













Torquetenovirus viral load is associated with anti-spike antibody response in SARS-CoV-2 mRNA BNT162b2 vaccinated kidney transplant patients

Sara Querido¹  | Teresa Adragão¹  | Iola Pinto^{2,3}  | Carolina Ormonde⁴  |
 Ana Luísa Papoila^{5,6}  | Maria Ana Pessanha⁷  | Perpétua Gomes^{7,8}  |
 Sílvia Ferreira⁹  | João Mário Figueira⁹  | Conceição Cardoso⁹  |
 João Faro Viana¹⁰  | André Weigert¹ 

¹Department of Nephrology, Unit of Renal Transplantation, Hospital de Santa Cruz, Centro Hospitalar de Lisboa Ocidental, Carnaxide, Portugal

²CMA, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Lisboa, Portugal

³ISEL, Instituto Superior de Engenharia de Lisboa, Lisboa, Portugal

⁴Department of Nephrology, Hospital do Divino Espírito Santo, Ponta Delgada, Portugal

⁵CEAUL, Centro de Estatística e Aplicações da Universidade de Lisboa, Lisboa, Portugal

⁶NOVAMedicalSchool, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisboa, Portugal

⁷Department of Clinical Pathology, Laboratory of Clinical Microbiology and Molecular Biology, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal

⁸Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), IUEM, Almada, Portugal

⁹Department of Clinical Pathology, Laboratory of Biochemistry, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal

¹⁰Department of Clinical Pathology, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal

Correspondence

Sara Querido, Nephrology, Hospital de Santa Cruz, Av. Prof. Dr. Reinaldo dos Santos, 2790-134 Carnaxide, Portugal.
 Email: saraqueridoconde@gmail.com

Abstract

Introduction: Kidney transplant patients (KT) are at high risk for severe COVID-19 and presented attenuated antibody responses to vaccination when compared to immunocompetent individuals. Torquetenovirus (TTV) has recently gained attention as a potential surrogate marker of the net state of immunosuppression. We evaluated the association between pre-vaccination TTV viral load and anti-spike total antibody response to SARS-CoV-2 vaccination in KT.

Material and Methods: The 114 adult KT recipients enrolled in this prospective single-center cohort study received two doses of SARS-CoV-2 mRNA BNT162b2 vaccine. Serum samples were collected immediately before vaccination at the days when patients received both the first (T0) and the second dose (T1) and 16–45 days after the second dose (T2). Primary endpoint was the development of anti-spike total antibodies after vaccination. Demographic, clinical, and laboratorial parameters were compared between patients with and without detectable SARS-CoV-2 antibodies at T2.

Results: Ninety-nine patients (86.8%) were naïve for SARS-CoV-2 before vaccination. Fifty-six (56.6%) patients developed anti-spike total antibodies at T2. The use of mTOR

inhibitors was associated with a favorable response ($p = .005$); conversely, mycophenolic acid (MPA) was associated with a negative response ($p = .006$). In a multivariable model, the presence of TTV at $T0 \geq 3.36 \log_{10}$ cp/ml was associated with unfavorable vaccine response (OR: 5.40; 95% CI: 1.47–19.80; $p = .011$), after adjusting for age and eGFR at $T0$.

Conclusions: Higher TTV viral loads before vaccination are associated with reduced anti-spike total antibody response in SARS-CoV-2 mRNA BNT162b2 vaccinated KT patients. The association between TTV viral load and vaccine response may be an added-value in the optimization of vaccination regimens in KT.

KEYWORDS

kidney transplantation, SARS-CoV-2, torquetenovirus, vaccine

1 | INTRODUCTION

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged as the etiologic agent of Coronavirus disease 2019 (COVID-19).¹ Kidney transplant patients (KT) have been considered high risk patients for severe COVID-19²; vaccination emerged as a crucial strategy, readily recommend by international guidelines. SARS-CoV-2 messenger-ribonucleic acid (mRNA) vaccines appear to be safe in KT recipients,³ but attenuated antibody responses were observed when compared to immunocompetent individuals. Seroconversion rates range from 0% to 17% following one COVID-19 vaccine dose and 3%–59% after two COVID-19 vaccine doses.⁴ Therefore, a third and even four dose of mRNA COVID-19 vaccine have been recommended.⁵ Evaluation of humoral response to SARS-CoV-2 vaccine is the best readily available tool to measure its efficacy. Different immunosuppressive protocols may influence the efficacy of SARS-CoV-2 vaccine. Mammalian target of rapamycin (mTOR) inhibitors were associated with a better immune response⁶ and the opposite effect was described with mycophenolic acid (MPA).⁷

No ideal marker reliably defines the immune function of KT patients. Torquetenovirus (TTV) has recently gained attention as a potential surrogate marker of the net state of immunosuppression.⁸ TTV can be detected in up to 90% of healthy individuals without association with any specific disease. Peripheral blood viral loads of TTV reflect the overall strength of innate and specific immunity in solid-organ transplant patients,⁸ as low TTV levels are associated with graft rejection and high TTV levels associate with a higher risk of infection.⁸ We recently reported a patient diagnosed with COVID-19 3 months after KT, whose TTV DNA load increased with COVID-19 onset and reduced after its resolution⁹ In addition, a Dutch group, demonstrated a better humoral response to SARS-CoV-2 vaccination in lung transplant patients with lower TTV loads before vaccination.¹⁰

Thus, we evaluated if TTV load before vaccination is associated with anti-spike (anti-S) total antibody response in response to SARS-CoV-2 mRNA BNT162b2 vaccine in KT patients.

