Torque teno virus viral load predicts SARS-CoV-2 vaccine response in kidney transplant recipients

Running title: TTV load and COVID-19 vaccine response in KTR

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Abstract

Transplant recipients display poor responses to SARS-CoV-2 mRNA vaccines. In this retrospective study, we investigate Torque teno virus (TTV) viral load (VL), a ubiquitous virus reflecting global immune response levels, as a predictive factor of vaccine response in kidney transplant recipients (KTR).

459 KTR having received two SARS-CoV-2 mRNA vaccine doses were enrolled, and 241 of them subsequently received a third vaccine dose. Anti–receptor-binding domain (RBD) IgG response was assessed after each vaccine dose and TTV VL was measured in pre-vaccine samples.

Pre-vaccine TTV VL >6.2 log10 copies (cp)/mL was independently associated with non-response to two doses (Odds Ratio (OR)=6.17, 95% confidence interval (CI95)=2.42–15.78) as well as to three doses (OR=3.62, 95% CI95=1.55–8.49). In non-responders to the second dose, high TTV VL in pre-vaccine samples or measured before the third dose were equally predictive of lower seroconversion rates and antibody titers.

High TTV VL before and during SARS-CoV-2 vaccination schedules are predictive of poor vaccine response in KTR. This biomarker should be further evaluated regarding other vaccine responses.

Keywords: biomarker, COVID-19 vaccine, humoral reponse, torque teno virus, immunocompromised

Introduction

Transplant recipients, including kidney transplant recipients (KTR), respond poorly to SARS-CoV-2 mRNA vaccines as a result of immunosuppressive treatments [1]. Under half of KTR respond to two doses of vaccine [2], and approximately 50% of patients who did not respond after a second dose seroconvert after a third dose [3]. This population remains at high risk for severe forms of COVID-19 and should be given early access to additional preventive and therapeutic strategies (antiviral drugs and monoclonal antibodies). However, predictive markers of vaccine response are lacking.

Torque teno virus (TTV) is a non-pathogenic ubiquitous DNA virus that accounts for 97% of Anelloviridae fraction in the virome of transplant recipients [4]. Previous studies have shown that plasma TTV viral loads (VL) correlate with the intensity of immunosuppression [5]. It was therefore suggested as a potential marker to predict infectious events or graft rejection in transplant recipients [6–10]. Low TTV VL, reflecting significant remaining immune activity, is predictive of graft rejection, whereas high TTV VL, reflecting poor immune levels, is associated with microbial infection [11]. In KTR, previous data suggest a high risk of rejection when TTV VL in the peripheral blood, as measured by in-house PCR [7], is below 6 $\log_{10}$ cp/mL, and a high risk of infection above 8 $\log_{10}$ cp/mL, between 3 to 12 months after transplantation. Re-analysis of this cohort by the authors, this time using TTV R-GENE® PCR, which is the technique applied in our center, showed lower values and thus corresponding thresholds of <4.6 $\log_{10}$ TTV cp/mL and >6.2 $\log_{10}$ TTV cp/mL for increased risk of rejection and infection, respectively [12,13]. Following these promising results, the randomized controlled phase II trial TTV GUIDE TX has been launched to ascertain the effectiveness of this TTV VL optimal range (4.6-6.2 $\log_{10}$ TTV cp/mL) using the TTV R-GENE® PCR technique [13,14]. Since TTV has been used to predict immunity-related events, it may also help anticipating how KTR could respond to an immune stimulation such as vaccine administration.

In this study, we investigated whether pre-vaccine TTV VL could predict SARS-CoV-2 mRNA vaccine response after two or three doses, using multivariable analyses, considering clinical criteria and immunosuppressive drugs. In non-responders to two doses of vaccine, we compared pre-vaccine and pre-third dose TTV VL to establish the consistency of its potential to predict vaccine response over time.

