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BRIEF COMMUNICATION

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# Changes in humoral immune response after SARS-CoV-2 infection in liver transplant recipients compared to immunocompetent patients

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**Abbreviations:** ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; ARB, angiotensin II receptor blockers; CI, confidence interval; COVID-19, coronavirus disease 2019; 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.

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The protective capacity and duration of humoral immunity after SARS-CoV-2 infection are not yet understood in solid organ transplant recipients. A prospective multicenter study was performed to evaluate the persistence of anti-nucleocapsid IgG antibodies in liver transplant recipients 6 months after coronavirus disease 2019 (COVID-19) resolution. A total of 71 liver transplant recipients were matched with 71 immunocompetent controls by a propensity score including variables with a well-known prognostic impact in COVID-19. Paired case–control serological data were also available in 62 liver transplant patients and 62 controls at month 3 after COVID-19. Liver transplant recipients showed a lower incidence of anti-nucleocapsid IgG antibodies at 3 months (77.4% vs. 100%, $p < .001$) and at 6 months (63.4% vs. 90.1%, $p < .001$). Lower levels of antibodies were also observed in liver transplant patients at 3 ($p = .001$) and 6 months ($p < .001$) after COVID-19. In transplant patients, female gender (OR = 13.49, 95% CI: 2.17–83.8), a longer interval since transplantation (OR = 1.19, 95% CI: 1.03–1.36), and therapy with renin–angiotensin–aldosterone system inhibitors (OR = 7.11, 95% CI: 1.47–34.50) were independently associated with persistence of antibodies beyond 6 months after COVID-19. Therefore, as compared with immunocompetent patients, liver transplant recipients show a lower prevalence of anti-SARS-CoV-2 antibodies and more pronounced antibody levels decline.

**KEYWORDS**
clinical research/practice, immune regulation, immunosuppressant, immunosuppression/immune modulation, infection and infectious agents-viral, infectious disease, liver transplantation/hepatology

## 1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) continues to raise uncertainties about the medium- and long-term clinical course after disease resolution. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection produces early detectable humoral immune responses in most cases reported to date; however, the duration and protective capacity of the humoral immune response are still unknown. Several studies have shown the appearance of neutralizing and protective anti-SARS-CoV-2 antibodies after infection, which confer protection against reinfection in the following 6 months.[1,2] Older age and a more severe course of the disease have been associated with a more rapid and intense appearance of antibodies.[3,4]

However, no studies have evaluated the medium-term humoral response and its protective role in liver transplant (LT) recipients. As immunosuppressed patients may show weakened immune response to infections, it is paramount to understand the extent and duration of humoral immunity after COVID-19 resolution to delineate surveillance and vaccination protocols. In this prospective nationwide study, we aimed to analyze the incidence, evolution, and conditioning factors of SARS-CoV-2 humoral response within the first 12 months post-SARS-CoV-2 infection in LT recipients as compared to immunocompetent individuals. We herein present preliminary results at 6 months post-SARS-CoV-2 infection.

## 2 | PATIENTS AND METHODS

### 2.1 | Study design

This was a prospective nationwide study endorsed by the Spanish Society of Liver Transplantation (SETH). 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.

LT patients with COVID-19 were prospectively enrolled as part of a nationwide study conducted from February 28 to April 7, 2020 in Spain.[5] A total of 101 LT recipients infected with SARS-CoV-2 from 23 centers were initially included. Serological data were available in 71 of 101 LT recipients at 6 months, and they were compared with an identical number of immunocompetent individuals who were diagnosed with COVID-19 at the Hospital Gregorio Marañón within the same timeframe (control group). Study exclusion criteria were as follows: death within the first 3 months after SARS-CoV-2 infection, active chemotherapy, previous therapy with immunoglobulins or convalescent plasma transfusions, and lack of willingness or ability to provide informed consent. In the LT group, clinical operational tolerance was an additional exclusion criterion, as LT recipients not receiving immunosuppression could be considered as immunocompetent. Cases and controls were matched by a propensity score analysis in a 1/1 ratio.[6] The propensity score was calculated by multiple logistic regression including variables with a well-known prognostic impact in COVID-19: age, gender, comorbidities (diabetes, arterial hypertension, and cardiovascular disease), hospital admission, requirement of mechanical ventilation, and admission to the intensive care unit. The nearest neighbor approach was used to match LT patients and immunocompetent controls to ensure that both groups were comparable in terms of clinical characteristics and severity of COVID-19.

