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## Cytotoxic CD8-positive T-lymphocyte infiltration in the lungs as a histological pattern of SARS-CoV-2 pneumonitis



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### Summary

Despite millions of PCR confirmed cases of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, the long-term pathophysiological changes induced by this infection in the lungs and their relationship with possible immune triggers remain incompletely understood. Acute respiratory distress syndrome and subsequent respiratory failure are the most common causes of mortality in hospitalised patients. Severe lung tissue destruction can be due to an overactive immune system that far exceeds the harm that would have been caused by direct virus replication. This study extends our previous investigation and presents detailed histopathological findings on cryotransbronchial biopsy in patients with persistent (range 31–182 days) pneumonitis and severe interstitial inflammatory infiltration in the lungs due to SARS-CoV-2 infection. We describe a novel lung injury pattern associated with SARS-CoV-2 pneumonitis, which manifests as a marked interstitial CD8-positive T-cell lymphocytic infiltration. These findings provide a better understanding of the changes in the lungs that ensue due to SARS-CoV-2 infection.

*Key words:* SARS-CoV-2; pneumonitis; CD8-positive T-lymphocytes; COVID-19.

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### INTRODUCTION

The 2019 novel coronavirus (COVID-19) has had a significant impact on practically everyone. Despite millions of polymerase chain reaction (PCR) confirmed cases of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, the long-term pathophysiological changes induced by this infection in the lungs and their relationship with possible immune triggers remain incompletely understood. This study extends our previous investigation<sup>1</sup> and describes the histological pattern in antemortem lung tissue after cryotransbronchial biopsy in an additional eight cases of SARS-CoV-2 infection with persistent (range 31–182 days) interstitial inflammatory infiltrates and pneumonitis.

### MATERIALS AND METHODS

Between August 2020 and March 2021, histological lung specimens were obtained from eight SARS-CoV-2-positive

patients for antemortem evaluation after bronchoscopy and cryotransbronchial biopsy. The clinical records, radiological, and pathological findings were retrospectively compiled. Transbronchial biopsy specimens were available in all patients, taken from the most prominent areas of the interstitial ground-glass opacities. The mean number of lung biopsy fragments per case was three (range 2–4).

Biopsy specimens were fixed in 10% formalin, embedded in paraffin wax, and sectioned at 5 µm. Sections were stained with haematoxylin and eosin. Each biopsy specimen was evaluated semi-quantitatively for the following parameters: the presence or absence of reactive pneumocytes; alveolar macrophages; lymphocytes; neutrophils; plasma cells, fibroblastic foci; peribronchiolar inflammation; and viral cytopathic effect.

Immunohistochemical labelling was performed with antibodies directed against the following proteins: CD3 (anti-CD3; Leica Biosystems, UK); CD4 (anti-CD4; Leica Biosystems); CD8 (anti-CD8; Leica Biosystems); CD20 (anti-CD20; Leica Biosystems); and SARS-CoV-2 nucleocapsid protein.<sup>2</sup> CD4 and CD8 staining were scored as percent-positive immune cell: 0, negative; 1, 1–30%; 2, 31–70%; and 3, 71–100% positive immune cell.

### RESULTS

#### Clinical findings

A summary of the clinical and endoscopic findings from eight patients (5 men and 3 women) is provided in [Table 1](#). The patients' age ranged from 41–69 years with a mean of 55 years. Three patients had underlying lymphomas but were in clinical remission, two patients had autoimmune disorders and were taking immunosuppressive therapy, and the remaining three patients did not have comorbid diseases related to the immune system. All eight patients tested positive for SARS-CoV-2 by real-time PCR using nasal swab samples at the time of clinical symptom onset and chest computed tomography (CT) exhibited peripheral patchy and heterogeneous ground-glass opacities consistent with SARS-CoV-2 pneumonia.

