Torque teno virus DNA load as a predictive marker of antibody response to a three-dose regimen of COVID-19 mRNA-based vaccine in lung transplant recipients

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KEYWORDS:
lung transplantation;
COVID-19 vaccine;
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vaccine response;
immunosuppression

BACKGROUND: Previous studies have reported that lung transplant recipients (LTR) develop a poor response to two doses of COVID-19 vaccine, but data regarding the third dose are lacking. We investigated the antibody response after three doses of mRNA vaccine in LTR and its predictive factors.
METHODS: A total of 136 LTR, including 10 LTR previously infected and 126 COVID-19-naive LTR, were followed during and after three doses of mRNA vaccine. We retrospectively measured anti-receptor-binding domain (RBD) IgG response and neutralizing antibodies. In a posthoc analysis, we used a multivariate logistic regression model to assess the association between vaccine response and patient characteristics, including viral DNA load (VL) of the ubiquitous Torque teno virus (TTV) (optimal cut-off set by ROC curve analysis), which reflects the overall immunosuppression.
RESULTS: After 3 doses, 47/126 (37.3%) COVID-19-naive LTR had positive anti-RBD IgG (responders) and 14/126 (11.1%) had antibody titers above 264 Binding Antibody Units/mL. None neutralized the omicron variant versus 7 of the 10 previously infected LTR. Nonresponse was associated with TTV VL $\geq$6.2 $\log_{10}$ copies/mL before vaccination (Odds Ratio (OR) = 17.87, 95% confidence interval (CI95) = 3.02-105.72), mycophenolate treatment (OR = 4.73, CI95 = 1.46-15.34) and BNT162b2 (n = 34; vs mRNA-1273, n = 101) vaccine (OR = 6.72, CI95 = 1.75-25.92). In second dose nonresponders, TTV VL $\geq$6.2 or <3.2 $\log_{10}$ copies/mL before the third dose was associated with low (0/19) and high (9/10) rates of seroconversion.
CONCLUSION: COVID-19-naive LTR respond poorly to three doses of mRNA vaccine, especially those with high TTV VL. Future studies could further evaluate this biomarker as a guide for vaccine strategies.
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Introduction

Lung transplant recipients (LTR) are at high risk for severe COVID-19 due to their lung disease and the high doses of immunosuppressive drug therapy they receive to prevent allograft rejection.[1,2] The highly transmissible omicron variant is resistant to several anti-SARS-CoV-2 monoclonal antibodies, and still represents a major issue in this population. Moreover, the combination of protease inhibitors nirmatrelvir/ritonavir (Paxlovid) is difficult to use in this population due to significant drug interactions. In this context, the COVID vaccine remains the safest strategy to protect LTR from severe disease.

By the end of 2020, solid organ transplant recipients were prioritized for COVID-19 mRNA vaccination. However, they have been shown to only develop a poor antibody response (34%-54%) to a 2-dose vaccine regimen compared to immunocompetent individuals,[3,4] with lower response observed in LTR (0-40%) than in kidney or liver transplant recipients since they receive higher doses of immunosuppressive drugs.[5-11] Previous studies showed that around half of solid organ recipients who were nonresponders to the second dose seroconverted after a third mRNA vaccine dose, given as booster.[12-15] However, only few studies have determined the effectiveness of a third vaccine dose in LTR,[16,17] and markers predictive of vaccine response are still lacking. In order to quantify the impact of immunosuppression on vaccine response and hopefully to predict response or nonresponse, one should be able to quantify the level of immunosuppression. Previous studies have reported that the composition of the virome in plasma is affected by immunosuppressant drugs and may therefore predict the state of immunosuppression.[18] In transplant recipients, the virome is mostly composed of *Anelloviridae* (68%) and *Herpesviridae* (13%).[18] Torque teno virus (TTV) accounts for 97% of *Anelloviridae* fraction in the virome,[18] and viral DNA loads (VL) in healthy individuals typically remain below 4 $\log_{10}$ cp/mL.[19] In the case of immunosuppression, this virus replicates strongly, and the level of its burden has made it possible to stratify rejecting and non-rejecting recipients.[18] This makes it a potential candidate for predicting vaccine response in transplant recipients. Among the *Herpesviridae*, increased VL of Epstein-Barr virus and Cytomegalovirus may also reflect immunosuppression status. However, antivirals used for prophylaxis after transplantation significantly decrease the load of these viruses and may prevent their use as effective markers of immunosuppression.

In this study, we characterized the antibody response in LTR after three doses of mRNA vaccine, by longitudinally analyzing anti-Receptor Binding Domain (RBD) IgG titers and neutralizing activity of sera against the ancestral strain D614G, B.1.617.2 (delta), and B.1.1.529 (omicron) variants. In posthoc analyses, we used logistic regression to assess for an association of demographic, clinical, and TTV VL variables with vaccine response.

Methods

Study design

We conducted a retrospective cohort study on LTR followed in the outpatient lung transplantation department of Strasbourg University Hospital. All LTR with serum samples available from 2 weeks to 2 months after the third booster dose of mRNA-based vaccine (administered between 1st April 2021 and 30th October 2021) were enrolled. To assess vaccine humoral responses and predictors in LTR without biases due to intercurrent events, patients who had received their first vaccine dose before lung transplantation and those infected between the first dose and the third dose, were excluded. Data including age, sex, body mass index, blood group, native lung disease, comorbidities, prior history of COVID-19, transplant type and date, and immunosuppressive drugs were collected just before vaccination, as well as information about COVID-19 vaccine types. The LTR cohort was divided into two groups according to previous SARS-CoV-2 infection status, as determined by serology performed in all patients before vaccination and by history of positive RT-PCR.

All patients provided informed written consent to the analysis of their samples included in the registered biobank n°DC2014-2222 for research purposes. This study was approved by the Institutional Review Board of the French learned Society for Respiratory Medicine: Société de Pneumologie de Langue Française (CEPRO 2022-009).

Antibody response

All available sera sampled within the three-month interval (median: 7 days, interquartile range [IQR]: 0-46) before vaccination and from 2 weeks to 2 months after each vaccine dose were retrospectively analyzed with the Abbott Architect SARS-CoV-2 IgG II Quant assay to assess the anti-receptor-binding domain (RBD) IgG response. All results were converted into Binding Antibody Units (BAU)/mL adapted to the World Health Organization standard for SARS-CoV-2 immunoglobulin by multiplication by a factor of 0.142 (quantification range: 1.0-11,360.0 BAU/mL, positivity threshold: 7.1 BAU/mL). Patients without history of COVID-19 who displayed positive IgG titers after a third vaccine dose were categorized as responders (vs seronegative patients who were classified as nonresponders). To exclude any humoral response following unidentified infection, the first seropositive sample of each COVID-19-naive vaccine responder was also examined for anti-nucleocapsid (N) IgG with the Abbott Architect SARS-CoV-2 IgG assay.

