Decreased Long-Term Severe Acute Respiratory Syndrome Coronavirus 2–Specific Humoral Immunity in Liver Transplantation Recipients 12 Months After Coronavirus Disease 2019

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Long-term humoral immunity and its protective role in liver transplantation (LT) patients have not been elucidated. We performed a prospective multicenter study to assess the persistence of immunoglobulin G (IgG) antibodies in LT recipients 12 months after coronavirus disease 2019 (COVID-19). A total of 65 LT recipients were matched with 65 nontransplanted patients by a propensity score including variables with recognized impact on COVID-19. LT recipients showed a lower prevalence of anti-nucleocapsid (27.7% versus 49.2%; $P = 0.02$) and anti-spike IgG antibodies (88.2% versus 100.0%; $P = 0.02$) at 12 months. Lower index values of anti-nucleocapsid IgG antibodies were also observed in transplantation patients 1 year after COVID-19

(median, 0.49 [interquartile range, 0.15-1.40] versus 1.36 [interquartile range, 0.53-2.91]; $P < 0.001$). Vaccinated LT recipients showed higher antibody levels compared with unvaccinated patients ($P < 0.001$); antibody levels reached after vaccination were comparable to those observed in nontransplanted individuals ($P = 0.70$). In LT patients, a longer interval since transplantation (odds ratio, 1.10; 95% confidence interval, 1.01-1.20) was independently associated with persistence of anti-nucleocapsid IgG antibodies 1 year after infection. In conclusion, compared with nontransplanted patients, LT recipients show a lower long-term persistence of anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies. However, SARS-CoV-2 vaccination after COVID-19 in LT patients achieves a significant increase in antibody levels, comparable to that of nontransplanted patients.

Liver Transplantation 28 1040–1050 2022 AASLD.
Received October 8, 2021; accepted December 4, 2021.

The coronavirus disease 2019 (COVID-19) pandemic has challenged liver transplantation (LT) programs worldwide and continues to cause significant morbidity and mortality. While LT recipients seem to have an increased risk of acquiring COVID-19, their mortality rates may be lower compared with the general population[1] and other solid organ transplantation (SOT) types.[2] However, evidence regarding long-term durability of immune response produced by primary severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in LT recipients is scarce. By contrast, knowledge about long-term SARS-CoV-2 immune response is essential to ascertain the predisposition to reinfection of LT patients and may help to delineate vaccination strategies in this population. Previous studies have revealed long-term persistence of immunoglobulin G (IgG) anti-SARS-CoV-2 antibodies[3,4] in immunocompetent patients after primary infection. Similarly, early[5] and medium-term humoral immune responses[6] have been described after COVID-19 in LT recipients. In addition, we have previously described a lower persistence of anti-nucleocapsid IgG antibodies within the first 6 months after infection and a more pronounced decline in antibody levels in LT patients as compared with immunocompetent individuals.[6] However, long-term humoral immunity in LT patients has not been elucidated.

We provide here the final results of a prospective nationwide study aimed at analyzing the incidence, evolution, and conditioning factors of SARS-CoV-2 humoral immune response at 12 months post-SARS-CoV-2 infection in LT recipients compared with carefully matched nontransplanted patients. Intermediate results have been published previously.[6]

Abbreviations: BAU, binding antibody units; COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; IQR, interquartile range; LT, liver transplantation; NA, not applicable; ns, not significant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SETH, Spanish Society of Liver Transplantation; SOT, solid organ transplantation.

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DOI 10.1002/lt.26389

Patients and Methods

STUDY DESIGN

A total of 111 LT recipients with COVID-19 were prospectively enrolled as part of a nationwide study advocated by the Spanish Society of Liver Transplantation (SETH) and conducted from February 28 to April 7, 2020, in Spain.[1] A total of 101 out of 111 LT recipients from 23 centers did not present any of the following exclusion criteria and were prospectively enrolled in this study (Fig. 1): death within the first 3 months after SARS-CoV-2 infection, previous therapy with Igs or convalescent plasma transfusions, active chemotherapy, and refusal or inability to provide informed consent. Clinical operational tolerance, defined as normal graft function in complete absence of immunosuppression, was also considered an additional exclusion criterion in the LT group. COVID-19 was confirmed in all patients by a real-time reverse transcriptase-polymerase chain reaction assay[7] of nasopharyngeal swab samples. Serological data were available in 65 of 101 LT recipients at 12 months and were compared with data from 65 nontransplanted

