; $P = .006$) was calculated for the prediction of an infection by TTV (Supplementary Figure 2). TTV levels above 3.1×10^9 copies/mL corresponded to a sensitivity of 90%, specificity of 20%, negative predictive value of 92%, and positive predictive value of 17% for the prediction of an infection.

Sensitivity analysis in a subgroup of patients with severe infections showed higher TTV levels preceding severe infections compared to follow-up periods without infection (n = 33; 6.4×10^8 [IQR 2.2×10^7–2.0×10^9] vs 2.9×10^7 [IQR 1.0×10^6–7.2×10^8]; $P = .012$). Such difference was also noted in a second subgroup analysis restricted to all bacterial infections (n = 26; 4.4×10^8 [IQR 5.8×10^7–2.8×10^9] for infections vs 2.9×10^7 [IQR 1.0×10^6–7.2×10^8] for follow-up periods without infection; $P = .003$).

Patient Characteristic Associated With Torque Teno Virus Level

In order to uncover possible confounder of TTV load we analyzed clinical baseline data at the time of transplantation in the context of TTV quantification (Table 4). Older and male allograft recipients, patients without detectable CMV IgG, recipients of a

Table 2. Characteristics of Infectious Disease Events

Type	Localization	Number	Pathogen	Number
Bacterial	Urinary tract infection	14	*Escherichia coli*	7[a]
			Enterococcus faecium	3
			Pseudomonas aeruginosa	2
			Klebsiella pneumoniae	2[b]
	Pneumonia	5	*Streptococcus pneumoniae*	2
			Aspergillus fumigatus	1
			Pneumocystis jirovecii	1
			Unknown	1[c]
	Soft tissue infection	4	*Staphylococcus aureus*	2
			Group A beta-hemolytic *Streptococcus*	1
			Unknown	1[c]
	Colitis	2	*Campylobacter jejuni*	1
			Clostridium difficile	1
	Otitis media	1	*Streptococcus pneumoniae*	1
Viral	PVN	11	BK virus	11
	Influenza A	1	Influenza A virus	1
Fungal	Oesophagitis	1	*Candida albicans*	1
Fever of unknown origin	Unknown	2	Unknown[d]	2

Abbreviation: PVN, polyomavirus nephropathy.

[a] Two infections were caused by multidrug-resistant bacteria.

[b] One infection was caused by a multidrug-resistant bacterium.

[c] Bacterial infection was diagnosed due to clinical and/or radiological findings.

[d] Clinical course was highly suggestive for bacterial infection.

Table 3. Clinical Findings at the Time of Torque Teno Virus Assessment Posttransplantation, Stratified According to the Occurrence of Infection in the Subsequent Follow-up Period

Characteristics	Infection (n = 41)	No Infection (n = 83)	*P* Value
Clinical data			
Infection, n (%)	13 (32)	4 (5)[c]	<.001
Allograft rejection,[a] n (%)	9 (22)	16 (19)	.813
Antiviral treatment, n (%)	1 (2)	0 (0)	.331
Laboratory data			
CMV load, copies/mL (IQR)	0 (0–140)	0 (0–20)	.082
Leukocytes, g/L (IQR)	6.3 (4.9–8.7)	6.6 (4.3–7.8)	.423
Neutrophils, g/L (IQR)	4.6 (3.8–7.5)	2.9 (4.5–6.0)	.326
Lymphocytes, g/L (IQR)	1.2 (0.7–1.5)	1.3 (0.8–1.8)	.229
eGFR, mL/min/m^2 (IQR)[b]	47 (36–65)	50 (34–68)	.563
Urinary protein/creatinine ratio, mg/mg (IQR)	201 (105–395)	192 (98–350)	.642
CRP, mg/dL (IQR)	0.66 (0.27–2.81)	0.27 (0.08–0.70)	.023
Immunosuppression			
Prednisolone, mg (IQR)	5 (5–10)	5 (5–11.25)	.174
Cyclosporine, n (%)	2 (5)	6 (7)	.719
Tacrolimus, n (%)	72 (87)	38 (93)	.384
Tacrolimus once per day, n (%)	11 (28)	13 (16)	.149
Tacrolimus trough level, ng/mL (IQR)	7.3 (5.8–9.7)	7.3 (5.6–8.8)	.524
Everolimus, n (%)	1 (2.4)	2 (2.4)	>.99
Belatacept, n (%)	1 (2.4)	4 (4.8)	.665
Mycophenolic acid, n (%)	40 (98)	80 (98)	>.99
Mycophenolic acid, g (IQR)	2 (1–2)	1 (1–2)	.842

Mann-Whitney U test was used for comparing continuous data and group comparisons were made using the X^2 test. Exact tests were used where applicable.