2 | MATERIAL AND METHODS

2.1 | Study design and population

A total of 114 adult KT recipients (at least 6 months post-KT and without rejection episodes in the previous 3 months) were enrolled in this prospective single-center cohort study. All KT patients received two doses of SARS-CoV-2 mRNA BNT162b2 vaccine with an interval of 21 days between doses in May and June 2021 at our Kidney Transplant Center.

All participants provided informed consent.

Serum samples were collected immediately before vaccination at the days when patients received both the first ($T0$) and the second dose ($T1$) and 16–45 days after the second dose ($T2$).

The cohort included KT patients previously infected with SARS-CoV-2 and virus-naïve subjects. Prior infection with SARS-CoV-2 was defined either by known infection diagnosed more than 6 months pre-vaccination or the presence of positive anti-S and/or anti-nucleocapsid (anti-N) SARS-CoV-2 total antibodies at $T0$.

Patients were then stratified considering anti-N or anti-S SARS-CoV-2 total antibodies positivity at $T0$ (positive patients—Group A; negative patients—Group B). Group B was thereafter stratified into two groups: patients with positive TTV DNA viral load at $T0$ (Group C) and patients with negative TTV DNA viral load at $T0$.

Since it was impossible to define if patients with negative TTV DNA had latent infection, with undetectable viral loads at $T0$, we excluded this subgroup of patients from subsequent analysis. Group A patients were excluded from the SARS-CoV-2 vaccine response analysis since all of them had previously formed anti-S antibodies.

Estimated glomerular filtration rate (eGFR) was computed using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.¹¹

All patients received triple immunosuppression, mainly with tacrolimus, MPA, and prednisolone or with tacrolimus, mTOR inhibitor, and prednisolone. No patient changed immunosuppression regimen during follow-up. Tacrolimus was administered orally at .15 mg/kg/day

divided into two doses and adjusted to maintain a target trough concentration between 4 and 10 ng/ml, depending on the time elapsed after KT. Prednisolone was prescribed since the fifth day after KT (.6 mg/kg) and was tapered to 5 mg/day during the first 3 months after KT. MPA (mycophenolate mofetil 1000 mg orally twice daily) was started after KT and was reduced if adverse events appeared; it was reduced to 1000–1500 mg daily dose after the first 3–6 months. Everolimus was started at 2.0 mg/day with dose adjusted to achieve target trough concentrations of 3–8 ng/ml throughout.

Anti-S and anti-N antibodies, TTV DNA viral load, Immunoglobulins G, A, and M and eGFR rate were determined at T0, T1, and T2.

The primary endpoint was the development of anti-S total antibodies after vaccination (favorable vs. unfavorable response). Demographic characteristics, type of donor, time since transplantation, maintenance immunosuppression, and length of follow-up collected at baseline, were compared between the groups with and without detectable anti-S antibodies at T2.

The study is in compliance with the Declaration of Helsinki, follows national and international guidelines for health data protection and was approved by the Ethics Committee of the “Centro Hospitalar Lisboa Ocidental” (aprobation code 2134).

2.2 | Anti-SARS-CoV-2 total antibodies detection

Electro-chemiluminescence immunoassay (ECLIA) was used to detect anti-N and quantify anti-S(RBD) (ElecsysAnti-SARS-CoV-2 S and Anti-SARS-CoV-2 N, Roche), on a Cobas e602 analyzer, with a positivity threshold of 1.0 (index) for anti-N and .8 U/ml for Anti-S(RBD), following manufacturer's instructions. These thresholds were validated in recent studies.^{12,13}

2.3 | Quantitative TTV DNA viral load

DNA extraction from serum samples, and amplification of DNA was performed as previously described.^{14,15} In brief, DNA extraction was carried out using the eMAG System (BioMerieux, Marcy, France). For DNA amplification and quantification, the Argene R-Gene TTV quantification kit (BioMerieux) was used on an Applied Biosystems 7500 (ThermoFisher, Waltham, MA, USA) according to the manufacturer's instructions. The R gene assay is designed to detect the majority of TTV genotypes.^{1,6–8,10,12,15,16,19,27,28} The threshold defining positivity was 100 copies/ml. Results are expressed in log₁₀ copies/ml.

2.4 | Statistical analysis

An exploratory analysis was carried out for all variables. Categorical data were presented as frequencies and percentages, and continuous variables as mean (standard deviation) or median and inter-quartile range (25th percentile;75th percentile), as appropriate. Nonparametric

tests (Chi-square, Fisher's Exact, Mann-Whitney U) were used to compare patients who responded to the vaccine and those who did not. Additionally, generalized additive regression models for the response to SARS-CoV-2 vaccination were also used. Model performance was assessed with Hosmer-Lemeshow goodness of fit test and with the area under the ROC curve (receiver operating characteristic). For TTV in T0 (TTV-T0), the best cut-point that discriminates patients regarding response to SARS-CoV-2 vaccination was obtained through the minimum *p*-value approach, and corresponding sensitivity and specificity estimated.

