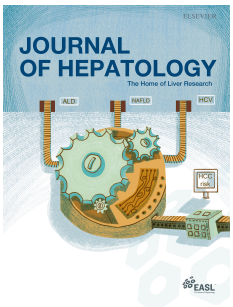


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Epidemiological pattern, incidence and outcomes of COVID-19 in liver transplant patients.

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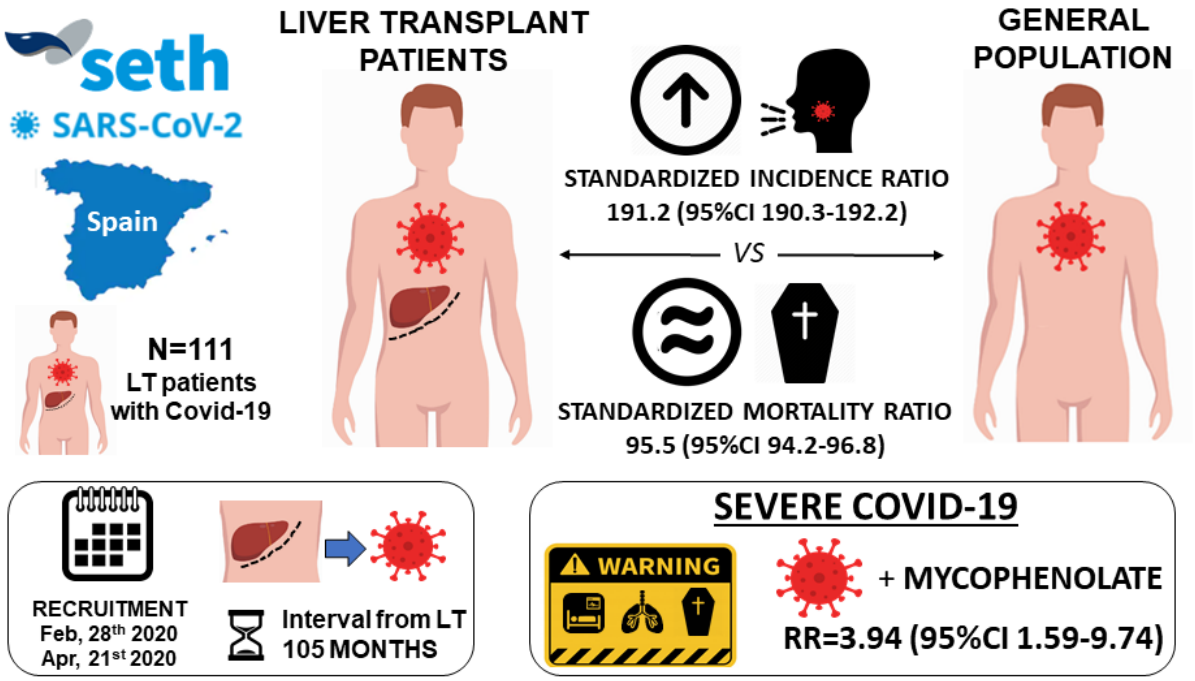
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seth
SARS-CoV-2
Spain
N=111
LT patients
with Covid-19
LIVER TRANSPLANT
PATIENTS
STANDARDIZED INCIDENCE RATIO
191.2 (95%CI 190.3-192.2)
VS
STANDARDIZED MORTALITY RATIO
95.5 (95%CI 94.2-96.8)
GENERAL
POPULATION
RECRUITMENT
Feb, 28th 2020
Apr, 21st 2020
Interval from LT
105 MONTHS
SEVERE COVID-19
WARNING
+ MYCOPHENOLATE
RR=3.94 (95%CI 1.59-9.74)

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TITLE PAGE

Epidemiological pattern, incidence and outcomes of COVID-19 in liver transplant patients.

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- MG has received speaker fees from and/or acted as advisor for Astellas, Novartis, Chiesi, Baxter, and Medtronic.

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Data availability statement

A raw data set underpinning the present research publication may be found in the following repository: http://dx.doi.org/10.17632/5ssscvfmxn.2

Authors contributions

- Jordi Colmenero*: Study design, statistical analysis and manuscript preparation.

- Manuel L. Rodríguez-Perálvarez*: Study design, statistical analysis and manuscript preparation.

- M. Salcedo: literature review, data recording and manuscript draft review for important intellectual content.

- A. Arias-Milla, A. Muñoz-Serrano, J. Graus, J. Nuño, M. Gastaca, J. Bustamante-Schneider, A. Cachero, L. Lladó, A. Caballero, A. Fernández-Yunquera, C. Loinaz, I. Fernández, C. Fondevila, M. Navasa, M. Iñarrairaegui L. Castells, S. Pascual, P. Ramírez, C. Vinaixa, M.L. González-Dieguez, R. González-Grande, L. Hierro, F.

Nogueras, A. Otero, J.M. Álamo, G. Blanco-Fernández, E. Fábrega, F. García-Pajares, J.L. Montero and S. Tome: identification and inclusion of patients, data recording and manuscript draft review for important intellectual content.

- G. De la Rosa: provided and analyzed data from the Spanish Liver Transplant Registry.

- J.A. Pons: Original concept, study design, literature review, and manuscript draft review for important intellectual content.

* These two authors contributed equally to the present work and may be considered co-first authors.

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ABSTRACT

Background & Aims: The incidence and outcomes of coronavirus disease 2019 (Covid-19) in immunocompromised patients are a matter of debate.

Methods: Prospective nationwide study including a consecutive cohort of liver transplant patients with Covid-19 recruited during the Spanish outbreak from February 28th to April 7th, 2020. The primary outcome was severe Covid-19, defined as the need for mechanical ventilation, intensive care, and/or death. Age- and gender-standardized incidence and mortality ratios (SIR and SMR) were calculated using data from the Ministry of Health and the Spanish liver transplant registry. Independent predictors of severe Covid-19 among hospitalized patients were analyzed using multivariate Cox regression.

