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Histological Findings in Transbronchial Cryobiopsies Obtained From Patients After COVID-19

To the Editor:

In cases of severe acute respiratory syndrome (SARS) caused by COVID-19, a broad spectrum of pulmonary sequelae can be observed.[1] Some preliminary reports described radiological and histological findings suggestive of pneumonitis and organizing pneumonia (OP),[2-4] two diffuse interstitial lung diseases (DILD) that can lead to pulmonary fibrosis (PF). However, only one report describes the long-term radiological evolution of pulmonary sequelae of COVID-19.[5] Similarly, follow-up studies after middle eastern respiratory syndrome-CoV and SARS-CoV described a prevalence of residual PF between 33% and 62%,[6,7] although the diagnosis of PF was never histologically confirmed.

We report the results of a complete investigation of patients with suspicion of DILD after COVID-19, including radiology (CT scan) and histopathology of lung biopsy specimens obtained from areas showing DILD to provide clinical guidance in the management of DILD.

Accordingly, we reviewed 757 of the 1,996 patients that were referred to our dedicated post-COVID clinic for the risk of pulmonary sequelae after hospitalization for COVID-19 between April and May 2020 based on severity of pneumonia (need of ICU or high or prolonged oxygen support) or persisting symptoms.

The ethics committee of the hospital approved the study (PR[AG]219/2020), and written informed consent was obtained from all patients.

Before their medical visit, all 757 patients underwent forced spirometry, diffusion capacity of the lung for carbon monoxide, 6-minute walking test, and chest CT scan. If radiological alterations suggestive of DILD were observed in addition to dyspnea (assessed by Modified Medical Research Council Dyspnea Scale) or functional criteria (FVC or diffusion capacity of the lung for carbon monoxide between 40% and 70% of predicted, or desaturation in 6-minute walking test), transbronchial cryobiopsy (TBC) through flexible bronchoscope (FB) was recommended by a multidisciplinary team as per an internal protocol. In fact, TBC through FB has been shown to be a safe and effective technique, with a diagnostic yield of approximately 80% in patients with DILD.[8] Because of the extreme infectiousness of SARS-CoV-2, disposable materials were used to perform TBC.

We describe the histological findings of the first 50 patients with suspected DILD who were requested to perform TBC through FB. None of the patients had received corticosteroids since hospital discharge.

All procedures were performed as previously described.[9] Single-use Ambu aScope 4 Broncho Large (Ambu Corp) and 2.4-mm single-use Erbe cryoprobes were employed, using cryosurgical technology ERBEKRYO 2 (ERBE Medizintechnik). According to radiological findings, the most affected lung was chosen for the TBC.

A negative polymerase chain reaction assay for SARS-CoV-2 RNA (nasopharyngeal swab) was required before all procedures. No patient developed serious complications.

Table 1 shows main characteristics of patients and biopsies.

All patients showed bilateral alterations at lung CT scan, with bilateral ground-glass opacities and reticulation the most common. The mean number of pulmonary segments showing interstitial changes at CT scan was 4.4 $\pm$ 2.9.

Different levels of TBC samples were obtained, staining 4 with hematoxylin and eosin and 1 with elastic fibers. All of them showed alveolate lung parenchyma, and 49 (98%) had representation of the airway, without artifacts.

Based on histological findings (Fig 1), patients were grouped as follows:

OP (at least focal Masson bodies) in 16 (32%) patients,
Diffuse-mild or moderate to severe lymphoplasmacytic interstitial infiltrate (in absence of Masson bodies or interstitial giant cells) in nine (18%) patients,
Lymphoplasmacytic interstitial infiltrate and interstitial giant cells in four (8%) patients

TABLE 1] Clinical, Demographic, and Biopsy Characteristics of the 50 Patients Included in the Study

