OPEN

Clinical Course, Immunogenicity, and Efficacy of BNT162b2 mRNA Vaccination Against SARS-CoV-2 Infection in Liver Transplant Recipients

Eunice X. Tan, MBBS, MMED, MRCP,[1,2,3] Wen Hui Lim, MBBS,[1] Elizabeth Thong, MBBS,[4] Jean-Marc Chavatte, PhD,[5] Jinyan Zhang, MBBS,[6] Jonathan Lim, MBBS,[5] Jocelyn Y. Jin, PhD,[5] Daniel R.X. Lim, MBBS,[5] Jaclyn Y.T. Kang, MBBS,[5] Ansel Shao Pin Tang, MBBS,[1] Kai En Chan, MBBS,[1] Caitlyn Tan, MBBS,[1] Shi Ni Tan, MBBS,[2] Benjamin Nah, MBBS, MRCP,[2] Daniel Q. Huang, MBBS, MMED, MRCP,[1,2,3] Lin-Fa Wang, PhD,[6] Paul A. Tambyah, MBBS, Diplomate ABIMID,[7] Jyoti Somani, AB, MD, Diplomate ABIMID,[1,7] Barnaby Young, MSc, MB, BChir, MRCP, DLSHTM,[5,8,9] and Mark D. Muthiah, MBBS, MRCP, MMed[1,2,3]

Background. Immunocompromised individuals have been excluded from landmark studies of messenger RNA vaccinations for severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). In such patients, the response to vaccination may be blunted and may wane more quickly compared with immunocompetent patients. We studied the factors associated with decreased antibody response to SARS-CoV-2 vaccination and risk factors for subsequent breakthrough infections in liver transplant (LT) patients undergoing coronavirus disease 2019 vaccination with at least 2 doses of messenger RNA vaccine from April 28, 2021, to April 28, 2022. **Methods.** All LT recipients received at least 2 doses of the BNT162b2 (Pfizer BioNTech) vaccine 21 d apart. We measured the antibody response against the SARS-CoV-2 spike protein using the Roche Elecsys immunoassay to the receptor-binding domain of the SARS-CoV-2 spike protein, and the presence of neutralizing antibodies was measured by the surrogate virus neutralization test (cPass) before first and second doses of vaccination and also between 2 and 3 mo after the second dose of vaccination. **Results.** Ninety-three LT recipients who received 2 doses of BNT162b2 were included in the analysis. The mean time from LT was 110 ± 154 mo. After 2-dose vaccination, 38.7% of LT recipients (36/93) were vaccine nonresponders on the cPass assay compared with 20.4% (19/93) on the Roche S assay. On multivariable analysis, increased age and increased tacrolimus trough were found to be associated with poor neutralizing antibody response ($P = 0.038$ and 0.022, respectively). The use of antimetabolite therapy in conjunction with tacrolimus approached statistical significance (odds ratio 0.21; 95% confidence interval, 0.180-3.72; $P = 0.062$). Breakthrough infection occurred in 18 of 88 LT recipients (20.4%). Female gender was independently associated with breakthrough infections ($P < 0.001$). **Conclusions.** Among LT recipients, older age and higher tacrolimus trough levels were associated with poorer immune response to 2-dose SARS-CoV-2 vaccination. Further studies are needed to assess variables associated with breakthrough infections and, hence, who should be prioritized for booster vaccination.

(*Transplantation Direct* 2023;9: e1537; doi: 10.1097/TXD.0000000000001537.)

Received 22 April 2023. Revision received 22 June 2023.

Accepted 7 July 2023.

[1] Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

[2] Division of Gastroenterology and Hepatology, Department of Medicine, National University Hospital, Singapore, Singapore.

[3] National University Centre for Organ Transplantation, National University Health System, Singapore.

[4] Department of Medicine, National University Hospital, Singapore, Singapore.

[5] National Centre for Infectious Diseases, Singapore.

[6] Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore.

[7] Division of Infectious Diseases, Department of Medicine, National University Hospital, Singapore.

[8] Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore.

[9] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore.

Correspondence: Eunice X. Tan, MBBS, MMED, MRCP, Division of Gastroenterology and Hepatology, Department of Medicine, National University Hospital, Singapore. (eunice_xx_tan@nuhs.edu.sg).

No writing assistance was obtained in the preparation of the article. The article, including related data, figures, and tables has not been previously published and that the article is not under consideration elsewhere.

E.X.T. and W.H.L. share co-first authorship.

B.Y. and M.D.M. share co-last authorship.

L.-F.W. and J.Z. from Duke-NUS Medical School received grants from the National Medical Research Council (STPRG-FY19-001, COVID19RF-003, COVID19RF-060, and OFLCG19May-0034).

L.-F.W. is a coinventor of the cPass assay used in this study. P.A.T. receives research support from Sanofi Pasteur, Arcturus, Institut Merieux and Roche. The other authors declare no conflicts of interests.

The study was conducted in accordance with the Declaration of Helsinki. All participants signed a written informed consent and study protocol was approved by the local institutional review board (DSRB 2012/00917).

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be submitted. All authors approve the final version of the article, including the authorship list and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. E.X.T., B.Y., and M.D.M. participated in conceptualization and design.

