Liver transplant recipients infected with SARS-CoV-2 in the early postoperative period: Lessons from a single center in the epicenter of the pandemic

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Abstract
The impact of coronavirus disease-19 (COVID-19) in liver recipients remains largely unknown. Most data derive from small retrospective series of patients transplanted years ago. We aimed to report a single-center case series of five consecutive patients in the early postoperative period of deceased-donor liver transplantation who developed nosocomial COVID-19. Two patients presented important respiratory discomfort and eventually died. One was 69 years old and had severe coronary disease. She rapidly worsened after COVID-19 diagnosis on 9th postoperative day. The other was 67 years old with non-alcoholic steatohepatitis, who experienced prolonged postoperative course, complicated with cytomegalovirus infection and kidney failure. He was diagnosed on 36th postoperative day and remained on mechanical ventilation for 20 days, ultimately succumbing to secondary bacterial infection. The third, fourth, and fifth patients were diagnosed on 10th, 11th, and 18th postoperative day, respectively, and presented satisfactory clinical evolution. These last two patients were severely immunosuppressed, since one underwent steroid bolus for acute cellular rejection and another also used anti-thymocyte globulin for treating steroid-resistant rejection. Our novel experience highlights that COVID-19 may negatively impact the postoperative course, especially in elder and obese patients with comorbidities, and draws attention to COVID-19 nosocomial spread in the early postoperative period.

KEYWORDS
COVID-19, liver transplantation, postoperative period, severe acute respiratory syndrome coronavirus 2

Abbreviations: ABTO, Brazilian Association of Organ Transplantation; ACR, acute cellular rejection; COVID-19, coronavirus disease-19; CT, computed tomography; DDLT, deceased-donor liver transplantation; HCC, hepatocellular carcinoma; ICU, intensive care unit; LT, liver transplantation; NASH, non-alcoholic steatohepatitis; NO, naso-oropharyngeal; OTI, orotracheal intubation; PMT, pulse methylprednisolone therapy; POD, postoperative day; RT-PCR, real-time polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TACE, transarterial chemoembolization.
| Case | Age, sex, and BMI | Liver Disease | Comorbidities | Donor data | Surgical data | Immunosuppression protocol | COVID-19 symptoms | COVID-19 treatment and outcome |
|------|------------------|---------------|---------------|------------|---------------|---------------------------|------------------|-------------------------------|
| 1    | 69 y, female     | HCV           | SAH           | Female, 45 y | GW: 1600 g    | Basiliximab, tacrolimus, mycophenolate, and intraoperative corticoid bolus and tampering | 9th POD: Fever | OTI on 12th POD: Azithromycin, Death on 13th POD due to refractory shock and acidosis. |
|      | BMI: 34.71 kg/m² | Downstaged HCC | Coronariopathy | BD: hemorrhagic stroke | TST: 285 min TIT: 585 min WIT: 35 min BBP: None |  | Mild dyspnea | Diarrhea |
|      |                  | MELD: 15      | Pulmonary      |             |               |                           |                  |                               |
|      |                  | Child-Pugh: A6| hyper tension  |             |               |                           |                  |                               |
| 2    | 67 y, male       | NASH          | SHA            | Female, 22 y | GW: 1475 g    | Basiliximab, tacrolimus, mycophenolate, and intraoperative corticoid bolus and tampering | 36th POD: Fever | OTI on 37th POD: Azithromycin, Hydroxychloroquine, Death on 56th POD due to secondary bacterial infection |
|      | BMI: 32.81 kg/m² | α1-antitripsin deficiency | Obesity       | BD: subarachnoid hemorrhage | TST: 435 min TIT: 445 min WIT: 40 min BBP: None |  | Hypoactive Delirium Progressive dyspnea | |
|      |                  | MELD: 13      |               |             |               |                           |                  |                               |
|      |                  | Child-Pugh: B7| 21 d ICU      |             |               |                           |                  |                               |
|      |                  | Hepatic encephalopathy |               |             |               |                           |                  |                               |
| 3    | 69 y, male       | Alcoholic cirrhosis | SHA            | Male, 54 y | GW: 1370 g    | Tacrolimus and intraoperative corticoids bolus with tampering | 10th POD: Fever | Venturi mask, Supportive care, Dryness worsened on 10th hospitalization day |
|      | BMI: 27.58 kg/m² | HCC           | DM            | BD: cranioencephalic trauma | TST: 425 min TIT: 405 min WIT: 35 min BBP: None |  | Watery diarrhea, Mild exertional dyspnea | Discharged home on 17th hospitalization day |
|      |                  | MELD: 13      |               |             |               |                           |                  |                               |
|      |                  | Child-Pugh: A6|               |             |               |                           |                  |                               |
| 4    | 59 y, male       | Cryptogenic cirrhosis | Hepatosplenic schistosomiasis | Female, 52 y | GW: 1450 g    | Tacrolimus, mycophenolate, and intraoperative corticoid bolus and tampering, Received PMT for acute cellular rejection treatment | 11st POD: Subfebrile temperature dry cough | Supportive care | Discharged on 27th POD |
|      | BMI: 24.38 kg/m² | Ascites        |               | BD: hemorrhagic stroke | TST: 360 min TIT: 460 min WIT: 35 min BBP: None |  |                                |                               |
|      |                  | Hepatic encephalopathy | Presenting cardiac arrest before organ recovery |             |               |                           |                  |                               |
|      |                  | MELD: 10      |               |             |               |                           |                  |                               |
|      |                  | Child-Pugh: B7|               |             |               |                           |                  |                               |
| 5    | 34 y, male       | Sclerosing primary cholangitis | None          | Female, 42 y | GW: 1425 g    | Tacrolimus and intraoperative corticoids bolus with tampering, Mycophenolate, PMT, anti-thymocyte globulin were later used due to steroid-resistant severe acute cellular rejection | 18th POD: asymptomatic | Supportive care, Needle thoracentesis drainage of pleural effusion |
|      | BMI: 22.38 kg/m² | MELD: 35      |               | BD: ischemic stroke | TST: 395 min TIT: 360 min WIT: 35 min BBP: None |  | 24th POD: Fever Mild dyspnea | Discharged home on 41st POD |
|      |                  | Child B7      |               |             |               |                           |                  |                               |