The level of significance $\alpha = .05$ was considered. All data were analyzed using SPSS 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows. Armonk, NY, USA: IBM Corp) and R software (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2014).

3 | RESULTS

3.1 | Patients' characteristics

One hundred fourteen KT patients (52 men; 62 women) were included in the study, with 46.54 ± 12.02 years and a median follow up after KT of 104.73 months ($P_{25} = 53.52$; $P_{75} = 170.33$). Only five patients (4.4%) received a kidney allograft within 1 year before vaccine administration. Sixty-eight patients (59.6%) received T-cell depleting as induction therapy. Two patients (1.8%) had one episode of acute rejection in the past year; none of them developed anti-S antibodies.

No patient developed anti-N antibodies between T0 and T2 or had symptoms compatible with COVID-19 during follow up.

The number of patients in each study group is presented in the flow chart of Figure 1.

3.2 | Vaccine response in positive SARS-CoV-2 patients before vaccination (Group A)

Fifteen patients (13.2%) had prior infection with SARS-CoV-2 before vaccination.

All these patients presented Anti-S at T0 despite immunosuppression regimen. Anti-S levels at T2 were significantly higher in patients with previously solved COVID-19, comparing with SARS-CoV-2 naïve patients: 23 253.0 (13 010.0–43 840.0) U/ml versus 57.3 (9.025–370.500) U/ml ($p < .001$). Moreover, 56.6% of SARS-CoV-2 naïve individuals had a positive response to vaccination against 100% of the patients with COVID-19 ($p = .001$).

3.3 | Vaccine response and associated factors (Group B)

Table 1 depicts demographic and laboratorial characteristics of SARS-CoV-2 naïve patients ($n = 99$). Fifty-six (56.6%) patients developed

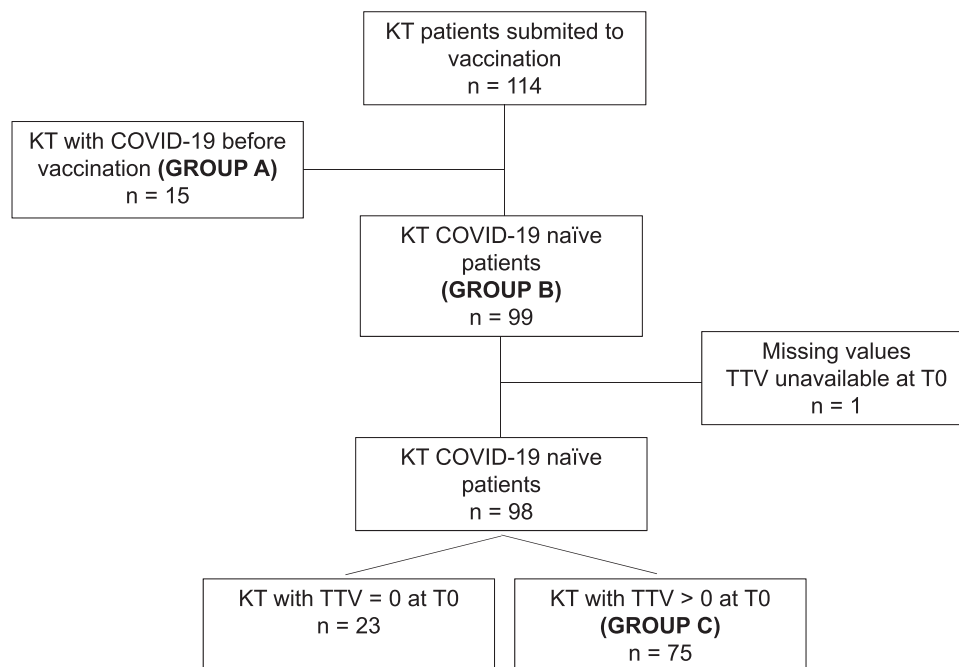

FIGURE 1 Study design flow chart

TABLE 1 Demographic and laboratorial characteristics of SARS-CoV-2 naive patients (Group B)