Results: A total of 111 liver transplant patients were diagnosed with Covid-19 (SIR=191.2 [95%CI 190.3-192.2]). The epidemiological curve and geographic distribution overlapped widely between liver transplant and general populations. After a median follow-up of 23 days, 96 patients (86.5%) were admitted to hospital and 22 patients (19.8%) required respiratory support. Twelve patients were admitted to the ICU (10.8%). The mortality rate was 18%, being lower compared to the matched general population (SMR=95.5 [95%CI 94.2-96.8]). Thirty-five patients (31.5%) met criteria of severe Covid-19. Baseline immunosuppression containing mycophenolate was an independent predictor of severe Covid-19 (RR=3.94; 95%CI 1.59-9.74; p=0.003), particularly at doses higher than 1,000 mg/day (p=0.003). This deleterious effect was not observed with calcineurin inhibitors or everolimus and complete immunosuppression withdrawal showed no benefit.

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Conclusions: Being chronically immunosuppressed, liver transplant patients have an increased risk of acquiring Covid-19 but their mortality rates are lower than matched general population. Upon hospital admission, mycophenolate dose reduction or withdrawal could help in preventing severe Covid-19. However, complete immunosuppression withdrawal should be discouraged.

LAY SUMMARY: In liver transplant patients, chronic immunosuppression increases the risk of acquiring Covid-19 but it could reduce disease severity. Complete immunosuppression withdrawal may not be justified. However, mycophenolate withdrawal or temporary conversion to calcineurin inhibitors or everolimus until disease resolution could be beneficial in hospitalized patients.

Abbreviations:

- Covid-19: coronavirus disease 2019
- CI: confidence interval
- ICU: intensive care unit
- IQR: interquartile range
- LT: liver transplantation
- PCR: real-time reverse transcriptase polymerase chain reaction assay.
- RR: relative risk
- SETH: Spanish liver transplant society, *Sociedad Española de Trasplante Hepático*.

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INTRODUCTION

Spain has registered the fastest spread of coronavirus disease 2019 (Covid-19) in Europe with 210,773 cases detected by April, 28th, 2020, and case-fatality rate of 11.3%[1], being much higher than those observed in China, the origin of the pandemic[2]. The liver transplantation (LT) network in Spain is composed by 25 institutions in which more than 13,000 alive LT patients are surveilled. Transplant recipients are considered by some authors as a high-risk sub-population for Covid-19 as they receive lifelong immunosuppressive therapy.

According to data on previous coronaviruses (MERS-CoV and SARS-CoV), it has been hypothesized that Covid-19 has an earlier phase of viral replication and a later phase characterized by viral clearance due to immune response. This second phase may eventually trigger a deregulation of CD4+ T cells, activation of CD8+ T cells and macrophages, and a cytokine storm[3, 4], ultimately producing the most severe forms of Covid-19. Immunomodulatory agents could ameliorate this harmful immune response[4] but this could lead to an increase in viral load and delayed disease recovery. Interestingly, the most frequently used immunosuppressive agents in LT recipients, namely calcineurin inhibitors[5], have shown intriguing capacities to inhibit the replication of coronaviruses[6]. Therefore, baseline immunomodulation could protect LT patients against the most severe clinical forms of Covid-19[7].

In this prospective nationwide study, we aimed to analyze the epidemiological pattern, clinical features and outcomes of LT patients diagnosed with Covid-19 as compared with those observed in the general Spanish population.

PATIENTS AND METHODS

This is a prospective nationwide study promoted by the Spanish Society of Liver Transplantation (SETH), which was launched after the outbreak of Covid-19 in Spain on February 28th, 2020. The study was performed according to the principles of the declaration of Helsinki and the European Union regulation 2016/679. The study was approved by the research ethics committee of the Hospital Clínic Barcelona (HCB 2020/0384) and the research protocol was registered at ClinicalTrials.gov (NCT04361591). Oral informed consent to participate in the study was obtained from all patients/relatives, and written informed consent was retrieved after viral clearance.

Identification and characterization of LT patients with Covid-19.

Patients receiving a LT in Spain undergo lifelong surveillance by the transplant team and they are instructed to contact for any health-related issue. All LT recipients with known Covid-19 were prospectively enrolled in the registry up to April 7th, 2020, when the national authorities informed that the epidemiological curve had reached a plateau. There were no exclusion criteria. LT patients with Covid-19 were declared to the SETH registry using an anonymized online platform. The transplant institution was responsible for declaring cases as soon as they were aware of them. Nurse transplant coordinators actively searched for Covid-19 patients by telephone contact.

Covid-19 was confirmed by a real-time reverse transcriptase polymerase chain reaction assay (PCR) of nasal and pharyngeal swab specimens[8]. In Spain, patients admitted to the hospital were tested onsite within the first 24 hours, while outpatients with mild symptoms were tested at home or at “drive-thru” testing sites within the next 72 hours after contacting a dedicated phone number. All clinical information was extracted by experienced transplant physicians from reliable electronic medical data sources.

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Demographic data, comorbidities (Charlson comorbidity index[9]), clinical features, laboratory parameters and transplant-related information including baseline immunosuppression (drugs and trough concentrations) were recorded. Chest X-rays were reported by a single member of the radiology department at each center. Modifications of immunosuppression therapy were registered as well as specific drugs prescribed for Covid-19.

Management protocols for Covid-19 were broadly similar within the national territory according to the SETH and the Ministry of Health recommendations. Patients were admitted to hospital if they had hypoxemia (arterial oxygen partial pressure <70 mmHg) and/or radiological chest X-ray abnormalities. Patients with significant co-morbidities or over the age of 60 were also admitted at the discretion of the responsible clinician even if they did not fulfill the above referred criteria. Patients could be treated with oral hydroxychloroquine and/or azithromycin for 5-7 days. In patients with moderate-severe Covid-19, antiviral therapy with lopinavir/ritonavir or remdesivir was allowed as compassionate use. Patients with acute distress respiratory syndrome could receive boluses of steroids and/or tocilizumab. Regarding immunosuppression, Covid-19 protocols encouraged clinicians to reduce, but not to withdraw immunosuppression in LT patients. Patients were followed until April 21st, 2020, thereby ensuring a minimum of 2 weeks of follow-up for clinical outcomes.

