Immunogenicity and safety of a third dose of anti-SARS-CoV-2 BNT16b2 vaccine in liver transplant recipients

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Abstract
Background & aims: A strategy to improve the low rate of anti-SARS-CoV-2 mRNA vaccine-induced immunogenicity in liver transplant recipients (LTs) is urgently needed.
Methods: We analysed the rate of positive (≥0.8 U/ml) anti-SARS-CoV-2 receptor domain-binding protein (RBD) antibody response 2 months after a third dose of the BNT16b2 vaccine in 107 LTs who completed the second vaccine dose 7 months earlier.
Results: A positive anti-SARS-CoV-2-s-RBD antibody response after the third vaccine dose was detected in 98 (91.6%) LTs compared to 82 (76.6%) after the second vaccine dose (p = .003). The median of anti-SARS-CoV-2 RBD antibody titres increased from 22.9 U/ml 6 months after the second to 3500 U/ml 2 months after the third vaccine dose (p < .001). Fourteen (14.3%) responder patients presented antibody titres <100 U/ml, 57 (58.2%) between 100 and 9999 U/ml and 27 (27.6%) ≥10000 U/ml. Seropositivity after the second dose was maintained after the third dose. Independent predictors of antibody response failure after the third vaccine dose were taking a higher daily dose of mycophenolate mofetil (MMF, p < .001) and had a lower (<60 ml/min/1.73 m²) estimated glomerular filtration rate (p = .007). Nine (9.1%) LTs experienced symptomatic SARS-CoV-2 infection after the third vaccine dose. Median antibody titres were not statistically different between infected and not infected LTs (1325 vs 3515 U/ml, p = .678).
Conclusions: The third dose of the BNT16b2 vaccine increased the number of LTs who developed a positive anti-SARS-CoV-2 s-RBD antibody response. A proportion of patients remained unresponsive, mainly for modifiable factors, such as the use of MMF or multiple immunosuppressants.

Keywords: COVID-19, immunosuppression, liver transplantation, mRNA vaccine, mycophenolate mofetil

Abbreviations: eGFR, estimated glomerular filtration rate.; LTs, liver transplant recipients.; MMF, mycophenolate mofetil.; s-RBD, spike glycoprotein-specific receptor-binding domain.

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The antibody response after two doses of the anti-SARS-CoV-2 mRNA Pfizer-BioNTech® BNT162b2 and Moderna®-1273 vaccines is excellent in the general population, but, it remains unsatisfactory in liver transplant recipients (LTs). The main factors responsible for the reduced vaccine-induced immunogenicity in LTs are the use of immunosuppressive agent combinations, particularly those containing mycophenolate mofetil (MMF). This fact implies that LTs should be considered a population remaining at high risk for SARS-CoV-2 infection despite having completed the vaccination course with two doses. A recent report indicated that severe cases of SARS-CoV-2 infection, with a mortality rate of 11%, occurred in LT patients who had a single vaccine dose and that approximately 20% of those who received a full vaccination course required hospitalization for severe respiratory failure.

These issues have prompted great interest in scientific societies in strongly recommending the administration of a third dose of the anti-SARS-CoV-2 vaccine, prioritizing patients at higher risk of infection, such as LTs. Currently, there are only a few reports indicating the immunogenicity and safety of a third dose of mRNA anti-SARS-CoV-2 vaccines in solid organ transplant recipients, and many of them included a very small number of LTs.

Therefore, the aim of the present study was to evaluate the immunogenicity and safety of a third dose of the Pfizer-BioNTech® BNT162b2 vaccine in LTs who had completed the full vaccination course with two doses of the same vaccine.

2 | METHODS

2.1 | Study protocol

All LTs followed at the hepatology and liver transplantation unit at the Academic Hospital of Udine, Italy, were enrolled in a centralized anti-SARS-CoV-2 vaccination program, adopting the Pfizer-BioNTech® BNT162b2 vaccine. The original vaccination study protocol provided the administration of two doses of the first vaccine, the basis of which took place in April 2021 and the second 3 weeks (19 days) thereafter. The results of this protocol have been recently reported. In the present study, data are presented regarding the antibody response to a third dose of the vaccine administered after a median time of 7 months (213 ± 10 days) following the second vaccine dose in a subgroup of LTs from the same cohort. The exclusion criteria were age at transplant < 18 years old, pregnancy, past known SARS-CoV-2 infection and liver transplantation performed < 3 months before vaccination. A vaccination self-reported side effects questionnaire was administered to participants within 30 days of receipt of the booster vaccination dose.

