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RESEARCH ARTICLE

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Torque teno virus (TTV): A gentle spy virus of immune status, predictive marker of seroconversion to COVID-19 vaccine in kidney and lung transplant recipients

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Abstract

To date, no comprehensive marker to monitor the immune status of patients is available. Given that *Torque teno virus* (TTV), a known human virome component, has previously been identified as a marker of immunocompetence, it was retrospectively investigated whether TTV viral load may also represent a marker of ability to develop antibody in response to COVID-19-BNT162B2 vaccine in solid organ transplant recipients (SOT). Specifically, 273 samples from 146 kidney and 26 lung transplant recipients after successive doses of vaccine were analyzed. An inverse correlation was observed within the TTV copy number and anti-Spike IgG antibody titer with a progressive decrease in viremia the further away from the transplant date. Analyzing the data obtained after the second dose, a significant difference in TTV copy number between responsive and nonresponsive patients was observed, considering a 5 $\log_{10}$ TTV copies/mL threshold to discriminate between the two groups. Moreover, for 86 patients followed in their response to the second and third vaccination doses a 6 $\log_{10}$ TTV copies/mL threshold was used to predict responsivity to the booster dose. Although further investigation is necessary, possibly extending the analysis to other patient categories, this study

Piergiorgio Roberto and Lilia Cinti contributed equally to the work.

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suggests that TTV can be used as a good marker of vaccine response in transplant patients.

KEYWORDS
BNT162B2 vaccine, kidney transplant, lung transplant, SOT patients, TTV

1 | INTRODUCTION

Immunocompromised individuals, in particular transplant recipients, represent a large amount of those deemed at risk of developing severe clinical forms of infectious diseases. For example, transplant patients have a high chance of acquiring SARS-CoV-2 infection, due to immunosuppressive therapies. To preserve the health status of these at-risk individuals, they have been included with the highest priority in vaccination campaigns worldwide. The antibody response to vaccines in this group of patients, however, is known to be below population average.[1] In particular, it was shown that they tend to develop poor antibody response after two doses of COVID-19 mRNA vaccine, compared to immunocompetent individuals. In addition, a lower antibody level was observed in lung transplant recipients with respect to kidney or liver transplant recipients, because of their heavier immunosuppressive therapies.[2] For these reasons, solid organ transplant (SOT) patients were selected as the first recipients for the administration of the booster doses. Immune status screening in these individuals is thus of uttermost importance. However, standardized and routinely used methods to monitor this parameter are not currently available.[3] According to new research, *Torque teno virus* (TTV) could be a reliable surrogate marker of immune status. In fact, in case of immunosuppression whatever the reason, TTV replicates strongly, and higher TTV DNA loads are observed compared to healthy individuals, which never exceed 4 $\log_{10}$ copies/mL.[4] TTV is a single-stranded DNA virus belonging to the *Anelloviridae* family discovered in 1997.[5] This virus is ubiquitous and clearly prevalent in the population worldwide with an infection rate around 80%, regardless of age, socio-economic status and health factors.[6] To date, it has not been implicated in the pathogenesis of any disease. It is spread through the major transmission pathways and is found in most of the host tissues and cells except for red blood cells and platelets.[7] TTV is the most abundant component of the human virome, in particular 68% of SOT recipient showed a blood virome composed by *Anellovirus*, of which 97% is represented by TTV.[8]

The focus of this paper is to investigate TTV as a possible marker of immune status in SOT patients, trough the correlation of its viral load with humoral response following the administration of COVID-19 mRNA vaccination (BNT162B2). Compared to previous studies that analyzed a single type of transplantation, this study aims to compare different categories of SOT patients (kidney and lung).

2 | MATERIALS AND METHODS

2.1 | Study design

A retrospective cohort study was conducted on 172 SOT patients including 146 kidney and 26 lung transplant recipients followed as outpatients at Polyclinic Umberto I Hospital of Rome. Serum collection was collected in median 55 days after the mRNA-based vaccine BNT162B2 administration. Two hundred and seventy-three total sera were analyzed: 161 after the second dose, 97 after the third, and 15 after the fourth. The non-adherence of some patients to the different phases of post-vaccine antibody screening explains the progressive decrease in sample size. In order for the data analyzed to be comparable, only samples from patients who were not infected with SARS-CoV-2 were included in the study. Furthermore, patients whose TTV load was never detected during the study were not included in the statistical analysis because a patient's colonization cannot be discarded from a single negative sample. Age, gender, type, and date of transplant were also included in the data. In addition, to obtain a baseline value of TTV genome copies in healthy patients, 72 blood donor patients with average age and gender comparable to the SOT patient group were recruited regardless of vaccine administration. The Local Ethics Board of Sapienza University of Rome approved the present study (CE 6338). Informed consent was also obtained for each patient included in the study.

