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TRANSPLANT INFECTIOUS DISEASE

BRIEF COMMUNICATION

Evolution of pathological findings in surveillance biopsies of lung transplant recipients infected with SARS-CoV-2

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Abstract

Background: Previous reports of coronavirus disease 2019 (COVID-19) following lung transplantation generally described a grim prognosis, but these were anecdotal case series of symptomatic patients. A systematic study of the outcomes and pathology of SARS-CoV-2 infection in a large cohort of lung transplant patients is lacking.

Methods: To determine the histopathologic evolution of COVID-19 in lung transplant recipients, we identified all patients who underwent surveillance transbronchial biopsies at our institution, tested positive for SARS-CoV-2, and had multiple pathology specimens available for evaluation. Histology was reviewed and immunofluorescence for SARS-CoV-2 nucleocapsid protein was performed.

Results: Ten patients met inclusion criteria. Half (5/10) had incidental diagnosis on routine respiratory pathogen testing at the time of transbronchial biopsy. Six patients were hospitalized, with three requiring intensive care unit (ICU) admission. One patient died. Two specimens showed new onset International Society for Heart and Lung Transplantation (ISHLT) Grade A2 rejection at or following diagnosis. One patient developed bronchiolitis obliterans 111 days following diagnosis and 1 year post transplant. Two patients had organizing pneumonia at diagnosis and three patients showed evolving lung injury following diagnosis. The SARS-CoV-2 nucleocapsid protein was detected in a subset of samples at diagnosis and up to 111 days following diagnosis.

Conclusions: Overall, the pathology of SARS-CoV-2 infection in lung transplant patients is varied, ranging from no pathologic alterations to organizing pneumonia and lung injury. The pathology findings did not necessarily correlate with clinical acuity, as one patient admitted to the ICU had normal pathology. These findings may be generalizable to non-transplant patients and require more follow-up regarding long-term outcomes.

KEYWORDS
COVID-19, lung transplant, pathology, SARS-CoV-2

Abbreviations: AMR, antibody-mediated rejection; BAL, bronchoalveolar lavage; COVID-19, coronavirus disease 2019; DSA, donor-specific antibodies; ICU, intensive care unit; ISHLT, International Society for Heart and Lung Transplantation; OB, obstructive bronchiolitis; TBBx, transbronchial biopsy

1 | INTRODUCTION

Lung transplant recipients are a unique patient population in the coronavirus disease 2019 (COVID-19) pandemic due to pre-existing conditions and immune modulation. The majority of previously published case series on COVID-19 in patients following lung transplantation have been anecdotal case series and reported poor outcomes and high mortality.[1–5] However, these studies are biased toward clinically symptomatic patients.

An unbiased analysis to examine clinicopathological characteristics of SARS-CoV-2 infection in a large cohort of lung transplant patients or the general population is lacking. Here, we report our single-institute experience of SARS-CoV-2 infection in lung transplant recipients, and examine the clinicopathologic evolution in routine surveillance transbronchial biopsies (TBBx).

2 | MATERIALS AND METHODS

This study was approved by the Washington University Institutional Review Board (#202005008), exempted from informed consent by the IRB, and conducted in accordance with the Declaration of Helsinki. All post-transplant TBBx between March 2, 2020 and February 24, 2021 were identified and the electronic medical records were reviewed to identify patients with SARS-CoV-2 infection. TBBx were categorized as (a) pre-diagnosis if the viral real-time reverse transcriptase polymerase chain reaction (RT-PCR) performed on the concurrent bronchoalveolar lavage (BAL) or hospital-required nasopharyngeal swab prior to biopsy was negative for SARS-CoV-2; (b) diagnosis if the first positive PCR result was from the concurrent BAL specimen; or (c) post-diagnosis if it was the first biopsy following a positive SARS-CoV-2 test. Patients were included in the cohort if they had biopsy specimens, which fit into two of these three time points. Hematoxylin and eosin stained slides were reviewed by a cardiothoracic pathologist (CYL) blinded to SARS-CoV-2 status or patient condition, with samples from the same patient mixed with others to maintain objectivity. The International Society for Heart and Lung Transplantation (ISHLT) lung transplant grading system was used for pathology review, and additional histological features were documented. All the TBBx are adequate for evaluation per ISHLT guidelines, with cartilaginous airways excluded for evaluation. Donor-specific antibody (DSA) testing was performed using the LABScreen single-antigen bead assay (One Lambda, West Hills, CA) on the Luminex platform according to manufacturer's instructions. For immunofluorescence stainingÿ paraffin-embedded sections were dewaxed in xylene and rehydrated. Endogenous peroxide activity was quenched in 10% methanol and 3% hydrogen peroxide before antigen retrieval by boiling in citrate buffer pH 6.0 for 15 min. Slides were blocked in 10% bovine serum albumin containing 0.05% Tween-20 and stained with the SARS-CoV-2 Nucleocapsid antibody (Sino Biological US Inc., 1:1000) for 1 h at room temperature. The primary antibody was detected using Opal Polymer HRP Ms + Rb. The PerkinElmer Opal Multicolor IHC kit was used for staining according to the manufacturer's protocol. Immunofluorescence was visualized with Zeiss Axio Scan Z1, and images were acquired and processed using Zeiss Zen Blue.