All LTs who developed during the follow-up after the third vaccine dose, respiratory and/or gastrointestinal symptoms, suggesting a potential acute SARS-CoV-2 infection, were evaluated for active SARS-CoV-2 infection via real-time reverse transcription (RT)-PCR performed on nasopharyngeal swabs.

3 | STATISTICAL ANALYSIS

Statistical analysis was performed by means of Stata statistical software, version 15.1 (StataCorp 2017. Stata Statistical Software: Release 15. College Station, TX, USA: StataCorp LLC). Because the Shapiro–Wilk test for normal data failed in more than half of the continuous variables, a nonparametric rank-sum (Mann–Whitney) test was used, and the data are presented as medians and interquartile (IQR) ranges. Pearson’s chi-square test was used for the comparison of categorical variables, and the data are presented as frequencies (%). To select independent predictors for the development of the anti-SARS-CoV-2 vaccine-induced humoral response, a stepwise logistic regression analysis with a forward approach was used considering the following cut-offs of antibody titres: 0.8, 100, 1000 and 10,000 U/ml. All variables showing a p ≤ 0.10 in the univariate analysis are included. Pseudo $R^2$ area under the ROC curve, and the percentage of correct classification are presented as quality estimations of the regression model. Multivariate linear regression analysis with a
stepwise forward approach was used to discriminate the best fitting variables in predicting the antibody response after vaccination, considering antibody titre as continuous variable. All variables significantly associated with antibody response post-vaccination at the univariate regression test, were selected to run in the multivariate linear model. \( R^2 \) and Akaike’s information criterion (AIC) are presented as estimators of the model.

4 | RESULTS

4.1 | Patients

Among the 143 LTs enrolled in the original vaccination protocol, in the present series, 12 patients were excluded since they tested positive at baseline for anti-SARS-CoV-2 N protein. Data on antibody response 6 months after the second vaccine dose were available in 123 of 131 (93.9%) patients. Thirteen of 123 (10.6%) patients refused to accept the third vaccination dose, and 3 (2.7%) of the remaining 110 patients did not undergo blood sampling for the measurement of antibody response two months after the third vaccine dose. Thus, 107 patients (77 men, median age of 67.3 years old) were finally enrolled in the present study. The median time from liver transplantation to vaccination was 91 months, and tacrolimus was the most used backbone immunosuppressive treatment after transplant. The main demographic and clinical characteristics of the enrolled patients are reported in Table 1.

4.2 | Anti-SARS-CoV-2s-RBD antibody response after the second and third doses of the BNT162b2 vaccine

None of the patients tested positive for anti-SARS-CoV-2-N protein antibodies at any time point prior to the third vaccination. In contrast, those who tested positive for anti-SARS-CoV-2-s-RBD after the second vaccine dose were as follows: 72 of 107 (67.3%) after 1 month (31 ± 2 days), 83 of 107 (77.6%) after 4 months (125 ± 5 days), and 82 of 107 (76.6%) after 6 months (165 ± 4 days). After the third dose of vaccine, 98 of 107 (91.6%) patients developed a positive anti-SARS-CoV-2-s-RBD antibody response (Figure 1). Among the 25 patients who were seronegative before the third dose, 16 (64%) turned positive. All the patients who were seropositive before the third dose were still seropositive 2 months after the second vaccine dose (p < .001, Figure 2). Fourteen (14.3%) responder patients presented antibody titres <100U/ml, 57 (58.2%) between 100 and 9999 U/ml and 27 (27.6%) ≥10 000 U/ml. Among the 51 responder patients who had anti-SARS-CoV-2s-RBD antibody titre <100U/ml 6 months after the second vaccine dose, only two (4%) maintained

