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Torque Teno Virus for Risk Stratification of Acute Biopsy-Proven Alloreactivity in Kidney Transplant Recipients

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Background. Drug-induced immunosuppression in kidney transplant recipients is crucial to prevent allograft rejection, but increases risk for infectious disease. Immunologic monitoring to tailor immunosuppressive drugs might prevent alloreactivity and adverse effects simultaneously. The apathogenic torque teno virus (TTV) reflects the immunocompetence of its host and might act as a potential candidate for a holistic monitoring.

Methods. We screened all 1010 consecutive patients from the prospective Vienna Kidney Transplant Cohort Study for availability of allograft biopsies and adequately stored sera for TTV quantification by polymerase chain reaction.

Results. Patients with acute biopsy-proven alloreactivity according to the Banff classification (n = 33) showed lower levels of TTV in the peripheral blood compared to patients without rejection (n = 80) at a median of 43 days before the biopsy. The risk for alloreactivity decreased by 10% per log level of TTV copies/mL (risk ratio, .90 [95% confidence interval, .84–.97]; $P = .005$). TTV levels $>1 \times 10^6$ copies/mL exclude rejection with a sensitivity of 94%. Multivariable generalized linear modeling suggests an independent association between TTV level and alloreactivity.

Conclusions. TTV is a prospective biomarker for risk stratification of acute biopsy-proven alloreactivity in kidney transplant recipients and might be a potential tool to tailor immunosuppressive drug therapy.

Keywords. immunologic monitoring; kidney transplantation; torque teno virus; rejection.

Immunosuppressive drugs are crucial to prevent allograft rejection after kidney transplantation, but increase risk for infectious disease. Immunologic monitoring relies mainly on the quantification of calcineurin inhibitor drug trough level in the peripheral blood, which correlates more closely with the risk of drug-related toxicity than the immunosuppressive efficacy [1]. Peripheral blood levels of the ubiquitous and apathogenic torque teno virus (TTV) mirror overall strength of the immune system [2] and thus represent a promising new strategy for guidance of immunosuppression to reduce alloreactivity and adverse effects at the same time. We hypothesize that a low level of TTV reflects an insufficient level of immunosuppression and thus precedes alloreactive episodes. The present study was designed to evaluate TTV as a prognostic biomarker for acute biopsy-proven alloreactivity after kidney transplantation.

MATERIALS AND METHODS

Patient Selection

We screened all 1010 consecutive renal allograft recipients from the prospective Vienna Kidney Transplant Cohort Study at the Medical University Vienna, Austria, who were transplanted between 1 January 2012 and 31 December 2017. Inclusion criteria for the current analysis were indication biopsy performed between months 4 and 12 posttransplantation, and adequately stored blood samples for retrospective TTV quantification taken between month 4 posttransplantation and the date of the transplant biopsy. If multiple biopsies per patient were available, we included the latest biopsy. For sensitivity analysis, the first biopsy was included. If >1 blood sample per biopsy was available, the sample taken at the earliest time point posttransplantation was selected. The study was approved by the institutional review board (approval number EK1785/2016).

Quantification of Torque Teno Virus

TTV DNA was quantitated by TaqMan real-time polymerase chain reaction (PCR), as described previously [3, 4].

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Transplant Biopsies and Clinical Management

Histomorphology was evaluated on paraffin-embedded sections. The primary outcome, acute biopsy-proven alloreactivity, included antibody-mediated rejection (ABMR), T-cell–mediated rejection (TCMR), and borderline changes suspicious for acute TCMR. Histopathological lesions were classified following the 2009 and 2013 updates of the Banff classification [5, 6]. Clinical management including initial immunosuppression, microbial prophylaxis, and rejection treatment are described in the Supplementary Data.

Statistical Analysis

Detailed statistical analyses are described in the Supplementary Data. The Mann–Whitney *U* test was used for comparing continuous data, and group comparisons were made using the χ^2 test. A generalized linear model was used to estimate the association between alloreactivity and log-TTV levels. Deviation from linearity was assessed using the likelihood ratio test. Excel 2010 (Microsoft), IBM SPSS Statistics 24.0 (SPSS Inc), and Stata 15 (StataCorp) software packages were applied for data analysis.

