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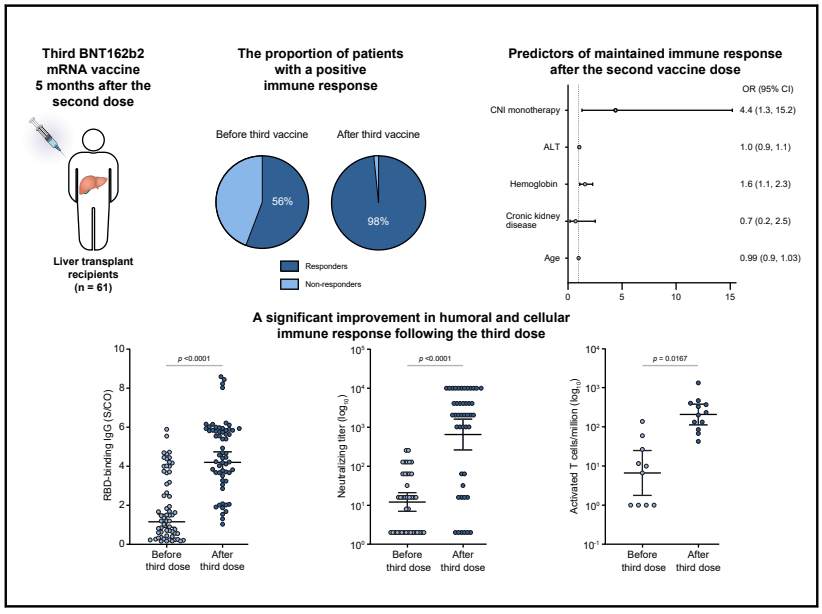
Graphical abstract



Highlights

- A third dose of the BNT162b2 mRNA vaccine significantly improved immune response among liver transplant recipients.
- The percentage of positive immune response 5 months after the second vaccine (56%) improved significantly after the third dose (98%).
- Geometric mean anti-RBD IgG levels, NA levels, and T-cell count increased significantly after the third dose.
- NA titers after the third vaccine negatively correlated with age, MMF treatment, and combined immunosuppression.

Authors

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Lay summary

The Pfizer-Biotech BNT162b2SARS-CoV-2 vaccine induced significant immunity among liver transplant recipients after a third dose. The majority of the patients developed sufficient levels of both humoral and cellular immune responses. Factors that predict non-response were older age and immunosuppressive medications.

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A third dose of the BNT162b2 mRNA vaccine significantly improves immune responses among liver transplant recipients

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Background & Aims: Immune responses of solid organ transplant recipients to 2 doses of the BNT162b2 mRNA anti-SARS-CoV-2 vaccine are impaired. The immunogenicity and safety of a third dose among liver transplant (LT) recipients are unknown. This work aimed to evaluate the immune response of LT recipients to a third dose of the BNT162b2 mRNA vaccine.

Methods: Consecutive LT recipients (n = 61) in follow-up at Sheba Medical Center were included. Receptor binding domain (RBD) IgG, neutralizing antibody (NA) titers, and T-cell levels before and 21-28 days after a third vaccine dose were determined. Adverse effects after the third dose were monitored.

Results: The median age of LT recipients was 65 years and 57.4% were male. The humoral immune response rate improved significantly, with 56% of patients showing a response before the third vaccine dose compared to 98% after the third dose. The cellular response in 12 evaluated patients improved significantly (p = 0.008). The geometric mean of anti-RBD IgG levels, NA levels, and T-cell count also increased significantly after the third dose. NA titers after the third dose negatively correlated with age (p = 0.03), mycophenolate mofetil treatment (p = 0.005), and combined immunosuppression as opposed to calcineurin inhibitor monotherapy (p = 0.001). After the third dose, adverse effects were reported by 37% of recipients and were mostly mild (local pain and fatigue).

Conclusion: After a third BNT162b2 mRNA vaccine, the immune response improved significantly among LT recipients, without serious adverse effects. Further studies are needed to evaluate immune response durability and to determine the optimal number and schedule of booster vaccine doses.

Lay summary: The Pfizer-Biotech BNT162b2SARS-CoV-2 vaccine induced significant immunity among liver transplant recipients after a third dose. The majority of the patients developed sufficient levels of both humoral and cellular immune responses. Factors that predict non-response were older age and immunosuppressive medications.

Keywords: BNT162b2 mRNA; third dose; liver transplant recipients; antibody response; immune response; side effects BNT162b2 mRNA vaccine.
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Introduction

The BNT162b2 mRNA vaccine against SARS-CoV-2 has been shown to be safe and effective in immunocompetent individuals.[1–3] In contrast, solid organ transplant (SOT) recipients were reported to have impaired immune responses after 2 doses of the vaccine,[4–8] with liver transplant (LT) recipients showing a better immune response to the mRNA vaccine than other SOT recipients.[4,9,10] The reduced immune response was more frequent in patients who received combined immunosuppression treatments (combination of calcineurin inhibitor [CNI] with mycophenolate mofetil [MMF], or sirolimus, or everolimus, and/or low-dose steroids) and patients with renal impairment.[4–7] We have reported a 72% positive immune response rate among 76 LT recipients after the second dose of the BNT162b2 mRNA vaccine.[10] Kamar *et al.* reported improved immune response after the third BNT162b2 mRNA dose, administered to SOT recipients (78 kidney transplant recipients, 12 LT recipients, 8 lung transplant or heart transplant recipients, and 3 pancreas transplant recipients) 61 days after the second dose.[11] Benotmane *et al.*[12] reported improved immune responses among 159 kidney transplant recipients who did not respond to 2 doses of the mRNA-1273 vaccine and who received a third dose 1 month after the second dose. Based on this evidence and the spread of the virus among the general population, the Israeli Ministry of Health recommended immunization of all groups of patients with reduced immune responses with a third (booster) BNT162b2 mRNA dose.