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001537

In the initial clinical trials investigating the efficacy and safety of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) vaccines, various immunocompromised or immunosuppressed patient populations were excluded.[1,2] Immunocompromised patients are at greater risk of severe infection and death from coronavirus disease 2019 (COVID-19) compared with those without existing chronic disease. Thus, despite the initial lack of data, vaccination of liver transplant (LT) recipients was recommended by professional societies.[3,4] Since then, various studies to measure the immunogenicity of the SARS-CoV-2 vaccination in LT recipients have been reported. Results from multiple centers have shown that LT recipients mount a reduced antibody response after a primary 2-dose vaccine series compared with immunocompetent patients.[5-8] Nevertheless, compared with unvaccinated immunocompromised patients, vaccinated immunocompromised people with breakthrough infections had a less severe disease course.[9]

Immunogenicity of the SARS-CoV-2 vaccines in most studies has been assessed by quantifying antibodies to the immune-dominant viral spike protein. However, these assays do not differentiate between general binding antibodies and neutralizing antibodies,[10] which are a subset of secreted antibodies that have been demonstrated to prevent SARS-CoV-2 viral entry into human cells.[11-14] Currently, the gold-standard virus neutralization test requires live cells and viruses in a BSL3 containment laboratory and results may require 2 to 4 d.[15] The cPass SARS-CoV-2 Neutralization Antibody Detection Kit (GenScript, NJ; cPass) is a novel surrogate virus neutralization test that can assess the presence of circulating antibodies that block the interaction of receptor-binding domain (RBD)–horseradish peroxidase with human angiotensin-converting enzyme 2 with high correlation to the gold-standard live-cell plaque reduction neutralization test (PRNT).[10] Therefore, cPass technology is complementary to antispike protein antibody tests, which do not measure neutralizing antibodies.

Immunocompromised patients are at greater risk of severe disease and possibly reinfections during this long, drawn-out COVID-19 pandemic. Although SARS-CoV-2–infected individuals may have detectable antibodies present for several months after seroconversion, the temporal persistence of neutralizing antibodies has been shown to decline.[10,16,17] The immune response of LT recipients to a primary course of COVID-19 vaccination and its relation to immunosuppression use and breakthrough infections has yet to be well reported. In this study, we described the neutralizing antibody responses of LT patients to 2 doses of the BNT162b1 BioNTech vaccination 21 d apart, using the cPass assay. We sought to identify the factors associated with antibody response to SARS-CoV-2 vaccination and breakthrough infections in these vaccinated LT recipients.

E.T., S.N.T., B.N., D.R.X.L., and J.Y.T.K. participated in the acquisition of data. W.H.L., A.S.P.T., K.E.C., C.T., D.R.X.L., and J.Y.T.K. participated in analysis and interpretation of data. E.X.T. and W.H.L. participated in writing the original draft. E.X.T., J.-M.C., J.Z., J.L., J.Y.J., A.S.P.T., L.-F.W., P.A.T., J.S., B.Y., and M.D.M. participated in writing, review, and editing.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

MATERIALS AND METHODS

Study Design

From March 2021, vaccination of LT recipients was recommended by the Ministry of Health, Singapore. This study includes 100 consecutive post-LT patients followed up in a single-center LT unit from April 29, 2021, to April 28, 2022. LT recipients in this center followed an immunosuppression protocol as previously described.[18] Inclusion criteria included the ability to provide informed consent. Exclusion criteria for this study included past or current infection with SARS-CoV-2 at the time of screening, multiorgan transplant, age younger than 21 y, contraindications to SARS-CoV-2 vaccination, such as previous anaphylactic reactions to components of the SARS-CoV-2 vaccination or immediate (<1 mo) post-LT, recent acute graft rejection (<1 mo), and inability to provide informed consent. All participants signed a written informed consent. The study protocol was approved by the local institutional review board (DSRB 2012/00917).

Patient Information

Clinical data such as cause of liver disease before LT; presence of chronic diseases such as diabetes, hypertension, and hyperlipidemia; type and dose of immunosuppression; and trough levels of immunosuppressants were obtained from patient medical records. Symptoms following vaccination, including graft rejection and symptomatic SARS-CoV-2 infection, if any, were collected. Breakthrough infection was defined as SARS-CoV-2 infection diagnosed at least 2 wk after the primary vaccination series (up to 3 doses of vaccination for post-LT recipients on immunosuppression).

Study Protocol

Blood samples were drawn from consenting patients at the baseline immediately before the first and second doses of vaccination, which were dosed 21 d apart and 8 to 12 wk after the second dose of vaccination. Data of liver tests, dosages of immunosuppression, and immunosuppression trough levels where available were collected at each study visit. At each study visit, patients were assessed for antibody response against the SARS-CoV-2 spike protein using the Elecsys (Roche, Basel, Switzerland) immunoassay to the RBD of the SARS-CoV-2 spike protein, and the presence of neutralizing antibodies targeting the viral spike protein RBD was measured by the cPass (Genscript, NJ) assay in the blood. During the study period from April 28, 2021, to April 28, 2022, the incidence of SARS-CoV-2 infection in Singapore ranged from 0.73 to 4773 new cases/million.[19] Notably, Singapore experienced a surge in the COVID-19 delta variant from September to December 2021 and the in the omicron variant from January to March 2022.

Quantification of Antibodies to SARS-CoV-2 Spike Protein and Surrogate Virological Neutralization Test cPass

Serological testing for total antibodies specific to the RBD of the SARS-CoV-2 S protein (anti-S) was performed using the Elecsys Anti–SARS-CoV-2 S electrochemiluminescence immunoassay with the Cobas e601 analyzer (Roche Diagnostics), according to the manufacturer's instruction. A test result of ≥0.8 U/mL was considered positive. Samples

with concentration >250 U/mL were diluted further (1:10, 1:100, and 1:1000) within the measurement range of the assay (0.4–250 U/mL).