Note: Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BBP, blood-borne products; BD, brain death; BMI, body mass index; DM, diabetes mellitus; GGT, gamma-glutamyl transferase; GW, graft weight; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ICU, intensive care unit; MELD, model of end-stage liver disease; NASH, non-alcoholic steatohepatitis; PMT, pulse methylprednisolone therapy POD, postoperative day; SHA, systemic artery hypertension; TIT, total ischemic time; TST, total surgery time; WIT, warm ischemic time.
1 | INTRODUCTION

Brazil is currently one of the most affected countries in the world by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and has become the epicenter of the coronavirus disease-19 (COVID-19) pandemic in Latin America. The impact of COVID-19 in liver recipients remains largely unknown, as most data derive from case reports and small retrospective series. Moreover, most of them address patients who were transplanted years ago. Information regarding patients in the early postoperative period is very scarce.

Many authors recommend weaning of immunosuppression and even advocate its complete withdrawal. Nevertheless, these suggestions are mostly based on patients in late postoperative period, for whom immunosuppression is already diminished and acute cellular rejection (ACR) is less likely. Another distinct feature is the impact of nosocomial infection, as these patients are generally infected in-hospital. When the pandemic starts its declining phase, community viral spread is expected to decrease; however, nosocomial infection may persist longer and affect more routinely hospitalized patients who just underwent transplantation.

São Paulo is Brazil’s largest city and the current epicenter of the disease in the country. Our institution is one of largest transplantation centers in Latin America, having performed more than 3000 liver transplants (LT), with an average of 160 LT per year. The aim of this study was to report a single-center case series of consecutive patients in the early postoperative period of deceased-donor liver transplantation (DDLT) who developed COVID-19.

2 | CASE REPORTS

During the first months of the city quarantine (March 24th to May 31st, 2020), we performed 19 DDLT in 18 patients and diagnosed 5 cases of COVID-19 in the early postoperative period. In all cases, SARS-CoV-2 infection was confirmed via real-time polymerase chain reaction (RT-PCR) in naso-oropharyngeal (NO) swab or tracheal secretion. Nucleic acid (RNA) was extracted with an automated method based on magnetic beads (mSample Preparation System RNA, Abbott). Reverse transcription, amplification, and detection were performed following an in-house protocol validated in the Laboratory Division (College of American Pathologists accredited) comprising an E gene assay as the first-line screening tool, followed by confirmatory testing with a N gene assay, as previously described. Endogenous gene RNAse P was used as internal control of extraction and amplification, as well as positive and negative external controls. Analytical sensitivity was 40 copies/mL, and specificity in samples containing other respiratory viruses RNA was 100%. Table 1 depicts a summary of all cases, and Table 2 shows laboratory assessment at time of COVID-19 diagnosis.