Materials and Methods

Study design

This study is a retrospective analysis of a prospectively sampled biobank, based on a monocentric longitudinal cohort study approved by the local Institutional Review Board (approval number: CE-2021-9) and was registered at Clinicaltrials.gov (registration number: NCT04828460). In the cohort study, 561 KTR followed in the outpatient Kidney Transplantation Department of Strasbourg University Hospital who were vaccinated with two doses of COVID-19 mRNA-1273 (Moderna) vaccine between February 16th, 2021 and April, 22th, 2021 were included. Anti-RBD IgG titers were measured one month after the second dose (median: 30 days, IQR: 28-35 days). Patients with incomplete follow-up (SARS-CoV-2 serology missing after the second dose) or prior COVID-19 infection (ascertained by history of positive COVID-19 testing by PCR or antigen test, or positive anti-RBD antibodies before vaccination) were excluded. For this study, 102 patients with no serum sample available before the first vaccine dose due to random inadequate sampling or storage were excluded. Of note, these 102 patients did not present any significant difference regarding patients' characteristics such as age, sex or response to the vaccine (data not shown). Patients displaying poor response to the two-dose regimen (as determined by an anti-receptor-binding domain (RBD) IgG titer under 143 BAU/mL), and not having developed SARS-CoV-2 infection after the second dose, were eligible for a third dose; those who presented for the third dose and had their antibody response subsequently assessed were retained in the study population. The threshold of 143 BAU/mL was previously considered as an insufficient response, based on preliminary studies in preprint at that time [15]. No monoclonal antibody prophylaxis was administered during the course of this study. All patients provided informed written consent for the analysis of their samples included in the registered biobank n°DC2014-2222 for research purposes.

SARS-CoV-2 IgG antibody testing

Anti-RBD IgG testing was performed using Abbott Architect SARS-CoV-2 IgG II Quant assay. To convert antibody titers into BAU/mL, adapted to the World Health Organization standard for SARS-CoV-2 immunoglobulin, a multiplication factor of 0.142 was applied (quantification range: 1.0–11,360.0 BAU/mL, positivity threshold: 7.1 BAU/mL). KTR who displayed positive IgG titers were classified as responders (versus seronegative patients who were categorized as non-responders).

Torque teno virus viral load

TTV VL was measured using the CE-IVD marked TTV R-GENE® kit (bioMérieux, Marcy l'Etoile, France) targeting the 5'UTR region and detecting all human TTV species. Extraction was performed on the EMAG® platform (bioMérieux, Marcy l'Etoile, France) and amplification on the LightCycler®

480 System II (Roche Diagnostics) according to the manufacturers' instructions. Inhibition controls were used to ensure adequate detection and quantification of TTV VL. The TTV R-GENE® kit provides standards to generate a standard curve, allowing to measure TTV VL values in copies/mL. The limit of detection is 250 copies/mL.

Statistical analysis

Continuous variables were compared using nonparametric Mann-Whitney U-test and categorical variables were compared using Fisher's exact test. Statistical tests were 2-tailed and significance was set at $p<0.05$. Post-hoc Receiver Operating Characteristic (ROC) curve analyses were carried out for antibody response after two or three doses. All of the above analyses were performed using Prism 6.6. Multivariable logistic regression was performed using SPSS 28.0 (IBM Statistics) to identify independent predictors of antibody response. Parameters associated with non-response with a p-value <0.2 in the univariate analysis were included in the model and results were expressed as adjusted Odds Ratios (OR) with 95% confidence intervals (CI95).

Results

Pre-vaccine TTV viral load predicts response to two doses of vaccine

Four hundred and fifty-nine patients who had serum samples available in the days before the first vaccine dose (median 0 days, interquartile range (IQR) 0–6), and did not experience SARS-CoV-2 infection before vaccination nor before the second vaccine dose, were included. After two doses, 208/459 (45.3%) KTR displayed positive anti-RBD IgG (responders) (Figure 1). Clinical criteria as well as immunosuppressive drug regimens of the 459 KTR having received two doses were compared between responders and non-responders in Table 1. Univariate analysis showed that diabetes, more recent transplantation, calcineurin inhibitor, mycophenolate mofetil/mycophenolic acid (MMF/MPA), belatacept, steroids, high creatinin levels and high TTV VL were associated with non-response. Indeed, higher pre-vaccine TTV VL was found in non-responders than in responders (4.23 vs 3.50 $\log_{10}$ cp/mL, $p<0.0001$) (Table 1, Figure 2A). Due to recent litterature mentioning the TTV VL value of 6.2 $\log_{10}$ cp/mL as the upper limit of the optimal range for KTR [12,13], and after finding that the same value applied to lung transplant recipient to predict SARS-CoV-2 mRNA vaccine response [16], we decided to use this threshold to assess its performances for distinguishing responders from non-responders (Figures S1 and S2 show this value on the ROC curves for response to the second or the third dose, respectively). We found that KTR displaying TTV VL below 6.2 $\log_{10}$ cp/mL before vaccination

seroconverted in 50.8% of cases (202/397) after two doses versus 9.6% (6/62) of KTR with higher VL ($p<0.0001$), corresponding to a negative predictive value (NPV) for seroconversion of 90.3% (Figure 2B).