RESULTS

Patient Characteristics

A total of 113 adult kidney allograft recipients, transplanted between 1 January 2012 and 31 December 2017 at the Vienna transplant unit, were enrolled in the present analysis. Baseline characteristics are displayed in Table 1. Laboratory parameters and immunosuppression at the time of TTV assessment (median of 127 days posttransplantation [interquartile range {IQR}, 105–174 days]) are shown in Table 2. Baseline characteristics of the study cohort were similar compared to the total population of all 1010 screened patients, transplanted consecutively during the same period at our center (Supplementary Table 1).

Kidney Allograft Biopsies

For each of the 113 patients, we included 1 indication kidney allograft biopsy, performed between months 4 and 12 posttransplantation (median, 186 days [IQR, 155–258 days]). Thirty-three (29%) biopsy samples showed significant features of acute alloreactivity (14 ABMR and 19 TCMR or borderline changes suspicious for acute TCMR). All 14 cases with ABMR were active ABMR, 3 were C4d-positive ABMR, and 2 showed mixed rejection, 1 with type I TCMR and 1 with borderline changes. Isolated TCMR and borderline changes were detected in 19 patients, with 1 type I, 3 type II TCMR lesions, and 15 borderline changes suspicious for acute TCMR. The most frequent pathologies described in biopsies without rejection were interstitial fibrosis/tubular atrophy or chronic vascular lesions (n = 46 [58%]; Supplementary Table 2).

Analyzing patient baseline characteristics in the context of biopsy results, transplant recipients with alloreactivity had more frequently preformed donor-specific antibodies (DSA) and were more often recipients of a retransplant (Table 1). Analyzing laboratory parameters and type and amount of immunosuppression at the time of TTV assessment, we did not detect any differences between patients with subsequent biopsy-proven alloreactivity and patients without rejection (Table 2).

Table 1. Baseline Characteristics of the Study Cohort and Stratified According to Kidney Allograft Biopsy Results

Characteristic	Study Cohort (n = 113)	Biopsy-Proven Alloreactivity (n = 33)	No Rejection (n = 80)	*P* Value
Recipient characteristics				
Age, y, median (IQR)	55 (43–66)	50 (36–65)	58 (44–66)	.119
Female sex	50 (44)	16 (49)	34 (43)	.678
Donor characteristics				
Living donor	22 (20)	5 (15)	17 (21)	.604
Donation after circulatory death	13 (12)	4 (12)	9 (11)	> .99
Donor age, y, median (IQR)	58 (50–69)	55 (45–68)	59 (51–71)	.097
Donor female	68 (60)	21 (64)	47 (59)	.629
Transplant characteristics				
Retransplantation	23 (20)	13 (39)	10 (13)	.002
ABO-incompatible transplantation	5 (4)	0 (0)	5 (6)	.319
HLA-A/B/DR mismatch, median (IQR)	3 (2–4)	3 (2–4)	3 (2–5)	.409
Donor-specific antibody	22 (20)	12 (36)	10 (13)	.005
CDCXM conversion[a]	4 (4)	3 (9)	1 (1)	.074
Cold ischemia time, h, median (IQR)	14 (8–18)	16 (11–19)	14 (7–18)	.199
Delayed graft function[b]	48 (43)	16 (49)	32 (40)	.407

Data are presented as No. (%) unless otherwise indicated. Mann–Whitney *U* test was used for comparing continuous data and group comparisons were made using the χ^2 test. Exact tests were used where applicable.

Abbreviations: CDCXM, complement-dependent cytotoxicity crossmatch; HLA, human leukocyte antigen; IQR, interquartile range.

[a]We allowed for peritransplant CDCXM conversion following a local protocol.

[b]Delayed graft function was defined by the necessity of >1 renal replacement therapy posttransplantation.