The development and validation of the cPass assay has been previously reported.[10] Briefly, a biochemical measurement of the amounts of neutralizing antibody present in the test sera was done by inhibition ELISA, in which the test sera were first preincubated with SARS-CoV-2 or SARS-CoV-2 RBD–horseradish peroxidase and then added to angiotensin-converting enzyme 2-coated plates. The cPass test achieves a 99.93% specificity and 95% to 100% sensitivity when using a cutoff of >30% compared with a PRNT.[10]

Statistical Analysis

Statistical analysis was conducted using STATA (version 16.1; StataCorp). Descriptive statistics were summarized as mean (SD) for continuous variables and as number of patients (percentage) in each group for categorical variables. Pearson chi-square statistic was used to assess the statistical significance for categorical data, whereas continuous variables were compared by the *t* test if normally distributed or by the Mann-Whitney test if abnormally distributed. Logistic regression with a robust variance estimator was used to estimate the odds of positive seroconversion after 2 doses of COVID-19 vaccination. Variables were checked for multicollinearity before multivariable regression was conducted with a robust variance estimator to assess factors associated with immunogenicity. A *P* value of <0.05 was considered statistically significant for all analyses.

RESULTS

Participant Baseline Characteristics

Ninety-three LT recipients were included in the analysis. Seven patients dropped out of the study before the second dose of vaccination and were thus not analyzed—6 withdrew because of inability to comply with multiple blood draws, whereas 1 patient transferred care to another hospital. All patients underwent 2 doses of the Pfizer BioNTech vaccination, and none of the patients had acute graft rejection or significant changes in liver function after immunization. The mean age of LT recipients was 55.5 ± 15.3 y, and the mean time from LT to first vaccination was 110 ± 154 mo. Most patients were of Chinese ethnicity, and the most common indication for LT was viral hepatitis (32.3%), followed by autoimmune disease (11.8%) and nonalcoholic fatty liver disease (8.60%). Almost three-quarters of the patients received deceased donor LT, and about a third had a history of hepatocellular carcinoma. Approximately two-fifths of patients (41.9%) had hypertension, half of the patients (47.3%) had diabetes, and about a third of patients (30.1%) had dyslipidemia. The mean time from the second dose of vaccination to the time of serology draw was 65.2 ± 20.4 d. Calcineurin inhibitors (CNIs) were used as the backbone of the immunosuppressive regimen in 92.5% of the patients. Everolimus was used in 11 patients (11.8%) and mostly in combination with reduced-dose CNIs (8/11; 72.7%). Forty-two percent of patients received mycophenolate mofetil or mycophenolic acid. Approximately three-fifths of patients (59.3%) had received basiliximab for induction immunosuppression at the time of LT. Approximately two-fifths were on single immunosuppressant (40.2%) and double immunosuppressant (43.5%), respectively, and one-sixth of the patients (16.3%) were on triple immunosuppressant at the time of first dose of vaccination (**Table 1**).

Baseline Characteristics and Humoral Response Measured Using the cPass Assay After 2-dose Vaccination

In total, 38.7% of LT recipients (n = 36) had insufficient neutralizing antibody response, whereas 61.3% of recipients (n = 57) had sufficient neutralizing antibodies postvaccination as measured by the cPass assay. Ethnicity, history of hepatocellular carcinoma, and type of transplant were not significantly different between those who had sufficient neutralizing antibodies (vaccine responders) and those who did not (vaccine nonresponders). Vaccine responders were younger (53.9 ± 15.9 y versus 60.0 ± 13.1 y, $P = 0.04$) and were more likely to have had underlying hepatitis B or alcoholic liver disease compared with nonalcoholic fatty liver disease ($P = 0.01$; $P = 0.02$, respectively) or autoimmune disease ($P = 0.001$; $P = 0.006$, respectively). Additionally, they were significantly further out from transplant surgery compared with vaccine nonresponders (147 ± 159 versus 56.6 ± 51.2 mo, $P < 0.001$; **Table 1**). A smaller proportion of vaccine responders had hypertension, ischemic heart disease, or stroke (55.6% versus 33.3%, $P = 0.03$; 38.9% versus 8.77%, $P < 0.001$; 11.1% versus 1.75%, $P = 0.05$) compared with nonresponders. The mean time from the second dose of vaccination to the time of blood draw was 65.2 ± 20.4 d, which was significantly different between both groups—70.0 ± 20.4 versus 57.9 ± 18.3 d, $P = 0.002$, in vaccine responders and vaccine nonresponders, respectively. However, this is unlikely to be clinically significant, given that the peak of immunogenicity after the second dose of vaccination studied in LT patients is reached in the first month after 2-dose vaccination.[20] There was a significantly lower proportion of patients on double and triple immunosuppressants in the vaccine responder compared with vaccine nonresponders (32.1% versus 61.1%, $P = 0.006$; 5.36% versus 33.3%, $P < 0.001$). The mean tacrolimus trough level at first and second doses of vaccination was also significantly lower in patients in vaccine responders compared with vaccine nonresponders (3.49 ± 1.63 versus 5.02 ± 2.11 ng/mL, $P < 0.001$; 3.46 ± 1.50 versus 5.24 ± 2.40, $P < 0.001$; **Table 1**).

Risk Factors for Vaccine Nonresponse After 2-Dose Vaccination

On univariate analysis, the presence of hypertension (odds ratio [OR]: 0.40; 95% confidence interval [CI], 0.169-0.947; $P = 0.037$) and ischemic heart disease (OR: 0.151; 95% CI, 0.048-0.474; $P = 0.001$) was found to be associated with decreased odds of positive immunogenicity on the cPass test (**Table S1, SDC,** http://links.lww.com/TXD/A566). Similarly, LT recipients who were on double- or triple-maintenance immunosuppressants had decreased odds of positive cPass test results (OR: 0.301, 95% CI, 0.125-0.726; $P = 0.007$; OR: 0.113; 95% CI, 0.029-0.442; $P = 0.002$). A combination of CNIs and antimetabolites such as mycophenolic acid or mycophenolate mofetil (OR: 0.071; 95% CI, 0.026-0.197; $P < 0.001$) and a combination of CNI and prednisolone (OR: 0.219; 95% CI, 0.068-0.701; $P = 0.011$) were also significantly associated with nonresponse to the vaccine. However, this was not seen with a combination of CNI and everolimus. Lower tacrolimus trough levels at a cutoff of ≤5 ng/mL (OR:

TABLE 1.