Most of the immune response evaluation after BNT162b2 mRNA vaccination concentrated on the humoral immune response. The role of T cell-mediated immune responses in protection against SARS-CoV-2 infection remains unclear and it is not known whether immunocompromised SOT recipients develop such responses. Mrak and coauthors reported that SARS-CoV-2-specific T cells were detected in 58% of rituximab-treated patients, independent of the humoral immune response.[13]

We aimed to prospectively assess, for the first time, the safety as well as the efficacy of the third Pfizer-BioNTech BNT162b2

mRNA vaccine dose among LT recipients by evaluating both humoral and cellular immune responses.

Patients and methods

This study was conducted at the Sheba Medical Center between 13 July 2021 and 5 September 2021 and was approved by the institutional review board. All participants agreed to participate in the study and signed written informed consent before any study-related procedures were conducted. The study population included 61 adult (age >18 years old) LT recipients routinely followed at the Liver Diseases Center.

Serum samples were collected from the LT recipients just before and at least 21 days after the third vaccination. A third standard intramuscular (IM) dose (30 μg) of the diluted Pfizer-BioNTech BNT162b2 mRNA vaccine was administered to all patients. Details regarding adverse reactions to the vaccine were reported by all participants. Side effects were monitored throughout the first 30 days post-vaccination. The side effects were categorized as local *vs.* systemic.

Levels of anti-RBD IgG and neutralizing antibody (NA) were available for 47 of 61 patients who had participated in our previous study,[10] which evaluated the humoral immune response of LT recipients to 2 doses of the BNT162b2 mRNA vaccine. We compared the humoral immune response separately among the 47 patients at 3 timepoints: after the second vaccine (median 38 [IQR, 21-52] days after the second dose), immediately before the third vaccine, and after the third vaccine (median 22 days [IQR 21-28] after the third dose). The median time between the second vaccine dose, serology after the second vaccine, and the third vaccine dose were 174 (IQR 168-182) and 135 (119-149) days, respectively.

Demographic, clinical, and laboratory data were extracted from electronic patient records. Blood tacrolimus or everolimus trough levels were determined, and routine blood tests were performed between the time the third vaccine was administered and before the serology test. Renal function was calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) creatinine equation. Chronic kidney disease was defined as estimate glomerular filtration rate <60 ml/min/1.73 m^2 for a duration of >3 months.[14]

All LT recipients who received the second vaccine dose at least 1 month prior to the third dose were considered for inclusion in this study. LT recipients who had recovered from SARS-CoV-2 or had an active SARS-CoV-2 infection up to 7 days after the third vaccine dose, were excluded from the study.

The study was approved by our institutional review board (8314-21-SMC). Written informed consent was obtained from each patient included in the study.

Serology assays

Antibody detection testing

Samples were evaluated with an "in-house" ELISA that detects IgG antibodies against the receptor binding domain (RBD) of SARS-CoV-2.[15,16] A SARS-CoV-2 pseudo-virus (psSARS-2) neutralization assay was performed, as previously described,[17] to detect SARS-CoV-2 neutralizing antibodies using a GFP reporter-based pseudotyped virus with a vesicular stomatitis virus backbone coated with the SARS-CoV-2 spike (S) protein, which was generously provided by Dr. Gert Zimmer (Institute of Virology and Immunology, Mittelhäusern, Switzerland). Sera not capable of reducing viral replication by 50% at 1 to 8 dilution or below were considered non-neutralizing. IgG antibody titers above 1.1 sample/cut-off (S/CO) were defined as positive (responders), while anti-RBD IgG <1.1 S/CO was defined as negative (non-responders). Patients who were still considered responders before receiving the third vaccine dose were defined as having a maintained immune response, while responders whose anti-RBD IgG levels dropped to below 1.1 S/CO prior to the third dose were defined as patients who failed to maintain an immune response.

Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using UNI-SEP+ (Novamed). Plasma was collected and spun at 1,000 × g for 20 min to remove platelets before collection of PBMCs. Following 1 wash with PBS and 1 wash with 4 Cell Nutri-T-Medium (Sartorius), cells were resuspended in 4Cell Nutri-T-Medium and counted using the Countess II Cell counter (Invitrogen).

T-cell response testing

Fresh PBMCs were used in all ELISpot assays using the Elispot IFNγ kit (AID). Briefly, fresh PBMCs were added in duplicate wells at $2x10^5$ cells in 50 μl per well and stimulated with 50 μl of SARS-CoV-2 peptide pools (S-complete, Miltenyi Biotech) (2 μg/ml per peptide). 4Cell Nutri-T-Medium was used as a negative control and phytohaemagglutinin was used as a positive control. After 16–20 h at 37 °C, 5% CO_2, 95% humidity, cells were removed and secreted IFNγ was detected by adding alkaline phosphatase-conjugated secondary antibody for 2 h. The plates were developed using BCIP/NBT substrate according to the manufacturer's instructions. ELISpot plates were scanned on an AID ELISpot Reader. The unspecific background (mean spot-forming units from negative control wells) was subtracted from experimental readings. It was only possible to perform the T-cell evaluation test over 2 days, so only the patients who agreed to participate during these days received a T-cell evaluation.