Pseudotyped virus-based neutralization assay

Neutralizing antibody titers were assessed by a pseudovirus-based assay in responders after the third vaccine dose against D614G, delta, and omicron variants, as described previously and in supplementary materials.[20] The neutralization efficiency expressed as the $\log_{10}$ of the median half-maximal effective dilutions (ED_{50}) was calculated using Prism 9.3.1. Sera were considered positive if they neutralized more than 50% SARS-CoV-2 pseudovirus at 1:40 dilution.

Viral genome amplification by real-time PCR

The TTV VL was retrospectively determined using the TTV R-GENE kit (bioMérieux) in the three months (median: 7 days, IQR:

0-46) preceding vaccination for all patients, as well as between the second and third dose if a blood sample was available.

Statistical analysis

We compared demographic, clinical and biological baseline characteristics between responders (patients displaying anti-RBD IgG ≥ 7.1 BAU/mL) and non-responder LTR to a three-dose vaccine regimen using the Fisher exact test and χ^2 test for dichotomous variables and the Mann-Whitney U-test for continuous variables. Correlation analyses between continuous variables were performed using Spearman rank correlation test. Statistical tests were 2-tailed and significance was set at $p < 0.05$. Posthoc Receiver Operating Characteristic (ROC) curve analyses were conducted on TTV VL data, and values presenting sensitivity or specificity over 95% and the highest Youden index were selected as cut-offs. All analyses were performed using Prism 9.3.1. Multivariable logistic regression analysis was performed to identify independent predictors of antibody response after the third vaccine dose. Relevant parameters associated with nonresponse with a p-value <0.2 in the univariate analysis were included in the model. Statistical analyses were performed using SPSS 28.0 (IBM Statistics), and results were expressed as adjusted Odds Ratios (OR) with 95% confidence intervals (CI95).

Results

Participants

This study was conducted on 479 blood samples from 136 LTR, including 10 patients with and 126 without a history of COVID-19 (Figure 1, Table S1). Median age was 62.5 years (IQR: 51.0-67.0), with 39% women in the cohort. Most patients had cardiovascular comorbidities, primarily hypertension (57.4%) and diabetes mellitus (49.3%). All received three doses of either BNT162b2 (Pfizer-BioN-Tech; n = 34, 25.0%) or mRNA-1273 (Moderna; n = 101, 74.3%) vaccines, except one patient who received a combination of both vaccines. The vaccination was carried out at a median of 5.5 years (IQR: 2.6-9.0) after lung transplantation. The first two doses were given 4 weeks apart, and the third dose was administered at a median interval of 59.0 days (IQR: 35.3-73.0) after the second dose. The maintenance immunosuppression regimen included glucocorticoids (94.1% of LTR), calcineurin inhibitors (97.0%; tacrolimus [86.0%] or cyclosporine [11.0%]), antimetabolites (91.9%) (mycophenolate mofetil/mycophenolic acid (MMF/MPA; 80.1%) or azathioprine [11.8%]), and everolimus (13.2%). Two patients received rituximab, 6.5 months
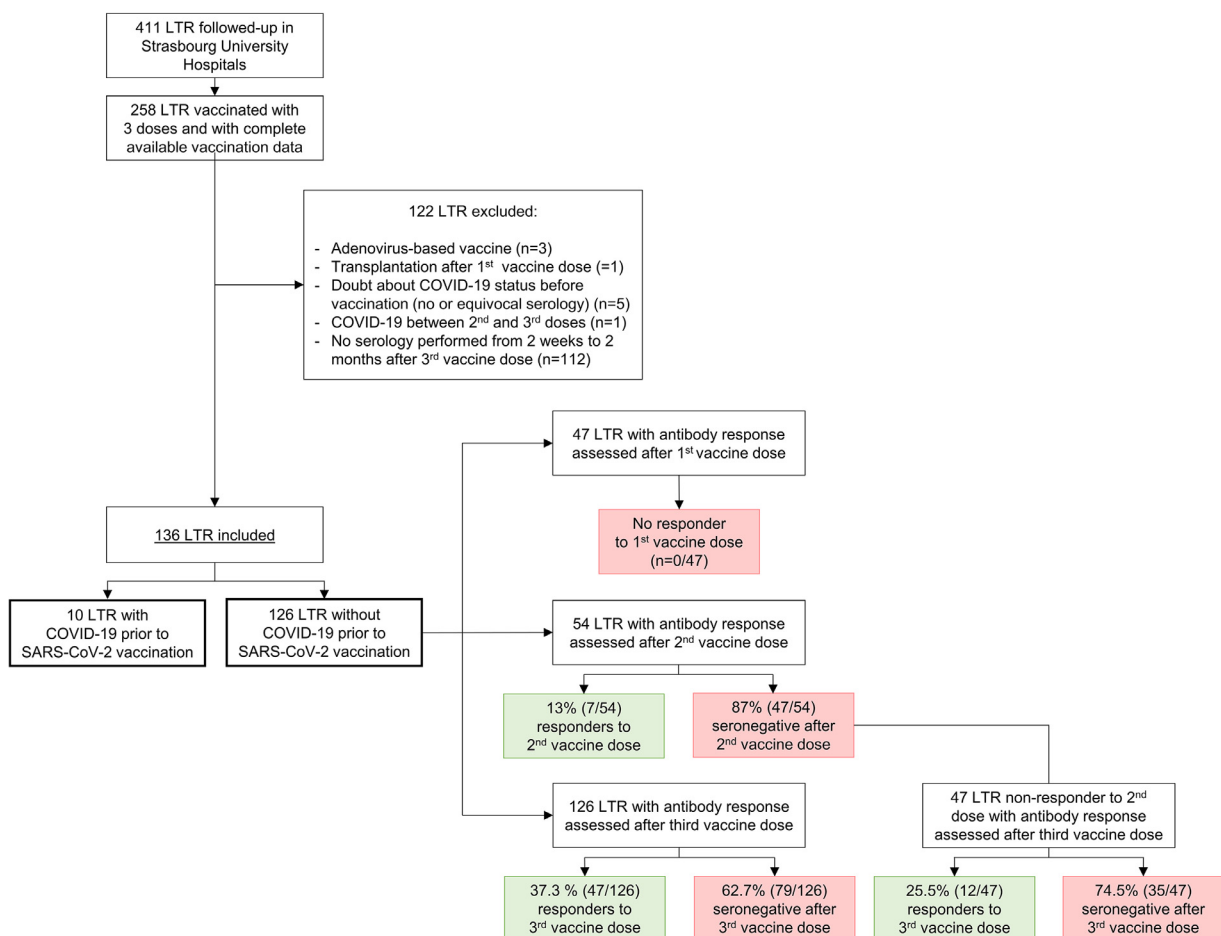
Figure 1   Flow chart of lung transplant recipients (LTR) recruitment and antibody response to COVID-19 mRNA-based vaccination. The study was conducted on 136 LTR, including 10 and 126 patients with and without history of COVID-19 before vaccination, respectively. Sera sampled after each vaccine dose in COVID-19-naive patients were analyzed to assess the anti−receptor-binding domain (RBD) IgG response, with seropositive patents defined as responders.

