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Decreased Long-term SARS-CoV-2-Specific Humoral Immunity in Liver Transplant Recipients 12-Months after COVID-19

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KEY WORDS: long-term antibody response, immunosuppression, vaccine immunogenicity.

ABBREVIATIONS

ACE, angiotensin converting enzyme
ACE2, angiotensin converting enzyme 2
ARB, angiotensin II receptor blockers
BAU, binding antibody units
CI, confidence interval
COVID-19, coronavirus disease 2019
IQR, interquartile range
LT, liver transplant
OR, odds ratio
RT-PCR, real-time reverse transcription-polymerase chain reaction
SARS-CoV-2, severe acute respiratory syndrome coronavirus 2
SD, standard deviation

SOT, solid organ transplant

DISCLOSURE

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

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ABSTRACT

Long-term humoral immunity and its protective role in liver transplant patients has not been elucidated. We performed a prospective multicenter study to assess the persistence of IgG antibodies in liver transplant recipients 12 months after coronavirus disease 2019 (COVID-19). A total of 65 liver transplant recipients were matched with 65 non-transplanted patients by a propensity score including variables with recognized impact on COVID-19. Liver transplant recipients showed a lower prevalence of anti-nucleocapsid (27.7% vs. 49.2%, P = 0.02) and anti-spike IgG antibodies (88.2% vs. 100.0%, P = 0.02) at 12 months. Lower index values of anti-nucleocapsid IgG antibodies were also observed in transplant patients one year after COVID-19 (0.49 [IQR 0.15-1.40] vs. 1.36 [IQR 0.53-2.91], P < 0.001). Vaccinated liver transplant recipients showed higher antibody levels compared to unvaccinated patients (P < 0.001); antibody levels reached after vaccination were comparable to those observed in non-transplanted individuals (P = 0.70). In liver transplant patients, a longer interval since transplantation (OR=1.10, 95% CI 1.01-1.20) was independently associated with persistence of anti-nucleocapsid IgG antibodies one-year postinfection. In conclusion, compared with non-transplanted patients, liver transplant recipients show a lower long-term persistence of anti-SARS-CoV-2 antibodies. However, SARS-CoV-2 vaccination after COVID-19 in liver transplant patients achieves a significant increase in antibody levels, comparable to that of non-transplanted patients.

1. INTRODUCTION

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 to the general population[1] and other solid organ transplant (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. On the other hand, 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 IgG anti-SARS-CoV-2 antibodies[3,4] in immunocompetent patients after primary infection. Similarly, early[5] and medium-term humoral immune response[6] has 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 to 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 to carefully matched non-transplanted patients. Intermediate results have been published previously[6].

2. PATIENTS AND METHODS

2.1 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 28th to April 7th, 2020 in Spain[1]. One hundred one out of 111 LT recipients from 23 centers did not present any of the following exclusion criteria and were prospectively enrolled in the present study (Figure 1). Study exclusion criteria were: death within the first 3 months after SARS-CoV-2 infection, previous therapy with immunoglobulins 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 (RT-PCR) assay[7] of nasopharyngeal swab samples. Serological data were available in 65 out of 101 LT recipients at 12 months and were compared with data from 65 non-transplanted 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.

2.2 Data collection

2.2.1 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). 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, Chicago, USA). 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.

2.2.2 Clinical evaluation

Clinical information was extracted from reliable electronic medical data sources and recorded in a Red-Cap database. Demographic data, comorbidities, clinical features, laboratory parameters, and transplant-related information were documented. Data regarding SARS-CoV-2 vaccination was 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 Spanish Society of Liver Transplantation and the Spanish Ministry of Health throughout the study period.

2.3 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 squared

test with Fisher 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 ≤ 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. Non-significant co-variates were removed from the model in a backward stepwise process, starting with those with the highest p value. Every hypothesis tested was two-tailed and considered significant at $P < 0.05$. Statistical analyses were performed using the Stata version 13.0 (StataCorp LP); graphs were generated using GraphPad Prism version 6.0 software (GraphPad Software).