Statistical analysis

Continuous variables are presented as mean (SD) for normally distributed data and as median (IQR) for skewed distributions. Categorical variables are expressed as count (percentage). Antibody titers were log-transformed prior to the statistical analysis. Concentrations of SARS-CoV-2 IgG against the RBD, NAs and number of IFNγ-secreting T cells per 10^6 PBMCs were calculated for all groups and presented as geometric mean titers and 95% CI. Patients were categorized as having a positive or negative immune response based on the level of IgG antibodies.

Categorical variables were compared using Chi-squared analysis and Fisher's exact test. Continuous measurements were compared by Student's *t* test or Mann-Whitney *U* test according to their distribution. Non-parametric Wilcoxon paired tests were conducted to compare quantitative data. The accuracy of predicting positive levels of anti-RBD IgG and NA was evaluated by the area under the receiver-operating characteristic curve. To assess predictors of maintenance of immune response after the second vaccine but before the third vaccine, patients were divided according to levels of anti-RBD IgG. A logistic regression analysis model was used to explore the factors associated with the maintenance of immune response after the second vaccine. Covariates for the multivariate models were selected using clinical judgment and variables that significantly differed between the groups. Correlations were estimated

between anti-RBD IgG, NA, and IFNγ-secreting T cells using the Spearman correlation test. p <0.05 was considered a statistically significant difference. All tests were 2-sided. Statistical analysis was performed with SPSS (IBM SPSS Statistics, version 25, IBM Corp., Armonk, NY, USA, 2016). Scatter plots of the analyzed data were produced using GraphPad Prism version 9.2.0 for windows (GraphPad Software, San Diego, USA).

Results

Baseline characteristics

Baseline demographics and clinical and laboratory characteristics of the 61 LT recipients vaccinated with the third dose are presented in Table 1. Their median age was 65 years (QR 52-70 years; range 24-81 years); 57.4% were males. Median time since LT was 7 years (IQR 4-18 years). Comorbidities were frequent, with hypertension (48.3%), diabetes mellitus (48.3%), chronic kidney disease (63.9%), and dyslipidemia (50%) being the most common.

CNIs were the principal immunosuppressive agent, administered to all 61 patients (52 tacrolimus and 9 cyclosporine). CNI monotherapy was administered to 30 patients (49.2%), 25 patients (41.0%) received double immunosuppression (combination of CNI and mycophenolate mofetil [MMF] – 12 patients; CNI and mTOR inhibitors (everolimus) – 7 patients; CNI and prednisone – 6 patients). Triple immunosuppression therapy was administered to 6 (9.8%) patients (combination of CNI, MMF, and prednisone) (see Table 1).

Humoral immunity to BNT162b2 mRNA vaccine

The immune response of all 61 LT recipients before and after the third BNT162b2 mRNA vaccine dose was evaluated. When compared to the immune response measured before the third dose, the third dose significantly improved immune response rates, as manifested by 56% responders before (34 of 61 responders prior to the third vaccine) *vs.* 98% after (60 of 61 responders after the third vaccine), vaccination (p <0.0001). The

Table 1. Baseline demographics and clinical and laboratory characteristics of patients with or without immunologic response to the third BNT162b2 mRNA vaccine dose.

	Total cohort, N = 61	Non-responders*, n = 1 (2%)	Responders*, n = 60 (98%)
Age, years	65 (52-70)	66	63 (51-70)
Male, n (%)	35 (57.4)	1	37 (59.7)
Indication for LT, n (%)			
Hepatitis C	14 (23)	1	13 (21)
NASH	14 (23)		14 (16.1)
Hepatitis B	4 (6.6)		4 (6.5)
PSC	4 (6.6)		4 (6.5)
PBC	5 (8.2)		5 (8.1)
Others¶	20 (32.8)		22 (35.5)
Age at transplantation, years	51 (41-63)	56	53 (41-63)
Time since liver transplantation, years	7 (4-18)	10	7 (4-19)
Comorbidities, n (%)			
Diabetes mellitus	25 (41.7)	1	24 (40.7)
Hypertension	29 (48.3)	1	28 (47.5)
Dyslipidemia	30 (50)	1	29 (49.2)
Chronic kidney disease	39 (63.9)	1	39 (65)
BMI, kg/m^2	25 (22-27)	24	25 (22-28)
WBC, 10^3/μl	5.6 (4.7-6.7)	6.1	5.6 (4.7-6.7)
Hemoglobin, g/dl	13.1 (12.0-14.3)	10.6	13.1 (12-14.3)
Platelets, x10^3/μl	167 (121-197)	197	166.5 (121-197)
Creatinine, mg/dl	1.04 (0.9-1.3)	1.9	1.0 (0.9-1.3)
ALT, IU/L	20 (15-27)	15	20 (15-27)
ALP, IU/L	96 (69-131)	93	97 (69-131)
Bilirubin, mg/dl	0.6 (0.5-0.9)	0.42	0.6 (0.5-0.9)
Albumin, g/dl	4.1 (4.0-4.3)	4.4	4.1 (3.9-4.3)
Tacrolimus dose, mg	2.8 (1.5-4)	1.5	3 (1.5-4.0)
Tacrolimus trough level, ng/ml	5.3 (4.1-6.4)	1.4	5.3 (4.2-6.4)
Prednisone, n (%)	12 (19.7)	0	12 (20)
Prednisone dose, mg	6 (5-10)		6 (5-10)
MMF, n (%)	18 (29.5)	1	17 (27.4)
MMF dose, mg	875 (500-1,000)	1,000	750 (500-1,000)
Everolimus, n (%)	7 (11.5)	0	7 (11.7)
Everolimus dose, mg	2 (2-3)		2 (2-3)
Everolimus trough level, ng/ml	3 (2-6)		3 (2-6)
Double‡/Triple‡‡ immunosuppression, n (%)	31 (50.8)	1	31 (50)
Time from third vaccine to serology collection, days	22 (21-28)	22	22 (21-28)