Abbreviations: CMV, cytomegalovirus; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; IQR, inter quartile range.

[a]Any episode of biopsy-proven allograft rejection or antirejection therapy preceding torque teno virus assessment.

[b]eGFR was calculated using the Mayo equation [20].

[c]Four patients had an infection at the time of torque teno virus assessment, but did not develop an infection in the subsequent follow-up period.

Table 4. Torque Teno Virus Levels Stratified According to Baseline Characteristics

Characteristics	TTV Copies/mL (IQR)		*P* Value
	Variable Positive[a]	Variable Negative[a]	
Recipient data			
Recipient age >56 years[b]	4.2×10^8 (1.8×10^7–2.9×10^9)	3.2×10^7 (3.6×10^6–4.0×10^8)	.050
Recipient female	9.3×10^6 (1.8×10^6–3.4×10^8)	3.4×10^8 (1.8×10^7–2.4×10^9)	.021
Recipient BMI >26 kg/m^2 [b]	4.2×10^8 (3.4×10^6–1.7×10^9)	6.8×10^7 (8.3×10^6–4.2×10^8)	.629
Renal replacement therapy >2.6 years[b, c]	5.2×10^7 (5.3×10^6–8.3×10^8)	1.8×10^8 (3.3×10^6–2.7×10^9)	.589
Diabetes mellitus	1.7×10^7 (2.8×10^6–4.3×10^8)	1.8×10^8 (5.0×10^6–1.5×10^9)	.243
Major comorbidity[d]	5.2×10^7 (4.0×10^6–1.5×10^9)	1.4×10^8 (3.9×10^6–1.1×10^9)	.978
CMV IgG positive	2.9×10^7 (3.4×10^6–5.3×10^9)	4.3×10^8 (1.8×10^8–2.9×10^9)	.003
EBV IgG positive	7.6×10^7 (4.1×10^6–1.4×10^9)	3.8×10^8 (3.5×10^8–NA)	.415
HBc antibody positive	7.3×10^6 (3.6×10^6–1.4×10^8)	2.6×10^8 (8.0×10^6–1.7×10^9)	.074
HIV antibody/antigen positive	NA	NA	.366
Donor data			
Living donor	9.9×10^7 (1.2×10^7–4.3×10^8)	1.8×10^8 (4.1×10^6–1.4×10^9)	.884
Donor after circulatory death	1.7×10^8 (1.6×10^5–7.6×10^8)	2.4×10^8 (7.3×10^6–1.8×10^9)	.203
Donor age >56 years[b]	4.2×10^8 (9.3×10^6–2.9×10^9)	3.0×10^7 (3.8×10^6–4.2×10^8)	.014
Donor female	3.1×10^7 (3.9×10^6–1.6×10^9)	2.2×10^8 (7.2×10^6–1.0×10^9)	.699
Donor CMV IgG positive	3.4×10^7 (3.8×10^6–2.1×10^9)	4.2×10^8 (1.1×10^7–1.6×10^9)	.164
Transplant data			
Retransplantation	1.0×10^7 (9.9×10^5–3.3×10^8)	2.6×10^8 (9.3×10^6–1.7×10^9)	.041
ABO incompatible transplantation	1.0×10^6 (6.3×10^5–1.8×10^9)	1.8×10^8 (7.3×10^6–1.3×10^9)	.633
Donor specific antibody	2.5×10^7 (1.8×10^6–2.1×10^8)	1.8×10^8 (5.3×10^6–1.4×10^9)	.238
HLA A, B, DR mismatch >3[b]	4.7×10^7 (2.8×10^6–1.3×10^9)	1.8×10^8 (7.3×10^6–1.3×10^9)	.486
ATG induction	1.9×10^7 (2.3×10^6–1.5×10^8)	1.8×10^8 (5.2×10^6–1.5×10^9)	.193
CMV prophylaxis	4.0×10^8 (4.0×10^7–1.8×10^9)	3.1×10^7 (3.7×10^6–1.2×10^9)	.067

Mann-Whitney U test was used for comparing continuous data and group comparisons were made using the X^2 test. Exact tests were used where applicable.