Baseline characteristics of LT recipients and comparison of recipients with negative and positive SARS-CoV-2 S1/S2 IgG serology based on cPass immunoassay after 2 doses of the Pfizer BioNTech vaccination

Parameter	All LT patients (n = 93)	Vaccine nonresponders (n = 36)	Vaccine responders (n = 57)	*P*
Biodata	55.5 (15.3)	60.0 (13.1)	53.9 (15.9)	0.04*
Age, mean (SD)				
Male gender, n (%)	61 (65.6)	22 (61.1)	39 (68.4)	0.47
Ethnicity, n (%)				
Chinese	67 (72.0)	29 (80.6)	38 (66.7)	0.545
Malay	11 (11.8)	3 (8.33)	8 (14.0)	
Indian	8 (8.60)	2 (5.56)	6 (10.5)	
Others	7 (7.53)	2 (5.56)	5 (8.77)	
Background medical history				
BMI, mean (SD)	25.7 (5.21)	26.1 (5.54)	25.8 (5.08)	0.88
HbA1c, mean (SD)	6.19 (1.40)	6.27 (1.50)	6.20 (1.39)	0.53
eGFR, mean (SD)	73.9 (31.2)	78.3 (32.9)	69.7 (29.3)	0.14
HTN, n (%)	39 (41.9)	20 (55.6)	19 (33.3)	0.03*
DM, n (%)	44 (47.3)	20 (55.6)	24 (42.1)	0.21
HLD, n (%)	28 (30.1)	14 (38.9)	14 (24.6)	0.14
IHD, n (%)	19 (20.4)	14 (38.9)	5 (8.77)	<0.001*
Stroke, n (%)	5 (5.38)	4 (11.1)	1 (1.75)	0.05*
Cause of liver disease, n (%)				
NAFLD	8 (8.60)	7 (19.4)	1 (1.75)	<0.001*
ALD	7 (7.53)	1 (2.78)	6 (10.5)	
Hepatitis B	23 (24.7)	6 (16.7)	17 (29.8)	
Hepatitis C	7 (7.53)	2 (5.56)	5 (8.77)	
Autoimmune[a]	11 (11.8)	10 (27.8)	1 (1.75)	
Others[b]	36 (38.7)	10 (27.8)	26 (45.6)	
Presence of HCC, n (%)	19 (32.2)	9 (40.9)	10 (27.0)	0.27
DDLT, n (%)	68 (73.1)	26 (72.2)	42 (73.7)	0.88
Months after first transplantation, mean (SD)	110 (154)	56.6 (51.2)	147 (159)	<0.001*
Induction immunosuppression, n (%)				
Methylprednisolone	22 (40.7)	10 (45.5)	12 (37.5)	0.56
Basiliximab	32 (59.3)	12 (54.5)	20 (62.5)	0.56
Immunosuppression				
Tacrolimus, n (%)	86 (92.5)	35 (97.2)	51 (89.5)	0.17
Antimetabolite, n (%)	39 (41.9)	28 (77.8)	11 (19.3)	<0.001*
Dose, mg, mean (SD)	767 (359)	849 (328)	550 (335)	0.008*
Cellcept, n (%)	10 (10.8)	4 (11.1)	6 (10.5)	0.93
Dose, mg, mean (SD)	790 (421)	1125 (250)	567 (363)	0.01*
Myfortic, n (%)	29 (31.2)	24 (66.7)	5 (8.77)	<0.001*
Dose, mg, mean (SD)	830 (356)	855 (349)	720 (360)	0.22
Prednisolone, n (%)	16 (17.2)	11 (30.6)	5 (8.77)	0.007*
Dose, mg, mean (SD)	5.98 (2.91)	6.56 (3.01)	4.70 (2.49)	0.22
Everolimus, n (%)	11 (11.8)	5 (13.9)	6 (10.5)	0.11
Dose, mg, mean (SD)	2.61 (1.16)	3.10 (1.24)	2.21 (1.16)	0.11
Single immunosuppression regime, n (%)	37 (40.2)	2 (5.56)	35 (62.5)	<0.001*
Double immunosuppression regime, n (%)	40 (43.5)	22 (61.1)	18 (32.1)	0.006*
Triple immunosuppression regime, n (%)	15 (16.3)	12 (33.3)	3 (5.36)	<0.001*
Tacrolimus trough levels, ng/mL, mean (SD)				
Day of first dose of vaccination	4.20 (1.98)	5.02 (2.11)	3.49 (1.63)	0.0006*
Day of second dose of vaccination	4.20 (2.10)	5.24 (2.40)	3.46 (1.50)	0.0004*
No. of days from dose 1 to final serology collection, mean (SD)	88.9 (19.3)	82.6 (17.0)	93.1 (19.7)	0.004*
No. of days from dose 1 to final serology collection, mean (SD)	65.2 (20.4)	57.9 (18.3)	70.0 (20.4)	0.002*

For all categorical variables, the chi-square statistic was used. Continuous variables were compared using the *t* test if normally distributed or the Mann-Whitney test if nonnormally distributed. $P < 0.05$ was considered statistically significant for all analyses.

[a]Autoimmune disease includes autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cirrhosis.