Study definitions and outcomes.

The main outcome evaluated was severe Covid-19, which was a composite endpoint including need for mechanical ventilation, admission to the intensive care unit (ICU), and/or death. This endpoint was used in a previous study describing the clinical features of Covid-19 in China[2]. Secondary outcomes were: respiratory insufficiency (arterial oxygen partial pressure <60 mmHg), need for mechanical ventilation, admission to the

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ICU and liver allograft dysfunction (conventionally defined as raising bilirubin >4 times from baseline values or international normalized ratio>1.4). Patients were considered cured of Covid-19 when discharged from the hospital or after two consecutive PCR-negative results separated by more than 48 hours, whichever occurred first.

Data sources, calculation of standardized ratios and statistical analysis.

Data regarding populations at risk were obtained from the National Statistics Institute (general population) and from the Spanish Liver Transplant Registry (LT population), both updated in 2019. The incidence and outcomes of Covid-19 epidemics in the general Spanish population were retrieved from the official publication of the Ministry of Health, which is updated on a daily basis (https://www.mscbs.gob.es/). To obtain the cumulative incidence of Covid-19, data was accessed on 7th April, 2020, whereas mortality data were obtained on 21st, April 2020, aligning with the periods of recruitment and surveillance respectively of LT patients. Relative risks of Covid-19 (incidence and mortality) in LT patients as compared with the general population were expressed as standardized incidence and mortality ratios, which result from the ratio of the observed to the expected events. Expected incidence and outcomes in LT patients were obtained from the numbers observed in the general population, after balancing for age and gender. Ninety-five percent confidence intervals (95%CI) were calculated under the assumption that the number of cases observed followed a Poisson distribution.

Continuous variables were reported as mean and standard deviations or as median and interquartile range (IQR), as appropriate. Categorical variables were summarized as counts and percentages. Among hospitalized patients, independent predictors of severe Covid-19 were identified using Cox regression analysis. Patients were censored when discharged or on April 21st. Variables available at Covid-19 onset showing a $p \leq 0.30$ in

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the univariate analysis entered the initial multivariate model. Non-significant co-variates were removed from the model in a backward stepwise process, commencing by those with the highest p value. All possible interactions were tested. A confounding factor was confirmed when its removal from the model motivated >15% variation in the remaining β coefficients. The statistical analysis was performed using SPSS version 22.0 (IBM Corp, Armonk, NY). Every hypothesis tested was two-tailed and considered significant if $p < 0.05$.

RESULTS

Standardized incidence of Covid-19 in LT patients.

Epidemiological curves in general and LT populations were broadly similar (figure 1). Peaks of new cases occurred between March 25th and April 1st, with subsequent stabilization, and incipient decline at database closure. The geographical distribution of Covid-19 in the general population and in LT recipients overlapped widely (figure 2). The autonomous regions with the highest absolute number of confirmed cases were Madrid and Catalonia, both in the general population and in the LT population. Within the study period, 146,690 persons were diagnosed with Covid-19 in Spain (cumulative incidence 311.93 cases/10^5 habitants). Cumulative incidence was increased in older people, particularly beyond 60 years old (supplementary table 1). On the other hand, the alive LT population in Spain comprises 13,255 patients, among whom there were 111 confirmed cases of Covid-19 (cumulative incidence 837.41 cases/10^5 patients). After adjusting for age and gender, the number of observed cases almost doubled the expected number of cases (suppl. table 1) thus resulting in a standardized incidence ratio of

191.22 (95%CI 190.28-192.16). In the subset of patients older than 60, the standardized incidence ratio was even higher (206.48; 95%CI 205.45-207.52).

Clinical characteristics and outcomes of LT patients with Covid-19.

A total of 111 LT patients with Covid-19 were included (table 1). The mean age was 65.34±10.96 and there was a male predominance (71.2%). The median interval from transplantation was 105 months (IQR 35-168). None of the patients had been infected by the liver donor. The most frequent comorbidity was hypertension (57.7%). The vast majority of patients (n=109; 98.2%) were receiving chronic immunosuppression: Calcineurin inhibitors were the predominant immunosupressants, either alone (n=34; 30.6%), or in combination with mycophenolate (n=29; 26.1%) or everolimus (n=9; 8.1%). The remaining 37 patients (33.3%) received calcineurin inhibitors-free immunosuppression, based on mycophenolate and/or everolimus (table 1). Covid-19 was symptomatic in 93% of patients. The most frequent symptoms were fever (74.8%) and cough (70.3%). Asymptomatic patients (n=7) were tested for Covid-19 because of incidental radiological findings (n=2) or after a high-risk contact (n=5). Regarding laboratory parameters, the absolute lymphocyte count was 670/µl (IQR 430-1,040) and D dimer was 600 ng/mL (IQR 345-1,630). The chest X-ray showed abnormalities in 78.4% of patients, with a bilateral diffuse pattern in 58.6% of patients.

The median follow-up after the diagnosis of Covid-19 was 23 days, with a minimum of 15 days for the last included patient. Ninety-six patients (86.5%) were admitted to hospital according to the following criteria: 18 patients (18.8%) had hypoxemia, 81 patients (84.4%) had radiological chest abnormalities, 77 patients (80.2%) were older than 60 years, and 51 patients (53.1%) had significant comorbidities (Charlson >3). Most of admitted patients met more than one criterion: 11 patients (11.5%) met 2 criteria, 32 patients (33.3%) met 3 criteria and 36 patients (37.5%) met 4 criteria. Only 6

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patients did not meet any of the recommendations described above and the reason for hospital admission was the presence of severe clinical symptoms. There was a significant elevation of transaminases (doubled nadir) in 16 patients (14.7%). Three patients developed liver graft dysfunction (2.7%) but none of them had graft loss. Respiratory insufficiency occurred in 44 patients (39.6%). Twenty-two patients (19.8%) required respiratory support after a median of 5 days (IQR 1.75-9.5) after hospital admission, being non-invasive in 13 patients and invasive 9 patients. Twelve patients were admitted to the ICU (10.8%). The mortality rate was 18% with a median interval from hospital admission of 7 days (IQR 5-9.75). At database closure, 81 patients (73%) had been discharged or remained asymptomatic at home, and 10 patients (9%) were still in-hospital. After a median of 17 days (IQR 13-27.5), a PCR-negative result for Covid-19 was confirmed in 18 patients.