| TABLE 1 Baseline demographic and clinical characteristics of the studied population. Categorical variables are presented as frequencies (%), continuous variables are presented as medians (interquartile range), and immunosuppressive drug serum levels are presented as the means (±SE) |
|---|
| Patients (\( N = 107 \)) |
| Age (years) | 67.3 (61.2–73.0) |
| Age at LT (years) | 57.8 (52.0–63.0) |
| Months between LT and vaccination | 91 (48–189) |
| Male gender | 77 (72.0) |
| BMI (kg/m\(^2\)) | 26.0 (23.7–28.7) |
| Aetiology (HCV, HBV, AH, AI, other) | 22, 19, 46, 11, 9 (20.6, 17.8, 43.0, 10.3, 8.4) |
| HCC | 41 (38.3) |
| DM | 35 (32.7) |
| Dyslipidaemia | 25 (23.4) |
| Alcohol consumption >40g/day | 9 (8.4) |
| HTN | 48 (44.9) |
| Presence of oesophageal varices | 6 (5.6) |
| Presence of ascites | 3 (2.8) |
| IS treatment |
| Tacrolimus | 71 (66.4) |
| Cyclosporine | 24 (2.4) |
| MMF | 46 (43.0) |
| Everolimus | 10 (9.4) |
| Prednisone | 12 (11.2) |
| Single IS | 54 (50.5) |
| T, C, MMF, E | 36, 8, 5, 5 (33.6, 7.5, 4.7, 4.7) |
| Double-triple IS including MMF | 41 (38.3) |
| MMF +T, +C,+E, +P, +C + P | 24, 13, 1, 1, 2 (22.4, 12.5, 0.9, 0.9, 1.9) |
| Double-triple IS excluding MMF | 12 (11.2) |
| T+ E, T+ A, T + P, C + P, T+E + P | 2, 1, 6, 1, 2 (1.9, 0.9, 5.6, 0.9, 1.9) |
| Any double IS therapy | 49 (45.8) |
| Any triple IS therapy | 4 (3.7) |
| IS levels with respect to reference \( ^a \) |
| Less than | 52 (48.6) |
| Greater than | 5 (4.7) |
| Serum IS drug levels or daily dose \( ^a \) |
| Tacrolimus (ng/ml) | 4.39 ± 0.75 |
| Cyclosporine (ng/ml) | 10.5 ± 2.3 |
| MMF (g/day) | 0.72 ± 0.09 |
| Everolimus (ng/ml) | 0.43 ± 0.16 |
| Prednisone (mg/day) | 0.58 ± 0.18 |
| Haemoglobin (g/dl) | 13.5 (12.4–14.7) |
| Leukocytes (n/µl) | 5830 (4500–6610) |
the same extent of response after the third dose, while 41 (71.9%) and 8 (29.6%) developed antibody titres in the ranges between 100 and 9999 U/ml and ≥10 000 U/ml respectively. Furthermore, among the 31 patients who presented anti-SARS-CoV-2s-RBD antibody titres in a range between 100 and 9999 U/ml 6 months after the second vaccine dose, 12 (21.2%) maintained the same range of antibody titres and 19 (70.4%) developed antibody titres ≥10 000 U/ml 2 months after the third vaccine dose (Table 2).

4.3 Factors influencing the anti-SARS-CoV-2s-RBD IgG response after BNT162b2 third vaccination dose

In the multivariate analysis, independent predictors of immune response failure (anti-SARS-CoV-2s-RBD IgG antibody titre <0.8 U/ml) 2 months after the third vaccine dose were taking a higher daily dose of MMF (p = .001) and had a lower estimated eGFR (p = .001), Table 3. Linear regression analysis confirmed that highly dose of MMF and lower eGFR were predictors of antibody response, Table 4. Any immunosuppressive treatment schedule employing two or three drugs (p = .034), a higher daily dose of MMF (p = .004) and lower eGFR (p = .012) were independent predictors of failed immune response to vaccination when the cut-off value of antibody titres was selected at 100 UI/ml (Table S1).