[b]Other causes include drug-induced liver injury, Wilson's syndrome, Budd-Chiari syndrome, hepatic artery thrombosis, acute liver failure, and cryptogenic liver cirrhosis.

ALD, alcoholic liver disease; BMI, body mass index; DDLT, deceased donor liver transplantation; DM, type II diabetes; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HCC, hepatocellular carcinoma; HLD, hyperlipidemia; HTN, hypertension; IHD, ischemic heart disease; LT, liver transplant; NAFLD, nonalcoholic fatty liver disease; SARS-CoV-2, severe acute respiratory syndrome coronavirus type 2.

*Statistically significant.

4.88; 95% CI, 1.80-13.2; $P = 0.002$) and antimetabolite daily dose equivalent of ≤500 mg/d of mycophenolate mofetil (OR: 5.52; 95% CI, 1.17-26.03; $P = 0.020$) were significantly associated with positive neutralizing antibodies (**Table S1, SDC,** http://links.lww.com/TXD/A566). However, this association was not observed with either prednisolone or everolimus use. Additionally, patients who were >5 y from transplant surgery were 7.69 times (95% CI, 2.99-19.82; $P < 0.001$) as likely to develop vaccine response on cPass after 2 doses of vaccination as compared with patients ≤5 y from transplant surgery (**Table S1, SDC,** http://links.lww.com/TXD/A566). On multivariable analysis, the variables associated with negative cPass results after 2 doses of vaccination were increased age and increased tacrolimus trough levels. The use of antimetabolite therapy in conjunction with tacrolimus approached statistical significance (OR: 0.21; 95% CI, 0.042-1.08; $P = 0.062$; **Table 2**).

Differences in Roche Antispike Serology and cPass Assay Results After 2-Dose Vaccination

At baseline, all patients had a negative SARS-CoV-2 N-protein IgG serology test. Only three-fifths of patients (57/93; 61.3%) developed antibody responses to 2 doses of the Pfizer BioNTech vaccination as measured by the cPass assay, with a cutoff of 30% inhibition.[10] The mean cPass level was 50% ± 35.2% after the second dose of vaccination. Comparatively, in the Roche S antibody measurement, four-fifths of patients (74/93; 79.6%) developed positive serology to 2 doses of vaccination, and mean antibody titers after the second dose was 597 ± 1084 U/mL (**Table 3**).

In total, 93 patients had valid cPass and Roche S antibody results after a 2-dose Pfizer BioNTech vaccination. Of these 93 patients, 76 (81.7%) had concordance between the cPass and Roche S assay, in which 57 patients were positive in both cPass and Roche S results and 19 had both negative cPass and Roche S results. The remaining 17 of 93 patients (18.3%) had discordant results from the 2 tests, in which all 17 patients had positive Roche S antibodies despite negative cPass results (**Table S2, SDC,** http://links.lww.com/TXD/A566).

Vaccine Response to 3-Dose SARS-CoV-2 Vaccination and Breakthrough Infections

Of the 93 patients included in the study, 88 patients have since completed 3 doses of SARS-CoV-2 vaccination, but only 69 patients at the time of writing have been tested for serological response after 3 doses of vaccination. Of these 69 patients, 34 patients had no vaccine response following the second dose of SARS-CoV-2 vaccination. Thirteen of 34 patients (38.2%) developed vaccine response measured by a cPass assay after 3 doses of SARS-CoV-2 vaccination (**Table 4**).

A total of 24 patients were diagnosed to have SARS-CoV-2 infection via polymerase chain reaction from nasopharyngeal swabs during a mean study period of 12 mo, of whom 18 patients had a breakthrough infection. In total, a fifth of 18 of 88 patients (20.4%) who received 3 doses of vaccination had breakthrough infections.

The baseline characteristics of patients with and without breakthrough infections were largely comparable. Of the 88 patients who were included in the final analysis, patients with breakthrough infections were further out from transplant (141 ± 102 versus 89.5 ± 69.1 mo, $P = 0.06$) post-LT, although not statistically significant. Notably, there were no significant differences in terms of immunosuppression use and trough levels between the 2 groups. Additionally, the time from the

TABLE 2.

Adjusted regression analysis for factors associated with positive seroconversion

Parameter	Multivariable OR	95% CI	*P*
Age	0.93	0.867-0.996	0.038[a]
CNI monotherapy	5.10	0.615-42.2	0.13
CNI + antimetabolite	0.21	0.042-1.08	0.062
CNI + prednisolone	0.82	0.180-3.72	0.80
Time from transplant to first dose of vaccination	1.00	0.991-1.02	0.53
Tacrolimus trough	0.57	0.358-0.922	0.022[a]
HTN	0.85	0.162-4.46	0.85
IHD	0.29	0.062-1.38	0.12
NAFLD	0.43	0.076-2.38	0.33

[a]$P < 0.05$ was considered statistically significant for all analyses.
CI, confidence interval; CNI, calcineurin inhibitor; HTN, hypertension; IHD, ischemic heart disease; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.

TABLE 3.

Serological response after first and second doses of the Pfizer BioNTech vaccination

	cPass			Roche S		
	Pre–dose 1	Post–dose 1	Post–dose 2	Pre–dose 1	Post–dose 1	Post–dose 2
No. of serum samples available for analysis, N	100	97	93	99	98	93
Seropositive, n/N (%)	0/100 (0.00%)	22/97 (22.7%)	57/93 (61.3%)	0/99 (0.00%)	39/98 (39.8%)	74/93 (79.6%)
Antibody level, mean (SD)	1.92 (5.79)	14.8 (18.2)	50.0 (35.2)	0.402 (0.0235)	10.3 (23.5)	597 (1084)

$P < 0.05$ was considered statistically significant for all analyses. Units of measurement for antibody levels for cPass and Roche S immunoassay were % and U/mL respectively.