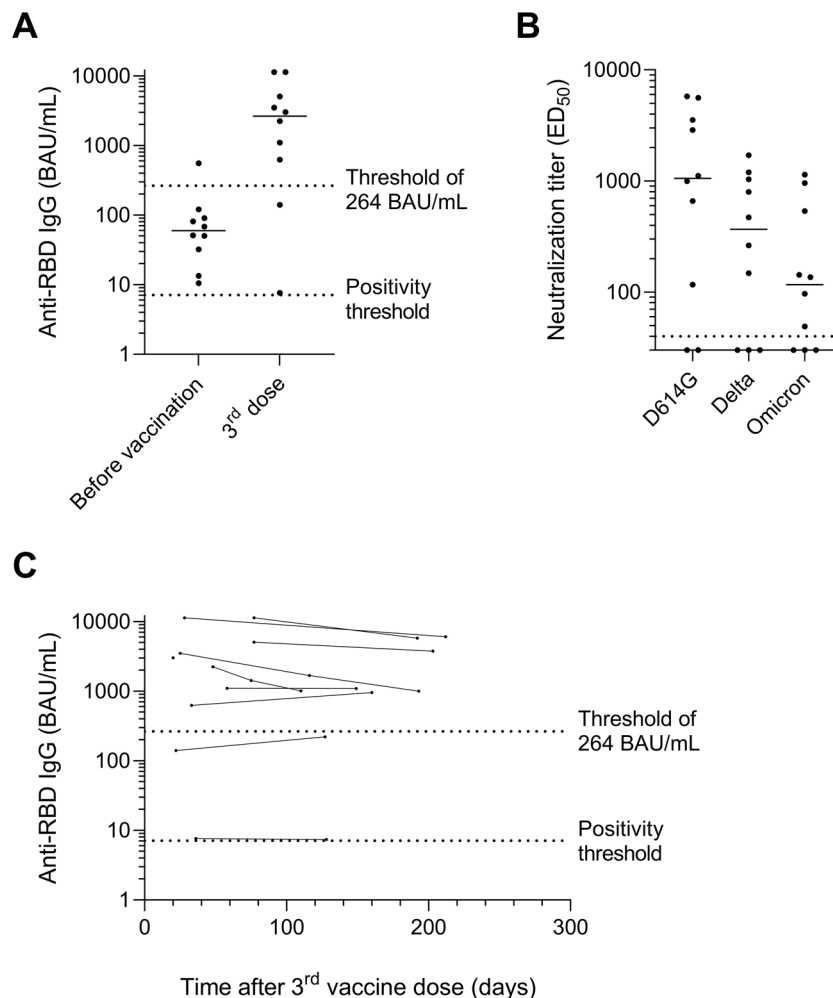before vaccination and three days after the first vaccine dose, respectively.

Antibody response to the three-dose vaccine regimen in LTR with prior COVID-19

The 10 LTR with a history of COVID-19 before vaccination (proven by positive serology for anti-RBD IgG: n = 10/10 or by RT-PCR: 3/10) had asymptomatic (n = 5/10), mild (4) or severe (1) disease. All were seropositive before vaccination with anti-RBD IgG titers ranging from 10.5 to 554.8 BAU/mL (median: 68.2, IQR: 50.4-90.3). Unexpectedly, in one patient, anti-RBD IgG titer decreased from 13.3 to 7.6 BAU/mL despite 3-dose vaccination, whereas an antibody rebound was observed in the nine other patients (median increase: 2,633.1 BAU/mL, IQR: 742.8-4,658.7) (Figure 2A). Among them, 8/10 patients harbored antibodies over 264 BAU/mL, which is the threshold now

considered in France as a decision-making tool for the choice between prophylaxis with a fourth vaccine dose or with monoclonal antibodies therapy.[21,22] Sera from these eight patients neutralized the D614G pseudovirus (log ED_{50}: 2.07-3.76, median 3.02), seven of them also neutralizing the delta (log ED_{50}: 2.17-3.23, median 2.55) and the omicron (log ED_{50}: 1.69-3.06, median 2.06) variants (Figure 2B, Figure S1). Seven patients were followed up to seven months after the third dose without intercurrent antigenic stimulation or monoclonal antibody therapy, and all of them maintained anti-RBD IgG titers over 264 BAU/mL (Figure 2C).

Antibody response to the 3-dose vaccine regimen in COVID-19-naive LTR

Anti-RBD IgG were available for 47, 54, and 126 COVID-19-naive LTR after the first, second, and third doses of

Figure 2 Antibody response after three vaccine doses in lung transplant recipients with history of COVID-19 (n = 10). (**A**) Anti-RBD IgG titers expressed in BAU/mL before vaccination and from two weeks to two months after the third dose. (**B**) Neutralizing antibody titers assessed by pseudovirus-based assay against D614G, delta and omicron variants. The dotted horizontal black line indicates the cutoff for positivity (1:40 dilution). (**C**) Dynamics of anti-RBD IgG titers expressed in BAU/mL over time after the third vaccine dose (n = 11 additional samples collected from 9 patients during follow-up). (**A** and **C**) The dotted lines indicate the positivity threshold (7.1 BAU/mL) and the threshold of 264 BAU/mL used in French recommendations to guide prophylactic strategy in immunosuppressed patients. BAU, Binding Antibody Units; ED50, half-maximal effective dilution; RBD, receptor-binding domain.

vaccine, respectively. None of the LTR seroconverted after the first dose, and 13% (7/54) were anti-RBD IgG positive after the second dose, with titers ranging from 13.2 to 585.8 BAU/mL (median: 54.6, IQR: 34.1-383.5; Figure 3A). After the third dose, 37.3% (47/126) LTR were seropositive with anti-RBD IgG ranging from 7.7 to 5,249.7 BAU/mL (median: 81.0, IQR: 32.0-274.1) including 14 patients with titers ≥264 BAU/mL (29.8% of responders and 11.1% of the whole cohort). Considering the 47 patients who tested seronegative after the second dose, the third dose led to a positive antibody response in 25.5% (12/47) patients, including one patient (representing 8.3% of these responders) with a titer over 264 BAU/mL (Figure 3B).