Table 2. Clinical Characteristics at the Time of Torque Teno Virus Assessment for the Study Cohort and Stratified According to Kidney Allograft Biopsy Results

Characteristic	Study Cohort (n = 113)	Biopsy-Proven Alloreactivity (n = 33)	No Acute Rejection (n = 80)	*P* Value
Laboratory parameter				
eGFR, mL/min/1.73 m^2, median (IQR)[a]	36 (29–48)	39 (33–50)	36 (28–48)	.620
Urinary protein:creatinine ratio, median (IQR)	199 (129–483)	193 (126–580)	207 (131–446)	.627
Microhematuria[b]	36 (36)	8 (29)	28 (39)	.334
Immunosuppression				
Triple immunosuppression	105 (93)	29 (88)	76 (95)	.232
Corticosteroid	112 (99)	33 (100)	79 (99)	.519
Prednisolone, mg, median (IQR)	7.5 (5–10)	7.5 (5–10)	5 (5–5)	.420
Mycophenolic acid	96 (85)	27 (82)	69 (86)	.570
Mycophenolic acid above median[c]	50 (54)	13 (48)	37 (57)	.442
Tacrolimus	99 (88)	26 (84)	73 (95)	.116
Tacrolimus trough level, ng/mL, median (IQR)	6.9 (5.4–9)	6.7 (4.8–10)	6.9 (5.5–9)	.558
Belatacept	5 (4)	2 (6)	3 (4)	.628
Assessment of primary outcome parameters				
Biopsy, days after transplantation, median (IQR)	186 (155–258)	186 (157–264)	186 (154–259)	.622
TTV, days after transplantation, median (IQR)	127 (105–174)	121 (107–174)	140 (103–174)	.877
TTV assessment to biopsy, d, median (IQR)	43 (22–96)	43 (22–97)	48 (15–89)	.786

Data are presented as No. (%) unless otherwise indicated. The Mann–Whitney *U* test was used for comparing continuous data and group comparisons were made using the χ^2 test. Exact tests were used where applicable.

Abbreviations: eGFR, estimated glomerular filtration rate; IQR, interquartile range; TTV, teno torque virus.

[a]eGFR was calculated using the Modification of Diet in Renal Disease equation [7]. Data were available from 100 patients.

[b]Microhematuria was assessed by dipstick analysis or light microscopy.

[c]Five hundred forty milligrams for enteric-coated mycophenolic acid and 1500 mg for non-enteric-coated mycophenolic acid. Data were available from 92 of 96 patients.

Torque Teno Virus Quantification

TTV was retrospectively quantified in the peripheral blood of all 113 patients. Median time between transplantation and blood sampling was 127 days (IQR, 105–174 days) and median TTV level was 6.1×10^7 copies/mL (IQR, 7×10^6–2.3×10^9 copies/mL). Patient baseline characters in the context of TTV

Table 3. Torque Teno Virus Level Stratified According to Baseline Characteristics of the Study Cohort

Characteristic	TTV, Copies/mL, Median (IQR)		*P* Value
	Variable Positive	Variable Negative	
Recipient characteristics			
Recipient age >55 y[a]	9.3×10^8 (3.2×10^7–3.7×10^9)	2.8×10^7 (1.8×10^6–3.0×10^8)	< .01
Recipient female	1.0×10^8 (1.7×10^6–3.8×10^9)	5.4×10^7 (8.5×10^6–1.7×10^9)	.835
Donor characteristics			
Living donor	1.2×10^8 (4.8×10^6–4.0×10^9)	5.8×10^7 (7.0×10^6–2.0×10^9)	.928
Donation after circulatory death	1.5×10^8 (9.3×10^6–3.1×10^9)	5.9×10^7 (6.9×10^6–2.2×10^9)	.815
Donor age >58 y[a]	5.0×10^8 (1.5×10^7–4.1×10^9)	3.2×10^7 (2.8×10^6–1.2×10^9)	.030
Donor female	5.6×10^7 (7.0×10^6–3.0×10^9)	8.5×10^7 (4.5×10^6–2.2×10^9)	.633
Transplant characteristics			
Retransplantation	3.1×10^7 (5.8×10^5–2.8×10^9)	1.3×10^8 (8.3×10^6–2.8×10^9)	.090
ABO-incompatible transplantation	4.9×10^9 (1.7×10^9–1.5×10^{10})	5.6×10^7 (6.1×10^6–2.0×10^9)	.036
HLA-A/B/DR mismatch >3[a]	7.1×10^7 (1.2×10^7–2.1×10^9)	6.1×10^7 (4.8×10^6–3.0×10^9)	.969
Donor-specific antibody	4.9×10^9 (1.6×10^9–1.4×10^{10})	5.8×10^7 (6.1×10^5–2.0×10^9)	.438
CDCXM conversion[b]	4.2×10^7 (8.1×10^6–1.3×10^9)	8.5×10^7 (6.9×10^6–2.6×10^9)	.482
Cold ischemia time >14 h[a]	5.6×10^7 (7.2×10^6–2.3×10^9)	1.2×10^8 (6.3×10^6–2.8×10^9)	.877
Delayed graft function[c]	1.4×10^8 (1.2×10^7–2.8×10^9)	5.7×10^7 (5.8×10^6–2.2×10^9)	.504

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

Abbreviations: CDCXM, complement-dependent cytotoxicity crossmatch; HLA, human leukocyte antigen; IQR, interquartile range; TTV torque teno virus.