TABLE 4.

Comparison of serological response between immunoassays in patients with breakthrough COVID-19 infection after the third dose of the Pfizer BioNTech vaccination

Post–dose 3	COVID-19 negative (n = 55)	COVID-19 positive (n = 14)	*P*
cPass seropositive[a]	39 (70.9)	13 (92.9)	0.089
Antibody level (%)	65.1 (37.5)	85.6 (24.7)	0.132
Roche S seropositive[a]	49 (98.0)[b]	14 (100.0)	0.594
Antibody level (U/mL)	7178(12 386)	14 521(26 311)	0.056

P < 0.05 was considered statistically significant for all analyses.
[a]Mean (SD); units of measurement for antibody levels for cPass and Roche S immunoassay were % and U/mL, respectively.
[b]Only n = 50 patients who were COVID-19 negative had valid Roche S serologies.
COVID-19, coronavirus disease 2019.

third dose of vaccination to the time of serological assessment was not significantly different between the 2 groups: 68.8 ± 32.3 versus 71.6 ± 28.5 d, *P* = 0.996 (**Table S3, SDC,** http://links.lww.com/TXD/A566).

Of the 88 patients who completed 3 doses of vaccination, serology levels were checked after the third dose of vaccination for 69 patients and 64 patients using cPass and Roche S, respectively. There were no significant differences found in the cPass and Roche S levels in both groups: 65.1 ± 37.5 versus 85.6 ± 24.7%, *P* = 0.132; 7178.25 ± 12 386.19 versus 14 521.02 ± 26 311.81 U/mL, *P* = 0.056, respectively (**Table 4**).

On multivariable analysis, only female gender was independently associated with breakthrough infection (**Table 5**). Age, number of immunosuppression, degree of renal impairment, and use of antimetabolite were not associated with the presence of breakthrough infections.

DISCUSSION

In this study, we assessed the antibody response after 2 doses of BNT162b1 Pfizer/BioNTech vaccine 21 d apart in LT recipients, where humoral response was measured by both cPass assay (GenScript) and Roche S. We found that approximately three-fifths of LT recipients had sufficient neutralizing antibodies after 2-dose vaccination. Using the Roche S immunoassay, this proportion was higher—whereby approximately four-fifths of the patients had positive immunogenicity to 2-dose BNT162b1 Pfizer/BioNTech vaccination (**Figure 1**). This could reflect differences in the accuracy of each test, although the gold-standard live-cell PRNT was not done in this study. Here, we found that increased age and increased tacrolimus trough levels were associated with insufficient neutralizing antibodies after 2 doses of vaccination. Concurrent use of antimetabolite and tacrolimus was also independently associated with negative immunogenicity, with a trend toward significance. Breakthrough infections were common, occurring in about 20% of the LT recipients in this study. However, none of the patients in this study had severe infection or required supplemental oxygenation or intensive care unit stay.

TABLE 5.

Adjusted regression analysis for factors associated with breakthrough infection

Parameter	Multivariate OR	95% CI	*P*
Age	0.965	0.907-1.03	0.265
Female gender	7.36	1.66-32.7	**0.009**
eGFR	1.01	0.968-1.05	0.689
Use of antimetabolite	0.165	0.0257-1.05	0.057
No. of immunosuppressants			
Double	3.72	0.625-22.2	0.149
Triple	0.201	0.050-8.01	0.384

P < 0.05 was considered statistically significant for all analyses.
CI, confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio.

Previous studies have largely quantified immunogenicity to SARS-CoV-2 vaccination based on antibodies to the RBD of the spike protein.[5-7,21,22] However, these cannot differentiate between general binding antibodies and neutralizing antibodies, a subset of secreted antibodies that prevents SARS-CoV-2 viral entry into human cells.[11-14] Conversely, the cPass assay is a surrogate virus-neutralizing antibody test with a high correlation to the gold-standard live-cell PRNT.[10] To our best knowledge, this is the first study in LT recipients that uses cPass technology to assess antibody response after 2-dose vaccination. As compared with the Roche S antibody, for which seropositivity was seen in 79.6% of our patients, only 61.3% of patients had sufficient neutralizing antibodies based on cPass. These findings could suggest that using antispike antibody serology alone may be insufficient to guide the decision-making process for vaccination in LT patients.

Existing studies have shown that age, higher doses of steroids, triple immunosuppression, use of antimetabolite therapy, and poorer renal function are associated with negative serology in post–solid organ transplant patients.[6,22,23] Older age has also been described as an important predictor of vaccine immune response and rate of waning after a 2-dose series of BNT162b2.[24] Our study, which measured neutralizing antibodies, reported results in keeping with these findings, where increased age and use of concurrent antimetabolite were associated with insufficient neutralizing antibodies on multivariable analysis. Moreover, our study also found that higher trough tacrolimus levels were associated with insufficient neutralizing antibodies. The cutoff tacrolimus trough level associated with improved vaccine response on univariate analysis was <5 ng/mL. This may have wider clinical implications and suggests that physicians may consider decreasing net immunosuppression to as low a dose as possible before initiation of vaccination to achieve improved immunogenicity to the vaccine.