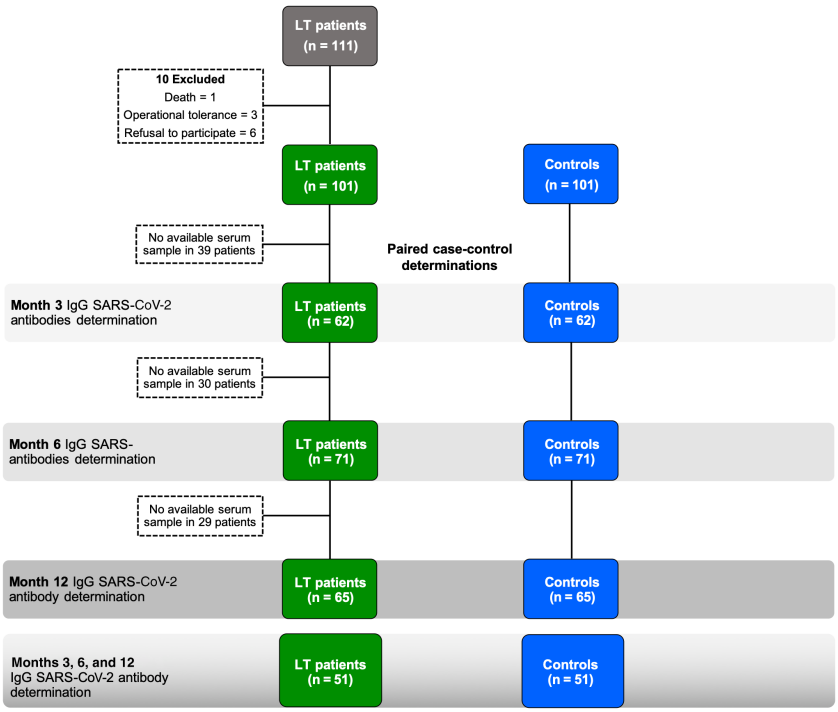

FIG. 1. Study protocol and follow-up. Serum samples were not available in all patients at 3, 6, and 12 months after COVID-19 due to logistic difficulties.

individuals who were diagnosed with COVID-19 at the Hospital Gregorio Marañón within the same time frame (control group). Cases and controls were matched by propensity score according to demographic features and severity of COVID-19 as described previously.[6] The main outcome of the study was the presence of anti-SARS-CoV-2-binding antibodies at 12 months after infection.

The study was approved by the Research Ethics Committee of the Hospital Gregorio Marañón (HGUGM 24 August 2020, 19/2020), and the research protocol was registered at ClinicalTrials.gov (NCT04410471). The study was performed according to the principles of the Declaration of Helsinki and European Union regulation 2016/679.

DATA COLLECTION

Anti-SARS-CoV-2 IgG Antibody Detection

Determination of anti-SARS-CoV-2 antibodies was performed at 3, 6, and 12 months after COVID-19 diagnosis. SARS-CoV-2 IgG antibodies targeting the nucleocapsid protein were detected in serum samples by a chemiluminescence technique (SARS-CoV-2 IgG Reagent Kit; Abbott, Chicago, IL). The detection method has been described in detail elsewhere.[6] SARS-CoV-2 IgG antibodies targeting the spike protein were additionally measured in serum samples by a quantitative chemiluminescent assay (SARS-CoV-2 IgG II Quant Reagent Kit) and expressed in binding antibody units per milliliter (BAU/mL). Detection of both anti-nucleocapsid and anti-spike antibodies was performed at the Microbiology Laboratory in the Hospital Gregorio Marañón, using the ARCHITECT i2000 INSTRUMENT (Abbott). Results above 7.10 BAU/mL were considered positive (detection range, 0.97-5680.00 BAU/mL). To assess the magnitude of the decline of antibody levels, we calculated an arbitrary index consisting of the ratio between the levels at months 12 and 6. Thus, a decrease of 50% is represented by an index value of 0.5.

Each local laboratory obtained and transported their specimens according to standard procedures. Serum levels of immunosuppressive drugs were determined in each participant center at the time of antibody determination.

Clinical Evaluation

Clinical information was extracted from reliable electronic medical data sources and recorded in a REDCap

database. Demographic data, comorbidities, clinical features, laboratory parameters, and transplantation-related information were documented. Data regarding SARS-CoV-2 vaccination were also specifically recorded. Severe COVID-19 was defined as admission to the intensive care unit, requirement of mechanical ventilation, or death, whichever occurred first, according to a previous study describing the clinical characteristics of COVID-19 in China.[8] Management protocols for COVID-19 in LT patients encouraged clinicians to reduce, but not to withdraw, immunosuppression. All patients were managed in accordance with COVID-19 protocols, following the recommendations of the SETH and the Spanish Ministry of Health throughout the study period.

STATISTICAL ANALYSIS

Continuous variables are reported as median and interquartile range (IQR). Categorical variables are described as absolute numbers and percentages. Antibody positivity rates in LT patients and controls at different time points were compared using the chi-square test with Fisher's correction when appropriate. Differences between antibody levels in both groups were compared by the Mann-Whitney U test.

Among LT patients, independent predictors of persistence of antibodies at 12 months after COVID-19 were identified using univariate and multivariate logistic regression analyses. Variables showing a P value $\leq$ 0.20 in the univariate analysis entered the multivariate model; age was excluded from the multivariate analysis due to potential collinearity with the time since LT. Nonsignificant covariates were removed from the model in a backward stepwise process, starting with those with the highest P value. Every hypothesis tested was 2-tailed and considered significant at $P < 0.05$. Statistical analyses were performed using the Stata version 13.0 (StataCorp LP, College Station, TX); graphs were generated using GraphPad Prism version 6.0 software (GraphPad Software Inc., San Diego, CA).