Data presented as median (IQR) or n (%); For categorical variables, the Chi-squared test was used. Continuous variables were compared by using a t test if normally distributed or the Mann-Whitney U test if non-normally distributed parameters. A p value of 0.05 or less was considered statistically significant for all analyses.
ALT, alanine aminotransferase; ALP, alkaline phosphatase; CNI, calcineurin inhibitors; LT, liver transplantation; MMF, mycophenolate mofetil; NASH, non-alcoholic steatohepatitis; OR, odds ratio; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; S/CO, sample/cut-off; WBC, white blood cell count.
*Patients with IgG antibody titers above 1.1 S/CO were defined as a responder; patients with titers below 1.1 S/CO as a non-responder.
¶Other indications for liver transplantation: Alcohol-related liver disease, Biliary atresia, Cystic fibrosis, Fulminant liver failure, Budd-Chiari syndrome.
‡Double immunosuppression denotes CNI and MMF (12 patients), CNI and everolimus (7 patients), CNI and prednisone (6 patients).
‡‡Triple immunosuppression was administered to 6 patients (combination of CNI, MMF, and prednisone).

geometric mean of anti-RBD IgG and NA levels increased significantly after the third dose compared to levels measured immediately before its administration (1.2 S/CO [95% CI 0.9-1.5] *vs.* 4.2 S/CO [95% CI 3.7-4.8], p <0.0001 and 12.1 [95% CI 7.0-21.0] *vs.* 650 [95% CI 263-1,608] p <0.0001, respectively; Table 2, Fig. 1A and Fig. 1B). Anti-RBD IgG titers positively correlated with NA titers both before (r = 0.6, p <0.0001) and after the third vaccine (r = 0.7, p <0.0001).

Due to the small sample size and the highly significant response to the third dose among the LT recipients (98.4%), predictors of negative immune response after the third dose could not be evaluated.

NA titers after the third dose negatively correlated with age (r = -0.32, p <0.03), MMF treatment (r = -0.4, p <0.005), and combined immunosuppression (double and triple therapy) compared to CNI monotherapy (r = -0.5, p <0.007). NA titers after the third dose also positively correlated with hemoglobin levels (r = 0.6, p <0.0001) and alanine aminotransferase levels (r = 0.5, p <0.0014). No correlation was found between NA titers following the third dose and sex, comorbidities (hypertension, diabetes mellitus, dyslipidemia, impaired renal function), or treatment with mTOR inhibitors or prednisone.

Monotherapy with CNI predicted response with an area ratio under the curve (ARUC) of 0.75 for IgG anti-RBD levels of 3.9 S/CO, with a sensitivity of 87% and specificity of 58%, or with a sensitivity of 50% and specificity of 84% for IgG anti-RBD levels of 5.8 S/CO, p = 0.001. Additionally, CNI monotherapy also predicted higher levels of NA with an ARUC of 0.76 for a cut-off value of 1,536; the sensitivity was 77% and the specificity 56% (p = 0.003; Fig. S1).

During the preparation of this manuscript, 2 of the 61 LT recipients became infected with SARS-CoV-2 two months after receiving the third dose and suffered from mild symptoms. Both patients were responders after the third vaccine, as shown by IgG anti-RBD levels of 2.1 S/CO for the first and 5.8 S/CO for the second. The first patient had undetectable NA levels, while the NA titer in the second patient was 4,096. However, the first patient was a non-responder prior to the third dose, with IgG anti-RBD levels of 0.2 S/CO and the second patient was a responder, with IgG anti-RBD levels of 3.2 S/CO.

T-cell immunity to the BNT162b2 mRNA vaccine

In 12 randomly selected patients, the T-cell response was evaluated before and after administration of the third vaccine dose. The clinical and laboratory characteristics of patients of all cohorts are presented in Table S1. There was no difference between patients who underwent T-cell evaluation *vs*. those who did not, in terms of age, sex, comorbidities, time since transplantation, or immunosuppression therapy, however the former had a longer period between the second and the third vaccine doses (186 days [IQR, 181-189] *vs.* 170 days [IQR, 163-177], p = 0.002) and significantly higher titers of anti-RBD-IgG (geometric mean 5.7 S/CO [95% CI 4.7-6.9] *vs.* 3.9 S/CO [95% CI 3.4-4.5], p = 0.03) and NA (geometric mean 3,334 [95% CI 1,339-8,305] *vs.* 358 [95% CI 114-1,127], p = 0.03).

The geometric mean of IFNγ-secreting T cells per 10^6 PBMCs following the third dose increased significantly compared to their levels before vaccination (7 [95% CI 2-25, range 0-137) *vs.* 206 (95% CI 112-378, range 43-1,320), respectively (p = 0.008, Fig. 1C). No correlation was found between anti-RBD IgG (r = 0.55, p = 0.06) or NA titers (r = 0.03, p = 0. 9) and T-cell counts.