Longitudinal serum samples collected from 6 to 8 months after the third dose were available for 21 responders. Anti-RBD IgG were still detectable for 16 of these responders (76.2%), but all titers were lower than 264 BAU/mL (Figure 3C). None of them had intercurrent infection, administration of a fourth vaccine dose, or monoclonal antibodies prophylaxis before the last serology performed during follow-up. Sera were not available for the remaining 26 responders at that time because their follow-up period after the third dose was shorter.

Analysis of the neutralizing activity of seropositive sera, collected at a median of 34 days post-third dose (IQR: 25.5-46.5 days), showed that 46.8% (22/47) neutralized the ancestral D614G strain with titers up to 2.99 $\log_{10}$ ED$_{50}$ (median 2.14 $\log_{10}$ ED$_{50}$) (Figure 3D). Only 31.9% (15/47) were able to neutralize the delta variant with 2.71 $\log_{10}$ ED$_{50}$ as a maximal neutralizing antibody titer (median 2.06 $\log_{10}$ ED$_{50}$; Figure 3D). Anti-RBD IgG titers over 132 BAU/mL and 748 BAU/mL were required to ensure neutralization of the D614G and the delta variants, respectively (Figure 3E). No serum was able to neutralize the omicron variant (Figure 3D, E).

During follow-up, seven (5.6%) patients including 5 nonresponders and 2 responders developed COVID-19 from 6.1 to 8.4 months after the third dose. These infections resulted in 3 asymptomatic, one mild and 3 severe diseases. The three severe cases were infected by the delta variant and were hospitalized in an intensive care unit, but with a good clinical outcome. Two of them were nonresponders and the third one displayed an antibody response lower than 264 BAU/mL 53 days after the third dose (132.5 BAU/mL), and a weak neutralizing activity against the delta variant (1.67 $\log_{10}$ ED$_{50}$).

Predictors of vaccine response

Baseline demographic and clinical characteristics of the 126 LTR without a history of COVID-19 were compared between responders and nonresponders in Table 1. Univariate analysis revealed that the 3-dose vaccine response is lower in LTR vaccinated with BNT162b2 (13.3%) compared to mRNA-1273 vaccine (44.2%, p = 0.004; Figure 4A), and that the vaccine response rate increases with the time elapsed between transplantation and the first

dose of vaccine. Nonresponse to vaccine was also associated with MMF/MPA treatment (p = 0.033), with high trough levels of MMF (p = 0.022) and tacrolimus (p < 0.001), and with high prednisone dosage (p = 0.039; Table 1, Table S2). Conversely, azathioprine treatment was associated with better vaccine response (p = 0.004). Of note, none of the 2 patients treated with rituximab seroconverted after the third dose.

To determine whether vaccine response varies with TTV replication, TTV plasma VL was assessed before the first vaccine dose administration in 125 LTR without a history of COVID-19. TTV VL ranged from 1.8 to 9.0 $\log_{10}$ cp/mL, with 6 patients displaying undetectable TTV DNA. Nonresponders displayed higher prevaccine TTV VL (n = 78, median: 4.9 $\log_{10}$ cp/mL, IQR: 3.4-7.7) than responders (n = 47, median: 3.8 $\log_{10}$ cp/mL, IQR: 2.5-5.4; p = 0.004; Table 1, Figure 4B). ROC curve analysis found the optimal TTV prevaccine VL threshold of 6.2 $\log_{10}$ cp/mL as predictive of overall lack of vaccine antibody response, with a sensitivity of 95.7% and a specificity of 37.2% (p<0.001; Figure 4C). LTR displaying TTV VL below this threshold before vaccination seroconverted in 47.9% of cases (45/94) after the third dose versus 6.5% (2/31) of LTR with higher VL (p < 0.001), corresponding to a negative predictive value (NPV) for seroconversion of 93.5%. Using TTV VL in conjunction with MMF/MPA treatment status further discriminated between responders and nonresponders, with 1/26 (3.8%) responders among LTR treated with MMF/MPA and harboring TTV VL ≥6.2 $\log_{10}$ cp/mL versus 13/19 (68.4%) in the opposite conditions of no MMF/MPA and TTV VL <6.2 $\log_{10}$ cp/mL (Figure 4D, E). A multivariable logistic regression analysis confirmed that LTR displaying TTV VL ≥6.2 log10 cp/mL before vaccination (OR = 17.87, CI95 = 3.02-105.72, p = 0.001) or treated with MMF/MPA (OR = 4.73, CI95 = 1.46-15.34, p = 0.010) have significantly lower odds of generating a positive antibody response after adjusting for other variables in the predictive model, as well as patients vaccinated with BNT162b2 compared to mRNA-1273 vaccine (OR = 6.72, CI95 = 1.75-25.92, p = 0.006; Figure 4F, Table 2, Table S3).

Longitudinal follow-up indicated that TTV VL measured before the third vaccine dose could also be predictive of antibody response. Considering LTR who remained seronegative after 2 doses (n = 47), none of the 19 patients with TTV VL ≥6.2 $\log_{10}$ cp/mL before the third dose seroconverted after the booster (NPV of 100%), versus 42.9% (12/28) of LTR with VL lower than this cutoff (p < 0.001; Figure 5A and B). Conversely, a TTV VL <3.2 $\log_{10}$ cp/mL was highly predictive of vaccine response (9/10 responders, versus 3/37 with TTV VL over this threshold, p < 0.001). This lower threshold was associated with a positive predictive value of 90.0% and a NPV of 91.9% for vaccine response in this cohort.

