Quantification of Torque Teno Virus Load to Monitor Short-term Changes in Immunosuppressive Therapy in Kidney Transplant Recipients

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Background. Quantification of torque teno virus (TTV) has been proposed as a surrogate parameter to monitor immunocompetence in kidney transplant recipients (KTRs) early after transplantation. However, its use in monitoring short-term changes of immunosuppression in KTRs late after transplantation requires further investigation. **Methods.** In this post hoc analysis, we quantified TTV load in sera of 76 KTRs, with 43 pausing mycophenolic acid (MPA) 1 wk before to 4 wk after COVID-19 vaccination to increase vaccine response. TTV load was quantified before, 4 wk, and 3 mo postvaccination. Results were compared to 33 KTRs with continued standard immunosuppressive therapy and with 18 hemodialysis as well as 18 healthy control subjects. **Results.** TTV load before vaccination was with a median (interquartile range) of 1.39×10^4 copies/milliliter (c/mL) (9.17×10^1–2.66×10^5 c/mL) highest in KTRs compared to 1.73×10^3 c/mL (1.07×10^3–1.31×10^4 c/mL) in hemodialysis patients and 1.53×10^2 c/mL (6.38–1.29×10^3 c/mL) in healthy controls. In KTRs with MPA withdrawal, TTV load decreased significantly from a median (interquartile range) of 1.11×10^4 c/mL (4.75×10^2–1.92×10^5 c/mL) to 5.24×10^3 c/mL (6.92×10^2–6.91×10^4 c/mL) 4–5 wk after initiation of MPA withdrawal ($P = 0.003$). In patients with MPA withdrawal, TTV load was significantly inversely correlated with COVID-19 or SARS-CoV-2–specific antibodies 4 wk and 3 mo postvaccination ($P = 0.009$ and $P = 0.004$). **Conclusions.** TTV load reflects changes in immunosuppressive therapy even late after transplantation, supporting its use to monitor immunocompetence in KTRs.

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INTRODUCTION

Kidney transplantation is the best option for patients with end stage kidney disease and substantially improves quality of life and overall survival.[1] Following transplantation, patients are treated with a combination of immunosuppressive drugs to prevent graft rejection. However, immunosuppressive therapy increases the risk for severe infections in transplanted patients and optimal dosing remains a challenge in posttransplant care. This challenge may be overcome by assessing the patient's individual immunosuppressive burden using surrogate parameters, such as torque teno virus (TTV) load monitoring.[2]

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TTV constitutes the most abundant component of the human virome.[2,3] It is a nonenveloped, single-stranded circular DNA virus and with up to nearly 100% highly prevalent in healthy people.[4] TTV load is strongly affected by the onset of immunosuppressive therapy but largely escapes antiviral drug therapy such as cytomegalovirus prophylaxis posttransplantation.[2] Lower TTV load is associated with subclinical alloreactivity in kidney transplant recipients (KTRs),[5,6] whereas higher TTV load predicts susceptibility to infection after kidney transplantation.[7]

Recently, Doberer et al[8,9] demonstrated the utility of TTV load measured 3 mo after kidney transplantation to predict graft rejection and infection within the first year posttransplant, affirming previous results obtained by Fernández-Ruiz et al.[10] The authors demonstrated that TTV load increased rapidly with the initiation of immunosuppressive therapy and peaked 3 mo posttransplantation, followed by a slow decline.[8] Additionally, Doberer et al[8] were able to demonstrate an association between a higher TTV load posttransplantation and subsequent opportunistic infections, BK viremia, cytomegalovirus disease, but also bacterial infections and infections without the need for hospitalization. These promising results prompted the development of an ongoing multicentric randomized controlled interventional trial to assess TTV-guided immunosuppression in KTRs during the first-year posttransplantation.[11]

Although the kinetics of TTV load of KTRs in the early posttransplant period are well known, data regarding TTV load in patients transplanted longer ago and the effect of short-term changes in immunosuppressive therapy on TTV load remain scarce. In December 2021, we developed a prospective interventional study to assess short-term withdrawal of mycophenolic acid (MPA) in COVID-19 vaccine nonresponder KTRs to increase vaccine immunogenicity in these patients.[12,13] Consecutively, we now investigated the impact of 4–5-wk MPA withdrawal to changes in TTV load in these KTRs.