### 2.2 | Data collection

#### 2.2.1 | Laboratory assays

COVID-19 RNA testing of nasopharyngeal/oropharyngeal swab specimens was performed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay[7] at 3 and 6 months after SARS-CoV-2 infection. The main outcome was the presence of anti-SARS-Cov-2 binding antibodies at 12 months after infection. Determination of anti-SARS-Cov-2 antibodies was additionally performed at 3 and 6 months. We herein present preliminary results at 6 months. Detection of SARS-CoV-2 IgG targeting nucleocapsid protein in serum samples was performed at the Microbiology Laboratory in the Hospital Gregorio Marañón using the Abbott ARCHITECT i2000 chemiluminescent microparticle immunoassay (Abbott). The resulting chemiluminescent reaction was measured as a relative light unit and used to calculate an index value. Specimens tested by ARCHITECT were considered reactive at a cutoff index of 1.4 or greater based on previous head-to-head evaluations of several immunoassays; the previously reported sensitivity and specificity of the anti-nucleocapsid assay was of 92.7% (90.20–94.8) and 99.9% (99.4–100), respectively.[8] Each laboratory from the different participating centers processed and transported specimens according to standard procedures. Serum levels of immunosuppressive drugs were determined in each participant center.

#### 2.2.2 | Clinical evaluation

All 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. 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.[9] Regarding immunosuppression, management protocols for COVID-19 in LT patients were broadly similar among the different centers following the recommendations of the Spanish Society of Liver Transplantation and the Ministry of Health throughout the study period. All patients were managed in accordance with COVID-19 protocols, which encouraged clinicians to reduce, but not to withdraw, immunosuppression in liver transplant recipients.

### 2.3 | Statistical analysis

Continuous variables are reported as mean and standard deviations (SD) or as median and interquartile range, as appropriate. Categorical variables are described as absolute numbers and percentages. To assess factors associated with persistence of antibodies at 6 months post-infection, the $X^2$ test was used, with the Fisher correction, whenever appropriate. Differences between antibodies levels in both groups were compared by the U Mann–Whitney test. Univariate and multivariate logistic regression analyses were performed to identify the independent predictors of persistence of antibodies beyond 6 months in the transplant group. Enter method was followed, including variables showing a $p \leq .10$ in the univariate analysis in the multivariable model. The statistical analyses were performed using the SPSS version 22.0 (IBM Corp). Every hypothesis tested was two-tailed and considered significant at $p < .05$.

## 3 | RESULTS

### 3.1 | Study population and baseline characteristics

The evaluation of SARS-Cov-2 humoral response at 6 months after COVID-19 included a total of 142 patients, with 71 in each study group. No patient was excluded from the study, however, due to

logistical difficulties, no serum sample was available in 30 of the 101 LT recipients. LT patients with and without available serum samples were comparable in terms of age distribution, sex, diabetes, hypertension, COVID-19 severity, and hospital admission (Table S1). COVID-19 was confirmed in all patients by a RT-PCR assay of nasopharyngeal swab specimens, between March 6, 2020 and April 7, 2020. In 116 cases (58 case–control pairs), serological data were available at both months 3 and 6 post-infection (Figure 1). According to propensity score matching, both groups were comparable in terms of age distribution, sex, comorbidities, COVID-19 severity, and hospital admission (Table S2). All patients presented symptomatic COVID-19. The majority of patients were from the Madrid area ($n$ = 43; 42.6%), which was the autonomous region with the highest absolute number of COVID-19 confirmed cases during the first wave in Spain.[5] The mean of participants per center was 4.39 (range: 1–13).