2.2 | TTV DNA detection and quantification

Total DNA was isolated from 300 µL of serum samples processed with NucliSens easyMAG extractor (bioMérieux) according to the manufacturer's instructions. The detection of TTV genome was performed by using CFX96 platform (Bio-Rad Laboratories, Inc.). The real-time PCR was optimized for the amplification of the TTV genome using appropriate primer pairs and temperatures. Specifically, primers were designed from a portion of the UTR region, which was found to be highly conserved among all TTV sequences available in GenBank. The oligonucleotide sequences are as follows: AMTS (forward primer 5'-GTGCCGIAGGTGAGTTTA-3'), AMTAS (reverse primer 5'-AGCCCGGCCAGTCC-3'), and AMTPTU (TaqMan probe 5'-TCAAGGGGCAATTCGGGCT-3'). The probe was labeled with 6-carboxy-fluorescein (FAM) and 6-carboxy-tetramethyl-rhodamine (TAMRA) at its 5' and 3' ends, respectively.[9] In each amplification, a base-10 dilutions (10^7 to 10^4) of synthetic oligonucleotide template

were used as positive controls to build the standard curves, and a negative control (no template) was included. Samples were analyzed after a single thaw to prevent loss of TTV genomic copies due to multiple thaws.

2.3 | Antibody response

The antibody response to the vaccine BNT162B2 was assessed using the chemiluminescence technology of LIAISON® SARS-CoV-2 TrimericS IgG immunoassay (DiaSorin S.p.A.), which allows the quantitative determination of specific IgG antibodies against the SARS-CoV-2 Trimeric spike protein in human serum or plasma samples. The clinical laboratory IgG titers were expressed in binding antibody units/mL (BAU/mL). The detection limit for antibody titer in serum is 4.81 BAU/mL. Values in the range of 4.81–33.8 BAU/mL, as reported by the manifacturer's instructions, are considered negative; values greater than or equal to 33.8 BAU/mL are positive. LIAISON® SARS-CoV-2 TrimericS IgG immunoassay provides a quantitative result with a sensitivity of 100% (95% CI: 85.1–100.0) and a specificity of 99.5% (95% CI: 99.0–99.7).[10,11]

2.4 | Statistical analysis

Data management, analysis, and plotting were performed in Python [https://www.python.org], relying on Pandas [https://pandas.pydata.org], Matplotlib [https://matplotlib.org], and SciPy [https://scipy.org] libraries. As detailed in the following sections, null hypothesis testing was performed using either Mann–Whitney–Wilcoxon or paired sample *t*-test methods, unless otherwise specified. The relative *p*-values are graphically annotated using the following scoring: $p > 0.05$ (ns), $p \le 0.05$ (*), $p \le 0.01$ (**), $p \le 0.001$ (***), $p \le 0.0001$ (****).

3 | RESULTS

3.1 | Study population

This study was conducted on a total of 273 serum samples from 172 SOT patients who received kidney ($n = 146$, 85%) and lung ($n = 26$, 15%) transplant, respectively. The median age of the tested population is 56 years (IQR: 48–65) with the following gender distribution: 41% females and 59% males. Among the 273 sera analyzed, 161 were collected after the second dose (from kidney [$n = 135$, 84%] and lung [$n = 26$, 16%] transplant patients), 97 after the third dose (from kidney [$n = 88$, 91%] or lung [$n = 9$, 9%] transplant patients), and only 15 samples are available after the fourth dose from kidney transplant patients. In 86 patients among the 172 recruited, samples were collected after both the second and third dose, thus allowing the same patient to be followed during the vaccination period. In addition, 72 blood donors were enrolled to crossmatch a healthy control population. They included 40% females and 60% males with a median age of 53 years (IQR: 35–59).