3 | RESULTS

Among 457 post-transplant TBBx performed on 200 patients during the study period, we identified 17 patients with SARS-CoV-2 infection. Ten patients with TBBx in at least two of the time points defined above were included in the cohort (Table 1). In five patients, the COVID-19 diagnosis was incidental as part of routine screening on the day of biopsy without significant symptoms or exposure/contact history. One additional patient was asymptomatic at diagnosis but was tested because his spouse had tested positive. For the eight patients with post-diagnosis TBBx available, five (63%) patients had detectable SARS-CoV-2 RNA in their concurrent BAL samples at the time of post-diagnosis TBBx sample collection (49–91 days after diagnosis).

The COVID-19-related treatments are listed in Table 1. Six of 10 patients were hospitalized, including three patients who required intensive care unit (ICU) admission. One patient died. He was diagnosed incidentally, but developed respiratory symptoms a month later. The repeat SARS-CoV-2 test was positive at the time of symptom development. He required admission to the ICU with mechanical ventilation, deteriorated, was transitioned to comfort care, and died on hospital day 7. The patient's family declined autopsy.

The histological features of TBBx of these 10 patients are summarized in Table 1 and representative images are shown in Figure 1. There is no meaningful increase of acute cellular rejection, antibody-mediated rejection (AMR) or chronic rejection in our cohort. Most TBBx showed no or only minimal acute cellular rejection (ISHLT grade A0 or A1, 20/22, 91%). Two patients had episodes of mild acute cellular rejection on diagnosis or post-diagnosis specimens (ISHLT grade A2). One of these had minimal acute cellular rejection (ISHLT grade A1) on the pre-diagnosis biopsy. New bronchiolitis obliterans (OB) was noted in one post-diagnosis TBBx (1 year post transplant, 111 days after positive SARS-CoV-2 testing). The OB could be caused by either chronic rejection (ISHLT grade C1) or prior airway injury due to infection. No TBBx showed lymphocytic bronchiolitis (ISHLT grade B). For these 10 patients, there was no concern or suspicion for AMR during the study period by a multidisciplinary approach based on histology, clinical manifestation, or development of de novo DSA as defined by mean fluorescence intensity above 1000 on flow cytometry-based testing. No patients in the study cohort were treated for AMR during the study period. While some of the histological findings in these patients can be seen in AMR, these findings are attributed to infection rather than rejection based on clinicopathological correlation. C4d immunohistochemical staining is not routinely performed at our institution on transplant lung biopsies based on institutional experience.[6]

Of the five patients with diagnosis TBBx (incidental SARS-CoV-2 infection without symptom or exposure history), two TBBx showed organizing pneumonia (OP, 40%). Both of these patients were admitted to the hospital following diagnosis and one required intensive care.