Thirty-five patients (31.5%) met criteria of severe Covid-19 (table 1). These patients were characterized by older age ($p=0.038$), more frequent dyspnea ($p<0.001$) and a reduced PaO2/FiO2 ratio ($p=0.044$) on admission. During hospital stay, severe Covid-19 patients presented reduced lymphocyte counts ($p=0.013$), increased D dimer ($p=0.032$) and increased serum ferritin ($p=0.004$). Regarding management, severe Covid-19 patients received more frequently tocilizumab ($p<0.001$) and boluses of corticosteroids ($p=0.007$). Patients receiving mycophenolate or attempting a complete withdrawal of immunosuppression were more prevalent in the severe Covid-19 group ($p=0.014$, and $p=0.016$ respectively). Conversely, tacrolimus-containing immunosuppression was more frequent in the non-severe Covid-19 group, although without statistical significance ($p=0.113$).

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Risk factors of severe Covid-19 among hospitalized patients.

Baseline risk factors of severe Covid-19 among hospitalized patients (n=96) were screened using Cox regression analysis (table 2). The multivariate analysis identified the following independent predictors: Charlson comorbidity index (relative risk [RR]=1.28 (95%CI 1.05-1.56), male gender (RR=2.49; 95%CI 1.14-5.41), dyspnea at diagnosis (RR=7.25; 95%CI 2.95-17.82), and baseline immunosuppression containing mycophenolate (RR=3.94; 95%CI 1.59-9.74). Age, estimated glomerular filtration rate and complete withdrawal of immunosuppression upon hospital admission were not significant, but they were kept in the analysis to be controlled as potential confounding factors. Kaplan-Meier curves illustrate the negative prognostic impact of mycophenolate, particularly at doses higher than 1,000 mg/day (figure 3). Among patients receiving full-dose of mycophenolate at baseline (ie. 2,000 mg/day), complete drug withdrawal had a trend towards reduced severe Covid-19 (41.7% vs 69.2%; p=0.16). Interestingly, baseline therapy with calcineurin inhibitors, mTOR inhibitors (everolimus), and maintenance corticosteroids, independently of their trough concentrations/doses, were not associated with increased risk of severe Covid-19. Therapy with hydroxychloroquine and/or azithromycin at hospital admission did not decrease the risk of severe Covid-19 (p=0.64 and p=0.45, respectively).

Standardized mortality ratio of Covid-19 in LT patients.

In the general population, a total of 21,717 deaths were registered by April 21st. Overall mortality rates were 14.8%, but were as high as 27.9% in patients older than 60 years (supplementary table 2). Among the LT population, 20 patients with Covid-19 died during the study period (mortality rate 18%). After adjusting for age and gender, the number of observed deaths among LT patients was slightly lower than the expected number of deaths (suppl. table 2), thus resulting in a standardized mortality ratio of

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95.55 (95%CI 94.25-96.85). When evaluating subgroups according to age strata, no death was registered among LT patients younger than 60. Adjusted mortality rates in patients older than 60 were similar in LT recipients and in the general population (standardized mortality ratio=100.29; 95%CI 98.93-101.66).

DISCUSSION

This is the first nationwide study evaluating the incidence and outcomes of Covid-19 in immunosuppressed patients as compared with age and gender-matched counterparts from the general population. Despite a similar epidemiological pattern, LT patients had almost doubled standardized incidence rates of Covid-19. Chronic exposure to immunosuppressive agents did not increase standardized mortality rates but our findings suggest that mycophenolate, particularly at doses higher than 1,000 mg/day, could increase the risk of severe Covid-19 among hospitalized LT patients. Since other immunosuppressants such as calcineurin inhibitors (tacrolimus and cyclosporine) and mTOR inhibitors (everolimus) did not show this association, the present study provides key evidence to modulate immunosuppression in order to improve outcomes in LT patients with Covid-19.

A similar epidemiological curve and geographical distribution of Covid-19 were observed between LT recipients and the general population. Despite routine recommendations given to LT patients regarding social distancing, they had almost doubled risk of developing Covid-19 as compared with age and gender-matched general population. The most plausible explanations are chronic immunosuppression and increased comorbidities, which would make them more vulnerable. Precautions should be intensified within a context of epidemy to prevent transmission. Regarding

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outcomes, patients had a minimum of 2 weeks of surveillance to ensure that the need for intensive care and mortality were adequately captured, as previous studies reported that these outcomes may occur earlier[2]. The current evidence of Covid-19 in solid organ transplant recipients is based on short and uncontrolled case-series with contradictory conclusions. Whereas some studies report aggressive Covid-19 with fatal outcomes[10, 11], other authors suggest that severe respiratory disease may be anecdotical[12]. Since transplant patients have more comorbidities than the general population[13], the expected severity of Covid-19 would be increased. Although standardized rates of severe Covid-19 could not be calculated (due to unreliable data from the general population), we demonstrated that standardized mortality rates were actually lower in LT patients as compared with the general population. The clinical features associated with severe Covid-19 were similar to those previously reported[2]: older age, male gender, increased co-morbidities, raised D dimer, serum ferritin and decreased absolute lymphocyte count. PaFiO2 and dyspnea at admission were also associated with an increased risk of severe Covid-19, but only the latter variable was included in the final multivariate model due to missing PaFiO2 information in 22 patients. Noteworthy, the interval from LT until SARS-CoV-2 infection had no impact on the risk of evolving to severe Covid-19. However, the number of patients diagnosed of Covid-19 within the first year after LT was limited (n=15) and further studies are required to confirm this finding.