Among the 142 patients, males predominated (74.6%), with a median age of 65.5 years old (range: 25–93) (Table 1). Arterial hypertension was the most common comorbidity (64.8%), followed by diabetes mellitus (44.4%), cardiovascular disease (12.7%), and chronic obstructive pulmonary disease (7%) (Table 1). Almost half of the patients (43.7%) received angiotensin-converting enzyme inhibitors (ACE) or angiotensin II receptor blockers (ARB), without differences between LT recipients and controls ($p$ = .398). All LT patients were receiving chronic immunosuppression. Tacrolimus was the predominant immunosuppressant ($n$ = 44; 62%), followed by mycophenolate mofetil ($n$ = 35; 49.3%) and everolimus ($n$ = 15; 21.1%). Eight patients (5.6%) were receiving prednisone as a part of the maintenance immunosuppressive regime when they were diagnosed with COVID-19 (Table S3).

The median time from LT to COVID-19 was 8.11 years (IQR: 2.87–13.26). Most of the patients required hospital admission (85.92%), but the rate of severe COVID-19 was 9.15%. COVID-19 therapy differed between LT recipients and controls. LT patients more frequently received azithromycin compared with controls (60.6% vs. 15.5%, $p$ < .001), whereas lopinavir therapy was much less frequent in LT recipients (28.2% vs. 95.8%, $p$ < .001) (Table 1).

## 3.2 | Incidence and quantitative assessment of antibodies against SARS-CoV-2.

LT recipients showed reduced humoral immune response to SARS-CoV-2 infection as compared with immunocompetent controls at 3 months (77.4% vs. 100%, $p$ < .001) and at 6 months (63.4% vs. 90.1%, $p$ < .001) after COVID-19 diagnosis (Table 2). The quantitative analysis also showed significantly lower levels of anti-nucleocapsid IgG antibodies in LT recipients at month 3 (4.28 vs. [IQR: 1.64–5.83] vs. 5.41 [IQR: 4.15–6.95], $p$ = .002) and at month 6 (1.94 [IQR: 0.51–4.26] vs. 4.33 [IQR: 1.98–6.15], $p$ < .001) post-infection (Figure 2). Although a decline of anti-nucleocapsid IgG levels was observed in both study groups, it was more pronounced in LT recipients as analyzed by the ratio between the index values at months 6 and 3 (0.627 vs. 0.784, $p$ = .001) (Figure 3).