Predictors of immune response maintenance after the second vaccine and its influence on response to the third vaccine

Prior to the third vaccination, 27 of 61 patients (44.3%) were non-responders, among whom 96.3% responded to the third dose. Analysis of the immune responses of patients who maintained a response prior to the third dose *vs.* those who did not, showed that the geometric mean of anti-RBD IgG and NA increased among responders from 2.7 (95% CI 2.3-3.2) and 27.7 (95% CI 13.9-55.2) S/CO before the third dose to 5.4 (95% CI 5.0-5.8) and 4,220 (3,040-5,858) S/CO after the third dose (p <0.0001). Among non-responders, the geometric mean of anti-RBD IgG and NA titers increased from 0.4 (95% CI 0.3-0.5) and 3.1 (2.5-3.8) S/CO to 2.81 (95% CI 2.15-3.69) and 39.2 (9.1-169) S/CO after the third dose (p <0.0001 and p = 0.1, respectively; Fig. 1D and 1E).

T-cell counts were available for 10 responders and only 2 non-responders. The geometric mean count of IFNγ-secreting T cells per 10^6 PBMCs following the third dose increased from 5.9 (95% CI 1.3-26.2) to 169 (95% CI 93-306) (p = 0.003) in responders. In the 2 non-responders, after the third vaccine dose, the number of T cells increased in both patients; before its administration, they had no T cells, while after the vaccination, the T-cell count increased to 233 and 1,320 T cells per 10^6 PBMC (p = 0.3, Fig. 1F).

Baseline demographics and clinical and laboratory characteristics of patients with a maintained immune response *vs.* those who did not maintain a response prior to the third dose, are presented in Table S2. Independent predictors of maintaining response after the second vaccine (median time after the second dose was 174 days [IQR 165-181] were CNI monotherapy (risk ratio 4.4, 95% CI, 1.3-15.2, p = 0.02) and higher level of hemoglobin (risk ratio 1.6, 95% CI, 1.1-2.3, p = 0.016). Age at vaccination, sex, time after transplantation, comorbidities, renal failure, and combined immunosuppression had no influence (Fig. 2, Table S3).

Comparison of the immune response immediately after the second vaccine and immediately after the third vaccine

The humoral immune response was evaluated in 47 of 61 patients after the second vaccine (median 38 [IQR, 21-52] days after

Table 2. Immune response before and after the third vaccine.

	Before third vaccine‡, n = 61	After third vaccine‡‡, n = 61
Positive anti-RBD IgG, n (%)	34 (56)	60 (98)
50% neutralization titer, GM (95% CI)	12.1 (7.0-21.0)	649.6 (262.5-1,608)
Anti-RBD IgG titer, S/CO, GM 95% CI)	1.2 (0.9-1.5)	4.2 (3.7-4.8)
Activated T cells/10^6cells, GM (95% CI)*	6.6 (1.8-24.8)	206 (112-378)

GM, geometric mean; RBD, receptor binding domain; S/CO, sample/cut-off.
‡Serum was collected before administration of the third vaccine dose (at the same day and in one patient one day before administration of third vaccine).
‡‡Serum was collected within a median 22 (IQR, 21-28) days after administration of the third vaccine dose.
*The T-cell response was evaluated before and after administration of the third vaccine dose in 12 randomly selected patients.