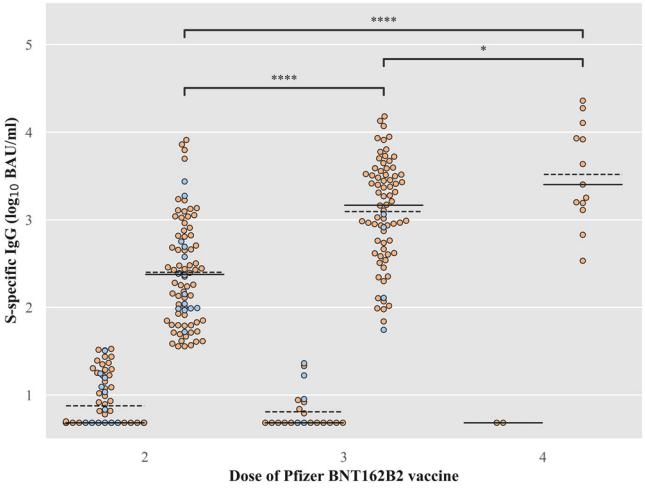

FIGURE 1 Overview of S-specific IgG titers observed in kidney (red dots) and lung (blue dots) transplant recipients after the administration of three BNT162B2 vaccine doses. For each dose of vaccine two columns are shown: on the left the nonresponsive patients and on the right the responsive ones. Median and mean lines are shown respectively with continuous and dashed lines. Mann–Whitney–Wilcoxon test *p*-values are reported according to the scoring system described in the text.

3.2 | Anti-SARS-CoV-2 antibody response

The antibody response to BNT162B2 vaccination in SOT patients was preliminary evaluated: a positive response was observed in 52% of patients after the second dose (median: 233 BAU/mL; IQR: 76–647), in 76% after the third (median: 1465 BAU/mL; IQR: 450–3282), and in 87% after the fourth (median: 2520 BAU/mL; IQR: 1550–8480), respectively. As shown in Figure 1, a progressive increase in anti-SARS-CoV-2 titer after each dose was detected in kidney transplant recipients. This is less evident in lung transplant ones. After the second dose, a comparable median value of antibody titer is observed in the two patient groups: 246 (IQR: 67–663) and 187 BAU/mL (IQR: 97–463) in the kidney and lung transplant subjects, respectively. The trend changes after the third dose where kidney transplant recipients show a marked increase in the median level of anti-Spike IgG (1735 BAU/mL; IQR: 552–3307) compared to the lung group (474 BAU/mL; IQR: 110–902). Interestingly, whereas after the second dose the percentage of responders is comparable in the two groups of transplant recipients (51% kidney and 54% lung transplant patients), after the third dose kidney transplant recipients who develop an antibody response to the vaccination are at 80% compared to only 44% of lung transplant ones (Table 1).

In both vaccine responder and nonresponder subjects, the median time from transplant to anti-SARS-CoV-2 antibody test was calculated. As shown in Table 1, the elapsed time is almost two times higher in the responder group (2379 days) than in nonresponder group (1340 days). To further investigate the relationship between the antibody response and the time from transplantation, patients were divided into two groups for each dose: those transplanted from less than 2000 days (group A) and those transplanted from more than

TABLE 1 Parameters of responsive and nonresponsive samples for each dose of SARS-CoV-2 vaccine.