2.1 | Case 1

A 69 years old female patient was presented with hepatitis C virus infection DDLT due to cirrhosis and hepatocellular carcinoma (HCC), which were downstaged to within Milan criteria after 4 transarterial chemoembolization (TACE). Her past medical history was remarkable for systemic arterial hypertension, coronary disease, and pulmonary hypertension. Myocardial perfusion scintigraphy did not show any inactive areas and ejection fraction was 69% on echocardiogram, even though coronary angiography identified important stenosis (>80%) in the right posterior descending artery, descending anterior artery and circumflex artery.

The procedure went on uneventfully, and the patient was extubated on the 1st postoperative day (POD). Renal function was worsened, and dialysis was required from the 2nd POD. She presented with mild dyspnea, fever, and diarrhea on 9th POD. A thoracic computed tomography (CT) scan showed multiple bilateral ground-glass pulmonary opacities, occasionally associated with interlobular septa thickening and fine reticulate, affecting approximately 50% of the lung. A RT-PCR in NO swab confirmed SARS-CoV-2 infection, and she was started on azithromycin. The patient clinically worsened on 12th POD, presenting hypotension and massive dyspnea, and had to undergo orotracheal intubation (OTT). Despite immunosuppression discontinuation, she developed important hemodynamic instability and died on 13th POD due to refractory shock and acidosis.

2.2 | Case 2

A 67 years old male patient underwent DDLT 10 days before the COVID-19 quarantine was declared. He was a cirrhotic patient due to non-alcoholic steatohepatitis (NASH) and alpha-1-antitripsin deficiency, who had additional points in waitlist because of hepatic encephalopathy. The postoperative course was remarkable for persistent need of hemodialysis. On 26th POD, liver enzymes were mildly elevated and he underwent a percutaneous liver biopsy, which did not show significant alterations, but cytomegalovirus infection was diagnosed (serum polymerase chain reaction: 213 458 IU/mL) and he was started on intravenous ganciclovir. On 36th POD, he was presented with fever, hypoxic delirium, and progressive dyspnea. Thoracic CT scan showed bilateral several ground-glass pulmonary opacities affecting approximately 50% of the lungs (Figure 1A,B). He underwent OTI due to persistent hypoxemia and respiratory discomfort. A RT-PCR in tracheal secretion was positive for SARS-CoV-2. He was then started on large-spectrum antibiotics (meropenem and vancomycin) for 14 days as well as oseltamivir, azithromycin, and hydroxychloroquine for 5 days. Mycophenolate was withdrawn, and tacrolimus serum level was maintained at around 5 μg/dL.

He remained on mechanical ventilation for 20 days. Ventilator-associated pneumonia was diagnosed on 53th POD with multiresistant Acinetobacter baumannii being identified on tracheal secretion, and he was started on ciprofloxacin. His condition continued to deteriorate and he died of persistent hypoxemia and hemodynamic instability on 56th POD.
2.3 | Case 3

A 69 years old male patient underwent DDLT due to alcoholic cirrhosis and HCC. Postoperative course was uneventful, and he was discharged home on 8th POD. He returned to the emergency department on 10th POD complaining of fever, watery diarrhea, dry cough, and mild exertional dyspnea. A thoracic CT scan showed bilateral multiple ground-glass pulmonary opacities (Figure 1C), affecting less than 50% of the lungs. He started on oseltamivir for 5 days, piperacillin/tazobactam for 7 days, and oral metronidazole for 10 days. An RT-PCR in NO swab confirmed SARS-CoV-2. Shortness of breath worsened on the 10th day of hospitalization. Another thoracic CT scan showed increase in number and dimensions of ground-glass opacities, now affecting more than 50% of the lungs (Figure 1D). He could nonetheless sustain adequate levels of $O_2$ saturation on Venture mask 50% and did not require further interventions, being discharged home on 17th hospitalization day.

2.4 | Case 4

A 59 years old male patient with cryptogenic cirrhosis underwent an uneventful DDLT. The patient was extubated on 1st POD, but required hemodialysis from the 4th POD. Pulse methylprednisolone therapy (PMT) was used on 6th POD due to ACR. On 11st POD, he developed subfebrile temperature and dry cough, without need of oxygen therapy. A thoracic CT scan showed bilateral ground-glass pulmonary opacities, sometimes associated with thickening of interlobular septa and fine reticulate. An RT-PCR in NO swab confirmed SARS-CoV-2. Meropenen and Vancomycin were used for 3 days, and mycophenolate was withdrawn. The patient presented satisfactory evolution and did not require further interventions, being discharged on 27th POD.

