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BRIEF COMMUNICATION

Successful liver transplantation from deceased donors with active COVID-19 infections with undetectable SARS-CoV-2 in donor liver and aorta

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Abstract

Background: The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has had unprecedented effects on society and modern healthcare. In liver transplantation, uncertainty regarding the safety of performing transplants during the early stage of the pandemic resulted in increased waitlist mortality. Additionally, concerns about disease transmission led to avoidance of deceased donors with COVID-19 infections. Several successful case reports describing incidental transplant of organs from donors with COVID-19 infections or intentional transplant of such donors into recipients with current or prior COVID-19 infections prompted the transplant community to re-evaluate that position. While excellent short-term results have been published, little is known about use of donors with active infections and the extent of COVID-19 organ involvement, which may affect long term outcomes.

Methods: We report the successful transplantation of three livers from deceased donors with active COVID-19 infections. Donor liver and aortic tissues were evaluated by sensitive molecular testing for SARS-CoV-2 RNA via in situ hybridization and real-time quantitative reverse transcription PCR.

Results: Postoperatively, all patients had excellent allograft function, without clinical or molecular evidence of SARS-CoV-2 transmission in donor tissues.

Conclusion: This evidence supports the use of liver donors with active COVID-19 infections.

KEYWORDS
COVID-19, in situ hybridization, liver transplantation, organ donors, PCR

1 | INTRODUCTION

The rapid spread of coronavirus disease 2019 (COVID-19) initially drastically restricted transplantation practices across the United States, with the United Network for Organ Sharing reporting a 25% decrease in liver transplantation during the first wave.[1,2] Many transplant centers faced the difficult decision to suspend life-saving transplant operations due to suspected high risks of COVID-19 transmission to immunosuppressed transplant recipients, risk of transmission to the healthcare workers, lack of medical resources and uncertainties of medical treatment effectiveness should immunosuppressed transplant recipients contract the disease postoperatively.[1,3] Interruption in

liver transplantation across the country led to increased waitlist mortality.[4] Despite scarcity of deceased donor organs, consensus in the transplant community was to defer the use of potential donors with COVID-19 infections, including those with mild or asymptomatic disease.[5]

However, a report in December 2020 established that hearts and livers from COVID-19 infected donors could be transplanted to severely ill transplant candidates with active or prior COVID-19 infections.[6] Subsequently, several reports described successful liver transplants from COVID-19 positive donors to COVID-19 naïve recipients.[7–9] More recently, a review of Organ Procurement and Transplantation Network (OPTN) data showed excellent short-term post-transplant patient outcomes in 269 recipients of organs from COVID-19 positive donors, but noted little information is known regarding viral involvement of donor organs, which may affect long-term outcomes.[10]

At our center, we started accepting organs from donors with resolved SARS-CoV-2 infection or positive tests if the organ was otherwise met center criteria in terms of liver function, steatosis and size for our matched recipient, regardless of the proposed recipient's history of SARS-CoV-2 infection. We report three cases from donors with active COVID-19 infections in which we were also able to evaluate donor tissue with very sensitive molecular testing for SARS-CoV-2 to evaluate viral involvement of the donor organs in addition to post-transplant patient and graft outcomes.

2 | MATERIALS AND METHODS

Donor liver tissue was procured via our standard practice of partial donor caudate lobe transection to facilitate side-to-side cavocavostomy. Aortic tissue procured with the liver was also studied given high expression of angiotensin-converting enzyme 2 receptors in the vasculature. To detect SARS-CoV-2 RNA (ribonucleic acid), in situ hybridization was performed on 5 μm-thick sections of formalin-fixed, paraffin-embedded donor liver tissue mounted on charged glass slides using the Leica Bond RXm automated system (Leica Biosystems, Richmond, IL).[11,12] Epitope retrieval was performed by heating to 95°C in an EDTA-based ER2 buffer (#AR9640, Leica Biosystems, Richmond, IL). Slides were treated in protease (#322102, Advanced Cell Diagnostics, Newark, CA) and probes hybridized to RNA. The SARS-CoV-2 probe (#848568, Advanced Cell Diagnostics, Newark, CA) was detected using the Leica RNAScope 2.5 LS Assay-RED kit and followed by a hematoxylin counterstain (#322150, Leica Biosystems, Richmond, IL). A section of lung from a golden Syrian hamster experimentally inoculated intranasally with SARS-CoV-2 B.1.1.529 (Omicron) served as a positive control for SARS-CoV-2 RNA detection. An RNApol2 probe (#310458, Advanced Cell Diagnostics, Newark, CA) confirmed RNA quality in donor samples. The area of liver evaluated by in situ hybridization was 218.5 mm^2 for Donor A, 331.5 mm^2 for Donor B, and 107.5 mm^2 for Donor C. Our Institutional Review Board does not require review of case series with three patients or fewer.