Results

STUDY POPULATION AND BASELINE CHARACTERISTICS

Serum samples were not available in 36 of the 101 LT recipients at month 12 due to logistic difficulties. Therefore, evaluation of SARS-CoV-2 humoral response at 12 months after COVID-19 was performed in a total of 130 patients (65 in each study group). There were no differences among LT patients with and without available serum samples regarding age, sex, prevalence of diabetes mellitus or arterial hypertension, COVID-19 severity, or hospital admission characteristics (Supporting Table 1). In 102 cases (51 case-control pairs) serological data were available at months 3, 6, and 12 after infection (Fig. 1). According to propensity score matching, the LT and control groups were comparable in terms of age, sex, comorbidities, COVID-19 severity, and hospital admission characteristics (Supporting Table 2).

The main clinical and demographic characteristics are shown in Table 1. All patients presented symptomatic COVID-19, most being nonsevere (90.0%), although hospital admission was frequently required (85.92%). Compared with control patients, LT recipients less frequently received interferon β (1.5% versus 41.5%; $P < 0.001$) and lopinavir (32.3% versus 96.9%; $P < 0.001$; Table 1).

All LT patients were receiving chronic immunosuppression. Tacrolimus was the immunosuppressive drug most frequently used at month 12 (n = 42; 64.6%), followed by mycophenolate mofetil (n = 23; 35.4%).

No symptomatic reinfections were observed in any of the study groups during follow-up.

PREVALENCE AND QUANTITATIVE ASSESSMENT OF IgG ANTIBODIES AGAINST SARS-CoV-2

Anti-nucleocapsid IgG Antibodies

LT recipients showed a lower prevalence of anti-nucleocapsid IgG antibodies as compared with non-transplanted patients at 12 months after COVID-19 (27.7% versus 49.2%; $P = 0.02$; Table 2). In addition, we detected significantly lower index values of anti-nucleocapsid IgG antibodies in LT recipients at the same time point (0.49 [IQR, 0.15-1.40] versus 1.36 [IQR, 0.53-2.91]; $P < 0.001$; Fig. 2). Similar results were observed at 3 and 6 months after COVID-19 (Fig. 2). Although a more pronounced decline of anti-nucleocapsid IgG index values was observed in LT recipients between months 3 and 6, LT recipients and control patients showed a comparable decline of anti-nucleocapsid IgG index values between months 6 and 12. Thus, the ratio between the index values at months 12 and 6 was similar (0.48 versus 0.47; $P = 0.95$).

TABLE 1. Clinical Characteristics of 130 Patients With Paired Case-Control Serological Determinations at Month 12 According to the Study Group

	LT Patients (n = 65)	Control Patients (n = 65)	*P* Value
Age, years	65 (61-69)	66 (57-72)	0.65
Sex, male	52 (80.0)	47 (72.3)	0.41
Previous medical history			
Diabetes mellitus	27 (41.5)	31 (47.7)	0.60
Hypertension	40 (61.5)	43 (66.1)	0.72
Angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers	23 (35.4)	30 (46.2)	0.28
Cardiovascular disease	9 (13.9)	10 (15.4)	>0.99
Chronic obstructive pulmonary disease	4 (6.2)	5 (7.7)	>0.99
Asthma	6 (9.2)	4 (6.2)	0.74
Clinical characteristics			
Non-severe COVID-19	58 (89.2)	59 (90.8)	>0.99
Hospital admission	54 (83.1)	56 (86.2)	0.81
Interval since transplantation, years	7.98 (2.43-13.26)	NA	NA
COVID-19–specific therapy			
Lopinavir	21 (32.3)	63 (96.9)	<0.001
Interferon β	1 (1.5)	27 (41.5)	<0.001
Hydroxychloroquine	58 (89.2)	62 (95.4)	0.32
Azithromycin	39 (60.0)	10 (15.3)	<0.001
Remdesivir	0 (0)	1 (1.6)	>0.99
Tocilizumab	5 (6.9)	9 (12.5)	0.40
Corticosteroids (boluses)	3 (4.6)	5 (7.7)	0.72
Immunosuppression at month 12			
Tacrolimus	42 (64.6)	NA	NA
Mycophenolate mofetil	23 (35.4)	NA	NA
Corticosteroids (maintenance)	2 (3.1)	NA	NA
Everolimus	15 (23.1)	NA	NA

NOTE: Data are expressed as median (IQR) or n (%). Severe COVID-19 was defined as a requirement for respiratory support, admission to the intensive care unit, and/or death. NA, not applicable.

Likewise, a similar frequency of loss of antibodies was observed at 12 months after the infection (51.4% versus 47.9%; $P = 0.82$; Supporting Table 3).

Anti-spike IgG Antibodies

We also assessed the prevalence and levels of anti-spike IgG antibodies at 3, 6, and 12 months after the infection. Although no differences were observed between unvaccinated LT recipients and controls regarding the prevalence of anti-spike IgG antibodies at 3 (94.8% versus 96.8%; $P = 0.12$) and 6 months after the infection (90.1% versus 94.4%; $P = 0.10$), LT patients showed a lower prevalence of anti-spike IgG antibodies at 12 months (88.2% versus 100.0%; $P = 0.02$; Table 3). Importantly, the anti-spike IgG antibody levels were similar between the 2 groups at all the time intervals considered (Fig. 2).