For all cases, bronchoscopy was performed to explore persistent extensive interstitial ground-glass opacities in the lungs on the CT studies that were associated with shortness of breath (minimum of 1 month and maximum of 6 months). Endoscopic examination of the respiratory system showed normal airway mucosa except for Case 8, where bronchoscopy

**Table 1** SARS-CoV-2 pneumonitis: clinical findings in eight cases of the present series

Case	Age, sex	Comorbids related to the immune system	Treatment prior to bronchoscopy <sup>a</sup>	SARS-CoV-2 IgG within 7 days of biopsy <sup>a</sup>	PCR at disease onset	PCR within 7 days of biopsy <sup>a</sup>	Chest CT at disease onset	Chest CT within 7 days of biopsy <sup>a</sup>	Bronchoscopy
1	49, M	CLL, in clinical remission	Oxygen, voriconazole, TMP-SMX, acyclovir	Non-reactive	Positive	Negative	Interstitial ground-glass opacities in the left midlung	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 31st day)
2	44, F	Sjögren and Hashimoto, on immune suppression	No indication for antimicrobial treatment rituximab, azathioprine	Non-reactive	Positive	Negative	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 93rd day)
3	61, F	N/A	No indication for antimicrobial treatment	Non-reactive	Positive	Negative	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 182nd day)
4	68, M	N/A	Oxygen, prednisone	Non-reactive	Positive	Negative	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 90th day)
5	41, M	Follicular lymphoma, in clinical remission	Dexamethasone, oxygen, convalescent plasma, TMP-SMX,acyclovir	Non-reactive	Positive	Negative	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 35th day)
6	53, M	N/A	Oxygen	Non-reactive	Positive	Negative	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 37th day)
7	58, M	Follicular lymphoma, in clinical remission	Dexamethasone, TMP-SMX	Non-reactive	Positive	Positive	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Normal airway mucosa (on 120th day)
8	69, F	Sarcoidosis, on immune suppression	Piperacillin/tazobactam, Prednisone	Non-reactive	Positive	Negative	Bilateral interstitial ground-glass opacities	Bilateral interstitial ground-glass opacities	Hyperaemic mucosa (on 97th day)

CLL, chronic lymphocytic leukaemia; CT, computed tomography; F, female; M, male; N/A, not applicable; PCR, polymerase chain reaction; TMP-SMX, trimethoprim-sulfamethoxazole.

<sup>a</sup> Within 7 days before or after biopsy.

**Table 2** SARS-CoV-2 pneumonitis: histological findings in 8 cases of the present series

Case	Reactive pneumocytes	Numerous alveolar macrophages	Lymphocytes	Neutrophils	Plasma cells	Fibroblastic foci	Peribronchiolar inflammation	Viral cytopathic effect
1	Present	Focally present	Moderate interstitial lymphocytic infiltrate	Absent	Rare	Present (severe)	Present	Absent
2	Present	Focally present	Moderate interstitial lymphocytic infiltrate	Absent	Rare	Absent	Present	Absent
3	Absent	Absent	Moderate interstitial lymphocytic infiltrate	Absent	Absent	Present (mild)	Present	Absent
4	Absent	Absent	Mild interstitial lymphocytic infiltrate	Absent	Absent	Absent	Absent	Absent
5	Absent	Absent	Mild interstitial lymphocytic infiltrate	Absent	Absent	Absent	Absent	Absent
6	Present	Focally present	Mild interstitial lymphocytic infiltrate	Absent	Absent	Present (severe)	Present	Absent
7	Present	Absent	Moderate interstitial lymphocytic infiltrate	Absent	Absent	Absent	Absent	Absent
8	Present	Focally present	Moderate interstitial lymphocytic infiltrate	Absent	Absent	Present (mild)	Present	Absent