To complement in situ hybridization assessments, total RNA was extracted from fresh frozen donor liver and aortic tissues using a homogenizer and TRIzol (Thermo Fisher Scientific, Waltham, MA). The Center for Disease Control and Prevention (CDC) qRT-PCR (real-time quantitative reverse transcription PCR) reactions for detecting SARS-CoV-2 genome and the internal control human RNase P gene were used as previously described.[13,14] Total RNA was extracted from frozen tissue after digestion with TRIzol using the PureLink RNA mini kit (#12183018A, Life Technologies).

3 | RESULTS

Recipient, donor, and transplant characteristics are summarized in Table 1. In accordance with transplant center policy, all recipients had been vaccinated against COVID-19. All donors had active COVID-19 infections by symptomatology and laboratory testing, diagnosed within 2 weeks of procurement. More proximate to the procurement, all donors tested positive for SARS-CoV-2 via nucleic acid detection on upper respiratory (e.g. nasopharyngeal swab) specimens obtained within 36 h of procurement. Donor A was admitted with hypoxia and COVID-19 infection 2 weeks prior to procurement; he suffered a respiratory arrest a week after admission after refusing intubation in the setting of declining respiratory status. He had laboratory evidence of active infection 36 h prior to cross clamp as evidenced by a cycle threshold of 24.6. Two weeks prior to procurement, Donor B was admitted with hypoxia in the setting of a positive COVID-19 test and required intubation and prone positioning prior to transfer to a quaternary care center for consideration of ECMO. Proximate to procurement, he had evidence of ongoing active infection as evidenced by a cycle threshold of 31.6 12 h prior to cross clamp. Donor C was admitted after calling emergency services for shortness of breath 2 days after testing positive for COVID-19 at an emergency department visit. She suffered a respiratory arrest en route to the hospital, 6 days prior to procurement. She did not have a cycle threshold available but had a positive BAL the day of the procurement in addition to a positive COVID-19 test 36 h prior to cross clamp.

Postoperatively, all recipients had negative tests for SARS-CoV-2 by nasopharyngeal swabs and no clinical evidence of disease transmission (Table 1). All three recipients had immediate and persistent excellent graft function postoperatively. Recipient A had a non-sustained seizure on postoperative day #4 without significant brain imaging findings as well as negative infectious and metabolic workups; etiology was attributed to tacrolimus use. She was transitioned to a maintenance immunosuppression regimen with the mammalian target of rapamycin (mTOR) inhibitor everolimus without further neurologic events. All three patients were doing well with normal liver enzymes and without signs or symptoms of COVID at postoperative days 493, 458, and 473, respectively.

Representative microscopic images of donor liver are shown in Figure 1. Hematoxylin and eosin staining showed minimal portal inflammation and steatosis without significant fibrosis (Figure 1A). In situ hybridization analyses did not detect SARS-CoV-2 RNA in any of

TABLE 1 Recipient and donor characteristics.