A direct correlation between having the eGFR >60 ml/min/1.73 m² and the achievement of increasing anti-SARS-CoV-2s-RBD antibody titre after the third vaccination dose was detected (Figure 3). In contrast, an inverse correlation between the antibody titre and the increasing daily dose of MMF was observed (Figure S1). The median anti-SARS-CoV-2s-RBD antibody titres evaluated after each time point following the second and the third doses of the vaccine were significantly lower in patients receiving immunosuppressive treatment schedules, including compared to those not taking
MMF. Moreover, while in patients receiving MMF, the median antibody titres remained stable from the first to the sixth month after the second vaccine dose, in patients not treated with MMF, the median antibody titres tended to progressively decrease over the same time frame. However, 2 months after the third vaccine dose, in patients both taking and not taking MMF, the median antibody titre increased by approximately two logs (Figure 4).

### 4.4 Patient-reported side effects of anti-SARS-CoV-2 third vaccination

No systemic symptoms, such as fever, asthenia or myalgia, were reported. Modest and transient pain at the vaccination injection site was reported in 12 of 107 (11.2%) cases. No significant liver test abnormalities were documented during routine postvaccination patient follow-up.

### 5 SYMPTOMATIC SARS-COV-2 INFECTIONS RECORDED IN LTS AFTER THE THIRD VACCINE DOSE

LTS were followed up to 125 days after the third vaccine dose. In the period between day 41 and day 120 of follow-up, 5 females and 4 males (8.4%) LTS tested positive for SARS-CoV-2 infection via RT-PCR performed on nasopharyngeal swabs. All patients reported mild symptoms and only five of them reported body temperature >38°C for up to 2 days, or diarrhoea with mild dysgeusia. Three of these patients (2 females) were treated as outpatients with the infusion of casirivimab (1200 mg) plus indevimab (1200 mg). Two further male patients have taken an oral therapy for 5 days: one with molnupiravir and one with nirmatrelvir/ritonavir. In the latter patient, tacrolimus dose administration was halved for 5 days, considering the relevant drug interaction with ritonavir. The remaining four patients were only clinically observed since their symptoms were milder. Besides the patient treated with nirmatrelvir/ritonavir, the immunosuppressive treatment schedule was maintained unaltered in all infected patients, and all of them tested RT-PCR negative within 3 weeks. No significant increases in serum transaminases were detected during the follow-up. Interestingly, no significant difference in the median anti-SARS-CoV-2 antibody titre between patients with acute SARS-CoV-2 infection (1325 U/ml) compared to those uninfected (3515 U/ml) was recorded. Similarly, no significant differences were recorded between SARS-CoV-2 infected and non-infected LTS regarding the mean daily dose of MMF assumption (0.83 vs 0.71 g/day, p = .778) and the median eGFR values (45.3 vs 61.3 ml/min/1.73 m² p = .145).

### 6 DISCUSSION

The anti-SARS-CoV-2s-RBD-positive antibody response 2 months after the third dose of the BNT162b2 vaccine was detected in 91.6% of LT patients in our series. This rate would appear to be higher than the rates ranging from 58% to 73% reported in recent studies. However, it should be emphasized that these studies enrolled solid organ transplant recipients and not specifically LT recipients, as in our series. Furthermore, the characterization of the antibody response was preferentially performed by means of assays measuring total or IgG antibodies against the SARS-CoV-2 spike protein, and not the IgG antibodies against SARS-CoV-2s-RBD, as adopted in our study. The SARS-CoV-2s-RBD protein is the target of vaccines in development and in use and thus might aid in more precisely characterizing the real immune response to vaccines. These features might also explain why 64% of our LTs non-responders to the second vaccine dose developed a positive response after the third vaccine dose compared to nearly 45% of the solid organ transplant recipients. In addition to the overall antibody response rate, our study attempted to identify the entity of the immunological response, considering the value of the antibody titre reached after the third dose of vaccine. We selected 100 U/ml as the antibody titre to define a potential clinically relevant antibody response. This assumption was derived from the observation that adoptive transfer of purified polyclonal IgG from convalescent macaques robustly protected naive recipient rhesus macaques against challenge with SARS-CoV-2 when the antibody titre was at least 100 U/ml. Interestingly, among the 98 LTS who developed a positive antibody response after the third vaccine dose, 84 of them (85.7%) presented an antibody titre ≥100 U/ml. This result is better than that obtained by Hall et al., who demonstrated an anti-s-RBD antibody level >100 U/ml 4 months after the third dose of vaccine in
Table 3: Association between prevaccination demographic and clinical characteristics of COVID-19 liver transplanted patients (N = 107) with regard to the development of a positive (≥0.8 U/ml) or negative (<0.8 U/ml) anti-SARS-CoV-2s-RBD antibody response, assessed 2 months (54 ± 9 days) after the third dose (booster) of the Pfizer BTN162b2 vaccine. Categorical parameters are presented as frequencies (%). and Pearson’s chi-square test was used for statistical comparisons. Continuous variables are presented as medians (interquartile range), and serum immunosuppressive drug levels are presented as the means (±SE). The rank-sum test (Mann–Whitney) was used for statistical comparisons. Stepwise regression with a forwards approach was used to identify independent predictive variables to achieve a positive antibody response after vaccination in a multivariate logistic model analysis.