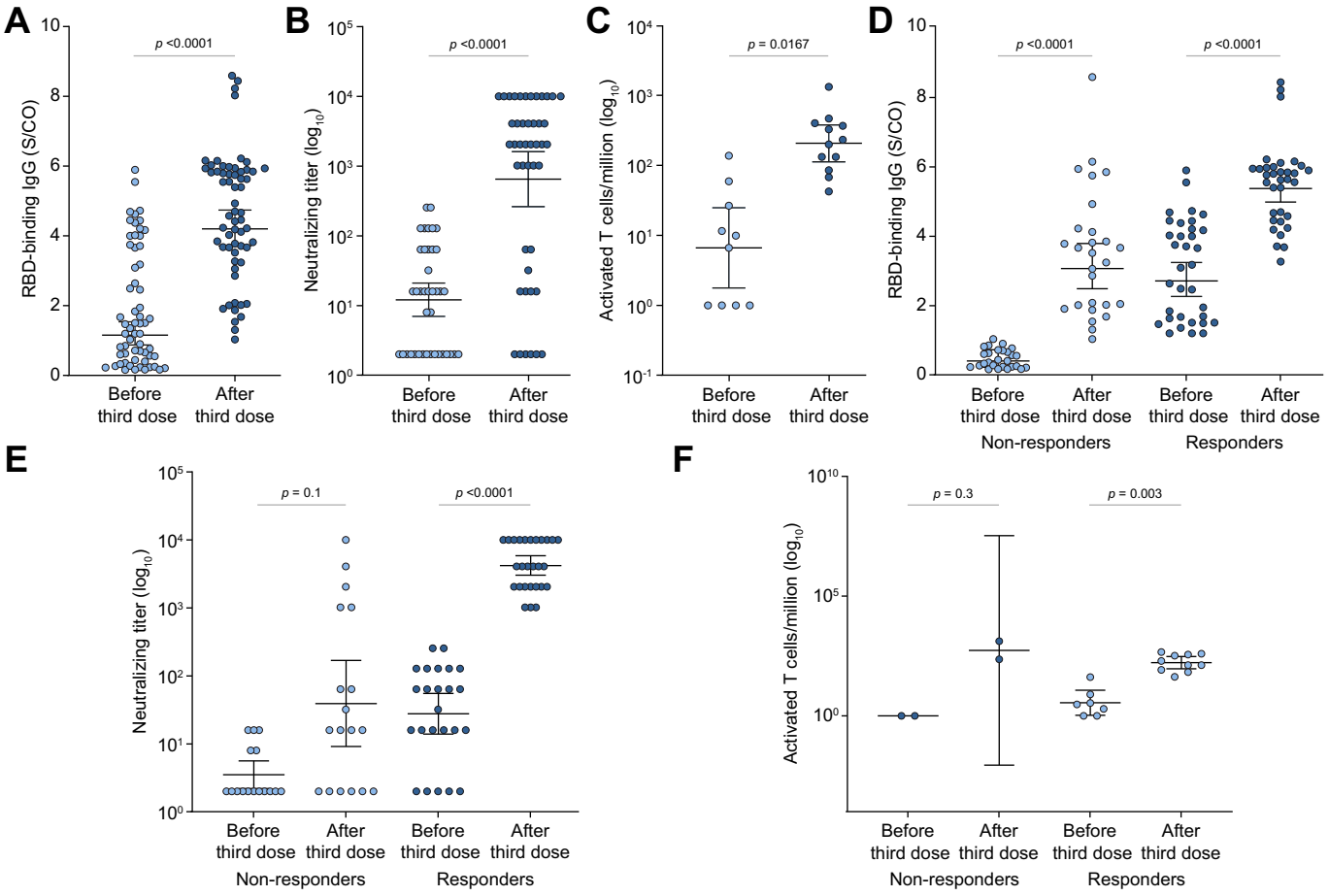

Fig. 1. Improved immune response following administration of the third BNT162b2 mRNA vaccine among LT recipients. Scatter plots presenting the changes in (A) anti-RBD IgG, (B) neutralizing antibody titers and (C) T-cell counts before and after the third vaccine dose. Changes in (D) anti-RBD IgG, (E) neutralizing antibody titers and (F) T-cell counts after administration of a third vaccine dose to patients who maintained and did not maintain an immune response after the second vaccine. The black horizontal line indicates geometric mean values with 95% CIs. Differences in paired samples were calculated using the Wilcoxon matched-pairs signed-rank test. LT, liver transplant; RBD, receptor binding domain.

the second dose). A positive immune response was detected in 32 of 47 patients (68%) after the second dose and in 27 of 47 patients (57%) prior to the third dose (median 174 [168-182] days after the second dose), increasing significantly to 98% (46 of 47 patients) after the third dose (median 22 days [IQR 21-28] after the third dose) (Fig. 3 and Table S4).

Adverse effects of the third BNT162b2 mRNA vaccine dose

Adverse effects (mostly mild) were reported by 50% of the patients after the second dose of BNT162b2 mRNA vaccine.[10] Following the third dose, adverse effects were mild and reported by 37% of patients. Among these patients, 29% had local adverse reactions (mostly localized post-injection pain) and 22% reported systemic adverse reaction (mostly fatigue).

Age, sex, comorbidities, and level of immune response (levels of anti-RBD, NA and T cells) did not correlate with the development of adverse effects.

Discussion

The third dose of the BNT162b2 mRNA vaccine significantly improved humoral and cellular immune response among LT recipients. The low immune response rate measured among LT recipients 38 days after the second vaccine (68%) further declined 5 months after the second dose (57%), but significantly improved, 3 weeks after the third dose (98%). The vast majority (96%) of LT recipients who had a negative immune response before the third dose, achieved a positive immune response after the third dose. Our findings are in line with a recent report by Naaber *et al.* who studied the dynamics of the antibody response in 122 healthy volunteers after 2 doses of the BNT162b2 mRNA vaccine.[18] While a robust antibody response to the Spike protein was noted after the second dose, the response waned over time, with a decline in antibody levels measured at 12 weeks and 6 months post-vaccination.[18] Several case series reported improved immunogenicity among SOT recipients after the third dose of the BNT162b2 mRNA vaccine approximately 1-2 months after the second dose.[11,18,19] Specifically, Westhoff *et al.* showed improved cellular (90%) and humoral (60%) immune responses among renal transplant recipients who failed to show a primary humoral response after 2 vaccine doses.[19] Kamar *et al.* reported improved immune responses after the third BNT162b2 mRNA dose, administered 61 days after the second dose to 101 SOT recipients, most of whom were kidney transplant recipients and only 12 were LT recipients.[11] The immune response rate prior to the third dose was 40% *vs.* 68% 4 weeks after the third vaccine. The lower immune response rate reported by Kamar *et al.*

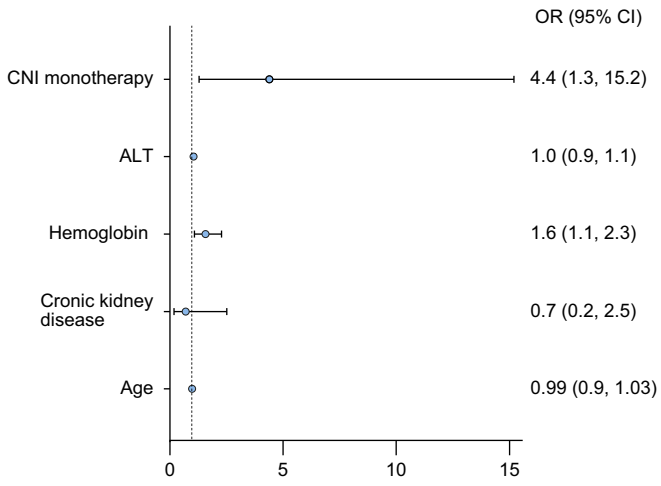

Fig. 2. Predictors of maintained immune response after the second vaccine dose. A forest plot showing the odds ratio and the 95% CI for a multivariable logistic regression analysis of predictors of immune response maintenance after the second vaccine dose. ALT alanine aminotransferase; CNI, calcineurin inhibitor.