Characteristic	N = 50
Women, No. (%)	24 (48)
Age, y, mean (range)	58.2 (38-76)
Smoker or former smoker; nonsmoker, No. (%)	18 (36%); 32 (64)
In-patient time, d, median (95% CI)	24.5 (18.5-34)
ICU time (n = 33), d, mean (range)	16.9 (2-33)
Time from diagnosis of COVID-19 to FB, d, median (IQR)	107.5 (93.5-151)
Time from discharge to FB, d, median, 95% CI	87.5 (84.7-99.9)
Comorbidities	
Cardiovascular, No. (%)	16 (32)
Metabolic syndrome, No. (%)	21 (42)
Malignancies, No. (%)	6 (12)
Immunosuppression, No. (%)	4 (8)
Pulmonary arterial hypertension, No. (%)	1 (2)
Clinical Criteria	
mMRC 0, No. (%)	9 (18)
mMRC 1, No. (%)	16 (32)
mMRC 2, No. (%)	21 (42)
mMRC 3, No. (%)	4 (8)
mMRC 4, No. (%)	0 (0)
Respiratory Function Test	
FVC, %, median (CI 95%)	79.6 (71.5-87.7)
FEV_1, %, median (CI 95%)	85.6 (74.7-93.5)
D_{LCO}, %, mean (range)	54.5 (29.6-89.1)
CT Scan	
Bilateral ground-glass opacities, No. (%)	44 (88)
Reticulation, No. (%)	36 (72)
Consolidation, No. (%)	11 (22)
Subpleural lines, No. (%)	11 (22)

(Continued)

TABLE 1] *(Continued)*

Characteristic	N = 50
Flexible Bronchoscopy	
TBC located on right lower lobe, No. (%)	39 (78)
No. of TBC, mean (range)	5 (3-5)
Total TBC volume per patient, cm^3, median (95% CI)	0.205 (0.152-0.294)
Mean volume of each TBC, cm^3, median (95% CI)	0.041 (0.032-0.059)
Moderate hemorrhage after TBC, No. (%)	20 (40)
Serious hemorrhage after TBC, No. (%)	0 (0)
Pneumothorax after TBC, No. (%)	0 (0)

D_{LCO} = diffusing capacity of the lungs for carbon monoxide; FB = flexible bronchoscope; IQR = interquartile range; mMRC = modified Medical Research Council Dyspnea Scale; TBC = transbronchial cryobiopsy

Patchy collagenous interstitial/alveolar scars in the absence of cellular infiltrate or fibroblastic foci in four (8%) patients

Emphysema or airspace pigmented macrophages alone in four (8%) patients

Normal or minimal nonspecific findings in 13 (26%) patients.

OP classification was based on the presence of specific histological findings in the absence of any other abnormality. There were no hyaline membranes or fibroblastic enlargement of the interstitium. Thrombi were not found, and vessels had no remarkable changes (no features of diffuse alveolar damage were observed at this point). The fibrosis observed in four patients was in the form of patchy collagenous scars that remanded in shape and distribution to "old OP." No clear irreversible fibrosis or any classic pattern (Usual or Nonspecific Interstitial Pneumonia) or smoking-related interstitial fibrosis were observed.

SARS-CoV-2 immunohistochemistry was performed and negative in 19 cases (SARS-CoV Nucleoprotein, Sino Biologicals). The same cases were also tested and negative with in situ hybridization (RNAscope VS Universal Assays). Also, the cultures of all samples (bronchoaspirate, BAL, and cryobiopsies) were negative, including polymerase chain reaction assay for SARS-CoV-2 RNA.