MATERIALS AND METHODS

Study Design

This is a post hoc analysis of a prospective, observational cohort study, where we enrolled 76 KTRs with no seroresponse after at least 3 COVID-19 vaccinations at the Department of Nephrology, Heidelberg University Hospital. Short-term MPA withdrawal was discussed for patients with a triple immunosuppressive maintenance therapy containing a calcineurin inhibitor (CNI), MPA, and corticosteroids (CSs) in case of stable graft function (S-creatinine ≤2.5 mg/dL and proteinuria ≤2 g/L) and no graft rejection during the past 12 mo (**Supplemental Methods, SDC**, http://links.lww.com/TP/C889). Following shared decision-making with the patient, MPA was withdrawn in 43 KTRs 5–7 d before vaccination and remained paused for an additional 4 wk. Serum for analysis of humoral response to COVID-19 vaccination as well as for quantification of TTV load was obtained immediately before vaccination and MPA withdrawal (t_0), a median (interquartile range [IQR]) of 27 d (27–30 d) (t_1) postvaccination at time of reintroduction of MPA, and a median of 89 d (85–91 d) (t_2) postvaccination (Figure 1). Patients with breakthrough infections (N = 22), of whom 13 had withdrawn MPA, were excluded from analysis. Additionally, 8 patients with no available sera at the second follow-up time point (t_2) were excluded from analysis (**Figure S1, SDC**, http://links.lww.com/TP/C889). Results on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–specific humoral response as well as safety data regarding development of donor-specific antibodies (DSAs) and changes in donor-derived cell-free DNA (dd-cfDNA) in patients with MPA withdrawal were published previously.[12,13] This additional, post hoc analysis focuses on changes in TTV load in long-term KTRs with short-term changes in immunosuppressive therapy.

To investigate a possible influence of COVID-19 vaccination on TTV load and to compare TTV load in KTRs with nonimmunocompromised individuals, we also quantified TTV load in 18 hemodialysis patients and 18 healthy controls before vaccination (t_0), a median (IQR) of 21 d (21–22 d) (t_1), and a median of 90 d (88–92 d) (t_2) postvaccination.

Quantification of Torque Teno Virus Load

TTV was quantified by using the TTV R-Gene assay (BioMérieux, Marcy-l'Étoile, France), a real-time

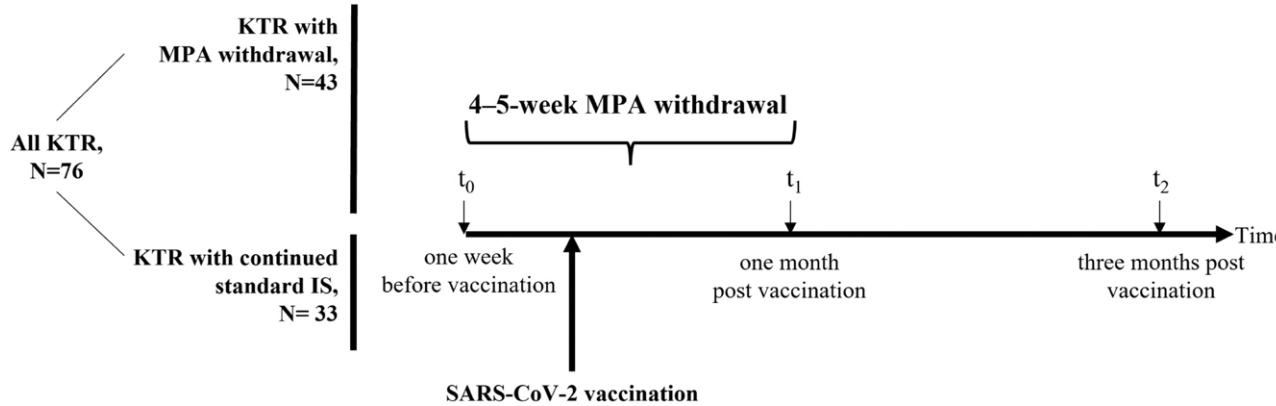

FIGURE 1. Study flow chart to assess TTV load in KTRs with short-time withdrawal of MPA to increase SARS-CoV-2 vaccine response. A total of 76 KTRs were enrolled into the study. In 43 KTRs, MPA was withdrawn 1 wk before to 4 wk after an additional SARS-CoV-2 vaccination. The other 33 KTRs continued their maintenance IS upon vaccination. TTV load and SARS-CoV-2–specific anti-S1 IgG antibodies were determined before vaccination (t_0), 1 mo (t_1), and 3 mo (t_2) postvaccination. IS, immunosuppression; KTRs, kidney transplant recipients; MPA, mycophenolic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TTV, torque teno virus.

polymerase chain reaction (PCR) assay targeting the TTV 5′ untranslated region with >90% identity between isolates.[3] The assay is validated and well-established for using plasma samples for the detection of TTV.[14] The dynamic range of TTV quantification by TTV R-Gene is from 250 to 10^9 copies/milliliter (c/mL), the limit of detection at 250 c/mL, according to manufacturer's protocol. The commercially available assay indicates a total TTV load and does not discriminate between different genotypes. To use serum samples as biomaterial for TTV quantification, we initially validated the protocol for serum as biomaterial using 134 paired plasma/serum samples (**Supplemental Methods** and **Figure S2, SDC**, http://links.lww.com/TP/C889).