SARS-CoV-2 VACCINATION IMMUNOGENICITY AFTER COVID-19

Patients in both groups received SARS-CoV-2 vaccination according to the Spanish Ministry of Health regulations. BNT162b2 SARS-CoV-2 was the most frequently administered vaccine in LT patients (58.1%), followed by the mRNA-1273 vaccine (38.7%). Half of the LT recipients vaccinated with BNT162b2 and 41.7% of those vaccinated with mRNA-1273 had received the second dose at 12 months. No LT patient was vaccinated with the Oxford-AstraZeneca AZD1222 vaccine. The vast majority of controls had received BNT162b2 SARS-CoV-2 vaccine (86.7%) followed by AZD1222 (13.3%; Table 4). Overall, the proportion of LT recipients receiving at least 1 dose of SARS-CoV-2 vaccination (either the Moderna

TABLE 2. Prevalence of Anti-nucleocapsid and Anti-spike IgG Antibodies Observed at 12 Months According to the Study Group

	LT Patients	Control Patients	
Month 12	n = 65	n = 65	*P* Value
Anti-nucleocapsid IgG detected	18 (27.7)	32 (49.2)	0.02
Anti-nucleocapsid IgG index values	0.49 (0.15-1.40)	1.36 (0.53-2.91)	<0.001
Anti-spike IgG detected	59 (90.8)	65 (100.0)	0.03
Anti-spike IgG levels, BAU/mL	386.99 (76.72-2287.34)	137.67 (76.95-419.44)	0.12

NOTE: Data are expressed as median (IQR) or n (%).

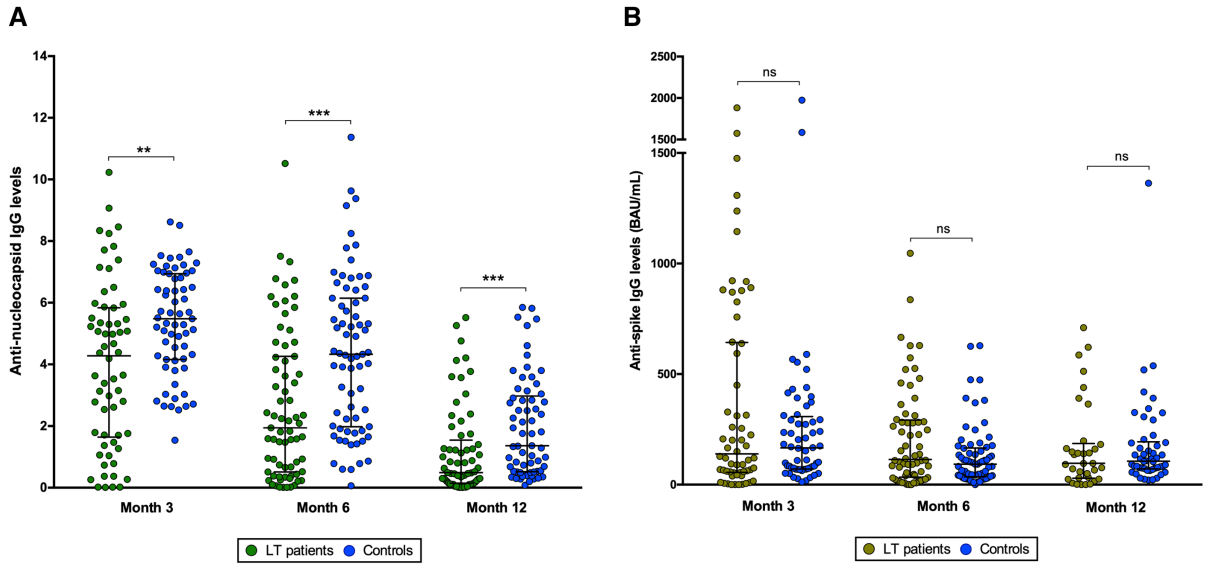

FIG. 2. Levels of (A) anti-nucleocapsid and (B) anti-spike IgG antibodies at 3, 6, and 12 months after COVID-19 in LT and control patients. Error bars indicate the IQR. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ (analyzed by Mann-Whitney U test). Anti-spike IgG antibodies levels at 12 months are shown only for nonvaccinated patients.

mRNA-1273 or the Pfizer-BioNTech BNT162b2 vaccine) at 12 months was greater than in non-LT patients (47.6% versus 23.1%; $P = 0.01$). There were no differences regarding age, sex, disease severity, and comorbidities between both groups (Supporting Table 5).

The median time from vaccination to the serological assessment at 12 months after COVID-19 was 2.71 weeks (IQR, 1.71-4.86 weeks) in LT patients. Moreover, the median interval from LT to vaccination was 11.42 years (IQR, 4.38-16.39 years). The vast majority (93.6%) of vaccinated LT recipients showed protective levels of anti-spike IgG antibodies at month 12 after COVID-19. The prevalence of anti-spike IgG antibodies was similar between vaccinated and unvaccinated LT recipients (93.6% versus 88.2%; $P = 0.67$; Table 5). However, vaccinated LT patients showed significantly higher levels of anti-spike IgG antibodies compared with unvaccinated patients (5414.55 BAU/mL [IQR, 1192.81-5680.00 BAU/mL] versus 96.10 BAU/mL [IQR, 30.12-182.14 BAU/mL]; $P < 0.001$). Similar results were observed in controls (Fig. 3).

Remarkably, LT recipients showed similar levels of anti-spike IgG antibodies after the first or second SARS-CoV-2 vaccine dose compared with controls (3248.24 BAU/mL [IQR, 630.89-5680.00 BAU/mL] versus 4050.56 BAU/mL [IQR, 2062.83-5680.00 BAU/mL]; $P = 0.70$) 12 months after COVID-19 (Supporting Table 6).