[a]Cutoff defined by median.

[b]We allowed for peritransplant CDCXM conversion following a local protocol.

[c]Delayed graft function was defined by the necessity of >1 renal replacement therapy posttransplantation.

Table 4. Torque Teno Virus (TTV) Levels Stratified According to Clinical Characteristics of the Study Cohort at the Time of TTV Assessment

Characteristic	TTV, Copies/mL, Median (IQR)		*P* Value
	Variable Positive	Variable Negative	
Laboratory parameter			
eGFR[a,b] >37 mL/min/1.73 m^2	3.3×10^8 (1.0×10^7–2.2×10^9)	2.5×10^8 (3.2×10^7–3.9×10^9)	.124
Urinary protein:creatinine ratio[b] >198	9.8×10^7 (3.1×10^7–5.1×10^9)	4.5×10^8 (2.2×10^7–5.1×10^9)	.484
Microhematuria[c]	8.5×10^7 (4.0×10^7–3.8×10^9)	3.5×10^8 (3.1×10^7–3.4×10^9)	.595
Immunosuppression			
Triple immunosuppression	6.1×10^7 (3.2×10^7–3.7×10^9)	4.1×10^8, [d]	.945
Prednisolone >5 mg[b]	6.1×10^7 (3.1×10^7–1.7×10^9)	2.7×10^9 (1.2×10^8–7.1×10^9)	.758
Mycophenolic acid	1.2×10^8 (3.2×10^7–3.7×10^9)	1.3×10^7, [d]	.046
Mycophenolic acid above median[b,e]	1.3×10^8 (3.2×10^7–3.0×10^9)	4.5×10^8 (2.0×10^7–3.9×10^9)	.969
Tacrolimus	1.5×10^8 (3.2×10^7–3.7×10^9)	1.6×10^7, [d]	.022
Tacrolimus trough level >7 ng/mL[b]	1.1×10^9 (4.8×10^7–3.9×10^9)	1.3×10^8 (6.4×10^6–2.5×10^9)	.712
Belatacept	1.9×10^6, [d]	9.9×10^7 (3.2×10^7–3.7×10^9)	.030
Virology			
BKV PCR positive	3.1×10^9 (5.1×10^7–1.5×10^{10})	8.5×10^7 (3.1×10^7–1.7×10^9)	.029
CMV PCR positive	1.3×10^8 (1.7×10^7–2.7×10^9)	8.6×10^8 (3.2×10^7–3.9×10^9)	.677
TTV detection time >127 d after transplantation[b]	2.5×10^8 (3.2×10^7–4.9×10^9)	5.3×10^8 (3.1×10^7–3.7×10^9)	.770

The Mann–Whitney *U* test was used for comparing continuous data. Exact tests were used where applicable.

Abbreviations: BKV, BK polyomavirus; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; IQR, interquartile range; PCR, polymerase chain reaction; TTV, torque teno virus.

[a]eGFR was calculated using the Modification of Diet in Renal Disease equation [7].

[b]Cutoff defined by median.

[c]Microhematuria was assessed by dipstick analysis or light microscopy.

[d]Interquartile range not applicable due to low number of events per group.

[e]Five hundred forty milligrams for enteric-coated mycophenolic acid and 1500 mg for non-enteric-coated mycophenolic acid.

levels are displayed in Table 3. Older patients, recipients of an older donor organ, and patients transplanted across a major ABO barrier had higher levels of TTV. Clinical parameters at the time of blood sampling for TTV analysis are shown in Table 4. Patients receiving mycophenolic acid– and tacrolimus-based immunosuppression had higher levels of TTV compared to patients without mycophenolic acid and without tacrolimus, respectively. TTV levels were associated with BK polyomavirus PCR positivity in the peripheral blood (Table 4).