Characteristics	Total (n = 99)	Anti-spike + Ab at T2 (n = 56)	Anti-spike – Ab at T2 (n = 43)	p value
Age in years – mean (SD)	46.1 (11.6)	44.3 (9.48)	48.3 (13.61)	.105 ^a
Gender – n (%)				1.000 ^b
Male	44 (44.4)	25/56 (44.6)	19/43 (44.2)	
Female	55 (55.6)	31/56 (55.4)	24/43 (55.8)	
Type of donor- (n/%)				.143
Deceased	64 (64.6)	39/64 (60.9)	25/64 (39.1)	
Living	35 (35.4)	17/35 (48.6)	18/35 (51.4)	
Time after KT, months	100.9 (54.0–169.9)	94.1 (51.4–159.2)	120 (64.3–178.9)	.280
eGFR ml/min, T0	59.0 (44.0–82.0)	71.5 (51.3–86.5)	49 (41–71)	.003
Calcineurinic inhibitors – n (%)	92 (92.9)	51/92 (55.4)	41/92 (44.6)	.696 ^b
mTOR inhibitors – n (%)	20 (20.2)	17/20 (85.0)	3/20 (15.0)	.005 ^b
MPA – n (%)	86 (86.9)	44/86 (51.2)	42/86 (48.8)	.006 ^b
Last available tacrolimus level ng/ml, mean (SD) n = 86	6.1 (1.5)	6.2 (1.6)	5.8 (1.4)	.252 ^a
Hemoglobin (IQR), g/dl, T0	13.3 (12.0–14.3)	13.7 (12.6–14.7)	12.8 (11.3–13.6)	.002
Immunoglobulin G, T0 (mg/dl)	958.0 (843.8–1175.0)	1030.0 (880.0–1220.0)	914.0 (786.0–1070.0)	.035
Immunoglobulin A, T0 (mg/dl)	202.5 (152.5–258.3)	199.0 (163.0–270.0)	212.0 (137.0–244.0)	.427
Immunoglobulin M, T0 (mg/dl)	96.6 (62.5–135.8)	96.2 (64.7–144.0)	97.0 (48.9–128.0)	.567

Abbreviations: eGFR, estimated glomerular filtration ratio; IQR, interquartile range; KT, kidney transplantation; MPA, mycophenolic acid; mTOR, mechanistic target of rapamycin; SD, standard deviation.

^ap-value obtained by Student's t test.

^bp-value obtained by Fisher exact test; other p-values were obtained by Mann-Whitney test.

TABLE 2 Demographic and laboratorial characteristics of SARS-CoV-2 naïve patients with positive TTV DNA viral load at T0 (Group C)

Characteristics	Total (n = 75)	Anti-spike + Ab at T2 (n = 44)	Anti-spike – Ab at T2 (n = 31)	p value
Age in years – mean (SD)	46.2 (11.1)	44.1 (8.8)	49.1 (13.4)	.072 ^a
Gender – n (%)				1.000 ^b
Male	40 (53.3)	23/44 (52.3)	17/31 (54.8)	
Female	35 (46.7)	21/44 (47.7)	14/31 (45.2)	
Type of donor- (n/%)				.440 ^b
Deceased	53 (70.7)	33/53 (62.3)	20/53 (37.7)	
Living	22 (29.3)	11/22 (50.0)	11/22 (50.0)	
Time after KT, months	86.7 (51.7–154.0)	86.5 (44.6–154.6)	93.2 (56.5–151.3)	.675
eGFR T0, ml/min	60.0 (43.0–83.0)	71.5 (52.8–85.0)	48.0 (41.0–70.0)	.005
Calcineurinic inhibitors – n (%)	71 (94.7)	40/71 (56.3)	31/71 (43.7)	.084 ^c
mTOR inhibitors – n (%)	14 (18.7)	14/14 (100)	0/14 (0)	<.001 ^b
MPA – n (%)	65 (86.7)	34/65 (52.3)	31/65 (47.7)	.004 ^b
TTV viral load T0, log copies/ml	4.05 (3.24–4.94)	3.71 (3.02–4.62)	4.46 (3.60–5.08)	.021
TTV viral load T1, log copies/ml	3.60 (2.71–4.78)	3.30 (2.71–4.49)	4.19 (3.01–4.78)	.088
TTV viral load T2, log copies/ml (n = 73)	4.29 (3.24–5.00)	4.0 (3.13–4.49)	4.57 (4.00–6.01)	.005
Last available tacrolimus level, ng/ml, mean (SD) n = 68	6.1 (1.6)	6.4 (1.7) (n = 39)	5.8 (1.4) (n = 29)	.121 ^a
Hemoglobin T0, g/dl	13.5 (12.30–14.60)	13.95 (13.10–14.78)	13.0 (11.90–13.90)	.008
Immunoglobulin G, T0, (mg/dl)	986.0 (878.0–1190.0)	1025.0 (890.5–1212.5)	957.0 (839.0–1120.0)	.237
Immunoglobulin A, T0, (mg/dl)	199.0 (159.0–263.0)	195.5 (163.3–274.5)	218.0 (125.0–248.0)	.767
Immunoglobulin M, T0, (mg/dl)	84.9 (49.8–124.0)	92.5 (68.3–140.8)	82.1 (47.1–104.0)	.199

Abbreviations: eGFR, estimated glomerular filtration ratio; IQR, interquartile range; KT, kidney transplantation; MPA, mycophenolic acid; mTOR, mechanistic target of rapamycin; SD, standard deviation; TTV, torquetenavirus.

^ap-value obtained by Student's t test.

^bp-value obtained by Fisher exact test.

^cp-value obtained by Pearson's chi-squared test; other p-values were obtained by Mann-Whitney test.

anti-S total antibodies at T2 with a median titer of 57.3 (P₂₅ = 9.03; P₇₅ = 370.50) U/ml.