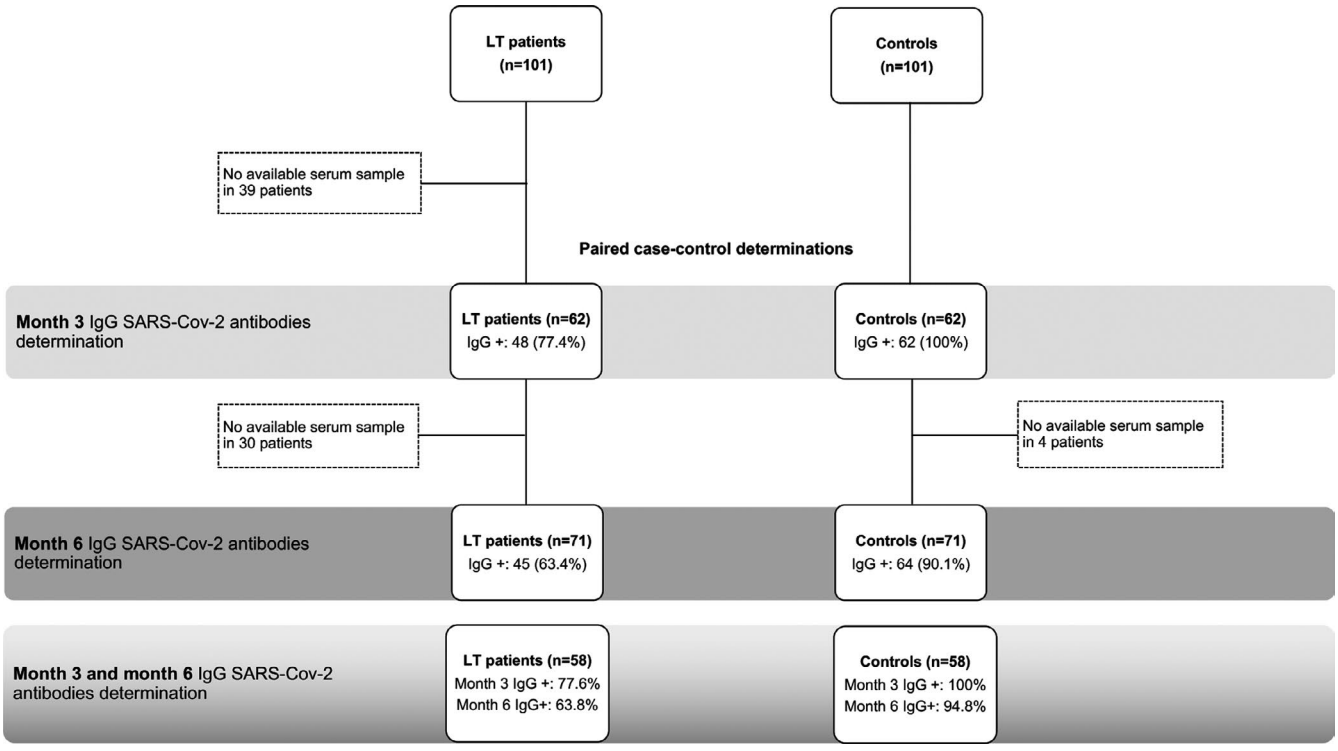


**FIGURE 1** Study protocol and follow-up. No patients were excluded from the study. Serum samples were not available in all patients at 3 and 6 months after COVID-19 due to logistical difficulties

**TABLE 1** Clinical characteristics of 142 patients with paired case–control serological determinations at month 6 according to the study group

| | Liver transplant patients (*n* = 71) | | Immunocompetent patients (*n* = 71) | | *p* |
|---|---|---|---|---|---|
| Age (years) | 65 | (60–71) | 66 | (57–73) | .931 |
| Sex (male) | 54 | (76.1) | 52 | (73.2) | .847 |
| ***Previous medical history*** | | | | | |
| Diabetes Mellitus | 30 | (42.3) | 33 | (46.5) | .736 |
| Hypertension | 44 | (62.0) | 48 | (67.6) | .598 |
| ACE inhibitors or ARB | 28 | (39.4) | 34 | (47.9) | .398 |
| Cardiovascular disease | 8 | (11.3) | 10 | (14.1) | .802 |
| Chronic obstructive pulmonary disease | 5 | (7) | 5 | (7) | 1.000 |
| Asthma | 7 | (9.9) | 5 | (7) | .764 |
| ***Clinical characteristics*** | | | | | |
| Non-severe COVID–19 | 63 | (88.7) | 64 | (90.1) | 1.000 |
| Hospital admission | 60 | (84.5) | 62 | (87.3) | .810 |
| Interval since transplantation (years) | 8.11 | (2.87–13.26) | NA | NA | NA |
| ***COVID–19-specific therapy*** | | | | | |
| Lopinavir | 20 | (28.2) | 68 | (95.8) | <.001 |
| Interferon beta | 1 | (1.4) | 28 | (39.4) | <.001 |
| Hydroxychloroquine | 64 | (91.1) | 67 | (94.4) | .532 |
| Azithromycin | 43 | (60.6) | 11 | (15.5) | <.001 |
| Remdesivir | 0 | (0) | 1 | (1.4) | 1.000 |
| Tocilizumab | 5 | (7) | 10 | (14.1) | .275 |
| Corticosteroids (boluses) | 4 | (5.6) | 5 | (7) | 1.000 |
| ***Immunosuppression at baseline*** | | | | | |
| Tacrolimus | 44 | (62) | NA | NA | NA |
| Cyclosporine | 4 | (5.6) | NA | NA | NA |
| Mycophenolate | 35 | (49.3) | NA | NA | NA |
| Corticosteroids (maintenance) | 8 | (11.4) | NA | NA | NA |
| Everolimus | 15 | (21.1) | NA | NA | NA |