3. RESULTS

3.1. Study population and baseline characteristics.

Serum samples were not available in 36 out 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, gender, prevalence of diabetes or arterial hypertension, COVID-19 severity or hospital admission characteristics (Supplementary Table 1). In 102 cases (51 case-control pairs) serological data was available at months 3, 6 and 12 postinfection (Figure 1). According to propensity score matching, the LT and control groups were comparable in terms of age, gender, comorbidities, COVID-19 severity and hospital admission characteristics (Supplementary Table 2).

The main clinical and demographic characteristics are shown in Table 1. All patients presented symptomatic COVID-19, most being non-severe (90.0%), although hospital admission was frequently required (85.92%). Compared with control patients, LT recipients less frequently received interferon beta (1.5% vs. 41.5%, $P < 0.001$) and lopinavir (28.2% vs. 95.8%, $P < 0.001$) (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.

3.2. Prevalence and quantitative assessment of IgG antibodies against SARS-CoV-2.

3.2.1. 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% vs. 49.2%, P = 0.02) (Table 2). Additionally, 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] vs. 1.36 [IQR 0.53-2.91], P < 0.001) (Figure 2). Similar results were observed at 3 and 6 months after COVID-19 (Figure 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 vs. 0.47, P = 0.95). Likewise, a similar frequency of loss of antibodies was observed at 12 months postinfection (51.4% vs. 47.9%, P = 0.82) (Supplementary table 3).

3.2.2. Anti-spike IgG antibodies.

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

3.3. 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 one dose of SARS-CoV-2 vaccination (either the Moderna mRNA-1273 or Pfizer-BioNTech BNT162b2 vaccine) at 12 months was greater than in non-LT patients (47.6% vs. 23.1%; P = 0.01). There were no differences regarding age, sex, disease severity and comorbidities between both groups (Supplementary 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) in LT patients. Moreover, the median interval between LT to vaccination was 11.42 years (IQR 4.38-16.39). 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% vs. 88.2%, P = 0.67) (Table 5). However, vaccinated LT patients showed significantly higher levels of anti-spike IgG antibodies compared to unvaccinated patients (5414.55 BAU/mL [IQR 1192.81-5680.00] vs. 96.10 BAU/mL [IQR 30.12-182.14], P < 0.001). Similar results were observed in controls (Figure 3).

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

We also assessed vaccination immunogenicity according to the number of vaccine doses administered and to 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] vs. 3914.66 BAU/mL [IQR 1915.68-5680.00], P = 0.23) (Figure 4). Regarding the type of vaccine administered, LT recipients showed higher

levels of anti-spike IgG antibodies after the mRNA-1273 vaccine compared to the BNT162b2 vaccine, although without reaching statistical significance (2104.48 BAU/mL [IQR 422.68-5149.10] vs. 5680.00 BAU/mL [IQR 1566.23-5680.00], P = 0.07) (Figure 5). Finally, only two LT patients did not respond to SARS-CoV-2 vaccination. These two patients presented anti-spike IgG antibodies at month 6 but lost them at month 12. Both patients had received only one dose of a SARS-CoV-2 mRNA-based vaccine at the time of antibody assessment.

3.4. Predictors of persistence of antibodies against SARS-CoV-2 in LT patients beyond 12 months.

Table 6 describes 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 [OR]=1.10, 95% confidence interval [CI] 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 (Supplementary Table 4).

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 to carefully matched non-transplanted individuals. Our results show that the majority of LT patients developed and maintained specific humoral immune response against SARS-CoV-2 one 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 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

one 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 postinfection in non-immunocompromised 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 transplant recipients[12]. Conversely, the proportion of patients who lost antibody response seems to be relevant. In fact, 20.7% of kidney transplant 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 to non-transplanted individuals at 3 and 6 months after COVID-19[6].