	Post two-dose vaccine regimen		Post three-dose vaccine regimen		Post four-dose vaccine regimen	
	Responders	Nonresponders	Responders	Nonresponders	Responders	Nonresponders ($n = 2$)
Age (IQR)	52 (45–60)	60 (50–69)	56 (48–63)	61 (56–67)	56 (49–65)	75 (75–75)
Female (%)	56	44	73	27	100	0
Male (%)	48	52	78	22	78	22
Total patients (%)	52	48	76	24	87	13
Kidney transplant recipients (KTR) (%)	51	49	80	20	86	14
Lung transplant recipients (LTR) (%)	54	46	44	56	/	/
Time of vaccine to antibody testing (days) median (IQR)	63 (37–97)	67 (35–135)	50 (36–68)	47 (30–66)	55 (37–86)	113 (97–128)
Time from transplantation (days) median (IQR)	2379 (1221–5488)	1340 (872–3772)	2356 (1337–5593)	1267 (731–2591)	2427 (1620–5300)	1732 (1520–1943)
Time from transplantation in KTR (days) median (IQR)	2329 (1208–5753)	1366 (877–3788)	2360 (1361–5715)	1756 (999–2603)	2427 (1620–5300)	1732 (1520–1943)
Time from transplantation in LTR (days) median (IQR)	3639 (1470–5250)	1430 (493–3916)	2080 (1178–2965)	598 (520–690)	/	/
BAU/mL median (IQR)	233 (76–647)	-	1465 (450–3282)	-	2520 (1550–8480)	-
BAU/mL in KTR median (IQR)	246 (67–663)	-	1735 (552–3307)	-	2520 (1550–8480)	-
BAU/mL in LTR median (IQR)	187 (97–463)	-	474 (110–902)	-	/	-
TTV copies/mL median (IQR) (log_{10})	4.6 (3.9–5.3)	5.6 (4.5–6.4)	4.6 (3.6–5.3)	5.7 (5.3–6.1)	4.5 (3.0–4.9)	5.4 (5.4–6.4)
TTV copies/mL in KTR median (IQR) (log_{10})	4.6 (3.8–5.1)	5.3 (4.4–6.1)	4.5 (3.5–5.2)	5.7 (5.0–6.0)	4.5 (3.0–4.9)	5.4 (5.4–6.4)
TTV copies/mL in LTR median (IQR) (log_{10})	4.7 (4.3–5.7)	6.3 (5.7–7.0)	5.4 (5.4–7.0)	6.1 (5.9–6.5)	/	/

Abbreviations: BAU, binding antibody units; IQR, interquartile range; KTR, kidney transplant recipients; LTR, lung transplant recipients; TTV, Torque teno virus.

2000 days (group B). After the second dose the median in group A is 20 BAU/mL (IQR: 4.81–63) and in group B is 89 BAU/mL (IQR: 7–463); after the third dose the values are: 284 (IQR: 8–1045) and 1620 BAU/mL (IQR: 361–3720), respectively. After the fourth dose the values are: 1550 BAU/mL (IQR: 672–1580) in group A and 3415 BAU/mL (IQR: 1412–11 645) in group B.

3.3 | TTV detection and analysis of viral load

The second point of the study focused on the evaluation of the association between TTV viral load and antibody response to SARS-CoV-2 vaccination. TTV was detected in 76% of healthy patients and 97.5% of transplanted subjects. The quantitative analysis of viral load showed that the median value of TTV copy number in healthy patients was 2.9 $\log_{10}$ copies/mL (IQR: 2.4–3.3 $\log_{10}$), whereas in transplanted recipients it was 3.9 $\log_{10}$ copies/mL (IQR: 3.2–4.8 $\log_{10}$). More specifically, a greater viral load of TTV is shown in lung transplant recipients (5.9 $\log_{10}$ copies/mL) than in kidney ones (4.8 $\log_{10}$ copies/mL).

Subsequently, TTV levels were evaluated considering their distance in time from transplantation. The results are shown in Figure 2. It can be seen that the TTV viral load decreases as the transplantation date moved away. It is worth noting that the decrease in TTV copy number is most pronounced during the first 2000 days after transplantation. Indeed, again dividing the patients' population into the above groups A and B, it can be seen a significant difference in viral load: 4.5 and 5.4 $\log_{10}$ copies/mL, respectively.

The specific analysis of relationship between TTV DNA load and post-vaccine IgG anti-spike titer expressed as BAU/mL showed that the median of TTV copies in the group of vaccine-responsive patients was significantly lower (4.6 $\log_{10}$ copies/mL) than in nonresponsive subjects (5.7 $\log_{10}$ copies/mL) ($p \leq 0.001$, Figure 3). This is true

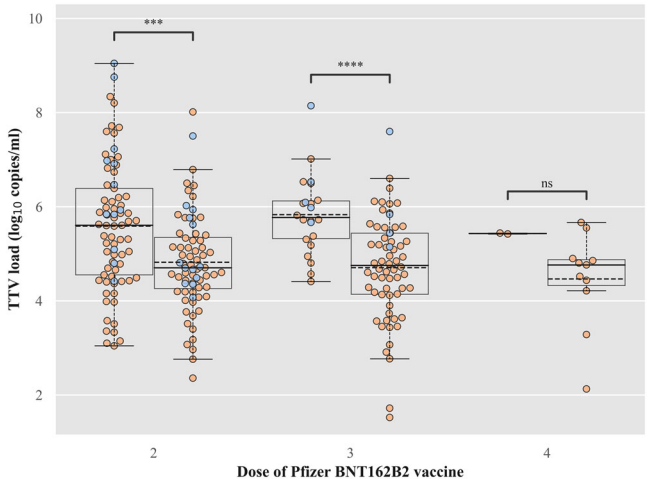

FIGURE 3 Distributions of TTV viral load observed in kidney (red dots) and lung (blue dots) transplant recipients after the administration of three vaccine doses, with superimposed Whisker plot. For each dose of vaccine two columns are shown: on the left the nonresponsive patients and on the right the responsive ones. Median and mean lines are shown respectively with continuous and dashed lines. Mann–Whitney–Wilcoxon test *p*-values are reported according to the scoring system described in the text. TTV, Torque teno virus.