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.

**TABLE 2** Observed incidence of anti-nucleocapsid IgG antibodies and levels according to the study group

| | Liver transplant patients | | Immunocompetent patients | | |
|---|---|---|---|---|---|
| **Month 3** | ***n* = 62** | | ***n* = 62** | | ***p*** |
| Anti-nucleocapsid IgG detected; n (%) | 48 | (77.4) | 62 | (100) | <.001 |
| Anti-nucleocapsid IgG levels; median (IQR) | 4.28 | (1.64–5.83) | 5.41 | (4.15–6.95) | .002 |
| **Month 6** | ***n* = 71** | | ***n* = 71** | | ***p*** |
| Anti-nucleocapsid IgG detected; n (%) | 45 | (63.4) | 64 | (90.1) | <.001 |
| Anti-nucleocapsid IgG levels; median (IQR) | 1.94 | (0.51–4.26) | 4.33 | (1.98–6.15) | <.001 |

Furthermore, a higher frequency of loss of antibodies at 6 months was observed in LT recipients compared to immunocompetent controls, although without reaching statistical significance (17.8% vs. 5.2%, $p$ = .055) (Table S4). This analysis was restricted to patients with available serum samples at 3 and 6 months and detection of antibodies at 3 months.

We also evaluated incidence and levels of anti-SARS-CoV-2 antibodies at 6 months excluding patients treated with interferon beta since its immunomodulatory activity could affect the immune response. However, LT recipients continued to show a reduced humoral immune response to SARS-CoV-2 infection as compared with immunocompetent controls (62.9% vs. 95.3%, $p$ < .001). The quantitative analysis also showed significantly lower levels of anti-nucleocapsid IgG antibodies in LT recipients (1.89 [IQR: 0.49–4.24] vs. 4.36 [IQR: 1.98–6.15], $p$ < .001; Table S5). The two study groups remained comparable in terms of age, sex, disease severity, and comorbidities (Table S6).

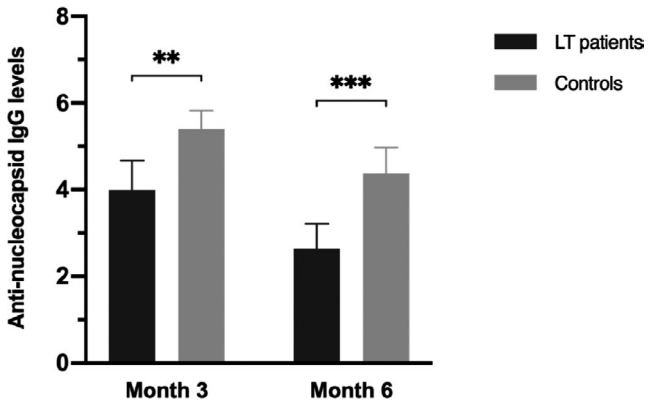


**FIGURE 2** Observed levels of anti-nucleocapsid IgG antibodies at 3 and 6 months post-infection in liver transplant patients (dark gray bars) and immunocompetent controls (light gray bars). Bars represent mean levels of antibodies. Error bars indicate the 95% confidence interval