Adjusted multivariable logistic regression analysis confirmed that KTR displaying TTV VL $\geq$6.2 $\log_{10}$ cp/mL before vaccination were less likely to seroconvert (OR=6.172, CI95=2.415–15.779, $p<0.001$) (Table S1, Figure 2C). This was also the case for patients over the age of 60 (OR=1.890, CI95=1.192-2.997, p=0.007), with diabetes (OR=1.620, CI95=1.029-2.551, p=0.037), treated with tacrolimus (OR=1.654, CI95=1.027-2.662, p=0.0038), MMF/MPA (OR=4.511, CI95=2.600-7.829, $p<0.001$) or belatacept (OR=6.412, CI95=1.285-32.004, p=0.023), or KTR with serum creatinin higher than 130 µmol/L (OR=2.007, CI95=1.295-3.109, p=0.002). Patients transplanted more than 6 years ago were more likely to seroconvert (OR=0.507, CI95=0.320-0.803, p=0.004). In KTR treated with MMF/MPA, a seroconversion rate of 45.0% (139/309) was achieved for patients with TTV VL <6.2 $\log_{10}$ cp/mL, whereas only 1.9% (1/54) of patients with TTV VL >6.2 $\log_{10}$ cp/mL seroconverted (Figure 2D).

Pre-vaccine and pre-third dose TTV DNA load predict response to three doses of vaccine

In total, two hundred and forty-one patients received a third dose 1–3 months after the second one and had a blood sample available to assess their third-dose antibody response. After three doses, 139/241 (57.7%) KTR responded to the vaccine (Figure 1). Responders and non-responders to the three-dose vaccine regimen were compared in terms of clinical criteria and immunosuppressive drug regimen (Table 1). Univariate analysis showed that age, sex, shorter time after transplantation, calcineurin inhibitor regimen, MMF/MPA, belatacept, steroids, high creatinin levels and high TTV VL were associated with non-response. Indeed, higher pre-vaccine TTV VL were also found in non-responders than in responders to the third dose (4.91 vs 3.58 $\log_{10}$ cp/mL, $p<0.0001$) (Figure 3A). KTR with TTV VL <6.2 $\log_{10}$ cp/mL before vaccination seroconverted in 65.5% of cases (127/194) after three doses versus 25.5% (12/47) of KTR with higher VL ($p<0.0001$) (Figure 3B), corresponding to a negative predictive value (NPV) for seroconversion of 74.5%.

Adjusted multivariable logistic regression analysis confirmed that KTR displaying TTV VL $\geq$6.2 $\log_{10}$ cp/mL before vaccination was independently associated with a decreased probability of seroconversion after three doses (OR=3.624, CI95=1.547-8.489, p=0.003) (Table S2, Figure 3C). This was also the case for patients over the age of 60 (OR=2.524, CI95=1.302-4.892, p=0.006), patients treated with MMF/MPA (OR=3.064, CI95=1.153-8.143, p=0.025), receiving belatacept (OR=24.563, CI95=2.488-242.473, p=0.006), steroids (OR=2.258, CI95=1.062-4.801, p=0.034), or KTR with serum creatinin higher than 130 µmol/L (OR=2.532, CI95=1.346-4.761, p=0.004). Male KTR (OR=0.361,

CI95=0.191-0.685, p=0.002) and patients transplanted over 6 years ago were more likely to seroconvert (OR=0.513, CI95=0.263-1.000, p=0.05). In KTR treated with MMF/MPA, a seroconversion rate of 62.5% (100/160) was achieved for patients with TTV VL <6.2 log_{10} cp/mL, whereas only 20.5% (9/44) of patients with TTV VL over this threshold seroconverted (Figure 3D). As previously stated, the 6.2 log_{10} cp/mL TTV VL threshold value was chosen due to recent litterature suggesting better outcomes for KTR below this threshold[12,13,16]. However, the cutoffs with the higher Youden indexes of the ROC curves (Figures S1 and S2) were 5.185 log_{10} cp/mL (Youden index= 0.254) for response to the second dose and 5.620-5.765 log_{10} cp/mL (Youden indexes= 0.227) for response to the third dose. These cutoffs generate NPVs of 81.5% and, 74.5%, respectively. Of note, KTR above these (lower) thresholds represent roughly one fourth of the study cohort. We also found a lower threshold when analysing the levels of BAU titers instead of seropositivity only. Indeed, the ROC curve for antibody titer >264 BAU/mL, a titer set by the French Vaccinal Strategy Orientation Board in November 2021 for eligibility to prophylactic monoclonal antibody treatment[17] presented the highest Youden index for the 5.0 log_{10} cp/mL TTV VL value (Figure S1). Despite "sufficient" vaccine response being currently problematic to establish in terms of BAU titers, due to more recent variants having appeared after the onset of vaccination campaigns, we analysed the performances of the 5.0 log_{10} cp/mL cutoff value for several former or arbitrary BAU targets (Table S3), generating high NPVs for these levels of response to the second or the third dose.