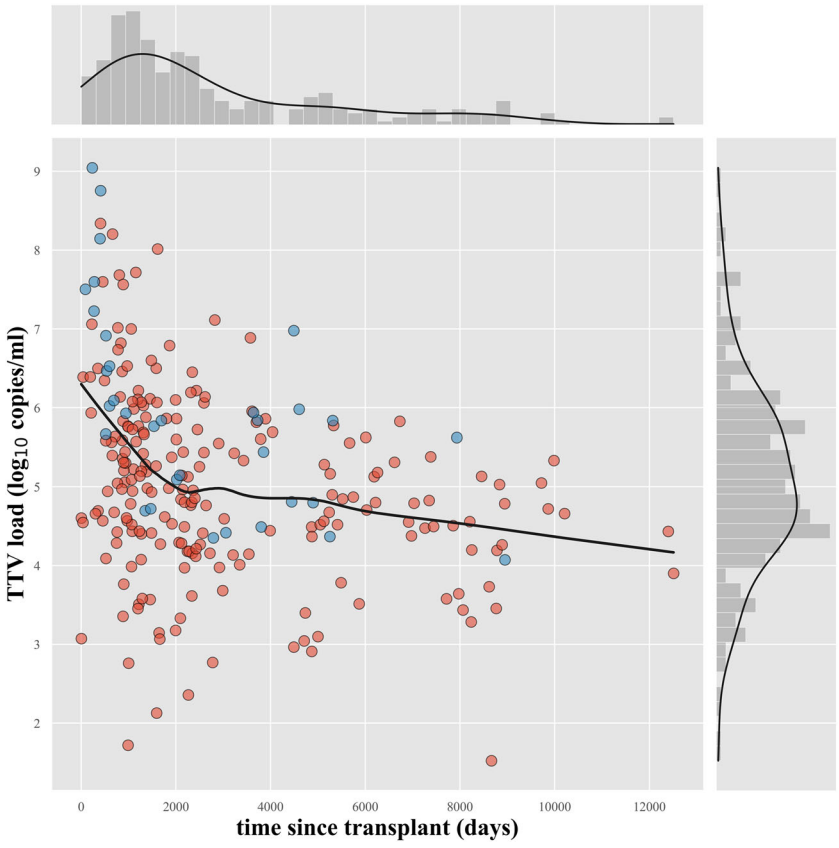

FIGURE 2 TTV load as a function of time elapsed since the transplant date with superimposed locally-weighted regression. Kidney transplant recipients are shown with red dots while lung transplant ones with blue dots. TTV, Torque teno virus.

independently of the doses considered in both kidney and lung transplant patients (Table 1). As proof of this, based on the values just shown, the samples were divided into two categories using 5 $\log_{10}$ TTV copies/mL as a discriminating value between responders and nonresponder patients. Kidney and lung transplant samples were nonresponsive for the category including those with a TTV copy number greater than 5 $\log_{10}$ copies/mL (16 BAU/mL; IQR: 5–71) and responsive for the category including those with a TTV copy number less than 5 $\log_{10}$ copies/mL (83 BAU/mL; IQR: 8–395). For the third and fourth doses, the analysis can only be conducted on kidney transplant patients with the respective values for the two categories:

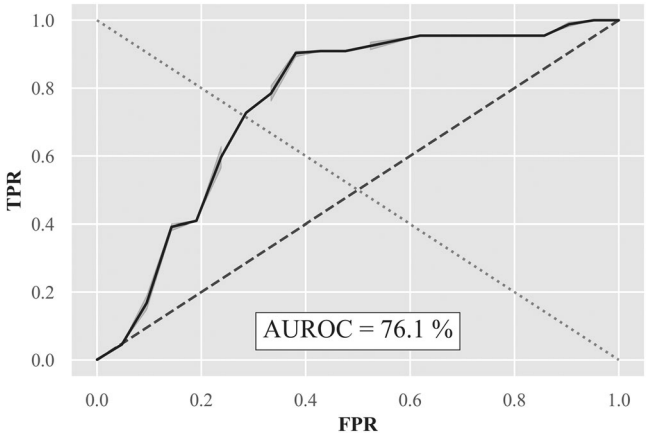

FIGURE 4 ROC (receiver operating characteristic) curve relative to the prediction of responsiveness to the third vaccination dose using a threshold value on the TTV load. TTV, Torque teno virus.