compared to our findings may be related to the type of immunosuppressants administered to the included patients; in the study by Kamar *et al.*, 63% of patients were receiving MMF, 87% prednisone, 30% mTOR and 12% belatacept,[11] while in our cohort 49% were receiving CNI monotherapy and only 29.5% received MMF. Peled *et al.* demonstrated an increase in the rate of immune responders from 23% to 67% following the third dose of the BNT162b2 mRNA vaccine among heart transplant patients.[20] Similar to our findings, MMF treatment and impaired renal function correlated with a negative immune response.[20] Taken together, like the higher immune response rate noted after the second vaccine dose in our LT recipients,[10] the third vaccine dose induced a substantially better immune response compared to heart[20] and renal transplanted recipients.[11,19]

While the immune response after the second vaccine dose was relatively low among all SOT recipients,[4–8] our results demonstrated a remarkably higher response rate (72%) among LT recipients.[10] Rabinowich *et al.*,[4] Rueter *et al.*[21] and Rashidi-Alavijeh[9] *et al.* reported 47.5%, 74% and 79% response rates, respectively, to 2 doses of the BNT162b2 mRNA vaccine among LT recipients (n = 80, 141, 43 patients, respectively).

After the second dose of the vaccine, negative immune responses among cohorts of SOT recipients that also included LT recipients, correlated with older age, impaired renal function, MMF treatment or combined (double or triple) immunosuppression.[4–6,8] Due to the very high response rate and relatively small sample size in our study, we were unable to identify immune response predictors. However, we found a significant negative correlation between anti-RBD IgG levels and MMF treatment, combined immunosuppression, older age and impaired renal function.

CNIs are the principal immunosuppression agents prescribed after LT. Among the available CNIs, tacrolimus is the drug of choice in almost 90% of LT recipients.[22] Due to the known nephrotoxicity that is associated with administration of CNI, patients are commonly prescribed reduced dosages, with or without renal-sparing agents such as sirolimus, or everolimus, low-dose steroids, or MMF.[22]

When compared to immunocompetent individuals, the reported anti-RBD IgG and NA titers among SOT recipients after the second dose of vaccine were significantly lower.[4–6] Bergwerk and coauthors[23] from our institute recently demonstrated that the occurrence of breakthrough SARS-CoV-2 infections (n = 39) among healthcare workers who had received 2 vaccine doses (n = 1,497) correlated with NA titers during the peri-infection period. In the present work, we found that the immune response after the third vaccine among primary non-responders to the second vaccine, increased considerably, reaching approximately the same level achieved by the responders to the second vaccine dose. Taken together, an additional fourth boost of the

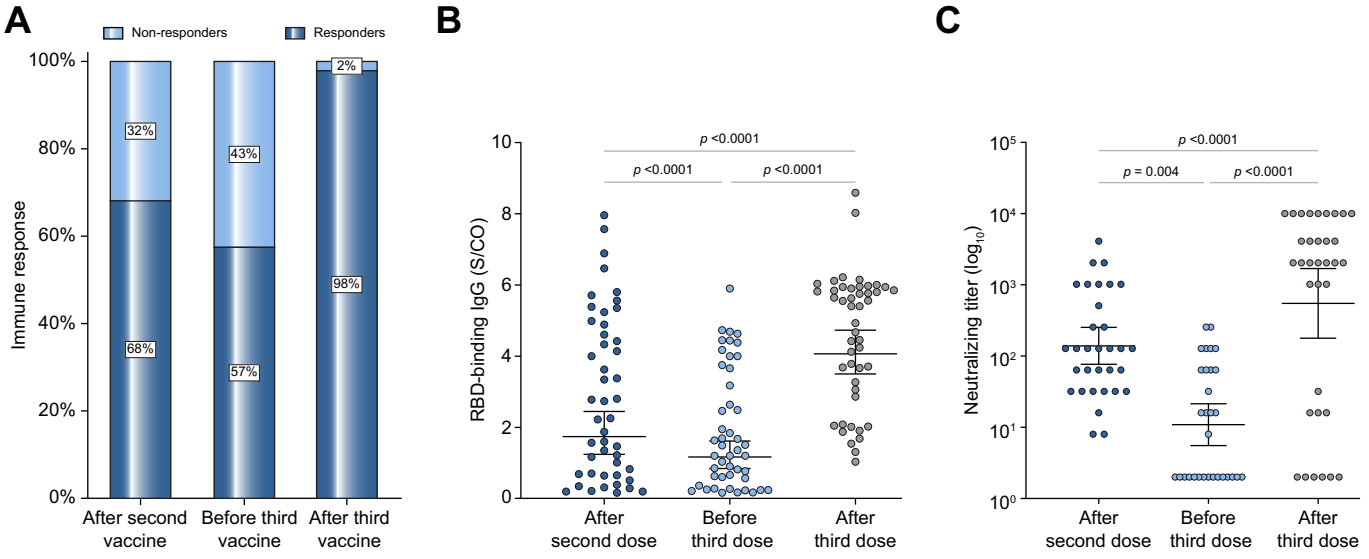

Fig. 3. Dynamics of the humoral immune response after the second and the third vaccine doses among 47 liver transplant recipients. (A) Changes in immune response after the second and third doses. Scatter plot presenting changes in (B) anti-RBD IgG and (C) neutralizing antibody titers after the second vaccine dose (median time after dose and serum collection was 38 [IQR 21-52] days), and before and after the third dose (median time between third dose and serum collection was 22 [IQR 21-28] days). The black horizontal line indicates geometric mean values with 95% CI. Differences in paired samples were calculated using the Wilcoxon matched-pairs signed-rank test. RBD, receptor binding domain; S/CO, sample/cut-off.

vaccine may be necessary in patients with a relatively low immune response.