### 3.3 | Predictors of persistence of antibodies against SARS-CoV-2 in LT patients beyond 6 months

Baseline predictors of anti-SARS-CoV-2 IgG antibodies in LT patients ($n$ = 71) were screened using binary logistic regression analysis (Table 3). Multivariate analysis identified the following independent predictors of persistence of anti-SARS-CoV-2 IgG antibodies at 6 months post-infection: female gender (odds ratio [OR] = 13.49, 95% confidence interval [CI]: 2.17–83.8, $p$ = .005), interval since LT (OR = 1.19, 95% CI: 1.03–1.36, $p$ = .018), and treatment with ACE inhibitors or ARB (OR = 7.11, 95% CI: 1.47–34.50, $p$ = .015). Interestingly, we failed to link the type of immunosuppressive therapy with the persistence of anti-SARS-CoV-2 IgG antibodies at 6 months post-infection.

## 4 | DISCUSSION

We report the first longitudinal study analyzing the specific humoral immune response (anti-nucleocapsid IgG antibodies) against SARS-CoV-2 in LT recipients after symptomatic SARS-CoV-2 infection compared with immunocompetent controls. Evidence of post-infection immunity was observed in LT patients regardless of chronic exposure to immunosuppressive agents; however, despite a similar epidemiological pattern and disease severity, LT patients showed an earlier and more pronounced decline of serum levels of anti-nucleocapsid IgG antibodies as compared with immunocompetent controls.

Seroconversion rates after SARS-CoV-2 symptomatic infection range from 91% to 99%.[2,10] Moreover, SARS-CoV-2 infection produces variable antibody durability[2,11] and may induce strong memory B-cell responses, despite low plasma neutralizing activity.[11,12] Chronic immunosuppression may influence immunological response against pathogens. Therapy with calcineurin inhibitors preferentially inhibits the primary immune response to new antigens,[13] and therefore a weaker response to SARS-CoV-2 would be expected as

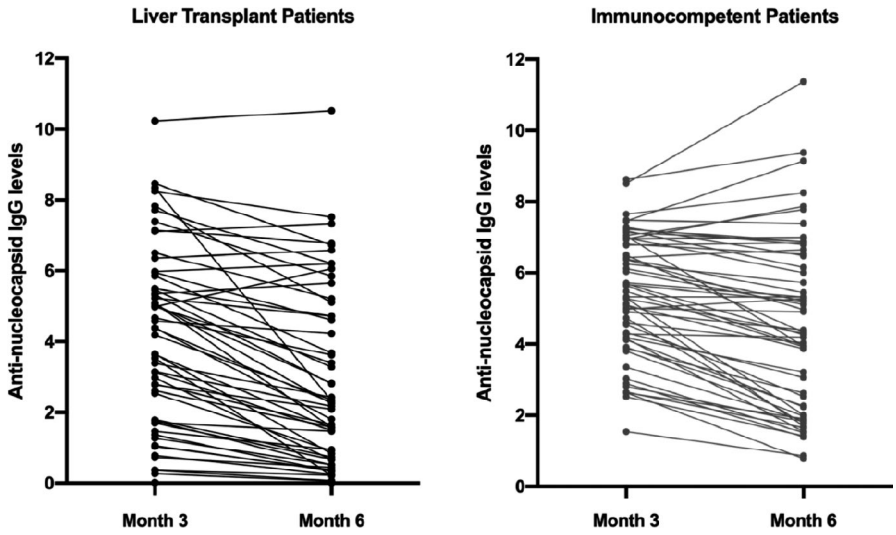


**FIGURE 3** Anti-SARS-CoV-2 IgG levels kinetics. The kinetic is presented for each liver transplant patient ($n$ = 58) and immunocompetent control ($n$ = 58)

**TABLE 3** Clinical predictors of detectable anti-SARS-CoV-2 IgG antibodies in liver transplant patients 6 months after COVID-19 ($n$ = 71)