TABLE 1 Data summary of the study cohort

	Age/ gender	T txpt to dx (days)	Incidental?	Hospitalized?	COVID-19 treatment	Survived?	Pre-diagnosis TBBx			Diagnosis TBBx				Post-diagnosis TBBx		
							ISHLT grade	Other findings	Interval[a]	ISHLT grade	Other findings	IF	Interval[b]	ISHLT grade	Other findings	IF/PCR
1	17/F	236	No	No	None	Yes	A0B0C0	None	63				49	A0B0C0	None	–/+
2	11/F	771	No	ICU	Remdesivir, dexamethasone, convalescent plasma	Yes	A0B0C0	Fibrin; hemosiderin-laden macrophages	224				55	A0B0C0	Acute bronchitis	+/–
3	42/F	407	Yes	No	None	Yes	A0B0C0	Acute and chronic bronchiolitis	42	A0B0C0	none	–				
4	64/M	238	Yes	ICU[c]	None	No	A0B0C0	None	37	A0B0C0	OP	+				
5	65/M	410	No	Floor	None	Yes	A0B0C0	Widened septa with mixed infiltrate	63				72	A0B0C0	OP; acute and chronic bronchiolitis	+/+
6	23/M	241	No[d]	No	None	Yes	A0B0C0	None	37				111	A0B0C1	None	+/–
7	64/M	122	Yes	ICU	Remdesivir, dexamethasone, convalescent plasma	Yes	A0B0C0	None	33	A0B0C0	None	–	84	A0B0C0	None	+/+
8	20/F	414	No	Floor	None	Yes	A1B0C0	None	18				89	A2B0C0	Fibrin; hemosiderin laden macrophages	+/–
9	62/F	65	Yes	Floor	remdesivir, dexamethasone, convalescent plasma	Yes				A1B0C0	OP	–	52	A0B0C0	Vasculitis; neutrophil margination; increased intraalveolar macrophages; widened septa	–/+
10	67/F	188	Yes	No	Bamlanivimab	Yes	A0B0C0	None	102	A2B0C0	None	–	91	A0B0C0	None	–/+

Note: Incidental is defined as positive multiplex PCR for SARS-CoV-2 without symptoms or clinical suspicion of infection.

Abbreviations: ICU, intensive care unit; IF, immunofluorescence microscopy; ISHLT, International Society for Heart and Lung Transplantation; OP, organizing pneumonia; PCR, polymerase chain reaction; T txpt to Dx, time from transplant to diagnosis; TBBx, transbronchial biopsy.

[a]Interval between pre-diagnosis sample and positive SARS-CoV-2 test, regardless of method of test.

[b]Interval between positive SARS-CoV-2 test and post-diagnosis sample, regardless of method of test.

[c]Initially asymptomatic, admitted 1 month after positive test and quickly decompensated.

[d]Asymptomatic, tested because spouse was positive.