TTV DNA was extracted from 200 µL of serum/plasma using the QIAsymphony SP platform (QIAGEN, Venlo, The Netherlands) following the extraction protocol provided by the assay manufacturer. PCR was performed at the Center of Infectious Diseases, Virology, Heidelberg University Hospital, on a Light Cycler 480 Instrument II (Roche Life Science), according to the manufacturer's instructions. Thermal cycling commenced 15 min at 95 °C, followed by 45 cycles at 95 °C for 10 s and at 60 °C for 40 s. PCR was terminated by cooling for 30 s at 40 °C. Virus load was calculated using a standard curve with known copy numbers. Specimens with undetectable virus load were assigned a value of 0.01 c/mL for analysis purposes, as previously done so by Fernández-Ruiz et al.[10]

Assessment of SARS-CoV-2–Specific Anti-S1 IgG Antibodies

We used the SARS-CoV-2 Total Assay (Siemens, Eschborn, Germany) to assess anti-spike S1 IgG antibodies and the Elecsys anti-SARS CoV-2 assay (Roche, Mannheim, Germany) for qualitative detection of anti-nucleocapsid antibodies. Seroconversion was defined as an anti-S1 IgG index >10, as we previously showed that this cutoff correlates significantly with the presence of wild-type SARS-CoV-2 neutralizing antibodies.[15,16]

Statistics

Quantitative data are shown as median with IQRs. Qualitative variables are expressed as absolute and relative frequencies. The Student *t* test or Mann–Whitney *U* test were applied for comparing continuous variables. When comparing >2 groups, the Kruskal–Wallis test with Dunn's posttest was applied for continuous data. Paired measures were compared with the Wilcoxon matched-pairs signed-rank test and with the Friedman test in case of >2 paired measures. To describe the correlation between TTV load and humoral response to COVID-19 vaccination, Spearman's ρ was calculated as a nonparametric measure of rank correlation. To examine the influence of other factors than MPA withdrawal on TTV load, TTV load was correlated to leukocyte count, and MPA dosage (before MPA withdrawal) and Tacrolimus dosage. Statistical analysis was performed using GraphPad Prism version 9.5.1 (GraphPad Software, San Diego, CA), and statistical significance was assumed at a $P < 0.05$.

Ethical Approval

We used serum samples obtained in previous studies to assess COVID-19 vaccine efficacy in KTRs (DRKS00024668). This study was approved by the ethics committee of the University of Heidelberg and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

RESULTS

Study Cohort

Seventy-six KTRs with no seroresponse after at least 3 prior COVID-19 vaccinations were enrolled to receive an additional messenger RNA (mRNA) vaccination. In KTRs, MPA was either paused to improve vaccine response (N = 43) or standard immunosuppressive therapy was continued (N = 33) (Figure 1). Median (IQR) age in the KTRs population was 57 y (47–63 y), and 29 of 76 (38%) participants were females. Baseline characteristics of all 76 KTRs participating in the study are shown in Table 1. Median (IQR) time since transplantation at t_0 was 4.3 y (2.0–9.3 y) and 5.3 y (2.5–10.9 y) in KTRs with MPA withdrawal and those with continued maintenance immunosuppression, respectively ($P = 0.56$). In KTRs with MPA withdrawal, changes in DSA were observed in 9 patients at t_1 (7 KTRs with increases in preexisting DSA, including 1 KTRs with de novo DSA; 2 KTRs with decreases in DSA) and in the same 9 patients at t_2 (3 increases in preexisting DSA with an additional de novo formation of DSA; 7 decreases in DSA; with 1 patient showing both increasing and decreasing DSA). dd-cfDNA at t_1 increased in 1 KTRs above >0.5%, a cutoff associated with graft injury, but decreased again by t_2, whereas the dd-cfDNA of another patient increased from 0.22% to 3.3% by t_2, most likely because of an infect-related graft deterioration.[12,13] No rejection was detected during 3-mo follow-up.