A serological response to the vaccine was more likely to occur with the use of mTOR inhibitors (p = .005). Seventeen of the 20 patients (85%) treated with mTOR developed positive anti-S antibodies at T2. Conversely, serological response to the vaccine was associated with immunosuppression schemes not containing MPA (p = .006). In fact, from 13 patients not treated with MPA, 12 (92.3%) responded to vaccination; only one patient (7.7%) not receiving MPA failed to develop anti-S antibodies. No patient received azathioprine.

Higher hemoglobin levels (p = .002), higher Immunoglobulin G levels (p = .035) and higher eGFR (p = .003), at T0, were also associated with an appropriate serological response (Table 1).

3.4 | Vaccine response and associated factors (Group C)

Table 2 depicts demographic and laboratorial characteristics of SARS-CoV-2 naïve patients with positive TTV-T0 (n = 75).

TTV DNA viral load was measured in serum at T0, T1 and T2, and the median TTV viral load was significantly different between T1 and T0 (p = .005) and between T1 and T2 (p < .001), with lower viral loads at T1 (Figure 2).

Immunosuppressive regimen was associated with antibody response. All 14 patients treated with mTOR inhibitors developed serological response at T2 (p < .001). A lower proportion for antibody response was associated with MPA therapy (n = 34;52.3%) and all 10 patients without MPA developed anti-S antibodies at T2 (p = .004). Time after KT was not associated with vaccination response (p = .675).

3.5 | TTV DNA viral load association with antibody response (Group C)

Table 3 displays the results of univariable logistic regression for Spike antibody response at T2.

We evaluated the association between TTV-T0 viral load and anti-S antibodies formation after SARS-CoV-2 vaccination. For each increase of 1 log TTV-T0 the chance of having an unfavorable vaccine response

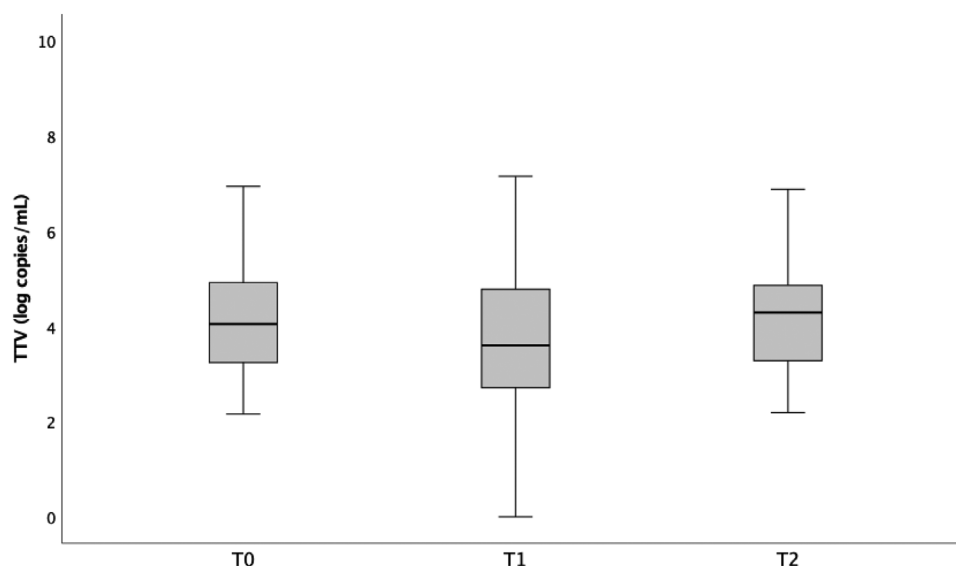


FIGURE 2 Evolution of TTV load at study time points

TABLE 3 Univariable logistic regression analysis for Spike antibody response at T2 (Group C)

Variables	Odds ratio estimate	95% CI		p-value
		Lower limit	Upper limit	
Actual age	1.04	.99	1.09	.057
Hemoglobin T0 (g/dl)	.65	.47	.91	.011
eGFR T0 (ml/min)	.98	.96	1.00	.025
Creatinine (mg/dl)	1.58	.82	3.05	.177
Immunoglobulin G T0, (mg/dl)	.999	.997	1.001	.247
Immunoglobulin M T0, (mg/dl)	.992	.982	1.002	.123
TTV T0 (\log_{10} cp/ml)	1.68	1.09	2.60	.020
TTV T0 > cutoff point 3.36 (\log_{10} cp/ml)	4.67	1.39	15.67	.013
Last available tacrolimus level (ng/ml)	.78	.56	1.07	.124

Dependent variable: Anti spike antibodies response at T2 (unfavorable vs. favorable vaccine response).

Abbreviations: eGFR, estimated glomerular filtration ratio; TTV, torquetenovirus.

increases 1.68 times ($p = .020$). A TTV-T0 cut-off value of 3.36 \log_{10} cp/ml was determined considering anti-S antibodies detection ($p = .013$), with a sensitivity of 87.1% and a specificity of 40.9% for discriminating antibody response.