We also assessed vaccination immunogenicity according to the number of vaccine doses administered and to

TABLE 3. Observed Incidence of Anti-Spike IgG Antibodies and Levels at 12 Months According to the Study Group and Anti-SARS-CoV-2 Vaccination

	LT Patients	Control Patients	
Unvaccinated Patients	n = 34	n = 50	*P* Value
Anti-spike IgG detected	30 (88.2)	50 (100.0)	0.02
Anti-spike IgG levels, BAU/mL	96.10 (30.12-182.14)	106.02 (72.15-190.35)	0.48
Vaccinated patients	n = 31	n = 15	
Anti-spike IgG detected	29 (93.6)	15 (100.0)	>0.99
Anti-spike IgG levels, BAU/mL	3248.24 (630.89-5680.00)	4050.56 (2062.83-5680.00)	0.70

NOTE: Data are expressed as n (%) or median (IQR).

TABLE 4. SARS-CoV-2 Vaccination at Month 12 According to the Study Group

Vaccination	LT Patients (n = 65)	Control Patients (n = 65)	*P* Value
Partial or complete SARS-CoV-2 vaccination	31 (47.6)	15 (23.1)	0.01
Pfizer-BioNTech BNT162b2 vaccine	18 (58.1)	13 (86.7)	0.09
First dose	9 (50.0)	8 (61.5)	0.72
Second dose	9 (50.0)	5 (38.5)	0.72
Moderna mRNA-1273 vaccine	12 (38.7)	0 (0.0)	<0.001
First dose	7 (58.3)	0 (0.0)	NA
Second dose	5 (41.7)	0 (0.0)	NA
Oxford-AstraZeneca AZD1222 vaccine	0 (0.0)	2 (13.3)	0.10
First dose	0 (0.0)	2 (100.0)	NA
Second dose	0 (0.0)	0 (0.0)	NA

NOTE: Data are expressed as n (%).

TABLE 5. Observed Incidence of Anti-spike IgG Antibodies and Levels According to the Study Group and Anti-SARS-CoV-2 Vaccination

	SARS-CoV-2 Vaccination	No SARS-CoV-2 Vaccination	
LT Patients	n = 31	n = 34	*P* Value
Anti-spike IgG detected	29 (93.6)	30 (88.2)	0.67
Anti-spike IgG levels, BAU/mL	5414.55 (1192.81-5680.00)	96.10 (30.12-182.14)	<0.001
Control patients	n = 15	n = 50	
Anti-spike IgG detected	15 (100.0)	50 (100.0)	NA
Anti-spike IgG levels, BAU/mL	3248.24 (630.89-5680.00)	106.02 (72.15-190.35)	<0.001

NOTE: Data are expressed as n (%) or median (IQR).

the type of vaccine. LT patients showed similar levels of anti-spike IgG antibodies after the first and second dose of the SARS-CoV-2 vaccine (1737.42 BAU/mL [IQR, 412.45-5680.00 BAU/mL] versus 3914.66 BAU/mL [IQR, 1915.68-5680.00 BAU/mL]; $P = 0.23$; Fig. 4). Regarding the type of vaccine administered, LT recipients showed higher levels of anti-spike IgG antibodies after the mRNA-1273 vaccine compared with the

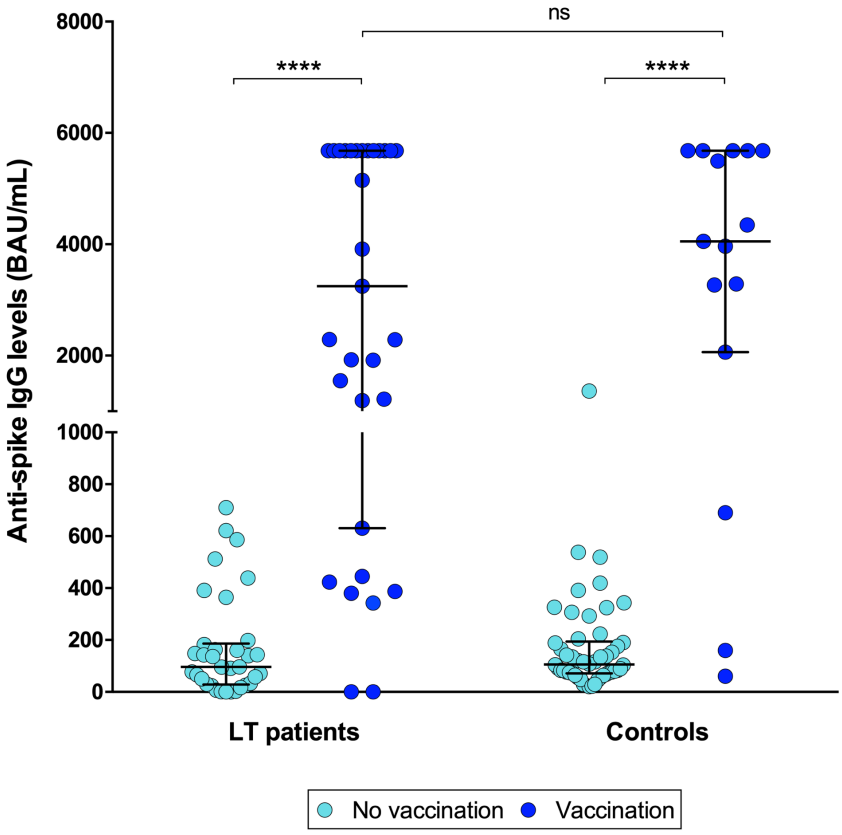

FIG. 3. Levels of anti-spike IgG antibodies at 12 months after SARS-CoV-2 infection in LT patients and controls according to the administration of COVID-19 vaccination. Error bars indicate the IQR. $^{****}P \leq 0.0001$ (analyzed by Mann-Whitney U test).