As controls, we enrolled 18 hemodialysis patients and 18 healthy controls that received an additional third mRNA vaccination. For the hemodialysis population, median (IQR) age was 76 y (57–81 y), and 4 of 18 (22%) were females. In the healthy control population, median (IQR) age was 48 y (42–59 y), and 13 of 18 (72%) were females.

Torque Teno Virus Load Before Vaccination

TTV load before vaccination was significantly higher in KTRs with a median (IQR) of 1.39×10^4 c/mL (9.17×10^1–2.66×10^5 c/mL) compared with TTV load in the healthy control population (median, 1.53×10^2 c/mL; IQR, 6.38–1.29×10^3 c/mL; $P < 0.01$; Figure 2A). When stratifying KTRs for MPA withdrawal, differences in TTV load to healthy controls remained significant ($P < 0.01$; Figure 2A). No significant differences in TTV load were seen between KTRs who continued immunosuppressive maintenance therapy including MPA (MPA+) compared with KTRs who withdrew MPA (MPA–) 5–7 d before vaccination (median, 5.72×10^4 c/mL; IQR, 6.38×10^1–1.12×10^6 c/mL for MPA+ KTRs and median, 1.11×10^4 c/mL; IQR, 4.75×10^2–1.92×10^5 c/mL for MPA– KTRs, respectively; $P = 0.38$; Figure 2A). With a median (IQR) of 1.73×10^3 c/mL (1.07×10^3–1.31×10^4 c/mL), TTV load in

TABLE 1.

Baseline characteristics of 76 kidney transplant recipients with no seroresponse after at least 3 COVID-19 vaccinations

Characteristic	MPA withdrawal (N = 43)	Continued maintenance IS (N = 33)	*P*
Age at enrollment (y), median (IQR)	58 (50–63)	56 (44–63)	0.42
Sex (female), N (%)	14 (33)	15 (45)	0.25
BMI (kg/m^2), median (IQR)	24.9 (21.6–28.6)	24.1 (22.1–30.1)	0.90
Transplant-related data			
Living donor kidney transplant	16 (37)	11 (33)	0.81
First transplant, N (%)	39 (91)	29 (88)	0.72
Kidney pancreas transplantation	1 (2)	4 (12)	0.16
Time since transplantation (y), median (IQR)	4.3 (2.0–9.3)	5.3 (2.5–10.9)	0.56
S-creatinine at t0 (mg/dL)	1.4 (1.3–1.8)	1.5 (1.2–1.8)	0.89
Immunosuppressive maintenance therapy			
Calcineurin inhibitor, N (%)	43 (100)	30 (91)	NA
Tacrolimus, N (%)	32 (74)	23 (77)	NA
Tacrolimus dose (mg), median (IQR)	3.5 (2–4.9)	4.0 (3.0–8.0)	0.06
Cyclosporine A, N (%)	11 (26)	7 (23)	NA
Cyclosporine A dose (mg), median (IQR)	150 (125–175)	120 (100–180)	0.43
Mycophenolic acid, N (%)	43 (100)	28 (85)	NA
MPA dose (g), median (IQR)	1.5 (1.0–2.0)	1.5 (1.0–2.0)	0.86
Everolimus, N (%)	0 (0)	3 (9)	NA
Sirolimus, N (%)	0 (0)	2 (6)	NA
Belatacept, N (%)	0 (0)	2 (6)	NA
Corticosteroids, N (%)	43 (100)	33 (100)	NA
Cause of end-stage kidney disease			
Vascular, N (%)	1 (2)	3 (9)	0.31
Diabetes, N (%)	1 (2)	6 (18)	0.04[a]
Glomerular disease, N (%)	20 (47)	11 (33)	0.35
PKD, N (%)	8 (19)	7 (21)	0.78
Systemic, N (%)	2 (5)	0 (0)	0.50
Reflux/chronic pyelonephritis	4 (9)	2 (6)	0.69
Other/unknown	7 (16)	4 (12)	0.74
Comorbidities, N (%)			
Arterial hypertension	35 (81)	22 (67)	0.18
Diabetes	7 (16)	4 (12)	0.74
CAD	12 (28)	6 (18)	0.42
Chronic lung disease	7 (16)	4 (12)	0.74
Chronic liver disease	1 (2)	4 (12)	0.16
Malignancy	11 (26)	7 (21)	0.79

[a]$P < 0.05$.

BMI, body mass index; CAD, coronary artery disease; IQR, interquartile range; IS, immunosuppression; MPA, mycophenolic acid; NA, Not applicable; PKD, polycystic kidney disease.

hemodialysis patients was in-between the TTV load quantified in KTRs and healthy controls, albeit without any statistical significance (Figure 2A).