2.5 | Case 5

A 34 years old male patient with sclerosing primary cholangitis and cirrhosis underwent DDLT with biliodigestive anastomosis. Postoperative evolution was complicated by severe ACR, which did not respond to PMT. He underwent percutaneous liver biopsy, which complicated with hemorrhage and required exploratory laparotomy to evacuate blood clots on 11st POD. Owing to the diagnosis of steroid-resistant severe ACR, mycophenolate and anti-thymocyte globulin were introduced. On 18th POD, an abdominal CT scan performed to evaluate the presence of retained clots showed some ground-glass opacities in the base of the left lung. He started with fever and mild dyspnea on 24th POD and RT-PCR in NO swab confirmed SARS-CoV-2. A thoracic CT scan showed numerous bilateral peribronchovascular ground-glass opacities, mainly in the upper

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**Table 2** Laboratory assessment on COVID-19 diagnosis

| Laboratory test                              | Case 1  | Case 2  | Case 3  | Case 4  | Case 5  |
|----------------------------------------------|---------|---------|---------|---------|---------|
| Alkaline phosphatase (U/L)                   | 571     | 398     | 103     | 646     | 906     |
| gamma-glutamyl transferase (U/L)             | 1288    | 1405    | 283     | 3211    | 2167    |
| Total Bilirubin (mg/dL)                      | 0.74    | 1.39    | 0.68    | 4.51    | 2.73    |
| Direct Bilirubin (md/dL)                     | 0.50    | 1.2     | 0.46    | 4.03    | 2.45    |
| Aspartate aminotransferase (U/L)             | 251     | 121     | 53      | 116     | 43      |
| Alanine aminotransferase (U/L)               | 150     | 137     | 106     | 209     | 65      |
| Albumin (g/dL)                               | 1.8     | 2.2     | 3.3     | 2.5     | 2.6     |
| INR                                          | —       | 1.1     | 1.07    | 1.06    | 1.10    |
| Total leukocytes (/mm$^3$)                    | 29 340  | 12 910  | 3130    | 24 850  | 14 740  |
| Lymphocyte count (/mm$^3$) and percentage    | 1174 (4%)| 387 (2.99%)| 330 (10.54%)| 1740 (7%)| 737 (5%)|
| Creatinine (mg/dL)$^a$                        | 5.84    | 6.36    | 1.0     | 3.04    | 1.07    |
| Urea (mg/dL)$^a$                              | 178     | 141     | 36      | 50      | 18      |
| C-reactive protein (mg/L)                     | 146.12  | 226.01  | 96.3    | 50.50   | 82.22   |
| D-dimer (ug/mL)                              | 8477    | 19 354  | 4626    | 2253    | 6489    |
| Lactate dehydrogenase (U/L)                  | 895     | 691     | 280     | —       | —       |
| Ferritin (ng/mL)                              | —       | 2896    | —       | —       | 1558    |
| Tacrolimus serum level (ng/mL)               | 10.8    | 13.1    | 13.3    | 13.4    | 13.7    |

$^a$Patients 1, 2, and 4 required renal replacement therapy.
lobes. Almost total atelectasis of the right lower lobe due to adjacent pleural effusion was also noted, which was eventually drained via needle thoracocentesis (Figure 1E,F). Despite the more aggressive immunosuppression, the patient remained well with adequate O₂ saturation on oxygen catheter and was discharged home on 41st POD.

3 | DISCUSSION

This report represents one of the largest series of liver transplant recipients in early postoperative with nosocomial COVID-19. Fever and respiratory symptoms were present in all cases, and gastrointestinal manifestations were observed in two. We could identify two patterns of clinical evolution: some patients presented mild disease while others required mechanical ventilation and eventually died. As appointed for post-transplant patients with long-term metabolic complications, comorbidities seem to outweigh immunosuppression in determining prognosis even in the early postoperative period. In fact, one patient with unfavorable outcome had severe coronary disease and the other one experienced prolonged postoperative course complicated with CMV infection and kidney failure. Both were also obese. Whereas the first patient developed SARS-CoV-2 infection and rapidly progressed to death, the second exhibited a more insidious course and ultimately died of secondary bacterial infection. Regarding laboratory assessment, a wide variation in liver enzymes was observed, probably related to the particular evolution of each case before COVID-19 diagnosis. However, inflammatory markers and D-dimer were elevated in all cases, even though they are not specific for COVID-19.