The quantification of virus-specific T-cell immune responses is technically more complex than performing serological analyses. As a result, limited data has been published regarding the role of T cells in the protection against SARS-CoV-2 infection. Recently, Painter *et al.* showed that mRNA vaccines activate SARS-CoV-2-specific T cells, which can contribute to durable immunity.[24] Moreover, a recent review emphasized the importance of the role of the spike-specific CD4 and CD8 T cells in reducing viral replication and subsequently limiting the pathogenicity of infection.[21] Rueter *et al.*[21] reported impaired cellular responses among LT recipients compared to healthy controls after the second mRNA vaccine dose (specifically LT recipients received the following types of vaccines: 79.2% BNT162b2 mRNA, 12.4% mRNA-1273 and 8.4% AZD1222). Similarly, Peled *et al.*[20] reported a discordance between humoral and cellular immune responses among heart transplant recipients after receiving a third dose of the BNT162b2 mRNA vaccine. Recently, Schrezenmeier *et al.* demonstrated improved cellular and humoral response following a third dose of the BNT162B2 mRNA vaccine among 25 kidney transplant recipients who failed to respond to the first 2 doses[25]; 36% of the patients exhibited seroconversion after the third dose (either ChAdOx1 (AstraZeneca) (n = 11) or BNT162b2 mRNA, n = 14). Schrezenmeier *et al.* also reported significant quantitative and functional changes within the spike-specific B cell and $CD4^+$ T-helper cell compartment, but did not perform a correlation analysis.[25] Moreover, Bange *et al.*[26] reported T-cell protection against severe COVID-19 disease among patients with hematological malignancies and insufficient humoral immune responses after anti-CD20 therapy. We identified a significant increase in T-cell counts after the third vaccine dose in all our LT recipients, however, no correlation was found between their NA and anti-RBD IgG titers. This can be explained by the low humoral response among non-responders after the second vaccine and the small sample size of T-cell samples evaluated in the non-responders group.

Despite the overall improved immune responses, 2 of the 61 patients with a positive immune response to the third vaccine dose had a breakthrough SARS-CoV-2 infection. A recent report on COVID-19 breakthrough infections among vaccinated healthcare workers at our center[23] suggested that peak antibody titers correlate with protection level. The predicted geometric mean titers of per-infection NA were 192.8 (95% CI 67.6-549.8) for cases and 533.7 (95% CI 408.1- 698.0) for controls and IgG 16.3 (95% CI 7.4–35.8) for cases and 32.2 (95% CI 28.6–36.2) for controls.[23] In our cohort, 2 patients became infected with SARS-CoV-2 after the third vaccination during the fourth wave of infection in Israel; neither patient developed severe disease. The breakthrough infection probably resulted from several factors, including waning immunity and the emergence of viral variants. The degree of protection provided by NA, IgG and T cells in immunocompromised patients remains to be determined. We can hypothesize that the humoral and immune responses seen in our cohort of patients 3 weeks after the third dose are likely to decline, similarly to the pattern observed 5-6 months after the second dose. Assessment of the durability of the immune response after the third dose is our next research goal; samples will soon be collected and analyzed.

The presented study was limited by its small sample size. In addition, although our findings revealed significantly improved humoral and cellular immune responses following the third vaccine dose among LT recipients, no comparative data was provided regarding such responses among healthy individuals. While 2 patients developed SARS-COV-2 infection and suffered from mild disease that did not require hospitalization, conclusions regarding protection against severe infection cannot be made at this time point.

In summary, the third BNT162b2 mRNA vaccine significantly improved humoral and cellular immune responses among LT recipients, particularly among non-responders to the second vaccine, and enhanced the immune response among LT recipients who showed a waning immune response after the second vaccine dose. The third vaccine dose was not associated with severe adverse effects. Further studies will be necessary to determine whether a 5-month period between booster doses is necessary or if scheduling vaccination in immunocompromised patients requires optimization, with particular emphasis on SOT recipients treated with combined immunosuppressive therapies.

Abbreviations

CNI, calcineurin inhibitors; LT, liver transplantation; MMF, mycophenolate mofetil; NA, neutralizing antibody; PBMCs, peripheral blood mononuclear cells; RBD, receptor binding domain; S/CO, sample/cut-off; SOT, solid organ transplantation.

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Conflict of interest

The authors of this manuscript have no conflicts of interest to disclose.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

YD, VI, KT, OCE, ML, GBY, RH, IL: patient recruiting and data collection. VI, OM, YL: laboratory assays. YD: statistical analysis. YD and ZBA: prepared the manuscript. YD, VI, NAL, GR, YL, ZBA, AA: reviewed the manuscript for scientific content and contributed to study design and laboratory assays.

Data availability statement

Data regarding patients included in the study are confidential. Some anonymous data can be made available upon request.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.03.042.

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Author names in bold designate shared co-first authorship

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