Fourteen breakthough infections were reported up to July 2021 which was the beginning of the administration of the fourth dose (Table S4). Although differences were not significant, the seropositivity rate of these 14 patients tended to be lower than the rest of the cohort (35.7% vs 45.3%) as well as the median BAU titer reached by those who were seropositive (36.3 vs 105.2 BAU/mL); their median pre-vaccinal TTV VL also tended to be higher than the rest of the cohort (4.44 vs 3.73 log_{10} cp/mL) with a higher proportion of patients with pre-vaccinal TTV VL higher than the thresholds of 6.2 log_{10} cp/mL (21,4% vs 13.3%) and 5.0 log_{10} cp/mL (35.7% vs 26.5%).

TTV VL may vary and what is true at one timepoint may not be so at another. To establish the consistency of TTV VL as a biomarker of COVID-19 vaccine response over time, we investigated the evolution of TTV VL, as measured before vaccination and before the third dose, in KTR who did not respond to two vaccine doses (n=172) (Figure 1). In these patients, TTV VL was remarkably constant between the two timepoints, with an absolute difference in median of 0.48 log_{10} cp/mL (Figure 3E). Within both groups, responders and non-responders to the third dose, TTV VL were not significantly different in pre-third dose samples versus pre-vaccinal samples (Figure 3F). At both timepoints, pre-vaccinal and pre-third dose, TTV VL of 6.2 log_{10} cp/mL was shown to be a predictor of vaccine

response after three doses, with an NPV of 77.8% and 82.9% for pre-vaccinal and pre-third dose, respectively (Figure S3).

Discussion

In this study involving 459 KTR, we demonstrate that i) high pre-vaccination TTV VL, using the threshold of of >6.2 $\log_{10}$ cp/mL, is independently predictive of non-response to two or three doses of mRNA COVID-19 vaccine and ii) the predictive potential of TTV VL regarding vaccine response is reliable and stable over time, with similar performances whether TTV VL is measured prior to vaccination, or between vaccine doses.

Factors such as age, sex, comorbidities or immunosuppressive drug type, combination and dosage have been described to impact vaccine response [1,18]. In our study, response to the second dose, or to a third dose for a subset of patients, was not strictly associated with the same factors. Indeed, age and sex were associated with response to the third dose, but not to the second dose, whilst the opposite was true for diabetes. These discrepancies may be explained by selection bias resulting from the criteria for receiving a third dose (having poorly responded to two doses and not been infected by SARS-CoV-2 subsequently). Immunosuppressive drugs such as MMF/MPA, tacrolimus, belatacept and steroids were associated with a lower rate of response, in agreement with previous studies [19], with MMF/MPA being the most significant and also the most frequently employed. Of note, TTV VL was able to discriminate between responders and non-responders in patients receiving MMF/MPA, with high TTV VL indicative of low rates of vaccine response.

This study describes for the first time clinically useful TTV VL cutoff values for risk stratification of lack of vaccine response in KTR. Indeed, the predefined TTV VL threshold of 6.2 $\log_{10}$ cp/mL was independently predictive of non-response to both the second and the third vaccine dose. Regarding response to the second dose, the threshold generated by our cohort data was 5.185 $\log_{10}$ cp/mL, close to the 5.0 $\log_{10}$ cp/mL value we found for "sufficient" response based on BAU titers used at that time. Due to selection bias for the third dose (i.e. KTR who did not respond to the second dose and thus had higher TTV values), TTV VL threshold for the third dose generated by our cohort data was higher, around 5.7 $\log_{10}$ cp/mL, approaching the initially chosen cutoff value of 6.2 $\log_{10}$ cp/mL. To determine whether TTV VL is an effective predictor of vaccine response throughout the vaccine schedule, we compared its predictive potential in two-dose non-responders with samples taken at pre-vaccinal and pre-third dose timepoints. TTV VL remained stable across time for the majority of patients, and its negative predictive values for seroconversion were similar at both timepoints. These results suggest

that TTV VL may be measured at various times in the vaccination schedule to predict response to an additional dose.