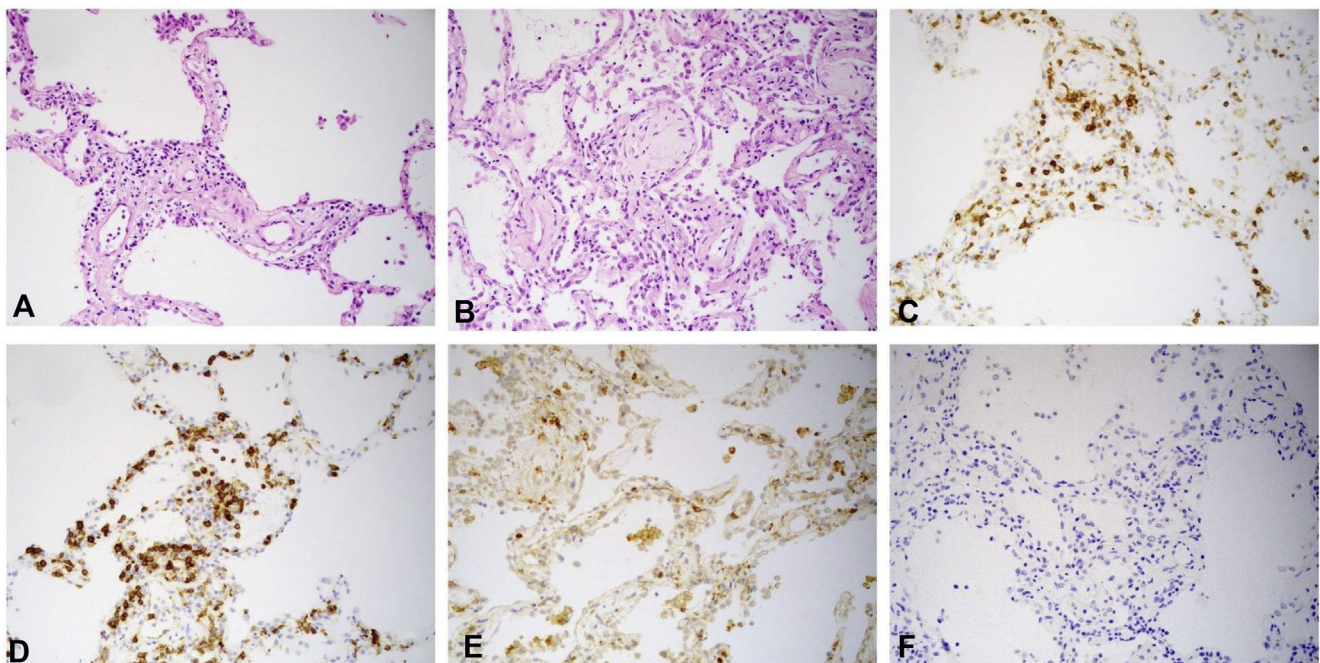
revealed hyperaemic mucosa with white mucus in the bronchial lumen. All patients re-tested negative for SARS-CoV-2 by PCR within 7 days before or after the day of their bronchoscopies except one (Case 7 showed presence of the virus on 120th day of the disease). SARS-CoV-2 IgGs were non-reactive in the same time frames for all patients (anti-nucleocapsid assays were performed on the Abbott Alinity; Abbott Diagnostics, USA).

### Pathology

Cryotransbronchial biopsy specimens for all eight cases of SARS-CoV-2 pneumonitis showed an interstitial inflammatory infiltrate (Table 2) that tended to be variable (mild to moderate) and was composed mostly of lymphocytes, with additional rare plasma cells in two cases (Fig. 1). Immunohistochemical studies demonstrated dominant prevalence of CD3 and CD8-positive T-lymphocytes with lesser numbers

of the CD4-positive T-cells (Table 3). There were virtually no CD20-positive B-lymphocytes and viral cytotoxic effects. A sensitive and validated immunohistochemistry test for the SARS-CoV-2 nucleocapsid protein, which we performed on lung biopsies, was negative in all cases, indicating that the virus had been cleared from the lungs (Fig. 2).