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| **Variables** | **OR (95% CI)** | ***p*** | **OR (95% CI)** | ***p*** |
| Age | 1.06 (1.00–1.10) | .026 | 1.01 (0.94–1.08) | .873 |
| Sex (female) | 3.46 (0.89–13.47) | .073 | **13.49 (2.17–83.80)** | **.005** |
| Interval since liver transplantation | 1.17 (1.06–1.28) | .002 | **1.19 (1.03–1.37)** | **.018** |
| Diabetes Mellitus | 0.78 (0.29–2.06) | .613 | | |
| Hypertension | 2.21 (0.82–5.99) | .117 | | |
| ACE inhibitors or ARB | 9.58 (2.51–36.57) | .001 | **7.11 (1.47–34.51)** | **.015** |
| Cardiovascular disease | 0.54 (0.12–2.36) | .410 | | |
| Severe COVID–19 | 1.85 (0.34–9.90) | .474 | | |
| Hospital admission | 0.60 (0.15–2.51) | .487 | | |
| Lopinavir | 3.03 (0.89–10.36) | .076 | 2.33 (0.46–11.76) | .365 |
| Hydroxychloroquine | 0.67 (0.12–3.71) | .643 | | |
| Azithromycin | 1.55 (0.58–4.15) | .380 | | |
| Tocilizumab | 0.86 (0.13–5.50) | .871 | | |
| Corticosteroids (boluses) | 1.79 (0.18–18.11) | .624 | | |
| Tacrolimus[a] | 0.25 (0.08–0.78) | .017 | 0.37 (0.08–1.67) | .193 |
| Cyclosporine[a] | 1.79 (0.18–18.11) | .624 | | |
| Mycophenolate[a] | 0.96 (0.36–2.51) | .928 | | |
| Corticosteroids (maintenance)[a] | 0.30 (0.07–1.38) | .122 | | |
| Everolimus[a] | 2.79 (0.71–11.00) | .143 | | |
| Month 6 tacrolimus | 1.02 (0.53–1.99) | .947 | | |
| Month 6 trough concentrations (tacrolimus) | 0.79 (0.59–1.07) | .131 | | |
| Month 6 mycophenolate | 1.18 (0.67–2.10) | .568 | | |
| Month 6 corticosteroids | 0.96 (0.57–1.64) | .888 | | |
| Month 6 everolimus | 1.27 (0.72–2.24) | .415 | | |

Bold values indicate the variables independently associated with antibody persistence at 6 months in the multivariate analysis.
a These variables pertain to active immunosuppression therapy at COVID-19 diagnosis.

compared with immunocompetent individuals. Interestingly, a more pronounced IgG decrease after SARS-CoV-2 infection has been described in kidney transplant patients treated with calcineurin inhibitors.[14] Mycophenolate mofetil has also been associated with a reduction in post-infection antibody production,[15] as well as decreased humoral immune response to the influenza vaccine.[16-18] Conversely, more intense humoral immune response has been described in older patients, particularly in those with a more severe COVID-19.[10,19,20] In our study, LT recipients and immunocompetent patients were matched according to these factors, being their immunocompromised status a possible conditioning factor of lower humoral immune response. Chronic immunosuppression is considered a double-edge sword in COVID-19. While it may facilitate viral replication in the early phase of the infection, it could also ameliorate the aberrant immune response which the most severe forms of the disease produce. This may explain why LT patients have twofold greater incidence rates of COVID-19 but slightly reduced mortality as compared with age- and gender-matched general populations.[5] However, not all immunosuppressive drugs may have the same effect in COVID-19. Mycophenolate mofetil therapy is a risk factor of severe COVID-19 in a dose-dependent manner[5] while tacrolimus can decrease mortality rates.[21] Regarding humoral immune response, we failed to demonstrate an association between the type of immunosuppression and the persistence of protective antibodies. This could be explained by the heterogeneity of immunosuppression protocols in our cohort and by the limited sample size.