In the multivariable model, the presence of TTV-T0 ≥ 3.36 \log_{10} cp/ml was associated with unfavorable vaccine response (OR: 5.40; 95% CI: 1.47–19.80; $p = .011$), after adjusting for age and eGFR at T0 (Table 4). Model's good calibration and discrimination abilities were obtained (Hosmer-Lemeshow test $p = .562$ and AUC = .76; 95%CI: .648–.865; $p < .001$), respectively.

4 | DISCUSSION

The association between TTV viral load at baseline and anti-S total antibody response after SARS-CoV-2 mRNA BNT162b2 vaccination at T2 was analyzed in a cohort of KT patients.

At baseline, 99 patients were naïve for SARS-CoV-2; only 56.6% developed anti-S antibodies after two vaccine doses, after a median of 30 days post second dose (T2). Affeldt et al.¹⁶ studied 86 SARS-CoV-2-naïve KT recipients using BNT162b2 with a median of 30 days after their second vaccination reporting a seroconversion rate of 39.5% and Nazaruk et al.¹⁷ found anti-S antibodies in 57.1% of KT patients, 4–8 weeks after second dose. Antibody response to mRNA vaccines in KT patients is inferior to the general population, ranging between 37.5% and 54% after two doses of vaccine.^{18–20}

Regarding immunosuppression, 92.3% of patients not treated with MPA responded to vaccine with antibody response. Our study is consistent with previous published studies where seroconversion rate was significantly lower in KT recipients on immunosuppressive regimens containing MPA.^{17,21,22} It inhibits inosine-5'-monophosphate dehydrogenase, suppressing antibody formation and proliferation and cell mediated immune responses.²³ Conversely, the presence of mTOR was associated with a better immune response to COVID-19 vaccine, since

TABLE 4 Multivariable logistic regression analysis for antibody response at T2 (Group C)

Variables	Odds ratio estimate	95% CI		p-value
		Lower limit	Upper limit	
TTVt0 ≥ 3.36 (log ₁₀ cp/ml) ^a	5.40	1.47	19.80	.011
Actual age (years)	1.05	1.00	1.10	.067
eGFR T0 (ml/min)	.98	.95	.99	.025

Dependent variable: Anti spike antibodies response at T2 (unfavorable vs. favorable vaccine response).

Abbreviation: eGFR, estimated glomerular filtration rate.

^aReference category TTVt0 < 3.36 (log₁₀ cp/ml).

87% of patients treated with mTOR inhibitors, developed anti-S antibodies at T2 and in consistent with findings by Netti et al.⁶ A possible mechanism for the enhanced immune response to the mRNA COVID-19 vaccine in KT treated with mTOR is its immunomodulatory effects in memory CD8+ and CD4+ T cells by promoting the enhancement of memory precursor effector cells that could differentiate into long-lived memory cells.²⁴

Patients with higher hemoglobin levels and a greater eGFR were more likely to develop an antibody response. Lower hemoglobin levels and lower eGFR are associated with allograft dysfunction, which could decrease humoral response.^{25,26}

Long period between transplantation and vaccination has been associated with positive humoral SARS-CoV-2 response.²³ In our study, time since transplantation was not associated with vaccine response as almost 90% of patients received the allograft at least 2 years before the study, with minimized doses of immunosuppression before vaccination.

Until now, no reliable marker has been identified to quantify the net state of immunosuppression in transplant patients. Kinetics of TTV DNA load is a potential marker of immune function⁸ and has also been investigated in the prediction of severe nosocomial infections and mortality in critically ill COVID-19-patients.²⁷

The interaction between TTV and viral antigens has already been described. In healthy individuals, TTV load slightly increases after immunologic stimulation with influenza and hepatitis B vaccination. We found a lower median TTV DNA viral load at T1, returning to baseline levels at T2. We postulated that vaccine stimulation of specific immunity could lead to a transient immune reconstitution, which would be associated with a lower TTV load.

In our study, 75.8% of patients had detectable TTV DNA, with a median viral load at baseline of 4.05 log₁₀ cp/ml. TTV load is associated with rejection and infection in solid organ transplant recipients and cut-off values for risk stratification of such events have been proposed for KT patients, but only along the first year after transplantation.⁸

We found that the development of anti-S antibodies after two doses of BNT162b2 vaccine was more frequent in KT recipients with lower TTV loads at baseline. The optimal cut-off value of TTV viral load, which best discriminates patients with positive from negative response, was 3.36 log₁₀ cp/ml. A TTV viral load higher than 3.36 log₁₀ cp/ml has a five-fold increase in the odds of non-development of antibodies. We reached a high sensitivity of 87.1%, so we expect that this cut-off is effective in identifying vaccine responders. This infor-

mation may become useful to identify the optimal time for an efficient boosting.²⁸

Our data was corroborated by a recent study¹⁰ of 103 lung transplant patients vaccinated with two doses of mRNA-123 SARS-CoV-2 vaccine. Authors showed that lower TTV loads at baseline correlated with higher S-specific antibodies 28 days after the second vaccination.