There remains a scarcity of data regarding breakthrough infections in LT recipients. In a smaller subset of patients (n = 59), we found that neither measures of immunogenicity nor number of immunosuppressant uses were associated with breakthrough infections. Surprisingly, there were also no significant associations between antimetabolite use, age, and comorbidities such as diabetes with breakthrough infections. This could be in part contributed by the small number of patients who had valid after the third dose of SARS-CoV-2 vaccination, which limited statistical power. The only variable that predicted breakthrough infection was the female gender. Indeed, there are existing studies that found similar findings, where the majority of patients with breakthrough infections were female, although the mechanism behind this is presently still unclear.[25,26] An important consideration is that patients with breakthrough infections had serology measurements of cPass levels at a mean of 2 mo after the third dose of vaccination but tested positive for SARS-CoV-2 infection at >4 mo after the third dose of vaccination. Serology assessment

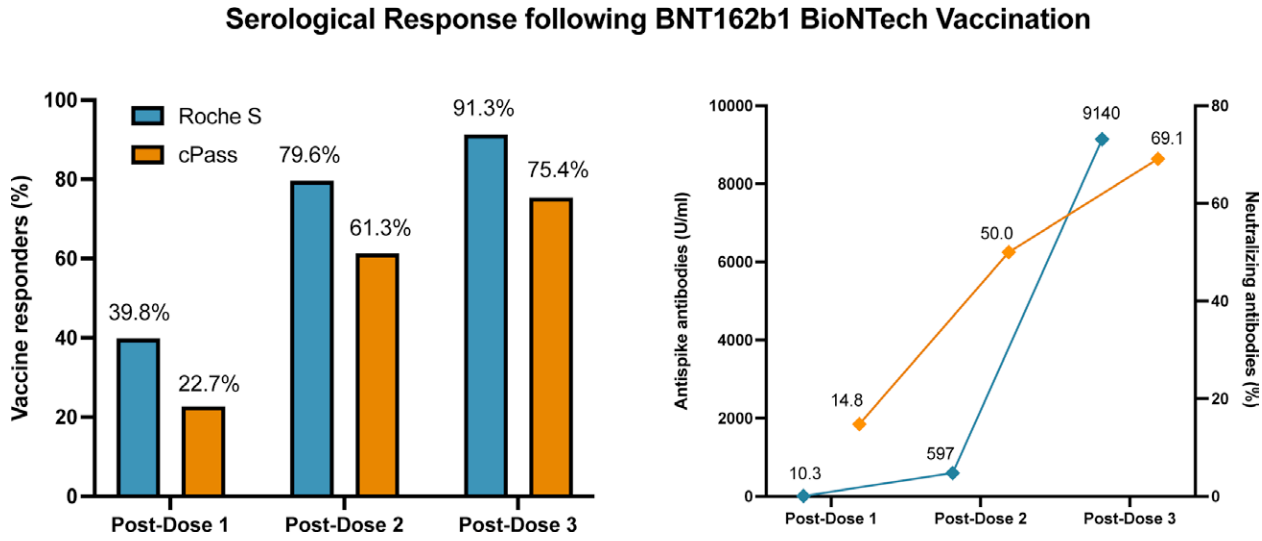

FIGURE 1. Overall serological response following Pfizer BioNTech vaccination.

of immunogenicity was also not performed at the time of infection. This is an important limitation, as even if SARS-CoV-2–infected individuals may have detectable antibodies present for several months after seroconversion, the temporal persistence of neutralizing antibodies has been shown to decline.[10,16,17] Our results may also suggest that measurement of antibody levels postvaccination may not directly correlate with the chance of breakthrough infection, which differs from studies performed in the general population.[27,28] Other factors such as the intensity of epidemiological exposures, individual T-cell responses, or innate immune responses may have contributed to the differences between those who had breakthrough infections and those who did not.

Strengths and Limitations

The strength of this study is the utilization of cPass technology as a measure of vaccine response in LT recipients. The application of cPass technology is more specific to neutralizing antibodies, thereby allowing for more robust results in the quantification of immunogenicity to SARS-CoV-2 compared with the use of Roche S antibody alone. This study also provides preliminary data demonstrating that tacrolimus trough level is independently associated with decreased vaccine response, which may guide the implementation of clinical strategies aimed at risk mitigation. Additionally, breakthrough infections were found to be common postvaccination among LT recipients and occurred at a mean of 4 mo postvaccination despite reasonable antibody levels in more than half of our patients. More studies with larger sample sizes and longer longitudinal assessments are warranted to confirm these findings.

Notwithstanding, the study findings should be interpreted in the context of its limitations. Firstly, we only included LT recipients, which does not reflect immunogenicity among other solid organ transplant recipients who are known to have a lower postvaccination response; nor does it allow comparison with immunocompetent individuals. Secondly, our study has a small sample size and a limited follow-up period. Thirdly, all our patients received 2 doses of the Pfizer BioNTech vaccination, and we did not assess the immunogenicity of other SARS-CoV-2 vaccinations. Furthermore, because this was a cohort study spanning 1 y, during which there were frequent changes in hospital protocols pertaining to immunocompromised patients with COVID-19 infection, there was substantial heterogeneity pertaining to the treatment patients received after breakthrough infections. Finally, although our study uses the cPass technology to assess neutralizing antibodies, we did not measure T-cell response, which is also essential in mounting an immune response to SARS-CoV-2 infection. Moreover, although the cPass technology has good specificity and sensitivity, it could still underestimate the patients who truly have neutralizing antibodies.[29] Nevertheless, this study reveals important insights into the predictors of vaccine response using more specific antibodies to spike protein and highlights that LT recipients have a suboptimal response to 2-dose vaccination. This calls for the prioritization of LT recipients in their receipt of a 3-dose primary vaccination series and probably for a fourth dose of messenger RNA vaccine as recommended by current guidelines.[4]

CONCLUSION

At present, effective measures to improve immunogenicity to the SARS-CoV-2 vaccine in post-LT patients remain unknown and are urgently needed. Results from our study support current recommendations to persist with vaccination in this population until better vaccines can be developed. We recommend that a decrease in net immunosuppression to augment immunogenic response must be considered, and emphasis must be placed on practicing personal protective measures while in the midst of completing their primary 3-dose vaccination series or 4-dose full vaccination schedule.