Spearman's ρ (95% confidence interval) to assess the correlation between TTV load and leukocyte count was –0.016 (–0.237 to 0.217; P = 0.89), 0.157 (–0.086 to 0.383; P = 0.19) for TTV load and MPA dosage, and 0.203 (–0.073 to 0.451; P = 0.14) for TTV load and Tacrolimus dosage.

Torque Teno Virus Load in Kidney Transplant Recipients With Short-term Withdrawal of Mycophenolic Acid

In KTRs in whom MPA was withdrawn to increase immunogenicity of an additional SARS-CoV-2 vaccination, TTV load decreased significantly from a median (IQR) of 1.11×10^4 c/mL (4.75×10^2–1.92×10^5 c/mL) to 5.24×10^3 c/mL (6.92×10^2–6.91×10^4 c/mL) when comparing levels before MPA withdrawal (t_0) to levels at reintroduction of MPA (t_1) (P = 0.003; Figure 2B). Upon recommencing MPA 4 wk after vaccination (t_1), median (IQR) TTV load at 3 mo postvaccination (t_2) increased again to 2.02×10^4 c/mL (7.79×10^2–2.75×10^5 c/mL), not significantly different from levels before MPA withdrawal (t_0) (P = 0.66; Figure 2B). SARS-CoV-2–specific anti-S1 IgG increased significantly from a median (IQR) of 0.18 (0.10–1.43, t_0) to 4.3 (0.22–78.76, t_1) 4 wk (t_1) and to a median (IQR) of 8 (0.19–36.82, t_2) 3 mo (t_2) postvaccination, as reported previously (P < 0.001 for all; Figure 2C).[12,13]

When correlating TTV load obtained 4 wk after vaccination (t_1), at the end of the 4–5-wk MPA withdrawal period, to SARS-CoV-2–specific anti-S1 IgG, higher TTV load was significantly inversely correlated to lower antibody titers with a Spearman's ρ of R = –0.42 four wk (t_1),