	Recipient characteristics					Donor characteristics				Perioperative characteristics		
	Age/sex	Cause of ESLD	MELD	Age/sex	BMI	Cause of death	Initial COVID diagnosis[a]	Proximate COVID test and cycle threshold[b]	Cycle threshold	CIT	Postoperative complications	Recipient postoperative COVID testing
A	63F	Alcoholic cirrhosis	27	59 M	32	COVID hemorrhagic stroke	Two weeks	36 h; 24.6	24.6	8h1	CNI-related seizure	Negative POD#7
B	70 M	NASH and HCC	18	50 M	31	Cerebrovascular stroke	Two weeks	12 h; 31.6 BAL negative	31.6 BAL negative	3h10	none	Negative POD#7, 36, 42, 58
C	38 M	Alcoholic cirrhosis	31	20 F	18	Anoxia	Six days	36 h; n/a BAL positive	n/a BAL positive	4h46	none	Negative POD#2, 10, 19

Note: All positive COVID tests from upper respiratory (e.g., nasopharyngeal swab) source, detected via nucleic acid detection (e.g., RT-PCR). BAL = bronchoalveolar lavage; BMI = body mass index; CIT = cold ischemia time; CNI = calcineurin inhibitor; ESLD = end stage liver disease; F = female; HCC = hepatocellular carcinoma; M = male; MELD = model for end-stage liver disease, n/a = not available; NASH = nonalcoholic steatohepatitis; POD = postoperative day.

[a]Timing of initial COVID test and diagnosis prior to procurement.

[b]Timing of most recent COVID test prior to procurement.

the donor liver samples (Figure 1B) compared to the SARS-CoV-2 RNA positive lung control (Figure 1C). Detection of human RNA pol2 by in situ hybridization in all samples confirmed integrity of RNA isolated from donor tissue (Figure 1D).

All tissues tested, from both donor liver and aorta samples, were below the limit of detection for SARS-CoV-2 by qRT-PCR. All donor tissue samples evaluated by qRT-PCR showed positive RNase P signal indicating appropriate RNA quality for PCR testing.

4 | DISCUSSION

We report three cases of successful liver transplants from deceased donors who tested positive for SARS-CoV-2 within 36 h of procurement, each of whom had either a low cycle threshold or a positive BAL sample as well. All donors had active and severe infections as demonstrated by their clinical courses. Postoperatively, all recipients had negative SARS-CoV-2 tests and no clinical evidence of disease transmission. Sensitive molecular testing of donor liver tissues did not yield evidence of SARS-CoV-2. All three recipients had immediate and persistent excellent graft function without signs or symptoms of COVID.

After initial concerns regarding organ transplantation from donors positive for COVID-19, several reports proposed the safety of these organs for non-lung recipients.[7,8,12,15–17] Data from these reports ultimately led to changes in the current organ recovery guidelines, leaving the decision to transplant organs from COVID-19 positive donors to transplant centers on a case by case basis.[18] Notably, however, many of these prior reports were of donors with incidental or remote infections or resolved infections with high cycle thresholds, indicating a low level of viremia. In our case series, we demonstrate safe use of organs from donors with active infections as measured by testing of nasopharyngeal samples and confirmed with either positive BAL testing or low cycle threshold. An early case reported incidental transplantation using a living donor later found to be infected with SARS-CoV-2; similar to our current data, this report showed negative PCR testing of the donor liver tissue for the virus.[19] However, given the timing of the donor's positive test on postoperative day 3, it is unclear that the donor would have tested positive preoperatively.

A recent systematic review found no viral transmission of SARS-CoV-2 among non-lung transplant recipients from 57 donors with recent or current COVID-19 infections, including 18 with persistent RNA positivity at the time of procurement.[20] Notably, the median cycle threshold of these donors was 32, a level approaching the >34 cycle threshold often cited as low level of virus.[21] In our study, we also report lack of transmissibility of the virus from donors with recent ongoing infections as well as absence of viral RNA in donor liver tissue, including from donors with a with a high viral loads (as demonstrated by low cycle threshold less than 34) and from a donor with positive BAL sample.