The relationship of immunosuppression and Covid-19 outcomes is frequently referred to as a double-edged sword[14]. Too much immunosuppression could result in increased viral load and delayed recovery whereas a competent immune system could be responsible for the most severe forms of the disease[15]**.** In the transplant setting, many authors advocate the potential benefit of immunosuppression in Covid-19 based

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on personal experiences or on uncontrolled clinical observations[12, 16-19]**.** In the present study, the vast majority of patients were receiving calcineurin inhibitors (tacrolimus or cyclosporine), mTOR inhibitors (everolimus), and/or mycophenolate. Whereas calcineurin and mTOR inhibitors were not associated with worse outcomes, at least not with their usual trough concentrations, mycophenolate therapy at baseline was an independent predictor of severe Covid-19 in a dose-dependent manner. This interesting observation may be explained by the different mechanisms of action. Mycophenolate produces a cytostatic effect on activated lymphocytes[20]. In Covid-19, the virus SARS-CoV-2 has a direct cytotoxic effect on lymphocytes, particularly CD8+[3, 21], thereby explaining the association between lymphopenia and worse outcomes[2]. Therefore, mycophenolate and SARS-CoV-2 may exert a synergic and deleterious effect on depleting peripheral lymphocytes, which would be responsible for an aberrant immune reconstitution as demonstrated with other viruses[22]. In contrast, mTOR inhibitors increase the quality and functionality of memory T cells and reduce the replication of a myriad of viruses including cytomegalovirus, herpes virus-8, Epstein-Barr, and human immunodeficiency virus[23]. Regarding calcineurin inhibitors, some studies have shown antiviral effects *in vitro* against coronaviruses[6] and they could also ameliorate the cytokine storm[15]. A randomized controlled trial with calcineurin inhibitors and/or mTOR inhibitors in immunocompetent individuals could be of great interest. An algorithm proposal to modify baseline immunosuppression in LT patients diagnosed with Covid-19 according to the study findings is presented in figure 4.

Given the lack of solid scientific evidence, the therapeutic approach against Covid-19 varied among participating institutions and usually included a combination of hydroxychloroquine, azithromyzin, antivirals, corticosteroids, and/or monoclonal

antibodies in a stepwise process. In the only randomized trial published hitherto, antiviral therapy with lopinavir/ritonavir failed to reduce mortality in patients with severe Covid-19[24]. In our cohort, hydroxychorloquine and/or azithromyzin were started early after diagnosis but we did not observe a clear benefit. Antivirals (lopinavir/ritonavir, remdesivir, and interferon), boluses of corticosteroids, and monoclonal antibodies (tocilizumab) were prescribed mainly in unresponsive cases in the present cohort and it is hard to extract valid conclusions. Caution should be taken in the absence of well-designed randomized trials[25].

Some limitations of the present study should be highlighted. Underdiagnosing of Covid-19 could be an issue given the suboptimal sensitivity of PCR of nasal and pharyngeal swab specimens and the limited access to diagnostic tests at some timepoints during the Covid-19 epidemy in Spain. The true cumulative incidence of Covid-19 may have been underestimated, although in a similar proportion in LT patients and in the general population, thus making standardized incidence ratio equally reliable. Under-reporting is another potential limitation in prospective registries but we believe it had a limited impact in the present study because LT patients/relatives are tightly connected by phone with the nurse transplant coordinator at each center to report any intercurrent health issue. The limited sample size may have weakened some analyses and results marginally not significant should be carefully interpreted as some could be clinically meaningful. Finally, the present study was neither designed nor powered to evaluate the effect of specific therapies against Covid-19 and no solid conclusion may be derived in this regard.

In conclusion, being chronically immunosuppressed and with increased co-morbidities, LT patients are more likely to develop Covid-19 within an outbreak scenario and therefore social distancing and other preventative measures should be enhanced in these

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patients. Mortality rates were lower than those observed in age- and gender-matched general population, thereby suggesting that chronic immunosuppression could exert a certain protective effect against the most severe forms of Covid-19. A complete immunosuppression withdrawal after the diagnosis of Covid-19 may not be justified. However, in patients receiving mycophenolate, dose reduction or temporary conversion to calcineurin inhibitors or everolimus may be considered until complete recovery from Covid-19.

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TABLES

Table 1. Clinical characteristics of 111 liver transplant recipients with coronavirus-19 disease (Covid-19) included in the Spanish Society of Liver Transplantation (SETH) registry. Severe Covid-19 was defined as need for mechanical ventilation, admission to intensive care unit and/or death.