In the present study, we also identified a lower positivity of both anti-nucleocapsid and anti-spike IgG antibodies in LT recipients compared to non-transplanted patients one year after SARS-CoV-2 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 one 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 non-transplanted individuals would have been even more pronounced in a larger unvaccinated cohort. Aligning with previous studies which have described an earlier decline of anti-nucleocapsid IgG antibodies compared to 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 post-infection in both study groups. Moreover, a similar trend in anti-nucleocapsid antibody decay compared to anti-spike antibody has been described in patients infected with SARS-CoV[15]. However, the cause of this disparity is largely unknown. Additionally, although the detection of antibodies against the nucleocapsid protein is more sensitive than the 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 since LT to COVID-19 was an independent predictor of sustained antibody response at 12 months postinfection. 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 since 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 to non-vaccinated patients. Of note, postvaccination antibody levels were similar after the first or second SARS-CoV-2 vaccine dose. Additionally, 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 non-transplanted 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 non-transplanted patients. Our results are in accordance with a recent study performed in kidney transplant 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 to non-infected 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 to 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 to 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 two 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 non-immunocompromised 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. Since a high proportion of patients presented 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 post-infection antibodies in LT patients. Moreover, long-term T-cell mediated immune response and its protective role against reinfection in the absence of detectable antibodies was not assessed in our study. Furthermore, since we did not observe any symptomatic re-infection, no solid conclusion may be derived regarding long-term clinical protective capacity of humoral immunity. Additionally, 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 to matched non-transplanted 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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FIGURE LEGENDS

Figure 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.

Figure 2. Levels of anti-nucleocapsid (panel A) and anti-spike IgG antibodies (panel B) at 3, 6 and 12 months after COVID-19 in liver transplant and control patients. Error bars indicate the interquartile range. **p ≤ 0.01, ***p ≤ 0.001 (analyzed by Mann-Whitney U test). Anti-spike IgG antibodies levels at 12 months are shown only for non-vaccinated patients.

Figure 3. Levels of anti-spike IgG antibodies at 12 months after SARS-CoV-2 infection in liver transplant patients and controls according to the administration of COVID-19 vaccination. Error bars indicate the interquartile range. ****p ≤ 0.0001 (analysed by Mann-Whitney U test).

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

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

TABLE LEGENDS

Table 1. Clinical characteristics of 130 patients with paired case-control serological determinations at month 12 according to the study group. 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. ACE, angiotensin converting; ARB, angiotensin II receptor blockers.

Table 2. Prevalence of anti-nucleocapsid and anti-spike IgG antibodies observed at 12 months according to the study group.

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. BAU/mL, binding antibody units; IQR, interquartile range.

Table 4. SARS-CoV-2 vaccination at month 12 according to the study group. Data are expressed as median (IQR) or n (%).

Table 5. Observed incidence of anti-nucleocapsid IgG antibodies and levels according to the study group and anti-SARS-CoV-2 vaccination.

Table 6. Clinical predictors of detectable SARS-CoV-2 IgG antibodies targeting nucleocapsid protein in liver transplant patients 12 months after Covid-19 (n=65). * These variables pertain to active immunosuppression therapy at COVID-19 diagnosis. † These variables pertain to active immunosuppression therapy at 12 months after COVID-19. ACE, angiotensin converting; ARB, angiotensin II receptor blockers; CI, confidence interval; OR, odds ratio.

	Liver transplant patients (n=65)		Control patients (n=65)		p
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
ACE inhibitors or ARB	23	(35.4)	30	(46.2)	0.28
Cardiovascular disease	9	(13.9)	10	(15.4)	1.00
Chronic obstructive pulmonary disease	4	(6.2)	5	(7.7)	1.00
Asthma	6	(9.2)	4	(6.2)	0.74
Clinical characteristics					
Non-severe COVID-19	58	(89.2)	59	(90.8)	1.00
Hospital admission	54	(83.1)	56	(86.2)	0.81
Interval since transplantation (years)	7.98	(2.43-13.26)	NA	NA	NA
COVID-19 specific therapy					
Lopinavir	21	(32.3)	63	(96.9)	<0.001
Interferon beta	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)	1.00
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	NA
Mycophenolate	23	(35.4)	NA	NA	NA
Corticosteroids (maintenance)	2	(3.1)	NA	NA	NA
Everolimus	15	(23.1)	NA	NA	NA