Another relevant finding of our investigation is that humoral response against COVID-19 was more intense in patients receiving ACE inhibitors or ARB. The angiotensin-converting enzyme 2 (ACE2) is a cellular receptor which is required for SARS-CoV-2 entry and propagation in host cells. Experimental evidence have shown that treatment with ACE inhibitors or ARB can increase ACE2 expression,[22] thus theoretically promoting SARS-CoV-2 susceptibility and replication, that could ultimately result in stronger humoral immune response. Female gender was another independent predictor of antibody development and persistence after COVID-19. This interesting finding could be explained by the stronger innate and adaptive immunity, and estrogen upregulated ACE2 expression in women.[23] Finally, we also found that the interval since LT was another independent predictor of antibody development. As a greater interval

since LT is generally associated with lower immunosuppression load and older age, these results were expected.

While SARS-CoV-2 antibodies seem to protect against reinfection, antibody levels following infection generally decline. The kinetics of waning antibodies appear to differ according to the severity of infection. A greater reduction of antibodies has been shown in less severe COVID-19 cases,[24] although in patients with greater severity of the disease and higher antibody levels, these levels will also eventually decline.[4] We observed persistence of anti–nucleocapsid IgG antibodies at 6 months after the onset of symptoms in most patients. Similar findings have recently been described in kidney transplant recipients.[14] Persistence of anti-spike IgG antibodies 6 months after COVID-19 has also been described in other studies,[11] although accompanied by a decline in SARS-CoV-2-specific $CD4^+$T cells and $CD8^+$T cells.

Other elements of innate or cellular immunity may confer protection to SARS-CoV-2 reinfection despite the absence of measurable antibodies. Interestingly, the incidence of reinfection has been inversely associated with baseline antibody levels, including those below the positive threshold.[1] Ongoing follow-up studies and future research are needed after infection and vaccination, including the evaluation of waning antibodies and persistence of B-cell and T-cell memory to SARS-CoV-2. Furthermore, given the lower humoral immune response we have demonstrated in LT recipients after COVID-19, it will be crucial to specifically assess the magnitude and duration of protection against reinfection by vaccination in this population. Additionally, as LT recipients present a higher risk of developing COVID-19[5] and can act as disease vectors, the effect of protection of vaccination on transmission should also be assessed in future studies.

Our study is not without limitations. The high incidence of post-infection antibodies in both study groups may be conditioned by the high proportion of patients with pneumonia that required hospitalization, which represents a more severe disease to that observed in the general population. This may be explained by the difficulties in accessing PCR diagnosis in the mildest or asymptomatic COVID-19 cases that existed during the first wave in Spain. On the other hand, we are aware that the test we used for the detection of antibodies, which is one of the most widely used, is not factually quantitative, and although it correlates well with the amount of antibodies present in the sample, it is not a titration technique in the strictest sense. Furthermore, the shorter half-life of antibodies targeting nucleocapsid compared with antibodies targeting spike protein[25] could have underestimated the proportion of seropositive patients in our study. Finally, COVID-19 therapies were heterogeneous in our cohort and a potential impact on subsequent humoral response cannot be ruled out. This heterogeneity could be explained by the potential pharmacological interactions of antiviral therapy with immunosuppression; consequently, this type of therapy was generally avoided in LT patients. Furthermore, as interferon beta therapy may lead to acute rejection and immune-mediated complications it was rarely used. However, when we analyzed humoral immune response excluding patients treated with interferon beta, we obtained similar results. Moreover, none of these agents were identified as predictors of antibody persistence in the multivariate analysis. Although caution must be exercised when attempting to generalize our results, this is the first study demonstrating a key aspect of the immune response against SARS-CoV-2 infection that may have implications for the vaccination of LT population.

In conclusion, LT patients exhibit a lower persistence of anti-nucleocapsid IgG antibodies within the first 6 months post-infection and more pronounced antibody levels decline. These results call for the need for specific studies regarding vaccination in solid organ transplant recipients receiving chronic immunosuppression to adapt dosing and surveillance protocols.

## DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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