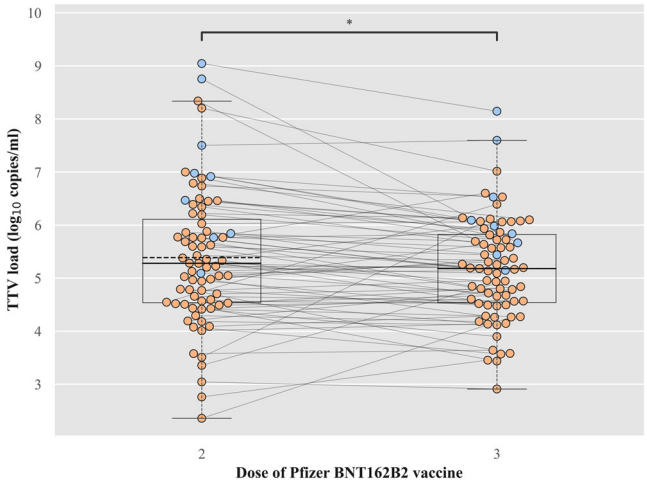

FIGURE 5 Overview of TTV viral load in kidney and lung transplant recipients followed-up in their response to the second and third vaccination dose, with superimposed Whisker plot. Kidney and lung transplant recipients are respectively indicated with red and blue dots. Median and mean lines are shown respectively with continuous and dashed lines. Mann–Whitney–Wilcoxon test *p*-values are reported according to the scoring system described in the text. Paired *t*-test *p*-values are reported according to the scoring system described in the text. TTV, Torque teno virus.

after three doses 383 (IQR: 4.81–1046) and 1850 BAU/mL (IQR: 401–3240) and after the fourth dose 792 (4.81–1630) and 4310 BAU/mL (1420–10590) (Supporting Information: Table S1).

Considering the 86 individual patients followed during vaccination, an attempt was made to understand whether TTV copies could predict the antibody response to the booster dose. To do this, the post-second dose viral load of each patient was considered as pre-third dose and it was related to the antibody response recorded following the booster dose. Figure 4 shows the ROC curve from which the 6 $\log_{10}$ copies/mL threshold was derived (76.1% AUROC). The application of this threshold resulted in the following scores for the prediction of the booster dose response based on TTV viral load: accuracy of 77%, precision of 72%, sensitivity of 91%, specificity of 62%, and negative predicted value of 87%. In fact, nonresponsive subjects at the first booster dose showed a TTV load above 6 $\log_{10}$ copies/mL (median: 6.2 $\log_{10}$; IQR: 5.6–6.9 $\log_{10}$) while in responsive subjects the TTV copy number was below this value (median: 4.8 $\log_{10}$; IQR: 4.1–5.4 $\log_{10}$). Importantly, it was possible to show and discuss this finding since no major differences in TTV copies were observed among the samplings of individual patients in the subsequent doses (Figure 5).

4 | DISCUSSION

It is well known that patients with various pathological conditions such as untreated solid cancer,[12] HIV-positive patients,[13] and after autologous[14] or allogeneic stem cell transplantation, can experience an increased viral replication of TTV.[15] The literature also contains evidence that TTV may serve as a SOT prediction marker for infection and rejection.[8,16,17]

This study's primary goal is to confirm and gather new knowledge of the idea that TTV load may be identified as measure of functional immunity, also known as response to vaccination against SARS-CoV-2. Indeed, the present study aims to evaluate whether the viral load can predict the seroconversion following COVID-19 vaccination in patients who have received a SOT. For the first time, two types of SOT have been considered in the same study: kidney and lung. It has been established that both transplanted patients, due to immunosuppressive therapies, develop a lower antibody response to COVID-19 vaccination compared to healthy subjects[1,2] and that TTV load is a predictor of antibody response to SARS CoV-2 vaccination.[18,19] This study expands on the above notions and details some characteristics of the antibody to SARS-CoV-2 response in transplanted patients specifically addressing its association with TTV load.