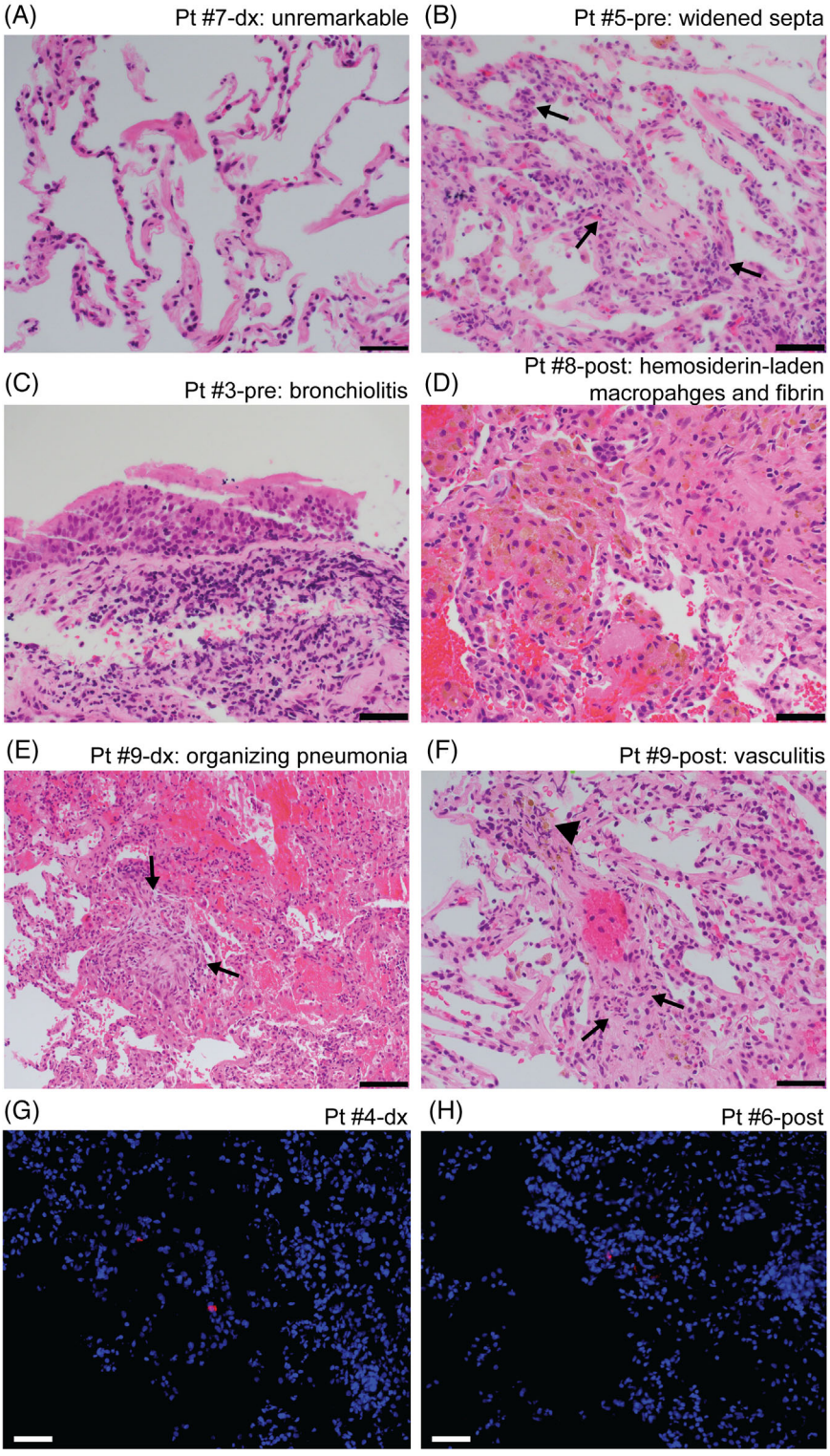

FIGURE 1 Representative pathologic findings and SARS-CoV-2 antigen in transbronchial biopsy (TBBx) specimens from lung transplant patients with SARS-CoV-2. Panels A–E are stained with hematoxylin and eosin and all scale bars are 50 μm, except for panel E, which is 100 μm. Abbreviations: pre, pre-diagnosis biopsy; dx, diagnosis biopsy; post, post-diagnosis biopsy. (A) Unremarkable lung parenchyma showing normal pneumocytes lining delicate septa without any significant abnormalities. (B) Widened alveolar septa (arrows) with mixed inflammatory infiltrates. No other features to support a diagnosis of organizing pneumonia were present in this specimen. (C) Bronchiolitis with mixed acute and chronic infiltrates in both epithelium and submucosa. (D) Hemosiderin-laden macrophages and fibrin deposition, suggestive of prior hemorrhage. No definite features of organizing pneumonia/subacute lung injury were present. (E) Organizing pneumonia with focal fibroblastic proliferation (arrows). (F) Vasculitis with neutrophils in the vessel wall (arrows) as well as hemosiderin deposition (arrowhead). Although vasculitis could be observed in high-grade acute cellular rejection, there is no perivascular lymphoid cuffing in this field or in the whole biopsy specimen to support a diagnosis of acute cellular rejection. (G and H) SARS-CoV-2 nucleocapsid protein (red) in representative diagnosis (G, patient 4) and post-diagnosis (H, patient 6) TBBx. Blue: DAPI

In post-diagnosis TBBx, three patients (38%) showed histological findings that could be consistent with acute/subacute lung injury pattern, including OP, focal fibrin deposit, and hemosiderin-laden macrophages. All three of these patients were admitted to the hospital without requiring intensive care. Notably, we did not identify other histological findings that have been reported in COVID-19, such as microthrombi, capillary proliferation, or viral cytopathic changes. With short-term follow-up, we did not observe increased interstitial fibrosis in post-diagnosis TBBx. Moreover, with this pilot cohort, the histologic findings did not necessarily correlate with disease severity. For instance, patient #7 required ICU admission, but the diagnosis and post-diagnosis TBBx showed no significant findings.