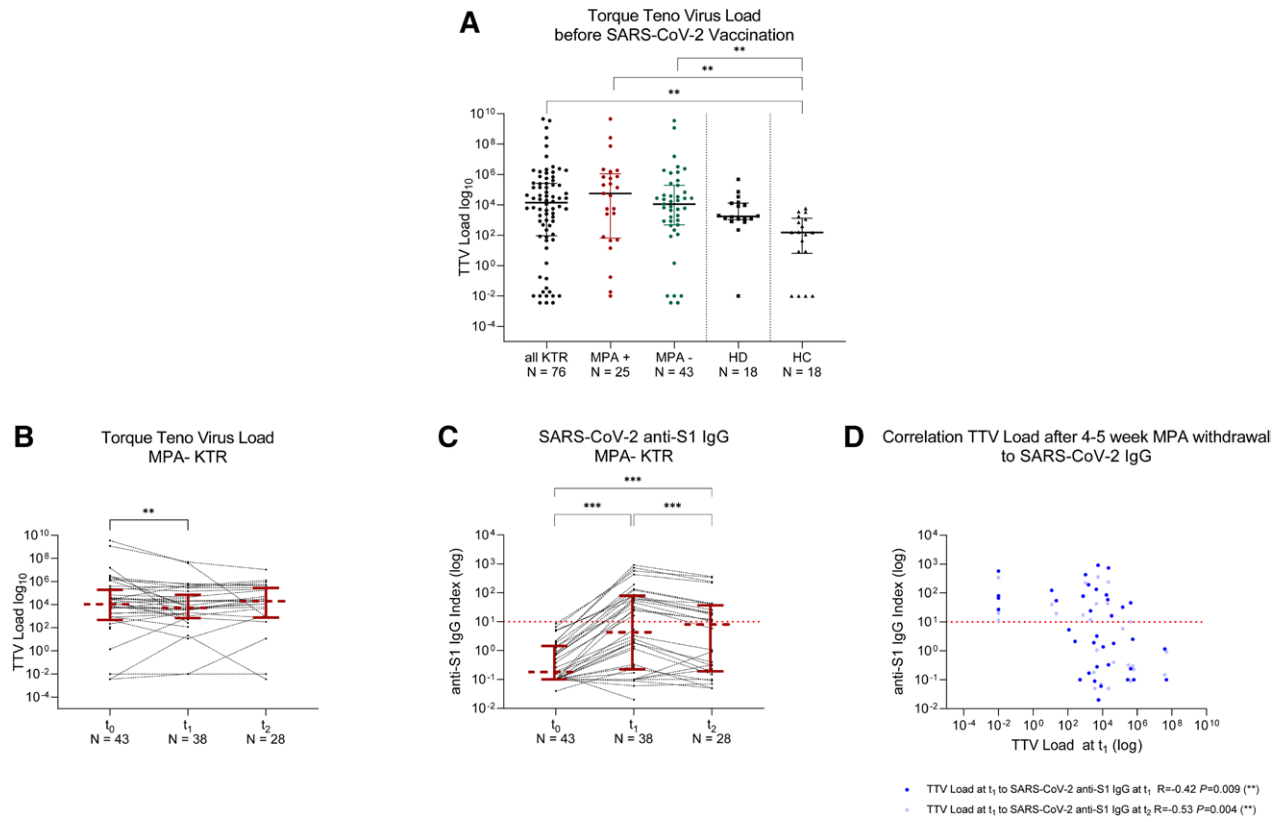

FIGURE 2. TTV load in KTRs with short-term withdrawal of MPA. A, TTV load before SARS-CoV-2 vaccination in all KTRs, KTRs who remained on a triple immunosuppressive therapy including MPA (MPA+), KTRs who withdrew MPA upon vaccination (MPA–), HD patients, and HCs. B, Development of TTV load in MPA– KTRs before MPA withdrawal and vaccination (t_0), upon reintroduction of MPA 4 wk after vaccination (t_1) and 3 mo after vaccination (t_2). C, Development of SARS-CoV-2–specific anti-S1 IgG in MPA– KTRs. D, Correlation of TTV load after 4–5-wk MPA withdrawal (t_1) to SARS-CoV-2–specific anti-S1 IgG 4 wk (t_1) and 3 mo (t_2) after vaccination. Bars indicate median, and brackets indicate interquartile ranges. The dashed red line at x = 10^1 in C and D indicates seroconversion for SARS-CoV-2–specific anti-S1 IgG. TTV load and anti-S1 IgG were log-transformed, and medians were compared by either Kruskal–Wallis test with Dunn's posttest or Wilcoxon matched-pairs signed-rank test. $^{**}P < 0.01$; $^{***}P < 0.001$. HCs, healthy controls; HD, hemodialysis; KTRs, kidney transplant recipients; MPA, mycophenolic acid; *R*, Spearman's ρ; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TTV, torque teno virus.

and of $R = -0.53$ three mo postvaccination (t_2) ($P = 0.009$ and $P = 0.004$, respectively; Figure 2D).

Torque Teno Virus Load in KTRs With Continued Immunosuppressive Maintenance Therapy, Hemodialysis Patients, and Healthy Controls

In 33 KTRs with continued immunosuppressive maintenance therapy (including 25 KTRs with a maintenance immunosuppression of CNI + MPA + CS and 8 KTRs with immunosuppression other than CNI + MPA + CS), no significant differences in the development of TTV load were found when comparing the results obtained at vaccination (t_0) with those obtained 4 wk (t_1) and 3 mo (t_2) postvaccination. The same applied to TTV load measurements obtained in hemodialysis and healthy controls (Figure 3A). SARS-CoV-2–specific anti-S1 IgG were significantly higher in all populations 4 wk after vaccination (t_1) with a slight decrease in anti-S1 IgG indices 3 mo after vaccination in the hemodialysis and healthy control populations (t_2; Figure 3B).

No significant correlation between TTV load before vaccination and antibody titers measured before (t_0), 4 wk after (t_1), and 3 mo after (t_2) vaccination was found, neither in KTRs excluding those where MPA was withdrawn, nor in hemodialysis patients or healthy controls (Figure 3C).

DISCUSSION

In the present study, we show that TTV load reflects short-term changes in immunosuppressive therapy in KTRs in whom MPA was withdrawn to increase SARS-CoV-2 vaccine immunogenicity. Decreases in TTV load 4–5 wk after initiation of MPA withdrawal, reflecting a lower immunosuppressive burden of the patient, were associated with better seroconversion rates and higher SARS-CoV-2–specific antibodies 4 wk after vaccination. As MPA was reintroduced 4 wk after vaccination, TTV load increased again in these patients, mirroring an increment of the patient's immunosuppression. In KTRs with short-term MPA withdrawal, TTV load correlated inversely to anti-S1 IgG antibody titers with a lower TTV load being associated with better seroconversion rates and higher anti-S1 IgG antibody titers. Changes in TTV load upon modifications in immunosuppressive therapy in KTRs have also been demonstrated by Regele et al,[17] who showed variations of TTV in 18 patients pausing MPA or azathioprine for 1 wk before and 1 wk after COVID-19 vaccination. However, the minimal variations in TTV loads following the withdrawal of MPA/azathioprine and the absence of a stronger seroresponse in KTRs with modified immunosuppressive therapy in their cohort may imply that a 2-wk cessation of