|                                   | Anti-s RBD IgG negative (N = 9) | Anti-s RBD IgG positive (N = 98) | p    | O.R.  | 95% C.I. | p    |
|-----------------------------------|---------------------------------|-----------------------------------|------|-------|---------|------|
| Age (years)                       | 66.5 (64.9–74.1)                | 67.5 (61.0–72.9)                  | .428 |       |         |      |
| Age at LT (years)                 | 60.4 (59.7–65.9)                | 57.5 (51.2–62.6)                  | .059 |       |         |      |
| Male gender                       | 6 (66.7)                        | 71 (72.5)                         | .712 |       |         |      |
| BMI (kg/m²)                       | 25.2 (23.7–29.4)                | 26.1 (23.7–28.7)                  | .814 |       |         |      |
| Months between LT and vaccination | 48.3 (20.1–72.1)                | 98.3 (51.2–189.2)                 | .203 |       |         |      |
| Aetiology (HCV, HBV, AH, AI, other) | 2, 2, 1, 1 (22.2, 22.2, 33.3, 11.1,11.1) | 20, 17, 43, 10, 8 (20.4, 17.4, 43.9, 10.2,8.2) | .980 |       |         |      |
| HCC                               | 1 (11.1)                        | 40 (40.8)                         | .079 |       |         |      |
| DM                                | 4 (44.4)                        | 31 (31.6)                         | .433 |       |         |      |
| Dyslipidemia                      | 4 (44.4)                        | 21 (21.4)                         | .118 |       |         |      |
| Alcohol consumption >40 g/day     | 0 (0.0)                         | 9 (9.2)                           | .342 |       |         |      |
| HTN                               | 3 (33.3)                        | 45 (45.9)                         | .468 |       |         |      |
| Presence of oesophageal varices   | 1 (11.1)                        | 5 (5.10)                          | .453 |       |         |      |
| Presence of ascites               | 0 (0.0)                         | 3 (3.1)                           | .594 |       |         |      |
| IS treatment                      |                                 |                                   |      |       |         |      |
| Tacrolimus                        | 8 (88.9)                        | 63 (64.3)                         | .135 |       |         |      |
| Cyclosporine                      | 1 (11.1)                        | 23 (23.5)                         | .395 |       |         |      |
| MMF                               | 8 (88.9)                        | 38 (38.8)                         | .004 |       |         |      |
| Everolimus                        | 0 (0.0)                         | 10 (10.2)                         | .314 |       |         |      |
| Prednisone                        | 1 (11.1)                        | 11 (11.2)                         | .992 |       |         |      |
| Any double-triple IS therapy      | 9 (100)                         | 44 (44.9)                         | .002 |       |         |      |
| Double-triple IS including MMF.   | 8 (88.9)                        | 33 (33.7)                         | .001 |       |         |      |
| (MMF + T + E; + C; + A; + P; + C + P); | 7, 1, 0, 0, 0 (77.8, 11.1, 0.0, 0.0, 0.0) | 17, 12, 1, 1, 2 (17.3, 12.2, 1.0,1.0, 2.0) |       |       |         |      |
| Double-triple IS excluding MMF    | 1 (11.1)                        | 11 (11.2)                         | .992 |       |         |      |
| MMF (T + E, T + A, T + P, C + P, T + E + P); | 0, 0, 1, 0, 0 (0.0, 0.0, 11.1, 0.0, 0.0) | 2.1, 5, 1, 2 (2.0, 1.0, 5.1, 1.0,2.0) |       |       |         |      |
| Serum IS drug levels or daily dose |                                 |                                   |      |       |         |      |
| Tacrolimus (ng/ml)                | 4.80 ± 0.69                     | 4.35 ± 0.81                       | .138 |       |         |      |
| Cyclosporine (ng/ml)              | 3.3 ± 3.3                       | 11.2 ± 2.54                      | .356 |       |         |      |
| Everolimus (ng/ml)                | 0.0 ± 0.0                       | 0.47 ± 0.17                      | .373 |       |         |      |
| MMF (g/day)                       | 1.54 ± 0.23                     | 0.64 ± 0.09                      | .004 | 0.211  | 0.082–0.542 | .001 |
| Prednisone (mg/day)               | 0.56 ± 0.56                     | 0.59 ± 0.19                      | .992 |       |         |      |
| IS levels with respect to reference |                                 |                                   |      |       |         |      |
| Less than                         | 5 (55.6)                        | 47 (48.0)                         | .663 |       |         |      |
| Greater than                      | 0 (0.0)                         | 5 (5.1)                           | .488 |       |         |      |