	Liver transplant patients		Control patients		p
Month 12	***n=65***		***n=65***		
Anti-nucleocapsid IgG detected; n (%)	18	(27.7)	32	(49.2)	0.02
Anti-nucleocapsid IgG index values; median (IQR)	0.49	(0.15-1.40)	1.36	(0.53-2.91)	<0.001
Anti-spike IgG detected; n (%)	59	(90.8)	65	(100.0)	0.03
Anti-spike IgG levels (BAU/mL); median (IQR)	386.99	(76.72-2287.34)	137.67	(76.95-419.44)	0.12

	Liver transplant patients		Control patients		p
Unvaccinated patients	***n=34***		***n=50***		
Anti-spike IgG detected; n (%)	30	(88.2)	50	(100.0)	0.02
Anti-spike IgG levels (BAU/mL); median (IQR)	96.10	(30.12-182.14)	106.02	(72.15-190.35)	0.48
Vaccinated patients	***n=31***		***n=15***		
Anti-spike IgG detected; n (%)	29	(93.6)	15	(100.0)	1.00
Anti-spike IgG levels (BAU/mL); median (IQR)	3248.24	(630.89-5680.00)	4050.56	(2062.83-5680.00)	0.70

	Liver transplant patients (n=65)		Control patients (n=65)		p
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)	0.00
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

	SARS-CoV-2 vaccination		No SARS-CoV-2 vaccination		p
Liver transplant patients	***n=31***		***n=34***		
Anti-spike IgG detected; n (%)	29	(93.6)	30	(88.2)	0.67
Anti-spike IgG levels (AU/mL); median (IQR)	5414.55	(1192.81-5680.00)	96.10	(30.12-182.14)	<0.001
Control patients	***n=15***		***n=50***		
Anti-spike IgG detected; n (%)	15	(100.0)	50	(100.0)	NA
Anti-spike IgG levels (BAU/mL); median (IQR)	3248.24	(630.89-5680.00)	106.02	(72.15-190.35)	<0.001

Variables	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p	OR (95% CI)	p
Age	1.16 (1.05-1.27)	0.00		
Sex (female)	1.21 (0.32-4.55)	0.78		
Interval since liver transplantation	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		
ACE inhibitors or ARB	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		