VARIABLE	Covid-19 SETH registry N=111	Non-severe Covid-19 N=76	Severe Covid-19 N=35	p
Age	65.34 ± 10.96	63.88 ± 11.94	68.51 ± 7.70	0.038
Gender (Women); % (n)	28.8% (32)	25% (19)	37.1% (13)	0.189
Previous medical history				
Diabetes; % (n)	47.7% (53)	43.4% (33)	57.1% (20)	0.179
Hypertension; % (n)	57.7% (64)	59.2% (45)	54.3% (19)	0.626
ACE inhibitors; % (n)	29.7% (33)	31.6% (24)	25.7% (9)	0.530
Cardiomyopathy; % (n)	19.8% (22)	17.1% (13)	25.7% (9)	0.290
Bronchopulmonary; % (n)	11.7% (13)	11.8% (9)	11.4% (4)	0.850
Charlson comorbidity index; median (IQR)	4 (2-6)	3 (2-4.7)	5 (4-7)	0.002
Interval since transplantation				
Months; median (IQR)	105 (35-168)	97.5 (30-160)	127 (57-208)	0.197
<12 months; % (n)	13.5% (15)	11.8% (9)	17.1% (6)	0.448
Aetiology of liver disease				
Alcohol; % (n)	30.6% (34)	28.9% (22)	35.3% (12)	0.571
Hepatitis C; % (n)	28.8% (32)	30.3% (23)	25.7% (9)	0.623
Hepatitis B; % (n)	10.8% (12)	11.8% (9)	8.6% (3)	0.750
Autoimmune; % (n)	8.1% (9)	6.6% (5)	11.4% (4)	0.459
Clinical presentation of Covid-19				
Fever; % (n)	74.8% (83)	75% (57)	74.3% (26)	0.936
Dyspnea; % (n)	41.4% (46)	25% (19)	77.1% (27)	<0.001
Cough; % (n)	70.3% (78)	67.1% (51)	77.1% (27)	0.282
Gastrointestinal; % (n)	34.2% (38)	36.8% (28)	28.6% (10)	0.394
Asymptomatic; % (n)	6.3% (7)	9.2% (7)	0% (0)	0.095
Laboratory parameters in hospitalized patients (n=96); median (IQR)				
PaO2/FiO2 ratio (onset)*	400 (309-436)	403 (333-444)	328 (279-420)	0.044
Lymphocyte (onset)-count/µl	670 (430-1,040)	715 (415-1,000)	600 (430-1,200)	0.449
Lymphocyte count (min)-count/µl	455 (275-755)	500 (380-840)	310 (200-500)	0.013
D dimer (onset)-ng/mL	600 (345-1,630)	540 (340-1,010)	1,100 (450-2,796)	0.127
D dimer (max)-ng/mL	1,050 (517-3,299)	800 (390-2,543)	2,229 (809-4,290)	0.032
Ferritin (max)-ng/mL	847 (376-1975)	511 (256-1,663)	1,459 (770-2,264)	0.004
Estimated glomerular filtration rate - ml/min**	62.5 (41.3-79.7)	63.2 (43.5-82.5)	53.3 (39-74)	0.396
Chest X-ray abnormalities				
Normal; % (n)	21.6% (24)	31.6% (24)	0% (0)	0.002

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Unilateral; % (n)	19.8% (22)	18.4% (14)	22.8% (8)	
Bilateral; % (n)	58.6% (65)	50% (38)	77.1% (27)	
Covid-19 specific therapy in hospitalized patients (n=96)				
Azithromycin; % (n)	62.5% (60)	63.9% (39)	60% (21)	0.702
Hydroxychloroquine; % (n)	91.7% (88)	90.2% (55)	94.3% (33)	0.482
Lopinavir/ritonavir; % (n)	41.7% (40)	36.1% (22)	51.4% (18)	0.142
Remdesivir; % (n)	1% (1)	0% (0)	2.9% (1)	0.365
Interferon beta; % (n)	3.1% (3)	1.6% (1)	5.7% (2)	0.552
Tocilizumab; % (n)	15.6% (15)	4.9% (3)	34.3% (12)	<0.001
Boluses of corticosteroids; % (n)	12.5% (12)	4.9% (3)	25.7% (9)	0.007
Immunosuppression at baseline (drugs)				
Tacrolimus; % (n)	59.5% (66)	64.5% (49)	48.6% (17)	0.113
Cyclosporine; % (n)	5.4% (6)	5.3% (4)	5.7% (2)	0.922
Mycophenolate; % (n)	51.4% (57)	43.4% (33)	68.6% (24)	0.014
Everolimus; % (n)	20.7% (23)	22.4% (17)	17.1% (6)	0.528
Corticosteroids (maintenance); % (n)	21.8% (24)	18.7% (14)	28.6% (15)	0.241
Immunosupression (combinations); % (n)				
CNI	30.6% (34)	32.9% (25)	25.7% (9)	0.374
CNI + mycophenolate	26.1% (29)	27.6% (21)	22.9% (8)	
CNI + everolimus	8.1% (9)	9.2% (7)	5.7% (2)	
Mycophenolate +/- everolimus	33.3% (37)	27.6% (21)	45.7% (16)	
None	1.8% (2)	2.6% (2)	0% (0)	
Immunosuppression baseline (dose- mg)				
Tacrolimus (n=66); median (IQR)	2.5 (1.5-5)	2.5 (1.7-5)	2 (1-4.25)	0.986
Cyclosporine (n=6); median (IQR)	75 (50-150)	125 (62.5-150)	125 (75-150)	0.400
Mycophenolate (n=57); median (IQR)	1,000 (1,000-2,000)	1,000 (1000-1,500)	1,500 (1,000-2,000)	0.056
Everolimus (n=23); median (IQR)	2 (1-2.1)	2 (1.1-2.4)	2 (0.87-2.25)	1
Corticosteroids (maintenance) (n=24); median (IQR)	10 (5-20)	5 (4.7-16.2)	10 (8.7-30)	0.673
Immunosuppression (trough concentrations onset- ng/mL)				
Tacrolimus (n=66); median (IQR)	4.95 (3.32-6.92)	5 (4-6.7)	4.2 (2.7-6.9)	0.772
Cyclosporine (n=6); median (IQR)	99 (51.5-148.6)	99 (88.5-125)	85.1 (25-145.2)	1
Everolimus (n=23); median (IQR)	3.5 (2.9-5.5)	3.5 (2.9-5.4)	3.5 (1.6-6.4)	1

* PaO2/FiO2 was not available in 22 patients. ** Estimated glomerular filtration rate calculated by the Modification of Diet in Renal Disease-4 (MDRD-4); IQR: interquartile range.

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Table 2. Clinical predictors of severe Covid-19 among patients admitted into the hospital (n=96). Severe Covid-19 was defined as requirement of respiratory support, admission in intensive care unit and/or death. Univariate and multivariate Cox´s regression analyses were used.