(Continues)
55% of solid organ transplant recipients. This discrepancy might be because of the different (mRNA-1273) vaccines used, to the presence of only 4 LTs and to the longer period elapsing from vaccination to the measurement of antibody titres compared to our study. This last difference could be particularly important since it has been recently demonstrated, in solid organ transplant recipients, that the anti-SARS-CoV-2 spike protein antibody titre decreased from the first month to the third month after the third vaccine dose.

In contrast, all our patients tested positive after the second vaccine dose and remained positive 2 months after the third dose, with the median antibody titre increased by approximately two logs. These results agreed with what was recently described in a group of 101 solid organ transplant recipients, among whom only 12 were liver transplanted.

Although in our series, the third administration of the vaccine recovered more than 50% of LT recipients who tested negative for anti-SARS-CoV-2s-RBD antibodies after the second dose, a proportion of non-responder patients remained, confirming the observations derived from studies conducted in solid organ transplant recipients, and more recently in LTs, although adopting different types of vaccines. Double or triple immunosuppressive regimens, particularly those containing MMF, in addition to the presence of renal failure, resulted in the main determinants significantly reducing the positive antibody response after the third vaccine dose. Our previous study demonstrated that MMF was the main determinant of failure of the antibody response after the second anti-SARS-CoV-2 mRNA-based vaccines in LTs and the same feature was confirmed in the present study after the third vaccine dose.

### Table 3 (Continued)

|                  | Haemoglobin (g/dl) | Leukocytes (n/μl) | Neutrophils (n/μl) | Albumin (g/dl) | Bilirubin (mg/dl) | eGFR (ml/min/1.73 m²) | AST (U/ml) | ALT (U/ml) | INR | 25-OH-Vitamin D (ng/ml) |
|------------------|--------------------|-------------------|-------------------|----------------|------------------|----------------------|------------|------------|-----|-----------------------|
| Anti-s RBD IgG negative (N = 9) | 12.4 (11.7–13.0) | 4440 (4010–5550) | 3060 (2770–3360) | 4.59 (4.07–4.61) | 0.57 (0.44–0.64) | 45.9 (39.1–49.7) | 14 (12–18) | 12 (9–14) | 1.06 (0.95–1.17) | 32.3 (29.0–34.0) |
| Anti-s RBD IgG positive (N = 98) | 13.6 (12.6–14.9) | 5940 (457–6610) | 3400 (2720–4300) | 4.26 (4.10–4.50) | 0.61 (0.40–0.92) | 65.0 (48.1–79.6) | 19 (15–24) | 17 (12–23) | 1.04 (0.99–1.11) | 31.0 (26.0–35.0) |
| p                | .031               | .087              | .252              | .617           | .686             | .007                 | .031       | .043       | .853 | .625                  |

### Table 4

Linear regression analysis evaluating the demographic and clinical contributors to the development of anti-SARS-CoV-2s-RBD antibody response, assessed 2 months (54 ± 9 days) after the third dose of the Pfizer BTN162b2 vaccine, in liver transplanted patients (N = 107). Stepwise multivariate linear regression with a forward approach was used to discriminate variables associated with the logarithm of the antibody titre response. All variables presented in Table 3, that were significantly associated with the univariate linear regression, have been selected to run in the multivariate model.