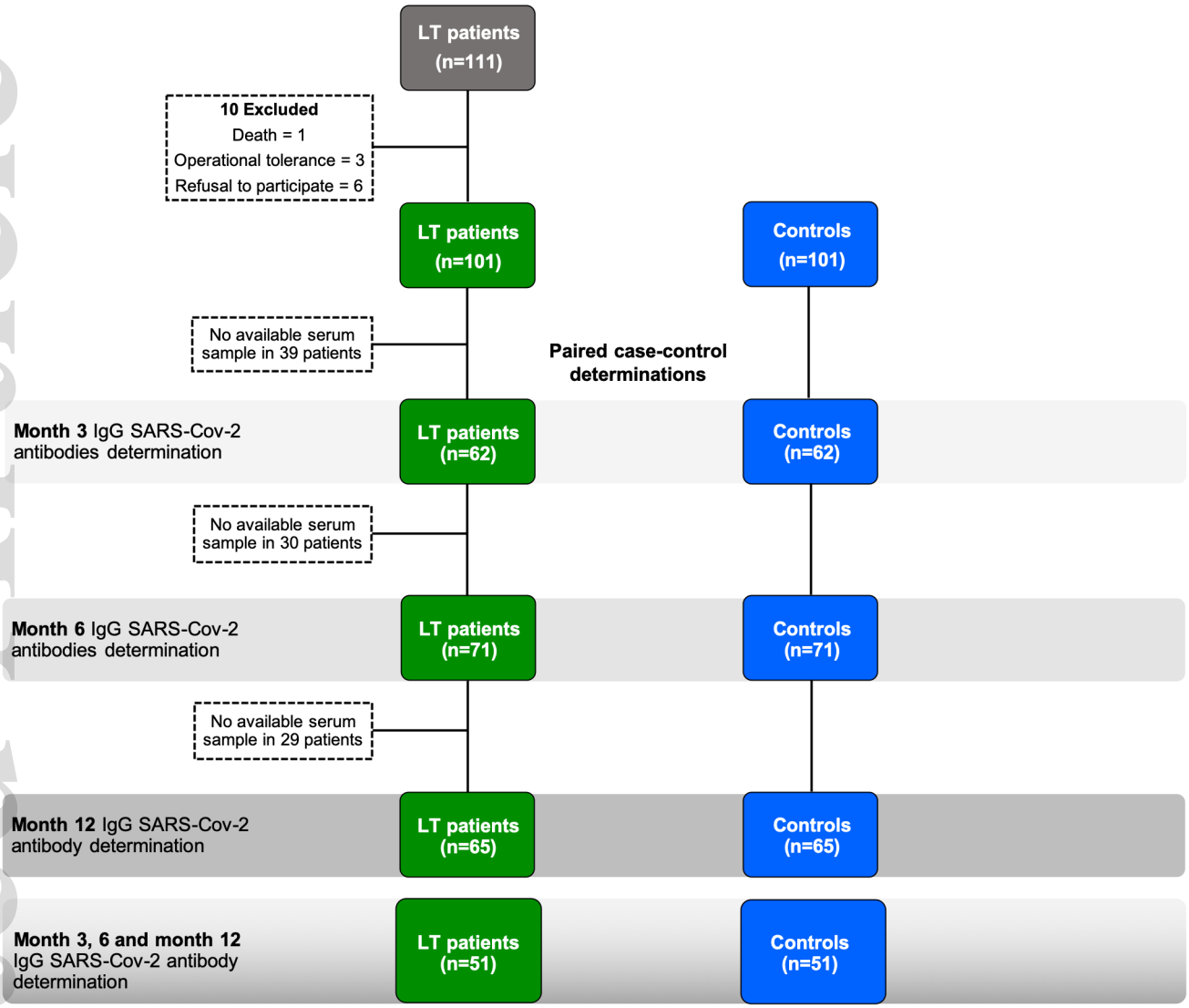

Figure 1. Study protocol and follow-up. Serum samples were not available in all patients at 3, 6 and 12 months after COVID-19 due to logistic difficulties.

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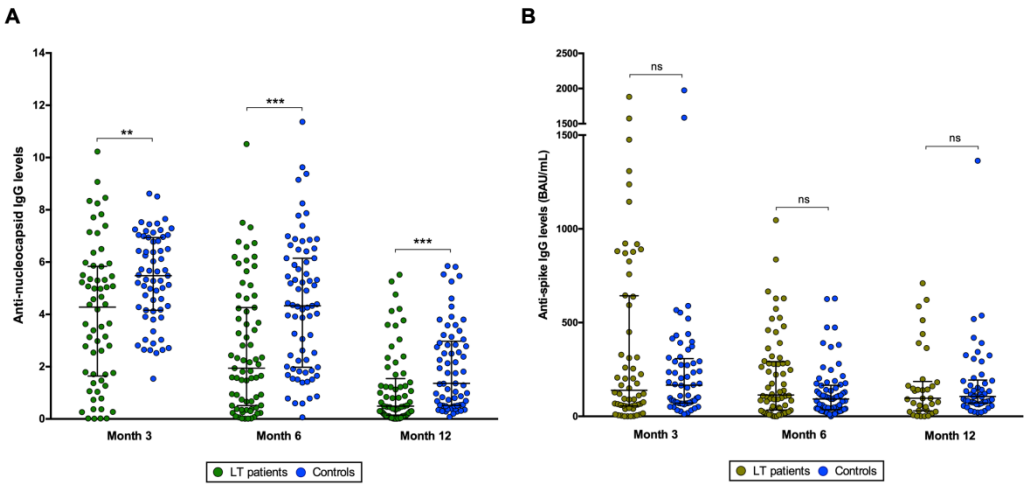