Discussion

This study revealed a substantially weak humoral immune response among COVID-19-naive LTR after a three-dose
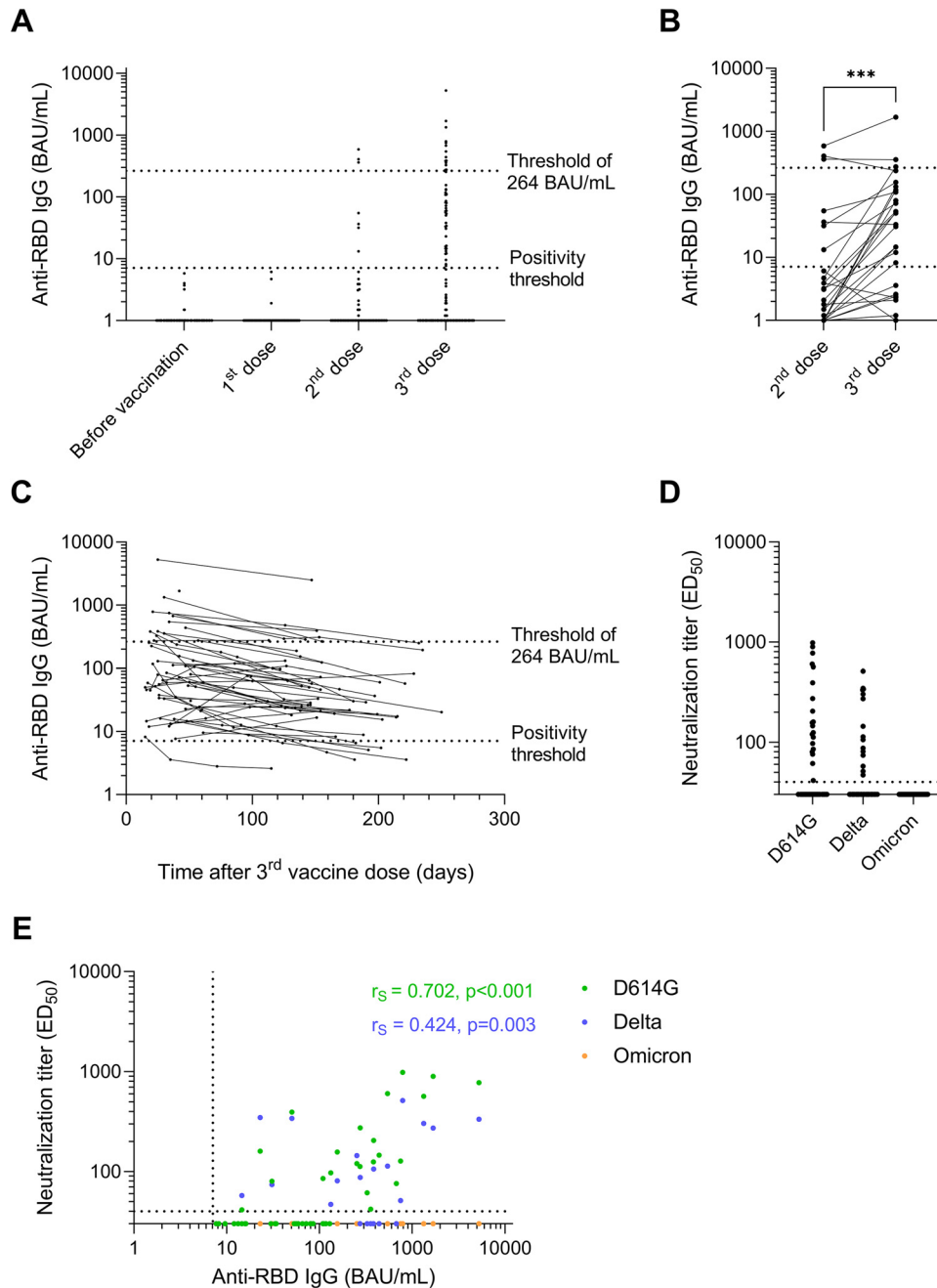
Figure 3   Antibody response after three vaccine doses in COVID-19-naive lung transplant recipients (n = 126). (**A**) Anti-RBD IgG titers expressed in BAU/mL before vaccination (n = 126) and from two weeks to two months after the first (n = 47), the second (n = 54) and the third (n = 126) vaccine doses. (**B**) Anti-RBD IgG titers before and after the third vaccine doses in the 54 patients with sera available after the second dose. (**C**) Dynamics of anti-RBD IgG titers expressed in BAU/mL over time after the third vaccine dose (n = 95 additional samples collected from 47 patients during follow-up). (**A, B** and **C**) The dotted lines indicate the positivity threshold (7.1 BAU/mL) and the threshold of 264 BAU/mL used in French recommendations to guide prophylactic strategy in immunosuppressed patients. (**D**) Neutralizing antibody titers assessed by pseudovirus-based assay against D614G, delta and omicron variants in the 47 patients seropositive after the third dose (responders). 22 (46.8%) and 15 (31.9%) sera neutralized the D614G and the delta variants, respectively, but none neutralized the omicron variant. The dotted horizontal black line indicates the cutoff for positivity (1:40 dilution). (**E**) Spearman correlation between anti-RBD IgG titers and neutralizing antibody titers against D614G (green dots), delta (blue dots) and omicron (orange dots) variants. Green dots are overlapping blue and orange dots on the x axis, and blue dots are overlapping orange dots. The dotted horizontal and vertical black lines correspond to the positivity thresholds of neutralizing antibody titers and anti-RBD IgG titers, respectively. Spearman correlation coefficients (r_S) and p-values related to the neutralization of the D614G mutant and the delta variant were calculated with the Graphpad Prism version 9.3.1. software and depicted in green and blue, respectively. BAU, Binding Antibody Units; ED50, half-maximal effective dilution; RBD, receptor-binding domain. ***p value <0.001.