TTV Quantification in the Context of Biopsy-Proven Alloreactivity

To define the value of TTV for risk stratification of biopsy-proven alloreactivity following kidney transplantation, TTV levels were analyzed in the context of subsequent biopsy findings. Median time between TTV quantification and allograft biopsies was 43 days (IQR, 22–96 days), with no difference according to rejection status (Table 2). There was no difference in timing of TTV assessment with regard to transplantation between patients with and without alloreactivity (Table 2). Patients with subsequent biopsy-proven alloreactivity (n = 33) had lower levels of TTV with a median of 3.1×10^7 copies/mL (IQR, 4.9×10^5–2.3×10^8 copies/mL) compared to patients without rejection (n = 80; 2.3×10^8 copies/mL [IQR, 1.4×10^7–3.6×10^9 copies/mL]) ($P = .004$; Supplementary Figure 1).

The risk for kidney transplant alloreactivity decreased by 10% per log level of TTV (risk ratio, .90 [95% confidence interval {CI}, .84–.97]; $P = .005$). A linear dose-response effect between TTV level and biopsy-proven alloreactivity was observed. A sensitivity analysis using results of the earliest biopsy in patients with >1 biopsy (n = 23) showed similar results (risk ratio, .90 [95% CI, .84–.96]; $P = .002$). Applying the receiver operating curve, an area under the curve of .67 (IQR, .56–.78; $P = .005$) was calculated to exclude rejection by TTV level (Supplementary Figure 2). A TTV level >1×10^6 copies/mL corresponded to a sensitivity of 94% and a specificity of 27% with 74% correct classification and a positive predictive value of 76% and a negative predictive value of 64%.

The subgroup of patients with borderline changes suspicious for TCMR (n = 15) had lower TTV levels compared to patients without rejection (1.2×10^7 copies/mL [IQR, 2.8×10^5–1.5×10^8 copies/mL]; $P = .001$; Supplementary Figure 1). Likewise, a trend toward lower TTV levels in patients with ABMR (n = 14) was noted compared to patients without rejection (1.2×10^7 copies/mL [IQR, 3.6×10^5–1.3×10^9 copies/mL]; $P = .154$; Supplementary Figure 1).

To test whether TTV was independently associated with alloreactivity, we applied a generalized linear model (Supplementary Table 3). Recipient sex, recipient age at transplantation, history of prior transplantation, preformed DSA, ABO-incompatible transplantation, donor age, time between kidney transplantation and TTV assessment, estimated glomerular filtration rate (calculated by the Modification of Diet in Renal Disease formula [7]), tacrolimus trough level, and mycophenolic acid, tacrolimus, and belatacept-based immunosuppression at the

time of TTV assessment were not confounding or interacting with the association of TTV levels and biopsy-proven alloreactivity applying univariate models. The final multivariate model including recipient sex, recipient age at transplantation age, preformed DSA, and history of prior transplantation confirmed a robust and independent association of TTV level and alloreactivity after kidney transplantation (Supplementary Table 3).

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 biopsy-proven alloreactivity. Patients with alloreactivity showed lower levels of TTV prior to the event compared to patients without rejection. In addition, we provided a clinically useful TTV level cutoff for risk stratification of allograft biopsy results. Most interestingly, TTV quantification could detect patients at risk for alloreactivity >1 month before the histologic diagnosis. Taken together, our data suggest low levels of TTV to reflect a state of insufficient immunosuppression after kidney transplantation leading to an increased risk of alloreactivity. Thus, TTV quantification might be a promising candidate to tailor immunosuppressive drugs after kidney transplantation and to reduce episodes of graft loss due to rejection.

Graft rejection due to insufficient immunosuppression represents the main cause of organ dysfunction following kidney transplantation. Currently, surveillance of immunosuppression is guided mainly via calcineurin inhibitor trough levels, although such measurements might not sufficiently mirror immune function [1]. "Functional" biomarkers, reflecting immunosuppression, have been studied, but until now, none has paved its way into clinical practice [8]. The ideal candidate for guidance of immunosuppression would detect both graft rejection and infectious disease. A test of leukocyte function, the T-SPOT.PRT assay (Oxford Immunotec), was prognostic for infectious events, but not for graft rejection in kidney, liver, and lung transplant recipients [9]. Tailoring of immunosuppression after liver transplantation via functional assay of $CD4^+$ lymphocytes, ImmuKnow (Cylex), in a randomized controlled setting, resulted in fewer infectious events, but had no influence on graft rejection [10].