Regarding the specific treatment of COVID-19, we followed our institution’s protocol, in which most novel drugs, such as umifenovir,
l洛匹那韦/ ritonavir, and tocilizumab,2,4,9,12,16 are used under clinical trials. Patients 1 and 2 developed severe COVID-19 and required OTI. They were started on azithromycin, but hydroxychloroquine was used only in the second case, because the first one rapidly progressed to death. Hydroxychloroquine was initially recommended, but is no longer used given the latest evidence. Large-spectrum antibiotics were used in patients 3 and 4 aiming to cover bacterial infection as well; however, as they presented satisfactory clinical evolution, no additional treatment was performed. Patient 5 experienced mild symptoms and successfully responded to supportive care.

Immunosuppression was completely withdrawn in case 1, due to the patient’s dramatic worsening. In case 2, mycophenolate was discontinued and tacrolimus serum levels were lowered. In case 4, mycophenolate was also discontinued, but no changes were made in tacrolimus serum levels. In cases 3 and 5, immunosuppression was not altered. The risk of ACR is higher in early transplanted patients and, in fact, it occurred in 2 early cases previously described in literature.7,13 It seems that immunosuppression must be tailored on a case-by-case basis according to the clinical evolution. For instance, patient 4 underwent PMT before COVID-19 diagnosis due to ACR and patient 5 underwent PMT and anti-thymocyte globulin infusion owing to steroid-resistant ACR. Despite being severely immunosuppressed, they presented only mild symptoms.

As a public quaternary center, our institution was prepared to be the main reference center of COVID-19 cases in São Paulo State at the beginning of April, 2020. As the number of transplants in the context of COVID-19 pandemic has decreased substantially,16,18,19 strategies have been developed for ensuring the availability of transplantation activity, such as establishment of hospital areas especially dedicated to non-COVID-19 patients.20,21 The main building of our institution was then designated exclusively for treating patients with COVID-19 when the quarantine was declared, while the nearby institutes were considered areas with low exposure to SARS-CoV-2. Organ transplantation activities were supposed to continue in wards and ICUs without SARS-CoV-2-infected patients. Furthermore, all deceased donors were screened to SARS-CoV-2 via RT-PCR in NO swab and all recipients performed a thoracic CT scan before hospital admission, in accordance with Brazilian Association of Organ Transplantation (ABTO) recommendations, except patient 2 who was transplanted immediately before the implementation of these measures. All donors tested negative for SARS-CoV-2, and no alterations were noted on recipients CT scans.

The incubation period for SARS-CoV-2 is usually 5-6 days; however, it may reach 14 days.21 Therefore, it is possible that 3 patients (cases 1, 3, and 4) might have been admitted to the hospital already infected. We consider this hypothesis unlikely, since all of them were clinically asymptomatic and with no radiological sign, and there was an ongoing nosocomial outbreak of COVID-19 in our institution at that time, with many non-transplant patients from others non-COVID-19 wards developing the disease. Therefore, it is reasonably safe to assume that we experienced a nosocomial spread of the virus, probably carried out by asymptomatic patients, or even by patient’s visitors and healthcare personnel. Due to these events, infection control protocols were tightened, including a temporary ban on family visits, strengthening of barriers precautions, and mass SARS-CoV-2 screening for healthcare professionals. We also included the RT-PCR for SARS-CoV-2 in NO swab in the recipients screening. Although those infection control protocols with strict barrier precautions may reduce SARS-CoV-2 nosocomial spread even in immunosuppressed patients,22 the challenge of maintaining large liver transplant programs in areas with high community transmission of SARS-CoV-2 will remain until a vaccine is developed.

In conclusion, we reported a case series of liver recipients who developed SARS-CoV-2 infection in the early postoperative period, probably related to a nosocomial outbreak. Our experience highlights that COVID-19 may impact negatively the postoperative course, especially in elderly and obese patients with comorbidities, and draws attention to COVID-19 nosocomial spread.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest with regards to the content of this manuscript.

AUTHOR CONTRIBUTIONS

DRW, EA, and LSN involved in conceptualization and wrote the original draft. VR, LNG, RBM, RSP, and RMA investigated the study. CSL and LMM involved in resources. LBH, LD, and DRT involved in data curation. FHG and WA wrote, reviewed, and edited the study. LAC involved in supervision.

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