REFERENCES

1. Polack FP, Thomas SJ, Kitchin N, et al; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med.* 2020;383:2603–2615.
2. Baden LR, El Sahly HM, Essink B, et al; COVE Study Group. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med.* 2021;384:403–416.
3. Cornberg M, Buti M, Eberhardt CS, et al. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. *J Hepatol.* 2021;74:944–951.

4. Fix OK, Blumberg EA, Chang K-M, et al; AASLD COVID-19 Vaccine Working Group. American association for the study of liver diseases expert panel consensus statement: vaccines to prevent coronavirus disease 2019 infection in patients with liver disease. *Hepatology.* 2021;74:1049–1064.
5. Guarino M, Cossiga V, Esposito I, et al. Effectiveness of SARS-CoV-2 vaccination in liver transplanted patients: the debate is open!. *J Hepatol.* 2022;76:237–239.
6. Rabinowich L, Grupper A, Baruch R, et al. Low immunogenicity to SARS-CoV-2 vaccination among liver transplant recipients. *J Hepatol.* 2021;75:435–438.
7. Rashidi-Alavijeh J, Frey A, Passenberg M, et al. Humoral response to SARS-CoV-2 vaccination in liver transplant recipients—a single-center experience. *Vaccines (Basel).* 2021;9:738.
8. Lee ARYB, Wong SY, Chai LYA, et al. Efficacy of COVID-19 vaccines in immunocompromised patients: systematic review and meta-analysis. *BMJ.* 2022;376:e068632.
9. Sun J, Zheng Q, Madhira V, et al; National COVID Cohort Collaborative (N3C) Consortium. Association between immune dysfunction and COVID-19 breakthrough infection after SARS-CoV-2 vaccination in the US. *JAMA Intern Med.* 2022;182:153–162.
10. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. *Nat Biotechnol.* 2020;38:1073–1078.
11. Garcia-Beltran WF, Lam EC, Astudillo MG, et al. COVID-19-neutralizing antibodies predict disease severity and survival. *Cell.* 2021;184:476–488.e11.
12. Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with high attack rate. Preprint. Posted August 14, 2020. *medRxiv*. doi:10.1101/2020.08.13.20173161
13. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol.* 2010;17:1055–1065.
14. Amanat F, Thapa M, Lei T, et al. The plasmablast response to SARS-CoV-2 mRNA vaccination is dominated by non-neutralizing antibodies and targets both the NTD and the RBD. Preprint. Posted March 9, 2021. *medRxiv*. doi:10.1101/2021.03.07.21253098
15. Yan Y, Chang L, Wang L. Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): current status, challenges, and countermeasures. *Rev Med Virol.* 2020;30:e2106.
16. Marot S, Malet I, Leducq V, et al; Sorbonne Université SARS-CoV-2 Neutralizing Antibodies Study Group. Rapid decline of neutralizing antibodies against SARS-CoV-2 among infected healthcare workers. *Nat Commun.* 2021;12:2824.
17. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol.* 2020;5:1598–1607.
18. Muthiah MD, Tan EY, Chua SHM, et al. Nucleoside analog monotherapy for prophylaxis in hepatitis B liver transplant patients is safe and efficacious. *Hepatol Int.* 2020;14:57–69.
19. Mathieu E, Ritchie H, Rodés-Guirao L, et al. Coronavirus pandemic (COVID-19). Our World in Data. 2020. Available at https://ourworldindata.org/covid-cases.
20. Toniutto P, Falleti E, Cmet S, et al. Past COVID-19 and immunosuppressive regimens affect the long-term response to anti-SARS-CoV-2 vaccination in liver transplant recipients. *J Hepatol.* 2022;77:152–162.
21. Herrera S, Colmenero J, Pascal M, et al. Cellular and humoral immune response after mRNA-1273 SARS-CoV-2 vaccine in liver and heart transplant recipients. *Am J Transplant.* 2021;21:3971–3979.
22. Ruether DF, Schaub GM, Duengelhoef PM, et al. SARS-CoV2-specific humoral and T-cell immune response after second vaccination in liver cirrhosis and transplant patients. *Clin Gastroenterol Hepatol*. 2022;20:162–172.e9.
23. Thuluvath PJ, Robarts P, Chauhan M. Analysis of antibody responses after COVID-19 vaccination in liver transplant recipients and those with chronic liver diseases. *J Hepatol*. 2021;75:1434–1439.
24. Renia L, Goh YS, Rouers A, et al; SCOPE Cohort Study Group. Lower vaccine-acquired immunity in the elderly population following two-dose BNT162b2 vaccination is alleviated by a third vaccine dose. *Nat Commun.* 2022;13:4615.
25. Dhumal S, Patil A, More A, et al. SARS-CoV-2 reinfection after previous infection and vaccine breakthrough infection through the second wave of pandemic in India: an observational study. *Int J Infect Dis.* 2022;118:95–103.
26. Jung J, Sung H, Kim S-H, et al. Covid-19 breakthrough infections in vaccinated health care workers. *N Engl J Med.* 2021;385:1474–1484.
27. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Preprint. Posted June 24, 2021. *medRxiv*. doi:10.1101/2021.06.21.21258528
28. Gilbert PB, Montefiori DC, McDermott AB, et al; Immune Assays Team§. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science.* 2022;375:43–50.
29. Mahmoud SA, Ganesan S, Naik S, et al. Serological assays for assessing postvaccination SARS-CoV-2 antibody response. *Microbiol Spectr.* 2021;9:e0073321.