Table 1   Lung Transplant Recipient Characteristics Stratified by Antibody Response to a Three-Dose Regimen of COVID-19 Vaccine

Variable	Nonresponders to three-dose vaccine regimen n = 79	Responders to three-dose vaccine regimen n = 47	p-value
Age, years, median (IQR)	62.6 (53.7-65.9)	63.0 (47.3-67.4)	0.901
Female, n (%)	26 (32.9)	21 (44.7)	0.253
Blood group			0.788
O, n (%)	35 (44.3)	22 (46.8)	
A, n (%)	30 (38.0)	18 (38.3)	
B, n (%)	10 (12.7)	3 (6.4)	
AB, n (%)	3 (3.8)	3 (6.4)	
Unknown, n (%)	1 (1.3)	1 (2.1)	
Primary disease			0.609
Chronic obstructive pulmonary disease, n (%)	40 (50.6)	20 (42.6)	
Cystic fibrosis, n (%)	10 (12.7)	8 (17.0)	
Interstitial lung disease, n (%)	8 (10.1)	3 (6.4)	
Other, n (%)	21 (26.6)	16 (34.0)	
BMI, kg/m^2, median (IQR)	23.5 (20.3-26.7)	23.9 (20.9-28.5)	0.448
Cardiovascular comorbidities			
Hypertension, n (%)	46 (58.2)	26 (55.3)	0.853
Diabetes mellitus, n (%)	42 (53.2)	22 (46.8)	0.581
Obesity, n (%)	8 (10.1)	6 (12.8)	0.771
Chronic heart failure, n (%)	2 (2.5)	2 (4.3)	0.629
History of transient ischemic attack or stroke, n (%)	9 (11.4)	5 (10.6)	>0.999
History of heart attack, n (%)	6 (7.6)	6 (12.8)	0.361
Transplant type			0.111
Double lung transplant, n (%)	65 (82.3)	39 (83.0)	
Single lung transplant, n (%)	6 (7.6)	0 (0.0)	
Cardiopulmonary transplant, n (%)	6 (7.6)	3 (6.4)	
Lung and liver transplant, n (%)	0 (0.0)	1 (2.1)	
Lung and kidney transplant, n (%)	2 (2.5)	2 (4.3)	
Lung and islet transplant, n (%)	0 (0.0)	2 (4.3)	
History of treated allograft acute rejection, n (%)	25 (31.6)	9 (19.1)	0.150
Chronic lung allograft dysfunction, n (%)	17 (21.5)	14 (29.8)	0.393
Maintenance immunosuppression			
Tacrolimus, n (%)	71 (89.9)	38 (80.9)	0.182
Dose, mg, median (IQR)	4.0 (3.0-7.0)	4.0 (3.0-4.6)	0.086
Trough level, μg/L, median (IQR)	7.5 (6.4-8.8)	6.1 (5.6-7.4)	<0.001
Cyclosporine, n (%)	8 (10.1)	6 (12.8)	0.771
MMF/MPA, n (%)	69 (87.3)	33 (70.2)	0.033
Dose, mg, median (IQR)	1,750 (1,000-2,000)	1,080 (1,000-2,000)	0.031
Trough level, mg/L, median (IQR)	2.8 (2.0-4.9)	2.2 (1.7-2.9)	0.022
Azathioprine, n (%)	3 (3.8)	10 (21.3)	0.004
Prednisone, n (%)	77 (97.4)	43 (91.4)	0.195
Dose, mg, median (IQR, range)	10 (10-10, 0-10)	10 (10-10, 0-40)	0.039
Everolimus, n (%)	9 (11.4)	8 (17.0)	0.424
COVID-19 vaccine			0.004
mRNA-1273, n (%)	53 (67.1)	42 (89.4)	
BNT162b2, n (%)	26 (32.9)	4 (8.5)	
mRNA-1273 + BNT162b2, n (%)	0 (0.0)	1 (2.1)	
Biology			
Creatinine, μmol/L, median (IQR)	102.9 (80.2-136.4)	109.9 (84.2-133.0)	0.732
Leucocytes, G/L, median (IQR)	6.0 (4.7-8.3)	6.5 (5.4-7.7)	0.578
Lymphocytes, G/L, median (IQR)	1.4 (0.9-1.7)	1.5 (1.1-1.9)	0.506
Monocytes, G/L, median (IQR)	0.6 (0.5-0.8)	0.6 (0.5-0.8)	0.453
CRP, mg/L, median (IQR)	<4.0 (<4.0-5.8)	<4.0 (<4.0-7.0)	0.965
Prevaccine TTV viral load, log$_{10}$ cp/mL, median (IQR)	4.9 (3.4-7.7)	3.8 (2.5-5.4)	0.004
Time between transplantation and first vaccination, years, median (IQR)	4.9 (1.7-8.3)	5.9 (4.2-9.3)	0.022
Time of 2nd vaccine from 1st vaccine, days, median (IQR)	28.0 (28.0-30.0)	28.0 (28.0-28.0)	0.145
Time of 3rd vaccine from 2nd vaccine, days, median (IQR)	56.0 (34.0-74.0)	62.0 (39.0-71.0)	0.945

Table 1 (*Continued*)

Variable	Nonresponders to three-dose vaccine regimen $n = 79$	Responders to three-dose vaccine regimen $n = 47$	p-value
Time of 3rd vaccine to antibody testing, days, median (IQR)	35.0 (24.0-47.0)	32.0 (25.0-42.0)	0.137
Maximum anti-RBD IgG concentration after third dose, BAU/mL, median (IQR)	<1.0 (<1.0-1.2)	108.0 (34.1-326.3)	<0.001

BAU, Binding Antibody Units; BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; MMF/MPA, mycophenolate mofetil/mycophenolic acid; TTV, Torque teno virus.

Age, biological parameters, and medication data (including dose and trough levels) were assessed just before the first vaccine dose. Patient characteristics were compared between responders (patients displaying anti-RBD IgG ≥7.1 BAU/mL) and nonresponders using the Fisher exact test and χ^2 test for dichotomous variables and the Mann-Whitney U-test for continuous variables with Graphpad Prism version 9.3.1. software. Statistical significance was set at $p < 0.05$ (shown in bold).

regimen of COVID-19 mRNA-based vaccine. In contrast with the correlation observed between anti-RBD IgG levels and neutralizing antibody titers for the D614G mutant, and to a lesser extent for the delta variant, no patient displayed neutralizing activity against the omicron variant in this cohort, including the few patients with high anti-RBD titers over 1,000 BAU/mL. We showed that these anti-RBD IgG titers decreased over time but persisted up to 8 months after the third dose, and identified TTV VL as a potential predictive marker of vaccine response in LTR.

Our results confirmed previous data observed in LTR and other types of solid organ transplant recipients where vaccine nonresponse was associated with mycophenolate treatment.[4,6,9,12,13,23] This may be due to a greater impairment of both T and B-cell functions with mycophenolate compared to azathioprine or no antimetabolites, which was already shown to impair the humoral response to influenza vaccine.[24]

A higher seroconversion rate was observed with mRNA-1273 compared to BNT162b2 vaccine in our cohort, similarly to findings reported after one or two vaccine doses.[4,5,7,23] Both vaccines contain mRNA encoding the spike protein stabilized into the pre-fusion conformation, but the higher dose of 100 μg in the mRNA-1273 versus 30 μg in the BNT162b2 preparation could explain these differences in immunogenicity in LTR.[25] Alter's group recently confirmed the superiority of mRNA-1273 vaccine over the BNT162b2 vaccine.[26] They showed that mRNA-1273 vaccine elicits higher concentrations of RBD- and N-terminal domain-specific IgA and higher levels of antibodies eliciting neutrophil phagocytosis and natural killer cell activation than the BNT162b2 vaccine.