VARIABLES	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS (INITIAL MODEL)		MULTIVARIATE ANALYSIS (FINAL MODEL)	
	RR (95% CI)	P	RR (95% CI)	P	RR (95% CI)	P
Age	1.05 (1.01-1.10)	0.017	1.05 (0.98-1.13)	0.166	1.03 (0.98-1.09)	0.182
Gender (male)	1.56 (0.77-3.17)	0.212	7.66 (2.20-26.69)	0.001	2.49 (1.14-5.41)	0.021
Diabetes	1.59 (0.80-3.17)	0.181	0.26 (0.10-0.68)	0.006		
Hypertension	0.93 (0.47-1.84)	0.838				
ACE inhibitors	0.23 (0.34-1.56)	0.414				
Charlson comorbidity	1.25 (1.11-1.40)	<0.001	1.34 (1.02-1.76)	0.031	1.28 (1.05-1.56)	0.015
Interval since liver transplantation (>12 months)	1 (0.99-1)	0.466				
Dyspnea	4.19 (1.89-9.30)	<0.001	15.91 (4.17-60.73)	<0.001	7.25 (2.95-17.82)	<0.001
Cough	0.96 (0.43-2.15)	0.934				
Fever	0.73 (0.34-1.58)	0.429				
Gastrointestinal symptoms	0.72 (0.34-1.52)	0.392				
PaFiO2	0.99 (0.99-0.99)	0.038	0.99 (0.99-0.99)	0.012		
Lymphocyte count	1 (0.99-1)	0.418				
D Dimer	1 (1-1)	0.409				
Estimated glomerular filtration rate	0.99 (0.98-1.01)	0.249	1.01 (0.99-1.04)	0.171	1.01 (0.98-1.03)	0.077
Tacrolimus*	0.54 (0.29-1.07)	0.079	0.19 (0.05-0.68)	0.011		
Cyclosporine*	0.76 (0.18-3.22)	0.708				
Trough concentrations (tacrolimus)*	1.01 (0.93-1.10)	0.762				
Trough concentrations (cyclosporine)*	1.04 (0.92-1.19)	0.485				
Mycophenolate*	2.62 (1.25-5.49)	0.011	5.32 (1.60-17.69)	0.006	3.94 (1.59-9.74)	0.003
Everolimus*	0.77 (0.32-1.88)	0.575				
Trough concentrations (everolimus)*	0.86 (0.59-1.32)	0.490				
Corticosteroids*	1.53 (0.72-3.22)	0.262	1.77 (0.59-5.27)	0.303		
Withdrawal of immunosuppression**	2.03 (0.91-4.51)	0.083	0.34 (0.08-1.51)	0.155	0.58 (0.24-1.40)	0.229
Hydroxychlorochine**	0.64 (0.33-5.88)	0.640				
Azithromycin**	0.77 (0.38-1.53)	0.454				

* These variables pertain to active immunosuppression therapy at Covid-19 diagnosis;

** These therapies were started at Covid-19 diagnosis. Other therapies against Covid-19 were not included as they were initiated selectively in unresponsive cases and would confound the analysis.

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FIGURE LEGENDS

Figure 1. Epidemiological curve of Covid-19 in Spain from February 28th, 2020 until the registry closure on April 7th, 2020. Absolute number of cases are shown for the whole Spanish population and for the liver transplant population.

Figure 2. Geographical distribution of Covid-19 among autonomous regions in Spain. Absolute number of cases are shown for the whole Spanish population and for the liver transplant population.

Figure 3. Kaplan Meier curves showing the impact of mycophenolate-containing immunosuppression (upper panel) and increased doses (lower panel) on the development of severe Covid-19. P values are from Log rank test.

Figure 4. Proposed algorithm to modify immunosuppression in liver transplant patients with Covid-19 according to the findings of the present study. The recommendations should be adapted to each patient taking into account the interval from liver transplantation and the individualized risk of rejection.

SUPPLEMMENTARY MATERIAL

Supplementary table 1. Calculation of standardized incidence ratio of coronavirus-19 disease during the Spanish epidemics from February 20, until April 7, 2020. Cumulative incidence of the general population was adjusted according to age and gender to match the liver transplant population.

Supplementary table 2. Calculation of standardized mortality ratio of coronavirus-19 disease during the Spanish epidemics from February 20, until April 7, 2020. Cumulative incidence of the general population was adjusted according to age and gender to match the liver transplant population.

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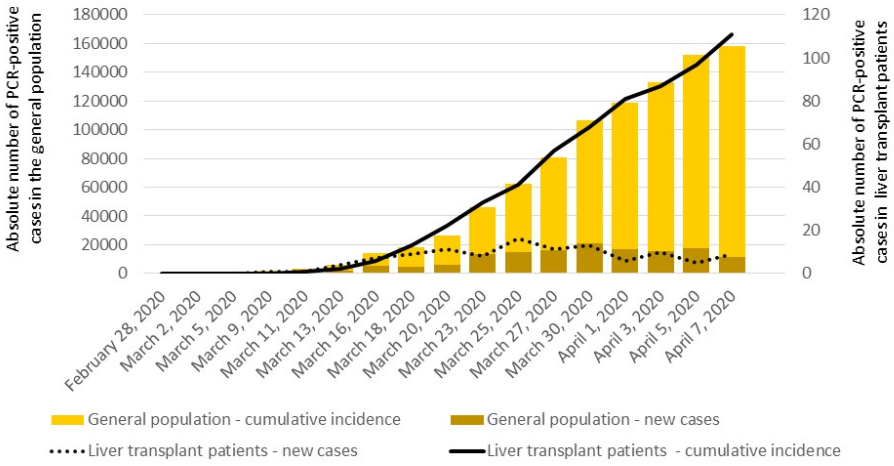
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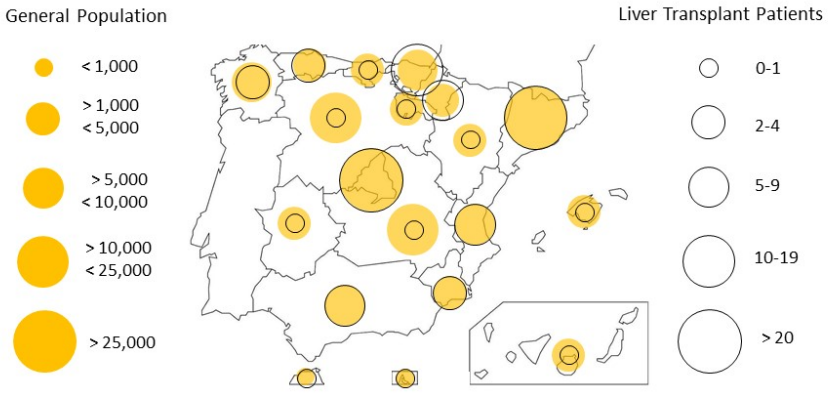
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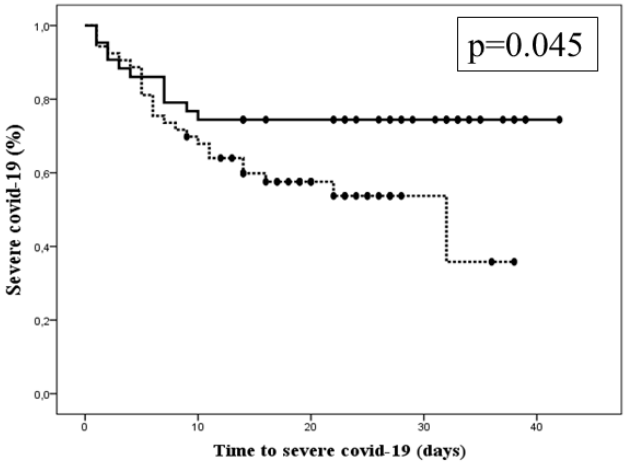
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Absolute number of PCR-positive cases in the general population
Absolute number of PCR-positive cases in liver transplant patients
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
120
100
80
60
40
20
0
February 28, 2020
March 2, 2020
March 5, 2020
March 9, 2020
March 11, 2020
March 13, 2020
March 16, 2020
March 18, 2020
March 20, 2020
March 23, 2020
March 25, 2020
March 27, 2020
March 30, 2020
April 1, 2020
April 3, 2020
April 5, 2020
April 7, 2020
General population - cumulative incidence
General population - new cases
Liver transplant patients - new cases
Liver transplant patients - cumulative incidence