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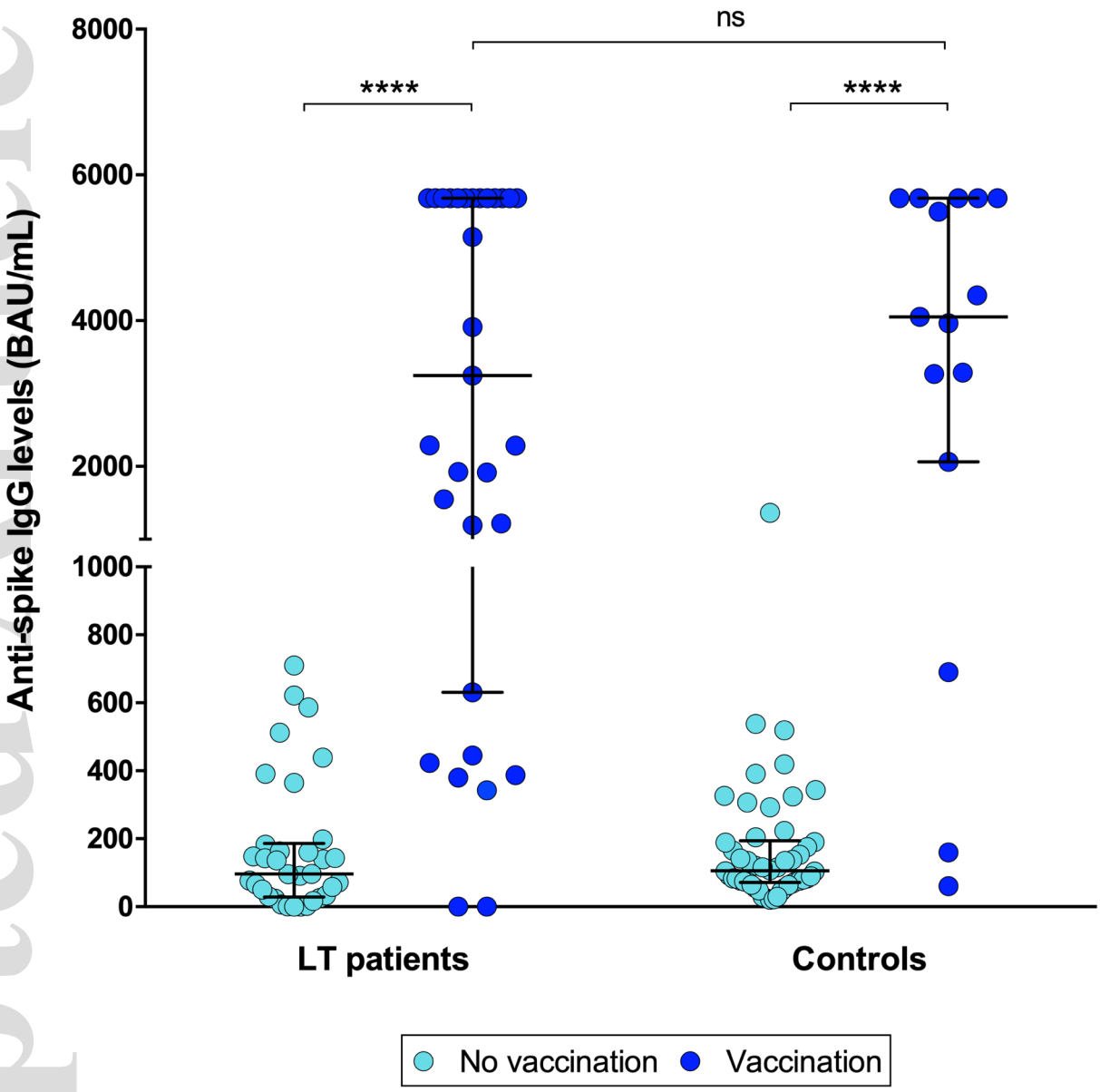