BNT162b2 vaccine, although without reaching statistical significance (2104.48 BAU/mL [IQR, 422.68-5149.10 BAU/mL] versus 5680.00 BAU/mL [IQR, 1566.23-5680.00 BAU/mL]; $P = 0.07$; Fig. 5).

Finally, only 2 LT patients did not respond to SARS-CoV-2 vaccination. These 2 patients presented anti-spike IgG antibodies at month 6 but lost them at month 12. Both patients had received only 1 dose of a SARS-CoV-2 mRNA–based vaccine at the time of antibody assessment.

PREDICTORS OF PERSISTENCE OF ANTIBODIES AGAINST SARS-CoV-2 IN LT PATIENTS BEYOND 12 MONTHS

Table 6 presents the logistic regression analysis of factors associated with persistence of anti-SARS-CoV-2 IgG antibodies targeting the nucleocapsid protein at 12 months after COVID-19 in LT patients (n = 65). Multivariate analysis identified the interval since LT (odds ratio, 1.10, 95% confidence interval, 1.01-1.20; $P = 0.02$) as the only independent predictor of persistence. Considering anti-spike IgG antibodies, multivariate analysis did not identify any independent predictor of persistence of these antibodies in LT patients (Supporting Table 4).

Discussion

In this prospective study, we investigated the long-term duration of SARS-CoV-2 humoral immunity among LT recipients after COVID-19 compared with carefully matched nontransplanted individuals. Our results show that the majority of LT patients developed and maintained specific humoral immune response against SARS-CoV-2 1 year after COVID-19. However, even with similar epidemiological characteristics and COVID-19 severity, LT recipients showed a reduced prevalence of anti-nucleocapsid and anti-spike IgG

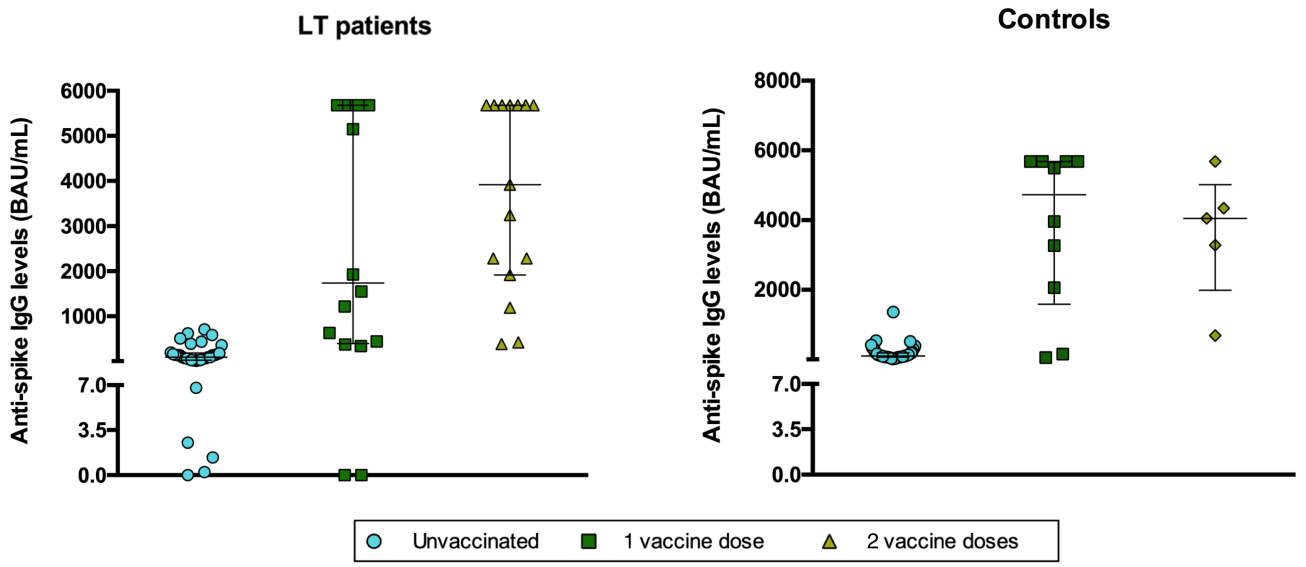

FIG. 4. Levels of anti-spike IgG antibodies at 12 months after SARS-CoV-2 infection in LT patients and controls according to the administration of COVID-19 vaccination and number of doses administered. Error bars indicate the IQR. Results above 7.10 BAU/mL were considered positive (detection range, 0.97-5680.00 BAU/mL).

antibodies at long term. These findings align with our previous study in which we also reported a significantly lower humoral immune response in LT recipients at 6 months after COVID-19.[6]