Studies in immunocompromised populations, especially transplant patients, have shown that TTV VL inversely reflects the strength of overall immune response. The use of TTV VL as a predictor of vaccine response has been investigated for COVID-19 vaccine schedules in lung transplant recipients (LTR) cohorts, with poor vaccine response to two doses observed in LTR with TTV VL >6.5 $\log_{10}$ cp/mL [20], or to three doses in LTR with TTV VL >6.2 $\log_{10}$ cp/mL [16]. SARS-CoV-2 vaccine response in KTR has also been shown to decrease when TTV VL increases [21]. These promising results on various cohorts with similar threshold values further reinforce our findings and the relevance of TTV VL as a biomarker for vaccine response. Indeed, lung transplant recipients and kidney transplant recipients are dissimilar in terms of comorbidities and immunosuppression (lung transplant recipients being more immunosuppressed, as reflected by higher TTV VL[7,16]) as well as in vaccine response, since in our cohorts only 13% of lung transplant recipients responded to two vaccine doses[16] compared to 45% of kidney transplant recipients. Finding that the same TTV VL threshold can apply to vaccine response (corresponding to a comparable level of immunosuppression, even if less kidney transplant recipients reach these TTV VL due to generally lower immunosuppression), can mean that above a certain state of immunosuppression, seroconversion is unlikely regardless of how this level of immunosuppression is achieved, broadening the potential use of TTV VL. In other populations, TTV VL and T-cell responses have been linked, with high TTV loads associated with poor T-cell proliferative capacity in allogeneic hematopoietic stem cell transplantation recipients [22], diminished CD4+ T-cell recovery in HIV patients [23], and inversion of the CD4/CD8 ratio showing an immune risk phenotype in healthy individuals [24]. Higher TTV VL have been shown to be a strong predictor of mortality in the elderly[25,26], as well as a marker of worse survival and complications in hematopoietic stem cell transplantation[27] and clinical deterioration in critically ill patients[28]. In patients with rheumatoid arthritis receiving immunomodulation with biological compounds, TTV levels helped predict clinical response[29]. In addition, low TTV VL also predicts antibody-mediated or mixed rejection in LTR [30,31] and KTR [6], suggesting that TTV VL, by its nature as an endogenous viral biomarker, may thus reflect the net state of immunosuppression[32].

Our study has several limitations, the first of which being its monocentric design. Furthermore, TTV VL were not measured in healthy vaccinated adults for comparison. Besides, anti-SARS-CoV-2 neutralizing antibody response was not assessed. Indeed, neutralizing activity is the more adequate humoral correlate of protection [33,34]. However, neutralizing techniques are not routinely used in clinical pratice and lack standardisation [35], while most serology methods have been standardised to the

international BAU standard. Additionally, anti-RBD IgG titers have generally been found to correlate with neutralizing activity [34,36]. Unfortunately, though they offer quantitative results with a large range, BAU results cannot now be analysed using a threshold other than seroposivity, since no correlate of protection is currently defined against Omicron subvariants. Anti-SARS-CoV-2 cellular responses were also not explored. Recent studies have shown poor vaccine response in other transplant populations above similar TTV VL thresholds [16,20]; however, further investigations, especially in multicentric settings, would be needed to confirm TTV VL threshold values for use in routine practice for transplant recipients, as dichotomous analyses can be prone to bias. Such studies may also be expanded to study vaccine response against various microorganisms, as well as in other patient populations.

The potential use of TTV VL as a predictive biomarker for vaccine response may help clinicians determine which patients may benefit the most from further vaccine doses, and for others head earlier towards supplementary strategies aiming to increase protection against SARS-CoV-2 in immunocompromised patients, such as monoclonal antibodies, immunosuppression modulation prior to the following dose or doubling of vaccine doses[37]. Additionally, COVID-19 vaccinations campaigns provided the opportunity for proof-of-concept regarding TTV viral load and vaccine response which could be confirmed for other vaccines, allowing to personalise vaccinal strategies in transplant recipients.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

MS and SFK conceived and designed the study. MS and IB collected clinical and biological data and organized the database. MS analysed the experiments. MS, IB, FG, SC and SFK contributed to data analysis and interpretation. MS and SFK wrote the manuscript. SFK and SC supervised this study. All authors listed have critically reviewed the manuscript for intellectual content and approved it for publication.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1. Flow chart of kidney transplant recipients (KTR) recruitment and antibody response to SARS-CoV-2 mRNA-1273 (Moderna) vaccination.