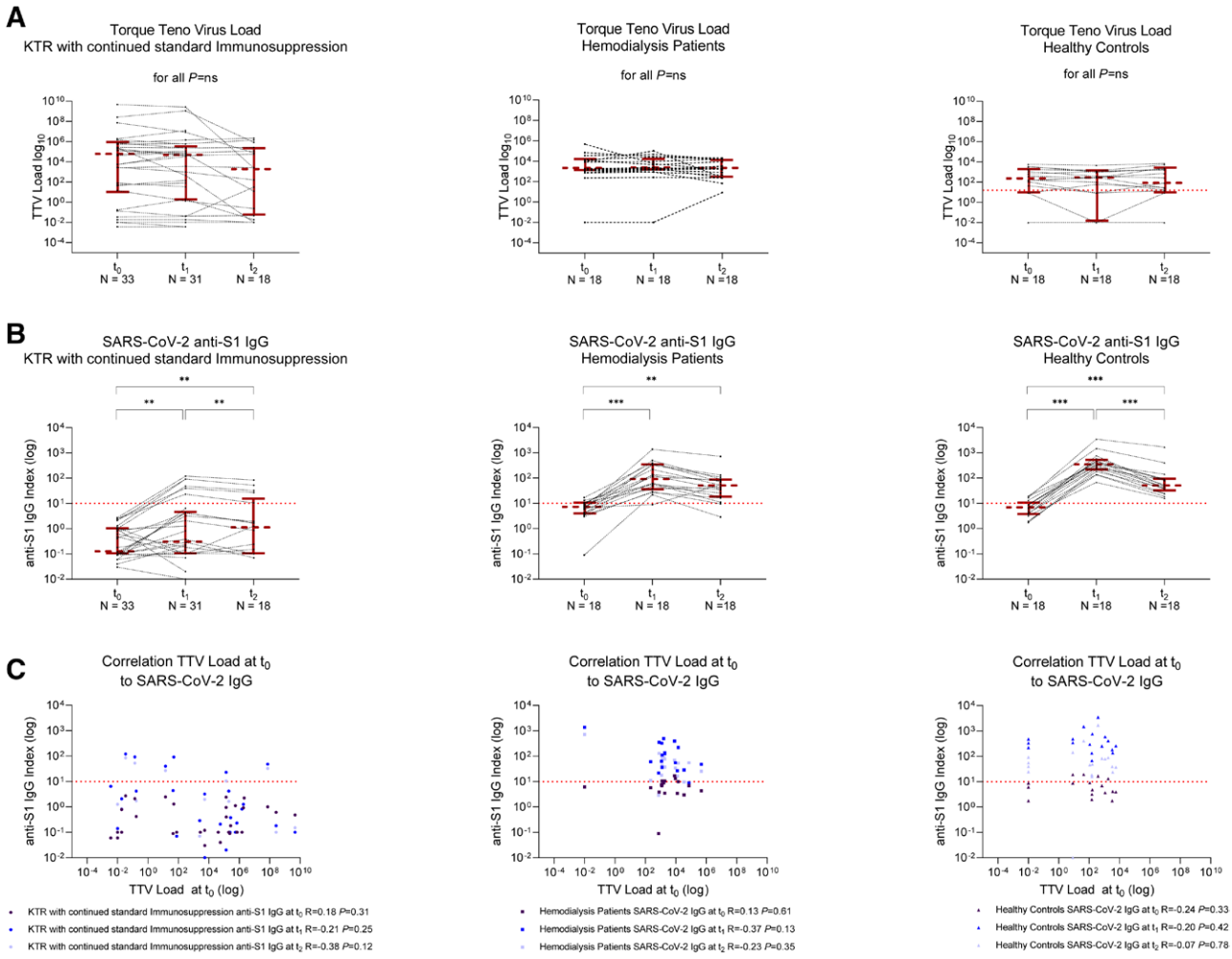

FIGURE 3. TTV load in KTRs without changes in immunosuppressive therapy, HD patients, and HCs. A, Development of TTV load in KTRs with continued standard immunosuppression, HD patients, and HCs, before vaccination (t_0), 4 wk (t_1), and 3 mo (t_2) after vaccination. B, Development of SARS-CoV-2–specific anti-S1 IgG in KTRs with continued standard immunosuppression, HD, and HC. C, Correlation of TTV load before vaccination (t_0) to SARS-CoV-2–specific anti-S1 IgG before vaccination (t_0), 4 wk (t_1), and 3 mo (t_2) after vaccination. Bars indicate median, and brackets indicate interquartile ranges. The dashed red line at $x = 10^1$ in B and C indicates seroconversion for SARS-CoV-2–specific anti-S1 IgG. TTV load and anti-S1 IgG were log-transformed, and medians were compared by Wilcoxon matched-pairs signed-rank test or Friedman's test. $^{**}P < 0.01$; $^{***}P < 0.001$. HCs, healthy controls; HD, hemodialysis; KTRs, kidney transplant recipients; MPA, mycophenolic acid; ns, nonsignificant; *R*, Spearman's ρ; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TTV, torque teno virus.

immunosuppressive medication may not provide sufficient time to promote vaccine immunogenicity.[17]

In KTRs who remained on standard immunosuppressive therapy, we did not observe any significant changes in TTV load upon vaccination. In these patients, we further did not observe a correlation between TTV load and anti-S1 IgG antibody titers. This contrasts with recently published data by Graninger et al[18] and Roberto et al[19] showing a significant inverse correlation between plasma TTV loads at time of first COVID-19 mRNA vaccination and humoral and cellular response after 2-dose vaccination in KTRs. These differences may be attributed to a selection bias, including solely nonresponder KTRs after at least 3 COVID-19 vaccinations in our current vaccine trial and to the low percentage of seroconverting KTRs (6/31, 19%) in the group of KTRs who remained on standard immunosuppressive therapy.

In hemodialysis patients and healthy controls, TTV load was generally lower than in KTRs, and no association between TTV load to vaccine response was observed. This reflects the lower immunosuppressive burden of dialysis patients and control subjects and may explain the better response to vaccination in these populations.[20] Additionally, our findings that are consistent with the results obtained by Graninger et al,[18] illustrate the usefulness of TTV to monitor immunosuppression in individuals with a more extensive immunosuppressive burden, such as solid organ transplant recipients, HIV-positive patients under therapy, and patients after autologous or allogeneic stem cell transplantation.[19]