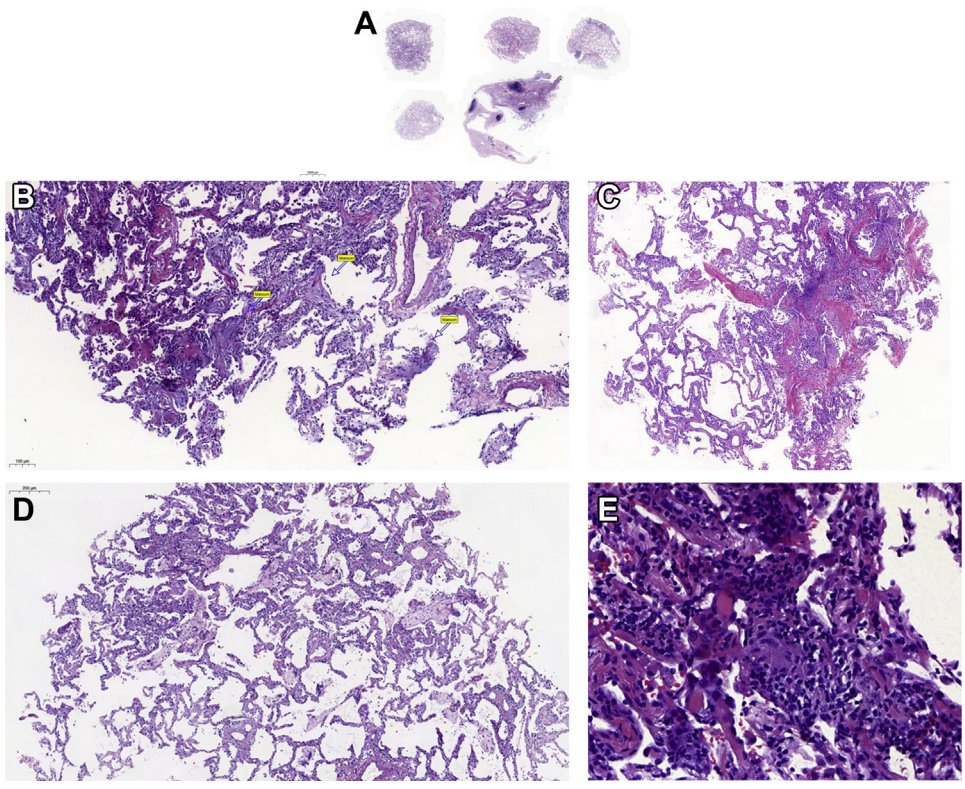

Figure 1 – *A, Low-magnification hematoxylin and eosin-stained sections of different cryobiopsied specimens from the same patient. Note the size and expansion of specimens. B, 6.2× hematoxylin and eosin-stained sections showing expanded interstitium with chronic inflammation and fibroblastic plugs in the alveolar spaces (arrows). C, Low-magnification hematoxylin and eosin-stained sections showing partially collagenized Masson bodies. D, Low-magnification hematoxylin and eosin-stained sections with mild-moderate interstitial inflammation. E, High-magnification hematoxylin and eosin-stained sections with moderate interstitial inflammation and interstitial giant cells.*

Histological patterns did not correlate with any of the factors that could have contributed to lung damage during hospitalization, such as invasive mechanical ventilation ($P = .659$, χ^2 test), ARDS ($P = .574$, χ^2 test), or length of hospital/ICU stay ($P = .729$, $P = .337$, respectively; analysis of variance) or time since diagnosis of SARS-CoV-2 infection ($P = .453$, analysis of variance). Likewise, none of the histological findings was significantly correlated with any specific radiological pattern ($P = .4011$, Prob $> \chi^2$ test).

After the histological confirmation of DILD, corticosteroid therapy was initiated in 30 (60%) patients, following an internal multidisciplinary protocol, and are currently on follow-up. In the remaining patients, only follow-up was decided because of clinical improvement (n = 3) or normal or unspecific histological findings (n = 17).

To our knowledge, this is the first study based on histological assessment of patients with suspected DILD after COVID-19. The observed findings can support the existence of interstitial involvement after some cases of severe SARS-CoV-2 pneumonia and the need to initiate a specific therapy or intervention to prevent disease progression.

OP after a viral infection has been previously described and often improves with corticosteroid treatment.[10] However, a long-term, high-dose steroid regimen is usually required, which can induce serious side effects. For this reason, we believe that a histological confirmation can likely avoid an empirical corticosteroid treatment when it is not evidence-based. Notably, in our study, 17 (34%) patients with initial empirical indication for corticosteroid treatment in the first visit were not initiated after results of TBC.

This study has also confirmed the safety and usefulness of TBC in expert hands. All obtained samples were of high quality, allowing a histological description of findings in all cases. Additionally, all procedures were performed using single-use material, avoiding the possibility of cross-contamination. However, a potential limitation of this kind of study is that none of the patients had a CT scan before COVID-19, making it

impossible to discard eventual preexisting DILD, although that is unlikely.

In summary, in severe SARS-CoV-2 pneumonia, some patients show pulmonary clinical, radiology, and functional criteria consistent with DILD with different histological patterns. Moreover, the TBC based on single-use FB and cryoprobe has shown to be a safe technique with a high diagnostic yield to investigate DILD during current and likely future pandemics.

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