First, following each booster dose of vaccination, an increment was found when the antibody response of the two categories of individuals investigated were assessed (Figure 1). This finding was expected, but it is worth noting that it applies mostly to kidney transplant patients, who had significant increases in their responsivity rate and anti-spike titer values. It's interesting to note that among patients who have had lung transplants, not only is the rate of seroconversion following the booster dosage lower, but the increase in antibody titers in the responder population is also less pronounced. This is most likely due to the various immunosuppressive treatments

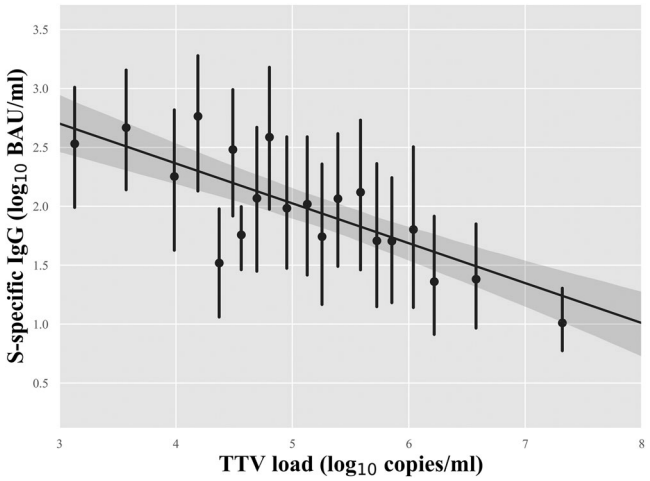

FIGURE 6 Overview of S-specific IgG titer for all patients as a function of the TTV load, with superimposed linear regression. TTV, Torque teno virus.

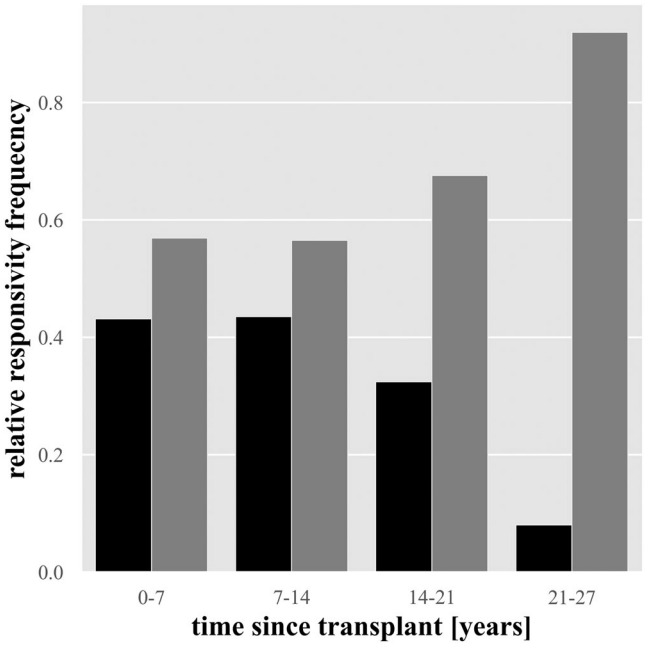

FIGURE 7 Relative frequency of responsiveness in patients as a function of time elapsed since the transplantation date. The nonresponsive fraction is shown in black, while the responsive one is in grey.

that the two groups of patients receive, with kidney transplantation being less immunosuppressive than lung transplantation.[2]

It was then investigated whether serum TTV levels could reflect the immune status of patients. As expected, healthy subjects have a much lower TTV load with respect to the totality of transplant patients presently considered. In addition, a higher viremia is observed in lung transplant recipients than in kidney transplant ones. This parallels the different course of antibody development in the two categories of SOT vaccinated subjects (see above). Indeed, the TTV load is inversely proportional to the development of anti-Spike antibodies (Figure 6). All together the findings confirm that a compromised immune system is more permissive to viral replication and so the TTV viral load may act as a spy virus for the antibody response to SARS-CoV-2 vaccine.