The study was conducted on 459 KTR without COVID-19 prior to SARS-CoV-2 vaccination. Sera sampled after each vaccine dose in COVID-19-naive patients were analyzed to assess the anti–receptor-binding domain (RBD) IgG response, with seropositive patients (titer ≥ 7.1 BAU/mL) defined as responders (green boxes). The proportion of seronegative patients is shown in red boxes. Of the initial 459 KTR, 241 KTR received a third dose, and had a blood sample available after this third dose (upper right). Of these 241 KTR, 172 were seronegative after the second vaccine dose (presented separately, bottom right).

Figure 2. Pre-vaccine TTV DNA load and vaccine response after two doses.

(A) TTV VL of non-responders (black) and responders after two vaccine doses (gray). Individual viral loads are represented by circles, whilst the median, upper and lower quartiles are represented by horizontal lines. The dotted line indicates the predictive threshold of 6.2 $\log_{10}$ cp/mL. **** = p<0.0001 **(B)** Chart representation of the proportion of non-responders and responders after two vaccine doses for patients with a TTV VL <6.2 log10 cp/mL (left) and >6.2 log10 cp/mL (right). **(C)** Forest plot showing adjusted Odds Ratios (OR) estimates (indicated by black dots) and 95% confidence intervals (indicated by whiskers) of association between patient characteristics and non-response to two doses of COVID-19 vaccine. Factors independently associated with poor vaccine response with a p-value < 0.001 are in bold. **(D)** Histogram representation of the percentage of responders to two vaccine doses according to TTV VL (> or < 6.2 $\log_{10}$ cp/mL) and whether the patient is under MMF/MPA treatment (+) or not (-) at the time of the first vaccination. The fraction of responders out of the total number of KTR in each category is mentioned above bars.

MMF/MPA: mycophenolate mofetil/mycophenolic acid; OR: Odds ratio; TTV: Torque teno virus; VL: viral load.

Figure 3. Pre-vaccine TTV DNA load and vaccine response after three doses.

(A) TTV VL of non-responders (black) and responders (gray) after three vaccine doses (n=241). Individual viral loads are represented by circles, whilst the median, upper, and lower quartiles are represented by horizontal lines. The dotted line indicates the predictive threshold of 6.2 $\log_{10}$ cp/mL. **** = p<0.0001 **(B)** Chart representation of the proportion of non-responders and responders after three vaccine doses for patients with a TTV VL <6.2 $\log_{10}$ cp/mL (left) and >6.2 $\log_{10}$ cp/mL (right) (n=241). **(C)** Forest plot showing adjusted Odds Ratios (OR) estimates (indicated by black dots) and 95% confidence intervals (indicated by whiskers) of association between patient characteristics and non-response to three doses of COVID-19 vaccine. Factors independently associated with poor vaccine response with a p-value < 0.005 are in bold. **(D)** Histogram representation of the percentage of responders to three vaccine doses according to TTV VL (> or < 6.2 $\log_{10}$ cp/mL) and whether the patient is under MMF/MPA treatment (+) or not (-). at the time of the first vaccination. The fraction of responders out of the total number of KTR in each category is mentioned above bars. **(E)** Pre-vaccinal and pre-third dose TTV VL of patients who were seronegative after two doses and had their response

to the third dose assessed (n=172). The TTV VL for each patient is represented by a black dot at each timepoint, linked by a solid gray line. Dotted lines indicate the predictive thresholds of 5.0 $\log_{10}$ cp/mL and 6.2 $\log_{10}$ cp/mL. **(F)** TTV VL at pre-vaccinal and pre-third dose timepoints of patients who were seronegative after two doses and had their response to the third dose assessed (n=172), shown as non-responders (black) and responders (gray) after three vaccine doses . Individual viral loads are represented by circles, whilst the median, upper and lower quartiles of each group are represented by horizontal lines.

**p* value <0.05, ****p* value <0.001, ns: not significant.

MMF/MPA: mycophenolate mofetil/mycophenolic acid; OR: Odds ratio; TTV: Torque teno virus; VL: viral load.