Our study revealed a strong association between high prevaccine TTV VL and lack of vaccine response, highlighting the potential of this virological marker to predict antibody response in the LTR population. This was particularly true when TTV VL was measured before the third dose for non-responders to the second vaccine dose: no vaccine response was observed in patients with VL ≥6.2 $\log_{10}$ cp/mL, whereas TTV VL <3.2 $\log_{10}$ cp/mL was highly predictive of seroconversion. This virological marker varies with the strength of humoral and cellular immunity and reflects the overall state of the immune system. TTV VL

could therefore help to identify patients who may not respond to three vaccine doses.

Limitations of our study include the absence of a control group of healthy vaccinated adults for comparison and the lack of serological follow-up after the first and second vaccine doses for some patients, which could limit the accuracy of vaccine response rates determined at these timepoints. Furthermore, memory T-cell response could not be explored in this retrospective study, whereas cases of T cell response without detectable antibodies have been reported in vaccinated LTR.[7,13,27] Finally, missing data about vaccine type and vaccination dates, and lack of sera available after third vaccine dose led us to exclude many LTR followed-up in our hospital. Despite these limitations, this study strongly suggests that COVID-19-naive LTR develop a poor antibody response to a 3-dose regimen of COVID-19 mRNA-based vaccine, in contrast to LTR infected with SARS-CoV-2 before vaccination. This study also revealed that BNT162b2 (vs mRNA-1273) vaccine, MMF/MPA treatment, and high TTV VL at the time of vaccination were associated with significantly reduced odds of seroconversion. This marker could help clinicians to decide which patients require additional vaccine doses. A very recent study conducted on LTR reported that prevaccine TTV VL over 6.5 $\log_{10}$ cp/mL was predictive of poor vaccine response to a second dose of mRNA-1273 vaccine, which strongly supports our findings.[11] Future studies could assess the validity of our predictive model in other cohorts of LTR, evaluate this virological marker as a guide for vaccine strategies, and explore its potential as a predictive tool of vaccine response in other types of transplanted and immunocompromised patients, as well as against other pathogens.

Author contributions
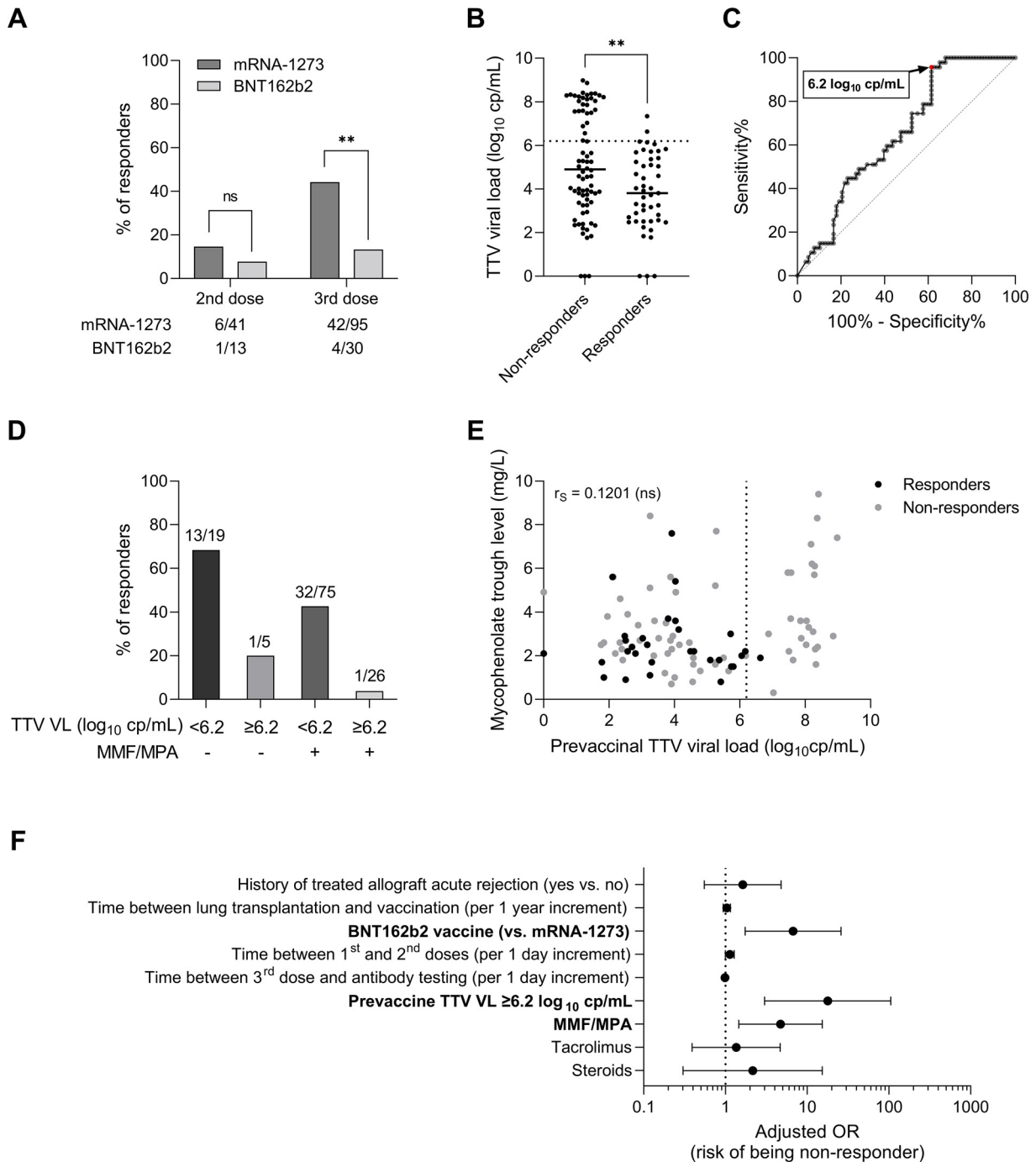
Figure 4   Investigation of predictors of vaccine response in COVID-19-naive lung transplant recipients (n = 125). (**A**) Vaccine response rates after the second and the third vaccine doses according to mRNA vaccine type. The number of responders to each vaccine dose is detailed below the bar chart. (**B**) Prevaccine TTV viral load in nonresponders (n = 78) and responders (n = 47) to a three-dose regimen of COVID-19 mRNA-based vaccine, with medians represented as solid horizontal lines. The dotted line indicates the predictive threshold of 6.2 $\log_{10}$ cp/mL. Comparison was computed with Mann-Whitney test using Graphpad Prism version 9.3.1. software. (**C**) Receiver operating characteristics (ROC) curve for prediction of vaccine response based on prevaccine TTV viral load. Area under the curve (AUC): 0.6533. The threshold of 6.2 log cp/mL is associated with a negative predictive value of 93.5% (in case of high TTV viral load) and a positive predictive value of 47.9% (in case of low TTV viral load) for vaccine response in this cohort. (**D**) Bar charts representing the percentage of responders after three vaccine doses according to the prevaccine TTV viral load and to MMF/MPA treatment at the time of the first vaccination. The number of LTR in each category of patients is mentioned above bars. (**E**) Spearman correlation between TTV viral load and MPA trough level measured at the same time before vaccination in responders (black dots) and nonresponders (grey dots) to three vaccine doses. The dotted line indicates the TTV viral load predictive threshold of 6.2 $\log_{10}$ cp/mL. Correlation coefficient (r_S) was calculated using the Graphpad Prism version 9.3.1. software. (**F**) Forest plot showing Odds Ratios (OR) estimates (indicated by black dots) and 95% confidence intervals (indicated by whiskers) of association between patient characteristics and lack of vaccine response to a three-dose regimen of COVID-19 mRNA-based vaccine. Multivariable logistic regression analysis was performed using SPSS 28.0 (IBM Statistics). Factors independently associated with poor vaccine response are in bold. MMF/MPA: mycophenolate mofetil/mycophenolic acid; OR: Odds ratio; TTV: Torque teno virus; VL: viral load. **p value <0.010, ns: not significant.