Abbreviations: ATG, antithymoglobulin; BMI, body mass index; CMV, cytomegalovirus; EBV, Epstein Barr virus; HBc, hepatitis B-core antigen; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IgG immunoglobulin G; IQR, inter quartile range; NA, not applicable; TTV, torque teno virus.

[a]TTV levels preceding the first documented event (infection or no infection) per patient were analyzed.

[b]Cutoff defined by median.

[c]Time between the start of renal replacement therapy and transplantation.

[d]Any history of major cardiovascular, gastrointestinal, pulmonary, or malignant disease.

graft from an older donor, and patients with a previous solid-organ transplant showed higher levels of TTV. TTV levels were not associated with frequency of diabetes mellitus or other major comorbidities, intensity of induction therapy, or CMV prophylaxis (Table 4).

Analysis of clinical data posttransplantation, at the time of TTV assessment, revealed an associated of CMV viremia, low lymphocyte counts in the peripheral blood, and the use of high-dose mycophenolic acid (> 1.5 G) as an immunosuppressive drug with higher TTV levels (Table 5). No differences in TTV levels stratified according to allograft rejection and antirejection therapy, virostatic treatment, or type and amount of immunosuppressive drugs other than mycophenolic acid and calcineurin inhibitor trough levels were noted (Table 5). Increased TTV levels before transplantation and timing of TTV assessment or documentation of events (infection or no infection) were not associated with TTV level (Supplementary Table 3).

To test whether TTV was independently associated with infection as the outcome we applied logistic regression models (Table 6). After inclusion of the clinically most relevant confounder of TTV load described in the literature or detected in the present study (recipient age, donor age, eGFR at the time of TTV assessment, and follow-up time), the OR for prediction of infection by TTV levels were only marginally changed. In addition, we tested for interaction with the presence of diabetes mellitus or recipient sex and found no significant influence on the association of TTV levels and infections. Taken together logistic regression confirmed a robust and independent association of TTV levels and infection after kidney transplantation.

DISCUSSION

In the present study we were able to demonstrate a linear and independent association of TTV levels in the peripheral blood of kidney transplant recipients and subsequent infectious diseases. Patients with infections showed higher levels of TTV prior to the event compared to patients without infections. This association was still present after adjustment of the most relevant confounder of TTV levels. Moreover, a sensitivity analysis restricted to bacterial infections and severe infections confirmed the association between TTV and infectious disease.

Table 5. TTV Levels Stratified According to Clinical Characteristics at the Time of Assessment

Characteristics	TTV Copies/mL (IQR) Variable Positive	TTV Copies/mL (IQR) Variable Negative	*P* Value
Clinical data			
Infection	1.3×10^8 (1.3×10^7–8.6×10^8)	9.9×10^7 (2.5×10^6–1.4×10^9)	.953
Allograft rejection[a]	5.2×10^7 (1.3×10^6–5.9×10^8)	1.7×10^8 (3.4×10^6–1.7×10^9)	.370
Antiviral treatment	NA	9.9×10^7 (2.5×10^6–1.1×10^9)	>.99
Diabetes mellitus	4.3×10^7 (2.6×10^6–8.8×10^8)	1.5×10^8 (2.7×10^6–1.4×10^9)	.806
Laboratory data			
CMV PCR positive	3.8×10^8 (2.5×10^7–2.8×10^9)	3.4×10^7 (1.3×10^6–8.5×10^8)	.014
Leukocytes > 6.6 g/L[b]	1.7×10^8 (3.4×10^6–1.1×10^9)	5.2×10^7 (2.4×10^6–1.2×10^9)	.841
Neutrophiles > 4.5 g/L[b]	3.5×10^7 (1.9×10^6–5.4×10^8)	2.4×10^7 (4.0×10^5–4.2×10^8)	.896
Lymphocytes > 1.3 g/L[b]	4.4×10^6 (2.4×10^5–1.8×10^8)	2.6×10^8 (1.2×10^7–1.3×10^9)	<.001
eGFR > 48 mL/min/m^2[; c]	3.0×10^7 (1.3×10^6–6.4×10^8)	3.1×10^8 (3.9×10^6–1.8×10^9)	.069
Urinary protein/creatinine ratio >192 mg/mg[b]	5.2×10^7 (2.9×10^6–9.6×10^8)	3.0×10^8 (6.9×10^5–2.5×10^9)	.776
CRP >0.3 mg/dL[b]	3.6×10^7 (2.4×10^6–6.4×10^8)	3.7×10^8 (3.4×10^6–2.5×10^9)	.080
Immunosuppression			
Prednisolone >5 mg[b]	1.7×10^8 (1.1×10^7–8.9×10^8)	9.9×10^7 (1.3×10^6–1.4×10^9)	.584
Cyclosporine	1.2×10^7 (2.0×10^5–9.2×10^8)	1.5×10^8 (3.4×10^6–1.2×10^9)	.290
Tacrolimus	1.1×10^8 (3.4×10^6–1.3×10^9)	1.0×10^8 (2.3×10^6–8.7×10^8)	.575
Tacrolimus trough level >7.3 ng/mL[b]	1.8×10^8 (2.2×10^6–1.9×10^9)	5.1×10^7 (3.5×10^6–8.8×10^8)	.585
Tacrolimus once per day	3.8×10^8 (3.8×10^6–3.4×10^9)	4.9×10^7 (2.0×10^6–8.5×10^8)	.098
Everolimus	1.8×10^7 (7.6×10^3 – NA)	1.3×10^8 (2.9×10^6 – 1.2×10^9)	.307
Belatacept	7.2×10^8 (1.0×10^8 – 1.8×10^9)	7.5×10^7 (2.3×10^6–1.1×10^9)	.263
Mycophenolic acid	1.5×10^8 (3.3×10^6–1.3×10^9)	1.8×10^7 (7.6×10^3 – NA)	.299
Mycophenolic acid > 1.5 g[b]	2.6×10^7 (1.8×10^7–9.6×10^8)	1.8×10^7 (4.8×10^5–1.3×10^9)	.040