In this respect, quantification of the ubiquitous and apathogenic TTV might be a promising strategy, as TTV levels have been associated with the global immunocompetence of its host [2]. Peripheral blood levels of TTV might mirror the overall strength of innate and specific immunity including cellular and humoral components of the immune system [11, 12]. Indeed, earlier work of our group analyzing kidney transplant recipients described an association of TTV level with both ABMR and infectious disease [4, 13]. However, this is the first report to demonstrate a prognostic value of TTV in the context of clinically significant biopsy-proven kidney graft alloreactivity. Jaksch and colleagues described lower TTV levels in the sera of lung transplant recipients subsequently developing rejections compared to stable patients in a retrospective study and recently confirmed their findings in a prospective setting [14]. TTV levels $>1 \times 10^7$ TTV copies/mL were associated with a low risk of subsequent graft rejection. Fernández-Ruiz and colleagues described an association between TTV levels, quantified before transplantation, and subsequent kidney allograft rejection in a prospective setting [15]. However, no analysis on the impact of posttransplant TTV levels was available. Both our present study and the report by Jaksch and colleagues described a high sensitivity and a low specificity of TTV to detect rejection. Therefore, TTV measurement is not sufficient for an accurate diagnosis of graft rejection after solid organ transplantation, but rather defines patients at low risk for rejection. Interventional studies are needed to test whether adaption of immunosuppressive drugs to reach a TTV level $>1 \times 10^6$ TTV copies/mL will reduce the occurrence of graft rejection after kidney transplantation.

It has been shown that TTV does not reach stable levels until month 3 after solid organ transplantation [13]. Analyses of TTV levels before stabilization do not allow for definition of clinically useful cutoff values. Therefore, we included patients only after month 3, and our findings cannot be translated into the early phase after transplantation. In addition, we restricted TTV measurements to the first year after transplantation. TTV levels experience a slow and constant decline from month 4 to year 3 after transplantation [4]. Therefore, our findings cannot be extrapolated beyond month 12 after transplantation. TTV levels were lower in patients experiencing biopsy-proven alloreactivity of any type, including ABMR, TCMR, and borderline changes suspicious for TCMR, compared to patients without rejection. Comparably low TTV levels were detected in subgroups of patients with borderline changes and patients with ABMR. Of note, differences in TTV levels in patients with ABMR compared to patients without rejection did not reach the predefined level of significance. In this context, it is important to note that earlier studies demonstrated an association between TTV levels and late ABMR in a large cohort of kidney transplant recipients [4]. One might speculate that we missed a true association between TTV levels and ABMR due to limited sample size. Future analyses have to focus on early ABMR as the primary outcome to confirm the hypothesis postulated by our subgroup analysis.

The major strength of the present study is its careful design to minimize selection, observer, and information bias and confounding, even though we are aware of the retrospective and observational nature of the analysis. All available biopsies of an unselected cohort of consecutive transplanted and prospectively followed recipients were included, and baseline variables of the study cohort did not differ substantially compared to the total cohort of patients transplanted at our center during the time selected for screening. Generalized linear modeling excluded

possible confounders, and sensitivity analysis demonstrated internal validity. The noninterventional design represents the major limitation of our study. The present data suggest low TTV levels to reflect insufficient immunosuppression and thus indirectly risk for graft rejection, but a causal relationship remains to be proven. A prospective protocol of TTV-guided personalization of immunosuppression is needed to determine whether TTV quantification has any advantage over current monitoring strategies. Second, our analysis was limited to a single European center and a time frame between months 4 and 12 after transplantation. Finally, the C statistic for risk stratification of rejection is limited due to the noninclusion of patients without allograft biopsy and stable graft function, respectively, and the limited sample size.

Taken together, our study provides evidence for the value of TTV quantification for risk stratification of biopsy-proven alloreactivity after kidney transplantation >1 month before clinical diagnosis was made. Moreover, we propose a TTV level cutoff for a prospective protocol to tailor immunosuppressive drugs. Interventional studies will have to prove the superiority of TTV-guided immunosuppression compared to standard of care.

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

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