At baseline, 13.2% of patients had COVID-19 prior to vaccination. All of them developed anti-S pre-vaccination and anti-S antibodies levels post-vaccination were significantly higher, independent of immunosuppressive regimen. In convalescent COVID-19 patients, antibody levels decline slightly after 6–8 months, whereas vaccine-induced immunity appears to decrease more rapidly.²⁹ Benning et al.³⁰ revealed that 18 convalescent KT recipients showed significantly higher anti-S antibody levels compared with 2-dose BNT162b2 vaccinated KT recipients. Consequently, additional boosting or a higher dose stimulus might need to be applied in non-responders.³¹ After the conclusion of this study, all solid organ recipients received a third dose of mRNA vaccine and are eligible for a fourth one.

This study has some limitations. First, we did not evaluate cellular responses to SARS-CoV-2. Assessment of humoral response alone may underestimate vaccine effect, since some studies demonstrated a significant T-cell immunity in cases of humoral non-response.³² Second, the analysis of anti-S titers might not reflect the global immunologic status, since the extent to which anti-S titer correlates with protection against infection and severe COVID-19 is still not understood. In addition, follow-up on waning antibody titers is unavailable.

In conclusion, this study established an association between higher TTV viral loads pre-vaccination and reduced anti-S response in SARS-CoV-2 mRNA BNT162b2 vaccinated KT patients. A TTV viral load higher than 3.36 log₁₀ cp/ml has a five-fold increase in the odds of absence of antibody response. In addition, TTV kinetics varies along SARS-CoV-2 vaccination, with lower DNA viral loads after the first vaccine inoculation.

The association between TTV viral load and vaccine response should be investigated in other cohorts of KT patients as a potential added-value strategy in the optimization of the COVID-19 vaccination regimens.

AUTHOR CONTRIBUTIONS

S. Querido: Concept/design, data collection, data analysis, drafting article. T. Adragão: statistics, critical revision of article. C. Ormonde: data

analysis, data collection. I. Pinto/ AL Papoila: statistics, critical revision of article. MA Pessanha/ P. Gomes/ JM Figueira/ C. Cardoso/ JF Viana: data analysis/interpretation. A. Weigert: critical revision of article/ approval of article.

ACKNOWLEDGMENTS

Diogo Francisco, Carla Rocha, Ana Casqueiro, Regina Oliveira, Ana Albuquerque, Lília Oliveira, Ana Margarida Casaca, Inês Sousa, and the remaining participating Nursing and Clinical Pathology teams of Hospital Santa Cruz for sample collection. Inês Costa from LBM for TTV viral load testing. Celia Pereira for invaluable secretarial assistance. Isabel Pereira, for statistical support and critical revision of the article.

DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST

None.