SARS-CoV-2 infection induces specific humoral immune responses that persist for over 1 year in more than 80% of immunocompetent individuals.[3,9,10] Indeed, antibody reactivity to the spike protein of SARS-CoV-2, neutralizing activity, and the number of spike-specific memory B cells remain relatively stable between 6 and 12 months after the infection in nonimmunocompromised convalescent individuals.[10] However, long-term SARS-CoV-2 humoral immunity after COVID-19 has not yet been thoroughly investigated in LT recipients. Acute and early SARS-CoV-2–specific humoral and functional T-cell immune responses have been assessed in SOT patients, being robust and similar to those observed in immunocompetent patients during early COVID-19 convalescence.[11] Similarly, persistence of anti-SARS-CoV-2 IgG antibodies and stable antibody levels have been described for up to 2 months after COVID-19 in kidney transplantation recipients.[12] Conversely, the proportion of patients who lost antibody response seems to be relevant. In fact, 20.7% of kidney transplantation recipients have been found to be seronegative at 6 months, with a median percentage decline of IgG antibody levels of 68%.[13] Furthermore, we have previously described a lower prevalence of anti-SARS-CoV-2 IgG antibodies targeting the nucleocapsid protein and a more pronounced decrease in antibody levels in LT recipients compared with nontransplanted individuals at 3 and 6 months after COVID-19.[6]

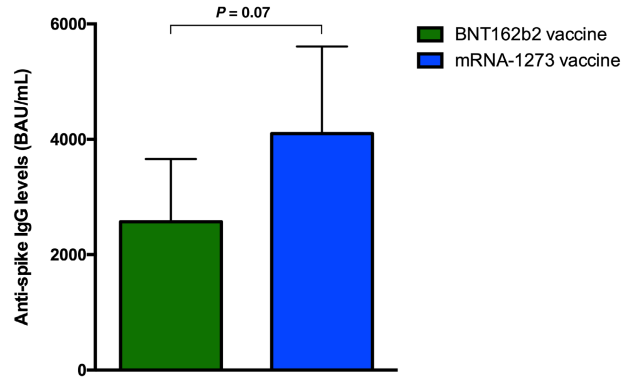

FIG. 5. Levels of anti-spike IgG antibodies observed at 12 months after the infection in LT recipients according to the type of COVID-19 vaccine administered. Bars represent mean levels of antibodies. Error bars indicate the 95% confidence interval.

In the present study, we also identified a lower positivity of both anti-nucleocapsid and anti-spike IgG antibodies in LT recipients compared with nontransplanted patients 1 year after SARS-CoV-2

TABLE 6. Clinical Predictors of Detectable SARS-CoV-2 IgG Antibodies Targeting Nucleocapsid Protein in LT Patients 12 Months After COVID-19 (n = 65)

	Univariate Analysis		Multivariate Analysis	
Variables	Odds ratio (95% confidence interval)	*P* Value	Odds ratio (95% confidence interval)	*P* Value
Age	1.16 (1.05-1.27)	<0.001		
Sex, female	1.21 (0.32-4.55)	0.78		
Interval since LT	1.11 (1.03-1.21)	0.01	1.10 (1.01-1.20)	0.02
Hypertension	0.98 (0.32-2.98)	0.97		
Angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers	3.27 (1.06-10.10)	0.04	2.56 (0.78-8.45)	0.12
Cardiovascular disease	1.37 (0.30-6.17)	0.69		
Severe COVID-19	1.85 (0.34-9.90)	0.47		
Hospital admission	0.61 (0.16-2.41)	0.48		
Tacrolimus*	0.31 (0.10-0.95)	0.04		
Mycophenolate*	0.56 (0.19-1.69)	0.31		
Everolimus*	0.84 (0.20-3.55)	0.82		
Month 12 tacrolimus†	0.31 (0.10-1.00)	0.95		
Month 12 mycophenolate†	0.65 (0.20-2.15)	0.48		
Month 12 everolimus†	0.61 (0.15-2.49)	0.49		

*These variables pertain to active immunosuppression therapy at COVID-19 diagnosis.
†These variables pertain to active immunosuppression therapy at 12 months after COVID-19.

infection. Interestingly, in LT patients who maintained humoral immune response, the 12-month levels of anti-nucleocapsid IgG antibodies were lower than those observed in non-LT patients. However, similar levels of SARS-CoV-2 IgG antibodies targeting the spike protein were observed in both groups at 1 year. Remarkably, both study groups had a similar proportion of anti-spike IgG antibody seropositive patients at 3 and 6 months. Therefore, our data suggest that the most relevant difference in the humoral immune response after COVID-19 between LT patients and non-LT individuals occurs in the long term. Furthermore, it is possible that the observed difference in antibody prevalence and levels between LT patients and nontransplanted individuals would have been even more pronounced in a larger unvaccinated cohort. Aligning with previous studies that have described an earlier decline of anti-nucleocapsid IgG antibodies compared with anti-spike IgG antibodies in immunocompetent individuals,[14,15] we observed a lower prevalence of SARS-CoV-2 antibodies targeting the nucleocapsid protein at 12 months after the infection in both study groups. Moreover, a similar trend in anti-nucleocapsid antibody decay compared with anti-spike antibody has been described in patients infected with SARS-CoV-2.[15] However, the cause of this disparity is largely unknown. In addition, although the detection of antibodies against the nucleocapsid protein is more sensitive than that observed against the spike protein within 14 days after onset of symptoms,[16] a substantial drop in the sensitivity of antibody responses specific to the nucleocapsid protein has been observed over time[14] in the postinfection phase.