Including monitoring of TTV load posttransplantation may allow for a more personalized immunosuppression in KTRs and may aid in predicting allograft rejection and infection in these patients.[5,7,8] The high in vivo replication capacity of TTV that seems to be controlled by the immune system may enable us to monitor even short-term changes in immunosuppressive therapy.[21] Our results demonstrate that TTV load can be quantified using either serum or plasma and therefore, may complement the

minimal-invasive monitoring of KTRs posttransplantation, aiding the clinician in decision-making of whether to biopsy a patient's allograft or not. Additionally, a more precise immunosuppression by continually monitoring TTV load may minimize the risk for infectious disease and development of malignancy and thus improve overall survival posttransplantation.[22] Of note, our results indicate that relative changes in a patient's TTV load seem more compelling than absolute TTV values or predefined cutoffs to monitor immunosuppressive therapy in KTRs.

Our study is subject to a few limitations, including a small sample size and a notable number of breakthrough infections, which necessitated the exclusion of 22 study participants from the analyses. However, the inclusion of all KTRs with breakthrough infections in a sensitivity analysis further confirmed changes in TTV load upon withdrawing MPA in KTRs (**Figure S3, SDC**, http://links.lww.com/TP/C889). Additionally, the enrollment of only KTRs who did not show seroconversion after receiving a minimum of 3 COVID-19 vaccinations may have introduced a potential bias to our study. Furthermore, it is important to acknowledge that our study did not involve matching for age and sex between KTRs and the healthy control and hemodialysis populations. This could potentially introduce bias to the results, given that TTV load is known to vary among different age groups and tends to be lower in younger and female individuals.[23,24] Consequently, it is important to consider our findings as observational in nature, potentially serving as a basis for future research. Another limitation of our study is the lack of data on cellular immunity postvaccination, which renders it impossible to draw conclusions regarding the relationship between changes in TTV and the cellular immune response.

In conclusion, this trial is among the first to quantify TTV load in KTRs with short-term changes of immunosuppressive therapy. The controlled withdrawal of MPA in KTRs in a prospective study design to increase vaccine immunogenicity featured a unique setting to assess the usability of TTV load to monitor short-term changes in immunosuppressive therapy. Our results emphasize the possible benefit of integrating TTV load in routine diagnostics after kidney transplantation to guide immunosuppression.

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