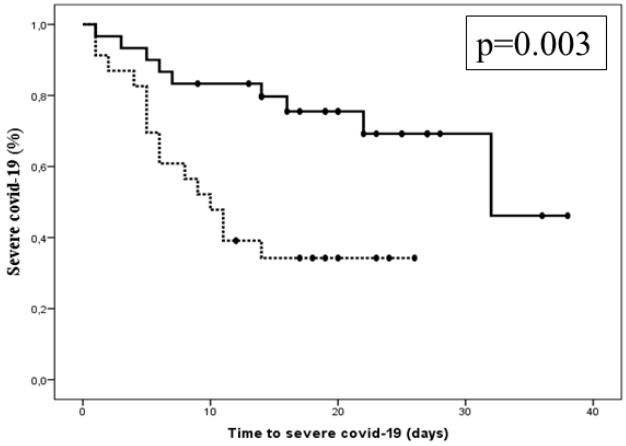
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General Population
< 1,000
> 1,000
< 5,000
> 5,000
< 10,000
> 10,000
< 25,000
> 25,000
Liver Transplant Patients
0-1
2-4
5-9
10-19
> 20

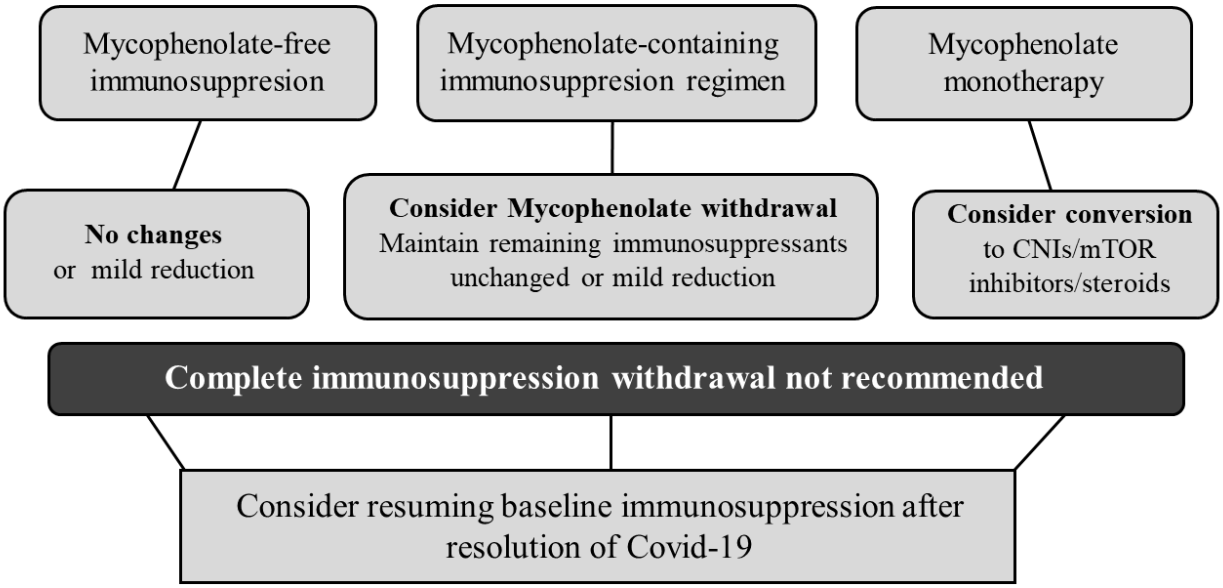
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Group; Number at risk	7 days	14 days	21 days
Mycophenolate	40	31	16
No mycophenolate	37	32	28



Group; Number at risk	7 days	14 days	21 days
Mycophenolate >1,000 mg/day	15	8	4
Mycophenolate ≤1,000 mg/day	25	23	12



CNIs: calcineurin inhibitors; mTOR: mammalian Target of Rapamycin

Journal Pre-proof

Journal Pre-proof

HIGHLIGHTS

- The incidence of Covid-19 is increased in liver transplant patients.
- Mortality rates are lower than those observed in matched general population.
- Immunosuppression withdrawal may not be justified.
- Mycophenolate may increase the risk of severe Covid-19 in a dose dependent-manner.
- Calcineurin inhibitors and everolimus are not deleterious for Covid-19.

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