Remarkably, we also found that the time from LT to COVID-19 was an independent predictor of sustained antibody response at 12 months after the infection. Considering that a longer interval since LT is usually associated with lower exposure to immunosuppressive drugs, these results were expected. This finding has been further substantiated in a recent study conducted in SOT recipients, which also identified a longer interval from transplantation to COVID-19 diagnosis with the presence of antibodies.[17] Overall, this temporal association potentially reflects the impact of immunosuppression on humoral immune response after COVID-19 in this population.

Substantially decreased immunological response to SARS-CoV-2 mRNA vaccination has been described in SOT recipients[18] and LT patients.[19] However, the question of whether this finding also applies to LT recipients with previous COVID-19 has not yet been

addressed. In our study, performed in LT patients with previous SARS-CoV-2 infection, we observed significantly higher antibody levels in vaccinated patients compared with nonvaccinated patients. Of note, postvaccination antibody levels were similar after the first or second SARS-CoV-2 vaccine dose. In addition, and despite their chronic exposure to immunosuppression and short median time from vaccination to serological assessment, the postvaccination antibody levels observed in LT recipients were similar to those of nontransplanted patients. This finding suggests that long-term memory B-cell response plays a major role in LT patients after COVID-19 and may be similar to that observed in nontransplanted patients. Our results are in accordance with a recent study performed in kidney transplantation recipients after COVID-19 showing a marked increase in antibody levels even after a single-dose SARS-CoV-2 mRNA–based vaccine.[20] This notably more potent immune response to SARS-CoV-2 vaccination observed in previously infected LT recipients as compared with noninfected LT patients[19] could raise the possibility of a single-dose vaccination strategy in this subpopulation. However, these data should be interpreted with caution given the limited sample size and the absence of comparative studies.

Another interesting finding of our study is the apparently stronger humoral immune response observed in LT patients vaccinated with the mRNA-1273 vaccine. Immunogenicity differences between different mRNA-based vaccines in LT patients have also been described in other studies, in which mRNA-1273 vaccine recipients were more likely to develop an antibody response after the first and second dose compared with the BNT162b2 vaccine recipients.[21] Similar findings have been reported in other immunocompromised populations, such as hemodialysis patients, in which the mRNA-1273 vaccine induced 2.98-fold higher anti-spike IgG antibody levels compared with BNT162b2-vaccinated patients.[22] Differences in antibody response between mRNA-based vaccine types in immunosuppressed patients may be related to several aspects: first, the possibility of a dose-response relationship considering the greater amount of RNA per dose used in the mRNA-1273 vaccine; second, the different timing of administration of each vaccine type could also influence their immunogenicity; and finally, it is conceivable that the presence of subtle differences between the 2 vaccines in the RNA and the lipid nanoparticles carriers may be responsible for the immune response observed. Immunogenicity discrepancies between different mRNA-based vaccines may go unnoticed in the general population, as they are highly immunogenic in nonimmunocompromised patients; however, these differences may be more apparent when evaluated in an immunosuppressed population such as LT recipients. Assessment of the efficacy of different vaccines types and vaccination strategies in LT patients is needed to establish whether additional vaccine doses are needed or whether specific vaccines are more effective in this setting.

To our knowledge, this is the first study that provides a precise evaluation of long-term SARS-CoV-2 humoral immune response in LT recipients after COVID-19. However, our study is not without limitations. Because a high proportion of patients presented with pneumonia and required hospitalization, the spectrum of mild and asymptomatic COVID-19 is probably not adequately captured. Therefore, it is possible that our results could overestimate the prevalence of postinfection antibodies in LT patients. Moreover, long-term T-cell–mediated immune response and its protective role against reinfection in the absence of detectable antibodies were not assessed in our study. Furthermore, because we did not observe any symptomatic reinfection, no solid conclusion may be derived regarding long-term clinical protective capacity of humoral immunity. In addition, we are aware that the method used for anti-nucleocapsid antibody detection, as opposed to that used to measure anti-spike antibodies, is not strictly quantitative. However, the index values offer an acceptable indirect approximation of antibody levels. Moreover, although we have not evaluated neutralizing antibodies, an adequate correlation between anti-spike IgG antibodies and the neutralizing activity has been described in previous studies in the general population.[23-25] Finally, although the present study was not specifically designed to assess the humoral response to SARS-CoV-2 vaccination in LT recipients after COVID-19, it may provide new insights into immune response after COVID-19 in LT patients and in the evaluation of the long-term efficacy of SARS-CoV-2 vaccines in this population.

In conclusion, LT recipients exhibit lower long-term persistence of SARS-CoV-2 IgG antibodies after COVID-19 compared with matched nontransplanted individuals. Vaccination boosts humoral response in LT patients, and it could be a valuable strategy to prolong immunogenicity against SARS-CoV-2. There is a need for further studies regarding long-term T-cell–mediated immunity after COVID-19 with and

without vaccination to determine the susceptibility to reinfection of this population.

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