To identify persistent viral antigens, immunofluorescence staining for the SARS-CoV-2 nucleocapsid protein was performed on biopsy

tissue from diagnosis (n = 5) and post-diagnosis (n = 8) specimens. Immunofluorescence microscopy highlighted viral nucleocapsid protein in one of five (20%) of the diagnosis samples and five of eight (63%) of post-diagnosis samples (Table 1 and Figure 1). Of the five patients with detectable SARS-CoV-2 nucleocapsid protein, two (40%) also had SARS-CoV-2 RNA detected by PCR of the concurrent BAL. Three (60%) of the patients with detectable SARS-CoV-2 nucleocapsid protein had negative PCR results of BAL and three (60%) patients with positive PCR results were negative for nucleocapsid protein by immunofluorescence.

4 | DISCUSSION

In this study, we report the heterogeneity of pathologic findings in surveillance TBBx of lung transplant recipients infected with SARS-CoV-2, including asymptomatic patients. The routine clinical practice of post-transplantation TBBx provided a unique opportunity to understand the spectrum of histological findings and tissue response over time. To our knowledge, no previous work has systematically evaluated serial pathology samples from COVID-19 patients, including pre-diagnosis samples and asymptomatic patients. Autopsy series, transplantation pneumonectomy specimens, and cancer resection specimens have provided only a single time point for examination, and were heavily biased toward patients with severe disease.[7–10] These works have found characteristic findings in the lungs of patients with severe COVID-19 such as diffuse alveolar damage, microthrombi, massive inflammatory infiltration, and increased megakaryocytes. Additional works examining biopsy specimens in symptomatic patients have found similar histopathologic alternations, including acute lung injury, interstitial pneumonia, type II pneumocyte hyperplasia, and septal thickening.[11–13]

Another work reported serial lung biopsies in a lung transplant recipient during and following hospitalization for severe SARS-CoV-2 infection and found diffuse alveolar damage, a lymphocytic and eosinophilic immune infiltrate, and fibrin clusters in the alveoli at the time of diagnosis.[14] These features were still present at follow-up bronchoscopy 2 weeks later. The authors of this work proposed that a diffuse pattern of lymphocytic infiltration was more characteristic of COVID-19 versus perivascular cuffing present in rejection. In our cohort, we did not observe diffuse lymphocytic infiltration in any of the specimens as described by this previous literature, possibly due to different patient inclusion criteria and disease severity. While we did not find any evidence of clinical AMR in our cohort, one other report described the onset of ISHLT A2B1R rejection with new DSAs in the context of COVID-19.[15]

Our case series establishes that COVID-19 can be mild or asymptomatic even in heavily immunosuppressed patients, with variable histological findings that evolve throughout disease course. Infection with SARS-CoV-2 did not necessarily lead to significant histopathologic abnormalities in TBBx specimens. Evaluation of post-diagnosis biopsies (49–111 days) generally showed evolution of pathologic abnormalities that could be considered on the spectrum of a normal healing response. Only one patient showed new onset OB in the post-diagnosis biopsy, which was at 1 year post transplant. While OB may begin to occur at that time, the role of COVID-19 in this finding cannot be excluded and more work is necessary to determine the long-term effects of COVID-19 on lung allografts. At least one other study has suggested that the immune response to allografts may be altered in patients recovered from COVID-19.[16] While none of the patients who remained outpatient during their illness showed any histopathologic abnormalities on TBBx, those who were admitted displayed a spectrum of findings ranging from normal lung histology to subacute lung injury pattern.

Immunofluorescence microscopy of diagnosis and post-diagnosis TBBx specimens showed only one diagnosis specimen with detectable SARS-CoV-2 nucleocapsid protein despite positive PCR testing on all five concurrent BAL specimens; however, the majority of post-diagnosis biopsies showed SARS-CoV-2 nucleocapsid protein by immunofluorescence. In fact, all post-diagnosis samples were positive for SARS-CoV-2 by immunofluorescence and/or PCR. This is in keeping with prior reports of extended SARS-CoV-2 viral replication in transplant recipients, which is believed to play a role in the evolution of multimutational variants.[17] The extended infection in these patients also complicates the evaluation of post-diagnosis biopsy specimens for acute cellular rejection, and recent COVID-19 should be considered when attempting to distinguish between the immune infiltrates of acute cellular rejection, AMR, and viral infection.

While our current work is limited to a small sample of lung transplant patients with short-term follow-up, it highlights disease heterogeneity that is likely generalizable to COVID-19 patients as a whole and warrants further studies with larger series and longer follow-up.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Alexander N. Wein and Chieh-Yu Lin designed the study, analyzed the data, and wrote the manuscript. Jing Liu performed the immunofluorescence staining.

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