Mann-Whitney U test was used for comparing continuous data and group comparisons were made using the X^2 test. Exact tests were used where applicable.

Abbreviations: CMV, cytomegalovirus; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; IQR, interquartile range; NA, not applicable; PCR polymerase chain reaction; TTV, torque teno virus.

[a]Any episode of biopsy-proven allograft rejection or antirejection therapy preceding torque teno virus assessment.

[b]Cutoff defined by median.

[c] eGFR was calculated using the Mayo equation [20].

In addition, we demonstrated a value of TTV quantification to predict infectious disease. Most interestingly, TTV quantification could detect patients at risk for infections almost 3 months before the infection was clinically apparent. Taken together, our data suggest high levels of TTV reflect a state of intense immunosuppression after kidney transplantation, leading to an increased risk of infectious disease. Thus, TTV quantification might be a promising candidate to tailor immunosuppressive drugs after kidney transplantation and to reduce complications by infections.

Infection is still the main cause of death [1] and organ rejection the main cause of graft dysfunction after kidney transplantation in the contemporary era [21], underlining the importance of improved tailoring of immunosuppressive drugs. Indeed, also in the present cohort, infection was the main cause of death. Currently, surveillances of immunosuppression is guided

Table 6. Logistic Regression Models to Test for an Independent Association of TTV With Infectious Disease

Method	Variables	Odds Ratio	95% CI	*P* Value
ln TTV				
Unadjusted		1.23	1.04–1.45	.014
Adjusted	Time to event	1.23	1.04–1.45	.011
	Recipient age, donor age, eGFR	1.24	1.04–1.49	.017
	Recipient age, donor age, eGFR, DM = 0	1.27	1.01–1.80	.043
	Recipient age, donor age, eGFR, DM = 1	1.26	0.90–1.76	.175
	Recipient age, donor age, eGFR, recipient female = 0	1.22	0.99–1.50	.066
	Recipient age, donor age, eGFR, recipient female = 1	1.37	0.96–1.95	.080

Logistic regression models were applied to assess whether TTV was independently associated with infection as the outcome. To allow for the correlation of more than 1 event per patient, a random-effect model was used. The likelihood ratio test was used to test for linearity and interaction.