ORCID

Sara Querido  <https://orcid.org/0000-0003-2325-1817>

Teresa Adragão  <https://orcid.org/0000-0003-3919-1070>

Iola Pinto  <https://orcid.org/0000-0002-2945-1441>

Carolina Ormonde  <https://orcid.org/0000-0002-4077-9405>

Ana Luísa Papoila  <https://orcid.org/0000-0002-2918-8364>

Maria Ana Pessanha  <https://orcid.org/0000-0002-1393-932X>

Perpétua Gomes  <https://orcid.org/0000-0003-3271-8255>

Sílvia Ferreira  <https://orcid.org/0000-0002-7953-6208>

João Mário Figueira  <https://orcid.org/0000-0003-2818-4828>

Conceição Cardoso  <https://orcid.org/0000-0002-8263-3340>

João Faro Viana  <https://orcid.org/0000-0002-8405-1221>

André Weigert  <https://orcid.org/0000-0001-9674-8862>

REFERENCES

- Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
- Caillard S, Chavarot N, Francois H, et al. Is COVID-19 infection more severe in kidney transplant recipients? *Am J Transplant*. 2021;21(3):1295-1303.
- Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov*. 2018;17(4):261-279.
- Bertrand D, Hamzaoui M, Lemée V, et al. Antibody and T-cell response to a third dose of SARS-CoV-2 mRNA BNT162b2 vaccine in kidney transplant recipients. *Kidney Int*. 2021;100(6):1337-1340.
- Caillard S, Thauinat O, Benotmane I, Masset C, Blancho G. Antibody response to a fourth messenger RNA COVID-19 vaccine dose in kidney transplant recipients: a case series. *Ann Intern Med*. 2022;175(3):455-456.
- Netti GS, Infante B, Troise D, et al. mTOR inhibitors improve both humoral and cellular response to SARS-CoV-2 messenger RNA BNT16b2 vaccine in kidney transplant recipients. *Am J Transplant*. 2022;22(5):1475-1482.
- Buchwinkler L, Solagna CA, Messner J, et al. Antibody response to mRNA vaccines against SARS-CoV-2 with chronic kidney disease, hemodialysis, and after kidney transplantation. *J Clin Med*. 2021;11(1):148.
- Rezahosseini O, Drabe CH, Sørensen SS, et al. Torque-Teno virus viral load as a potential endogenous marker of immune function in solid organ transplantation. *Transplant Rev (Orlando)*. 2019;33(3):137-144.
- Querido S, Calça R, Weigert A, et al. Kinetics of torquetenovirus DNA load in a recent kidney transplant recipient with mild SARS-CoV-2 infection and a failed antibody response. *Transpl Infect Dis*. 2021;23(2):e13524.
- Hoek RA, Verschuuren EA, de Vries RD, et al. High torque tenovirus (TTV) load before first vaccine dose is associated with poor serological response to COVID-19 vaccination in lung transplant recipients. *J Heart Lung Transplant*. 2022;41(6):765-772.
- Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2011;155(6):604-612.
- Riester E, Findeisen P, Hegel JK, et al. Performance evaluation of the Roche Elecsys anti-SARS-CoV-2 S immunoassay. *J Virol Methods*. 2021;297:114271.
- Faro-Viana J, Bergman ML, Gonçalves LA, et al. Population homogeneity for the antibody response to COVID-19 BNT162b2/Comirnaty vaccine is only reached after the second dose across all adult age ranges. *Nat Commun*. 2022;13(1):140.
- Gore EJ, Gomes-Neto AW, Wang L, et al. Torquetenovirus serum load and long-term outcomes in renal transplant recipients. *J Clin Med*. 2020;9(2):440.
- Solis M, Velay A, Gantner P, et al. Torquetenovirus viremia for early prediction of graft rejection after kidney transplantation. *J Infect*. 2019;79(1):56-60.
- Affeldt P, Koehler FC, Brensing KA, et al. Immune responses to SARS-CoV-2 infection and vaccination in dialysis patients and kidney transplant recipients. *microorganisms*. 2021;10(1):4.
- Nazaruk P, Monticolo M, Jędrzejczak AM, et al. Unexpectedly high efficacy of SARS-CoV-2 BNT162b2 vaccine in liver versus kidney transplant recipients-is it related to immunosuppression only. *Vaccines (Basel)*. 2021;9(12):1454.
- Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. *JAMA*. 2021;325(21):2204-2206.
- Grupper A, Rabinowich L, Schwartz D, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. *Am J Transplant*. 2021;21(8):2719-2726.
- Marinaki S, Adamopoulos S, Degiannis D, et al. Immunogenicity of SARS-CoV-2 BNT162b2 vaccine in solid organ transplant recipients. *Am J Transplant*. 2021;21(8):2913-2915.
- Heldman MR, Limaye AP. SARS-CoV-2 vaccines in kidney transplant recipients: will they be safe and effective and how will we know. *J Am Soc Nephrol*. 2021;32(5):1021-1024.
- Benotmane I, Gautier-Vargas G, Cognard N, et al. Low immunization rates among kidney transplant recipients who received 2 doses of the mRNA-1273 SARS-CoV-2 vaccine. *Kidney Int*. 2021;99(6):1498-1500.
- Allison AC, Eugui EM. Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection. *Transplantation*. 2005;80:S181-S190.
- Ye L, Lee J, Xu L, et al. mTOR promotes antiviral humoral immunity by differentially regulating CD4 helper T cell and B cell responses. *J Virol*. 2017;91(4):e01653-16.
- Marion O, Del Bello A, Abravanel F, et al. Predictive factors for humoral response after 2-dose SARS-CoV-2 vaccine in solid organ transplant patients. *Transplant Direct*. 2021;8(1):e1248.
- Chukwu CA, Mahmood K, Elmakki S, et al. Evaluating the antibody response to SARS-COV-2 vaccination amongst kidney transplant recipients at a single nephrology centre. *PLoS One*. 2022;17(3):e0265130.

27. Forqué L, Albert E, Giménez E, et al. Monitoring of Torque Teno virus DNAemia in critically ill COVID-19 patients: may it help to predict clinical outcomes. *J Clin Virol.* 2022;148:105082
28. Reza Hosseini O, Nielsen SD. Torque-Teno virus to optimize timing of vaccination against severe acute respiratory syndrome coronavirus 2 in solid organ transplant recipients. *J Clin Virol.* 2021;143:104967.
29. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science.* 2020;370(6521):1227-1230.
30. Benning L, Morath C, Bartenschlager M, et al. Natural SARS-CoV-2 infection results in higher neutralization response against variants of concern compared with 2-dose BNT162b2 vaccination in kidney transplant recipients. *Kidney Int.* 2022;101(3):639-642.
31. Stumpf J, Siepmann T, Lindner T, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: a prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. *Lancet Reg Health Eur.* 2021;9:100178.
32. Hall VG, Ferreira VH, Ierullo M, et al. Humoral and cellular immune response and safety of two-dose SARS-CoV-2 mRNA-1273 vaccine in solid organ transplant recipients. *Am J Transplant.* 2021;21(12):3980-3989.

How to cite this article: Querido S, Adragão T, Pinto I, et al. Torquetenovirus viral load is associated with anti-spike antibody response in SARS-CoV-2 mRNA BNT162b2 vaccinated kidney transplant patients. *Clin Transplant.* 2022;36:e14825. <https://doi.org/10.1111/ctr.14825>