|                  | t      | Coef.  | 95% C.I. | p       |
|------------------|--------|--------|----------|---------|
| MMF quantitative treatment (g/day) | -3.38  | 0.626  | -0.993 to 0.259 | .001    |
| eGFR (ml/min/1.73 m²) | 3.51   | 0.018  | 0.008 to 0.028 | .001    |

Note: Linear model estimation parameters $R^2 = .3653$, $p < .0001$. Akaike's information criterion (AIC) = 351.6.

Abbreviations: eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil.
agrees with a recent report including 248 LTs in whom the assumption of MMF in addition to taking a non-mRNA based vaccine was associated with a significantly reduced rate of anti-SARS-CoV-2 antibody response after the third vaccine dose. Furthermore, our results confirmed recent observations in LTs regarding the negative influence of decreased kidney function in conditioning the antibody response.
response rates after the third vaccine dose in solid organ transplant recipients. No safety concerns have been recorded for the third dose of vaccine in our series, as recently demonstrated in solid organ and liver transplant recipients. Although our study was not designed to evaluate the protective effect of the third vaccine dose on the development of new SARS-CoV-2 infections, we detect 9 (9.1%) LTs who developed a symptomatic SARS-CoV-2 infection during the follow-up period. This is to our knowledge the first report on this issue in LTs. The rate of new SARS-CoV-2 infections after three doses of mRNA-BNT162b2 vaccine in actively treated cancer patients has been reported in 6.3% of cases, which is very similar to that reported in our series. None of the SARS-CoV-2 infection in cancer patients was clinically severe as we observed in LTs. Since the median anti-SARS-CoV-2 antibody titres between LTs who experienced or not symptomatic infection was not statistically different, further well-conducted studies must be performed to detect, if possible, the minimum post-vaccination anti-SARS-CoV-2 antibody titre protecting LTs from new SARS-CoV-2 infection.

Our study has some limitations. First, we did not assess the prevalence of neutralizing antibodies, or the cell-mediated immunity induced by the vaccine. It has been demonstrated that, in the presence of anti-SARS-CoV-2s-RBD antibody titres >100 U/mL after the third dose of mRNA-1273 vaccine, the median per cent virus neutralization was 71%. Thus, we hypothesize that, in our study, in which the frequency of responsive patients presenting antibody titres >100 U/mL was higher, the per cent virus neutralization could be similar or higher. Furthermore, the correlation between humoral and cellular immune responses to anti-SARS-CoV-2 vaccination remains unclear. In a recent report evaluating 138 LT recipients, there was no evidence of a spike-specific T cell response in the majority of subjects without any detectable antibody response, suggesting that, in some cases, humoral and cellular immune responses could overlap. Second, we enrolled patients with a long interval between transplant and vaccination; thus, our results might not be comparable to those obtainable when vaccination has been scheduled closer to liver transplant.

In conclusion, in LTs, the administration of a third dose of the BNT162b2 vaccine was safe and significantly improved the immunogenicity of the vaccine, inducing a significant increase in the median anti-SARS-CoV-2s-RBD antibody titres in most patients. A proportion of LT patients remained unresponsive to vaccination, mainly because of potentially modifiable factors, such as the use of MMF and the intensity of immunosuppressive regimens. Despite the positive response to the third vaccine dose, near 10% of LTs experienced symptomatic SARS-CoV-2 infection, which was clinically mild, followed by complete recovery without the need for hospitalization.

**AUTHOR’S CONTRIBUTION STATEMENT**

Pierluigi Toniutto, Annarosa Cussigh, Sara Cmet, Davide Bitetto, Ezio Fornasiere, Elisa Fumolo, Martina Fabris, Federica D’Aurizio, Carlo Fabris, Lucrezia Grillone, Assunta Sartor, Francesco Curcio and Edmondo Falletti have the required criteria for having the role of author.

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**CONFLICT OF INTEREST**

The authors have no conflicts of interest to disclose regarding this work.

**DATA AVAILABILITY STATEMENT**

Data are available on request because of privacy/ethical restrictions.

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**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

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