Table 2 Multivariable Analysis on Antibody Response in LTR to a Three-Dose Regimen of COVID-19 mRNA Vaccine

Variable	B	SE^B	e^B (adjusted odds ratio)	95% CI	p-value
Intercept	−8.758	2.599	-	-	-
History of treated allograft acute rejection	0.487	0.553	1.627	0.550-4.808	0.379
Time between lung transplantation and vaccination (per 1 year increment)	0.038	0.052	1.039	0.939-1.150	0.463
BNT162b2 vaccine (vs mRNA-1273)	1.906	0.688	6.724	1.745-25.916	0.006
Time between first and second doses (per 1 day increment)	0.123	0.064	1.131	0.998-1.283	0.055
Time between third dose and antibody testing (per 1 day increment)	−0.011	0.017	0.989	0.957-1.022	0.516
Prevaccine TTV viral load ≥6.2 log$_{10}$ cp/mL	2.883	0.907	17.866	3.019-105.716	0.001
MMF/MPA	1.554	0.600	4.730	1.458-15.343	0.010
Tacrolimus	0.303	0.633	1.354	0.391-4.684	0.632
Steroids	0.769	1.001	2.157	0.303-15.335	0.442

B, regression coefficient; CI, confidence interval; MMF/MPA, mycophenolate mofetil/mycophenolic acid; SE, standard error; TTV, Torque teno virus.

Multivariable logistic regression analysis of the risk to be a non-responder was performed using SPSS 28.0 (IBM Statistics). Factors independently associated with poor vaccine response ($p < 0.05$) are in bold. Overall model statistics: χ^2 = 42.407, p-value <0.001.
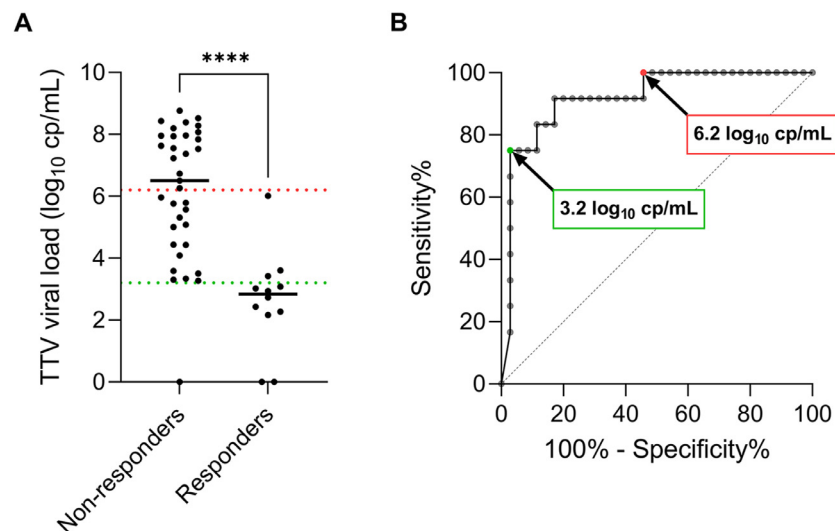
Figure 5 Investigation of TTV viral load as potential predictive biomarker of response to a third vaccine dose in COVID-19-naive lung transplant recipients determined as nonresponders to the second dose. **(A)** TTV viral load measured after the second vaccine dose in nonresponders (n = 35) and responders (n = 12) to the third vaccine dose, with medians represented as solid horizontal lines. The dotted lines indicate the predictive thresholds of 6.2 log$_{10}$ cp/mL and 3.2 log$_{10}$ cp/mL associated with low and high rates of vaccine response, respectively. Comparison was computed with Mann-Whitney test using Graphpad Prism version 9.3.1. software. **(B)** Receiver operating characteristics (ROC) curve for prediction of vaccine response to the third dose based on TTV viral load measured in seronegative LTR after the second vaccine dose. Area under the curve (AUC): 0.9190. The upper threshold of 6.2 log copies/mL is associated with a negative predictive value of 100% (in case of high TTV viral load) and a positive predictive value of 40.0% (in case of low TTV viral load) for vaccine response. Conversely, the lower threshold of 3.2 log copies/mL is associated with a negative predictive value of 91.9% (in case of high TTV viral load) and a positive predictive value of 90.0% (in case of low TTV viral load) for vaccine response in this cohort. TTV: Torque teno virus. ****p value <0.0001.

Disclosure Statement

Declaration of Competing Interest

None.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.healun.2022.07.008.

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