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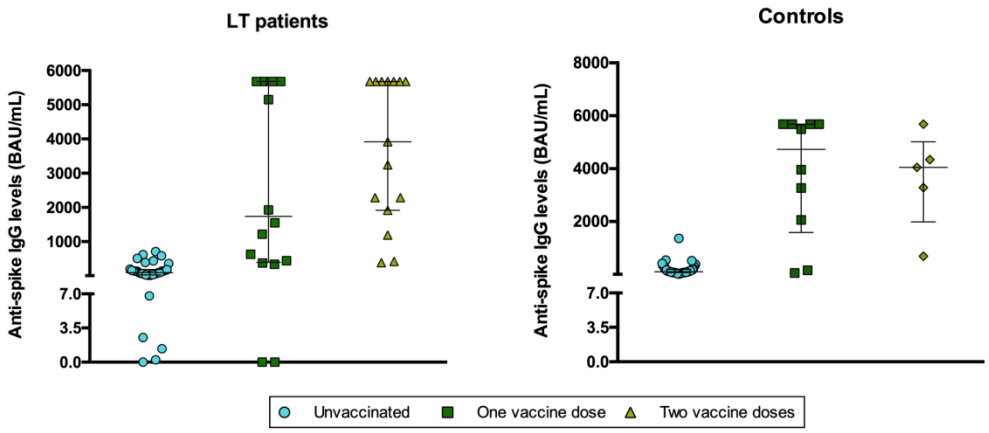

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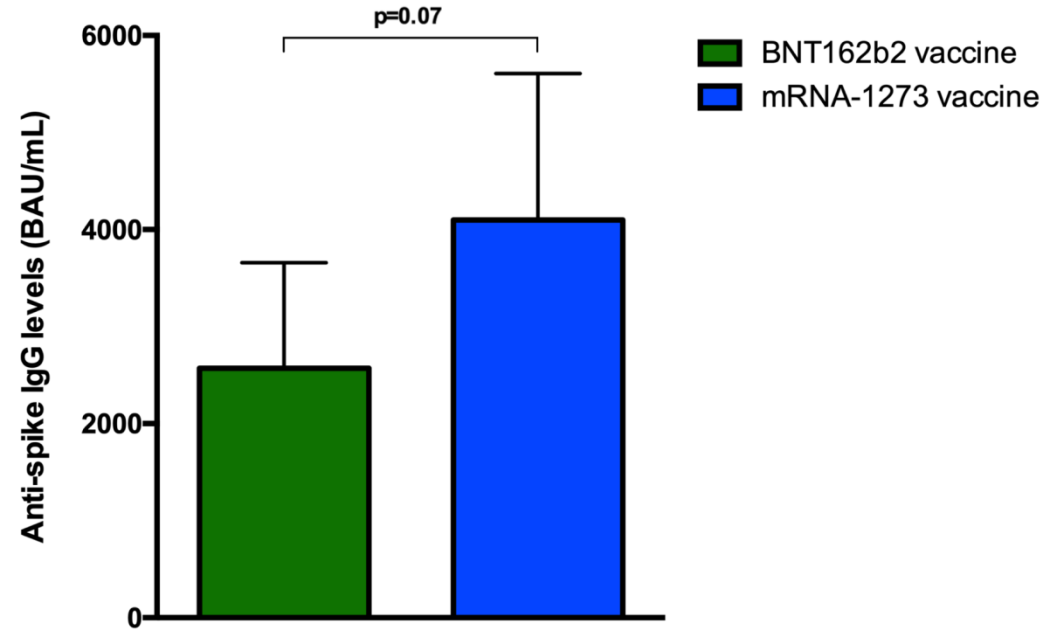

Figure 5. Levels of anti-spike IgG antibodies observed at 12 months post-infection in liver transplant recipients according to the type of COVID-19 vaccine administered. Bars represent mean levels of antibodies. Error bars indicate the 95% confidence interval.

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