Nationally, the reports of successful transplantation from these donors have resulted in increased use of deceased donors with previous or final testing indicating COVID-19 infections, though utilization

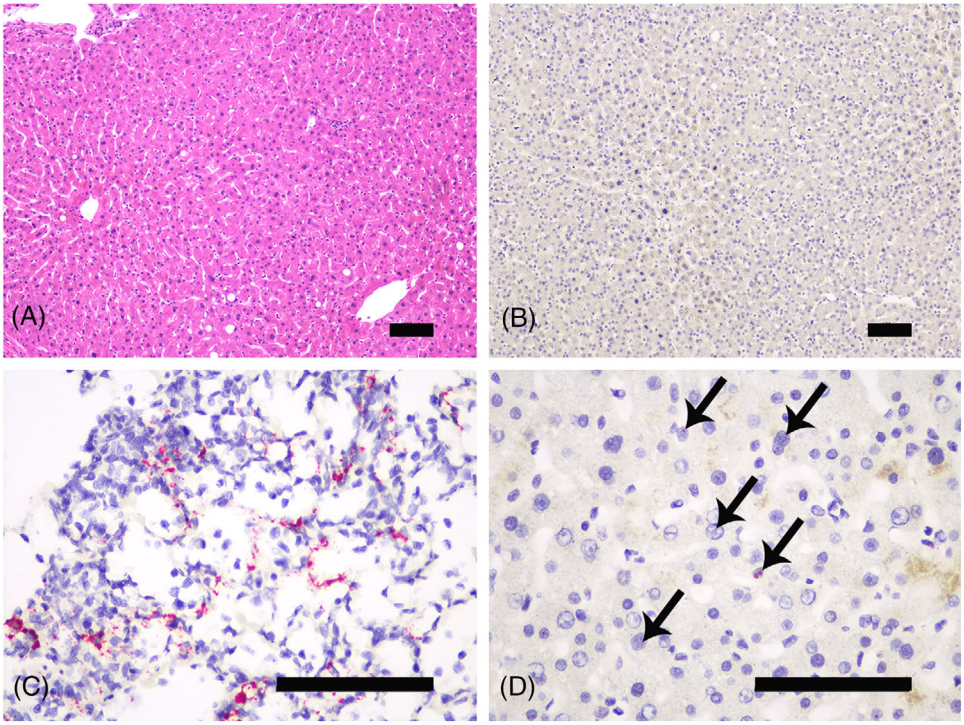

FIGURE 1 Representative donor liver biopsy findings with in situ hybridization for SARS-CoV-2 RNA (A) Representative hematoxylin and eosin staining of donor liver demonstrates minimal portal inflammation and steatosis and no significant fibrosis. (B) In situ hybridization for SARS-CoV-2 RNA in the donor liver samples showed negative staining in all donor biopsy samples. (C) Positive COVID-19 lung control demonstrates abundant staining for SARS-CoV-2 RNA by ISH in alveolar pneumocytes. (D) RNA control for the donor liver using RNA pol2 probe for ISH to verify RNA integrity in liver samples (red punctate staining in nuclei, arrows). Scale bars = 100 μM.

remains low compared to donors without recent or current COVID-19 infection.[22] Two studies examining the OPTN data on transplanted organs from COVID-19 positive deceased donors both showed similar short-term graft and patient survival in recipients of these organs compared to the recipients of COVID-19 naïve donors.[10,22] Based on the limitations of the OPTN data, however, little information was known regarding the viral involvement of donor organs and viremia levels of the donors. In our report, we show successful transplantation of organs from donors with ongoing COVID-19 infections, without evidence of viral involvement in liver or aortic tissue.

While evidence supporting the use of organs from SARS-CoV-2 infected donors is still limited, our findings suggest that liver transplant from these donors is feasible. Utilization of high-quality organs from donors with mild, asymptomatic or resolved SARS-CoV-2 infection, or those donors with symptomatic disease but high cycle SARS-CoV-2 thresholds should be strongly considered. The lack of virus in tissues from donors with clinically severe infections as well as even low cycle thresholds or positive BAL samples in our current study suggests even active and/or severe infection should not be an absolute contraindication to liver donation. These donors represent a suitable source of organs. We advocate for the routine use of donors with mild, symptomatic or resolved SARS-CoV-2 infection including those with marginal grafts as well as consideration of liver grafts from donors with active and/or severe infections which are otherwise of good quality. Larger scale studies focused on long-term patient and graft outcomes are required to confirm these observations. In the setting of persistent COVID-19 pandemic, however, deferral of non-lung donors who have tested positive for SARS-CoV-2 risks needless deferment of otherwise suitable organs for transplantation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Available on request from the authors.

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