Furthermore, in an attempt to establish whether a threshold on TTV viral load could be indicative for the prediction of antibody response in vaccinated SOT subjects, it was found that a genomic virus copies number greater than 5 $\log_{10}$ copies/mL could reflect with good power, a negative outcome in response to the COVID-19 vaccination; vice versa, values around 4 $\log_{10}$ TTV copies/mL characterize responsive patients (Figure 3). This mirrors the values of kidney transplant patients after all doses. In lung transplant recipients, on the other hand, the TTV copy number baseline appears to be higher after the third booster dose. Again, this could correlate with the higher immunosuppression status of these patients due to the therapy they receive. However, the small cohort size ($n = 9$) does not allow to reach definite conclusions.

Next, in analyzing possible differences that could influence the outcome of the vaccination response in the various subjects, an important parameter arises: the time elapsed since transplantation. In fact, by calculating the median number of days passed from the transplant, an increasing antibody response (Figure 7) and, as a result, a decrease in TTV copy number can be recorded gradually as one moves away from the date of transplantation (Figure 2). Once again, the explanation for these data comes from the post-transplant treatment protocols. The immunosuppressive therapy is heavier in the early post-transplant periods resulting in greater immunosuppression and therefore a lower response to vaccination concomitant with a high TTV copy number. This is exacerbated in lung transplant recipients where it takes a longer time from the date of transplantation to appreciate both a positive outcome to COVID-19 vaccination and a fall below 5 $\log_{10}$ TTV copies/mL. The immunosuppressive therapy of this category of patients is in fact maintained for a longer period at higher doses than that of kidney transplant recipients.

Finally, a threshold, derived from the ROC curve (Figure 4), on the TTV viral load (6 $\log_{10}$ copies/mL) relative to the first serum collection, was used to predict responsiveness to the booster dose, considering the 86 patients followed in all vaccination steps. This threshold value showed excellent sensitivity (91%) and good specificity (62%) in predicting the response to booster dose vaccination.

Although a pre-COVID-19 vaccination sampling is not available, it is possible to assume that the immune status of a transplant patient does not vary much over a short period of time. Consequently, the TTV copy number would presumably remain similar to the value found after the second dose (Figure 5). Thus, based on the values obtained, 5 $\log_{10}$ copies/mL of TTV could also predict the response to the second dose. In fact, in SOT recipients a threshold value of 5 $\log_{10}$ TTV copies/mL distinguishes, with a high probability, responsive from nonresponsive subjects. Furthermore, this value would have a good diagnostic validity with 73% sensitivity and 65% specificity in kidney transplant recipients and 78% sensitivity and 65% specificity in lung transplant recipients.

This study also has limitations. First, being a retrospective study, no pre-vaccination samples are available and not all patients were

followed-up regularly over time. Furthermore, although all samples were analyzed using the same assay thus validating the findings, the TTV copies shown are not expressed in absolute value as no standardized assay was used.

This study, however, beyond its limitations, confirms that TTV viremia correlates with the patients' immune status and reveals a correlation with the immune response to COVID-19 vaccination too, allowing the prediction of the antibody response.

In the future one might consider extending the study to other patient populations and evaluating if TTV can be useful in adjusting the dosage of immunosuppressive therapies for SOT recipients and whether it can predict the risk of all viral reactivations in immunocompromised subjects.

AUTHOR CONTRIBUTIONS

Piergiorgio Roberto: conceptualization, investigation, data curation, formal data analysis, writing – original draft. **Lilia Cinti**: conceptualization, investigation, data curation, formal data analysis, writing – original draft. **Anna Napoli**: formal data analysis, investigation, writing – original draft. **Daniele Paesani**: statistical analysis, formal data analysis, data curation, revision. **Rodolfo J. Riveros Cabral**: investigation. **Fabrizio Maggi**: revision, visualization. **Manuela Garofalo**: data resources. **Renzo Pretagostini and Federico Venuta**: clinical investigation. **Anastasia Centofanti**: data resources and clinical investigation. **Carolina Carillo**: clinical investigation. **Aurelia Gaeta**: editing and revision, visualization, supervision. **Guido Antonelli**: conceptualization, validation, writing – original draft, editing and revision, visualization, supervision, project administration, funding acquisition. All authors approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are not publicly available due to them containing information that could compromise research participant privacy/consent. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The Local Ethics Board of Sapienza University of Rome approved the present study (CE 6338). Informed consent was obtained for each patient included in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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