Abbreviations: CI, confidence interval; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; TTV, torque teno virus.

mainly via measuring of drug trough levels of calcineurin inhibitors, although such measurements might not sufficiently mirror immune function [2]. Accordingly, increased calcineurin inhibitor levels were not associated with infections in our present study. Likewise other known risk factors for infection, including diabetes mellitus, were not useful for selection of patients at risk in our cohort. In this respect, quantification of the ubiquitous and apathogenic TTV might be an interesting strategy, as TTV levels have been associated with immunocompetence of the host [7, 8]. Indeed, in an earlier study we demonstrated an inverse association of TTV levels and allograft rejection at the time of diagnosis in a retrospective analysis of kidney transplant recipients [13]. In addition, Görzer and colleagues described higher TTV levels in the sera of lung transplant recipients developing infections compared to stable patients in a small retrospective study [11]. They calculated a high sensitivity of 90% and a low specificity of 50% for the diagnosis of infections for a cutoff of 2×10^9 TTV copies/mL. In our prospective study a sensitivity of 90% for the diagnosis of infections following kidney transplantation was calculated for a similar cutoff of 3.1×10^9 TTV copies/mL. Both our present study and the report by Görzer and colleagues [11] described a low specificity of TTV to detect infections. Therefore TTV measurement is not sufficient to accurately predict infectious disease after solid-organ transplantation, but rather define patients with a low risk for infection. Interventional studies are needed to test whether tapering of immunosuppressive drugs to reach a TTV level below 3.1×10^9 TTV copies/mL will reduce the occurrence of infectious disease after kidney transplantation.

TTV has a high prevalence of >90% in healthy subjects [4] and up to 100% in the transplant population [9]. Indeed >99% of patients in our cohort were TTV positive after transplantation. Kinetics of TTV levels in our study were similar to those observed after liver [14], heart [10], and lung [9] transplantation, that is a steep increase of TTV load up to month 3 after transplantation, followed by a smooth decline thereafter. Absolute TTV levels were higher in lung and lower in liver transplant recipients compared to patients after kidney transplantation [9, 14]. Such differences might reflect a more intense immunosuppression following lung and a less intense immunosuppression following liver transplantation compared to immunosuppression administered in kidney transplant patients.

TTV levels following kidney transplantation in our study were associated with recipient age and sex, a finding that has been described in previous literature [22]. Interestingly, in our cohort, patients without CMV IgG before transplantation and positive CMV viremia after transplantation had higher levels of TTV, suggesting an association between primary CMV infection and TTV infection. A link between CMV and TTV infection might be supported by earlier findings in healthy individuals, where higher TTV levels were described in CMV IgG-positive compared to IgG-negative individuals [22]. Conflicting data have been presented on the association of CMV and TTV levels in patients early after hematopoietic stem cell transplantation. One study described lower levels [23] and 2 studies higher levels of TTV in patients developing CMV infection [24, 25]. In our cohort TTV was not affected by antiviral treatment with valganciclovir, a finding that has been observed in recipients of a lung and heart allograft also [10]. Recipients of older kidney allografts showed higher TTV levels in our cohort, which might be explained by higher TTV loads within kidneys derived from older donors already prior to transplantation. The higher TTV levels detected in recipients of a retransplant in our cohort might reflect a more intense immunosuppression used in this population to counteract the increased risk for allograft rejection due to HLA presensitization. Higher levels of TTV were also described in our study in a group of patients treated with high doses of mycophenolic acid, which might reflect a more intense immunosuppression due to higher drug dosing. Indeed influence of antimetabolite dosing on TTV following kidney transplantation has been described in an earlier study of our group [13].

The major strength of the present study is the prospective design, the unselected cohort of consecutive kidney transplant recipients, the longitudinal TTV evaluation at predefined time points and the strict definition of the primary end point. Detailed and thorough recording of clinical characteristics allowed for the exclusion of possible confounder. One of the limitations of the study is the short follow-up. TTV levels for prediction of infection were included after TTV viremia stabilization at month 3 posttransplantation and the follow-up was limited to 14 months after transplantation. Secondly, the present data suggest high TTV levels to reflect intense immunosuppression, but did not prove causality.

Taken together our study provides evidence for the value of TTV quantification to predict infectious disease after kidney transplantation. Moreover, we defined a TTV level cutoff for tailoring of immunosuppressive drug dosing. Interventional studies would be required to prove the value of TTV guided immunosuppression compared to calcineurin inhibitor trough level guided immunosuppression to prevent infectious disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Authors' contributions. R. S., M. S., K. D., F. E., Z. K., G. A. G., M. G. V., and S. R. participated in the data collection and writing the manuscript. E. P. and I. G. participated in TTV analysis and writing the manuscript. H. H. and G. A. B. participated in data analysis and writing the manuscript. G. B. participated in the research design, data analysis, and writing the manuscript.

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