Additionally, focal epithelial reactive changes were present in five cases, such as pneumocytes with nuclear enlargement. These reactive changes were associated with a focal increase of macrophages in the alveoli. The inflammatory infiltrates were accompanied by mild fibroblastic foci in two (Cases 3 and 8) and severe fibroblastic foci consistent with organising pneumonia in two other cases (Cases 1 and 6) (Fig. 1). Also, peribronchiolar inflammation in some small airways were noticed in five cases. There was no evidence of granuloma, aspiration, and vasculitis. Stains for acid-fast bacilli (AFB) and fungal organisms (GMS) were negative.

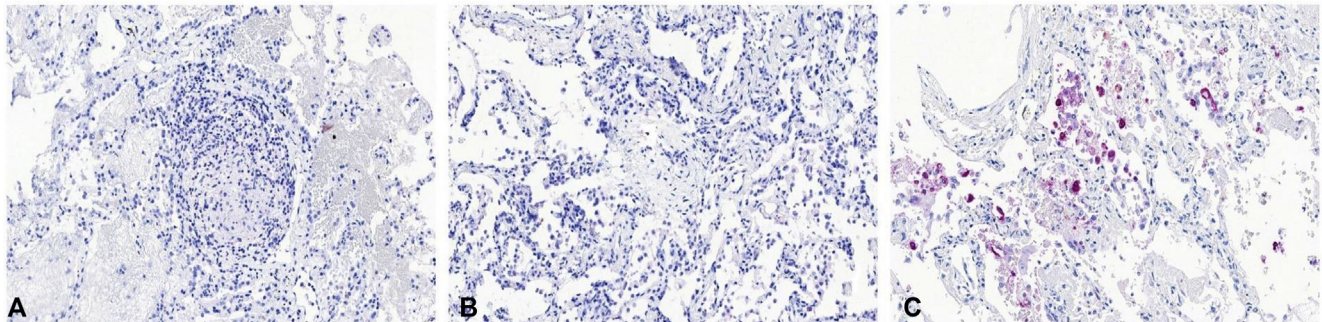


**Fig. 1** (A) Interstitial lymphocytic infiltration of the lung (H&E). (B) Interstitial lymphocytic infiltrate and fibroblastic foci (H&E). The inflammatory infiltrate consists of (C) CD3-positive T-lymphocytes, (D) predominantly CD8-positive cytotoxic cells, and (E) a minor component of CD4-positive helper cells. (F) CD20-positive B-cells were absent.



**Table 3** SARS-CoV-2 pneumonitis: clinical findings on immunohistochemistry in 8 cases of the present series

Case	CD3	CD4	CD8	CD20	Plasma cells	SARS-CoV-2 nucleocapsid protein
1	Present	1	3	Absent	Rare	Absent
2	Present	1	3	Absent	Rare	Absent
3	Present	1	3	Absent	Absent	Absent
4	Present	1	2	Absent	Absent	Absent
5	Present	1	3	Absent	Absent	Absent
6	Present	1	3	Absent	Absent	Absent
7	Present	1	3	Absent	Absent	Absent
8	Present	1	3	Absent	Absent	Absent

**Fig. 2** No residual SARS-CoV-2 was detected in post-COVID-19 lung biopsy from the patients. (A,B) Immunohistochemistry of lung tissue is negative for SARS-CoV-2 nucleocapsid protein; (C) positive immunohistochemistry control for SARS-CoV-2 nucleocapsid protein.

Based on these observations, we concluded that the main histopathological finding, such as markedly severe interstitial CD8-positive T-cell lymphocytic infiltration, can be one of the characteristic patterns of SARS-CoV-2 pneumonitis.

## DISCUSSION

The duration of COVID-19, level of SARS-CoV-2 viral replication, and intensity of immune response to viral invasion are important factors in assessing the risks, prognosis, and guiding decisions regarding treatment during the acute and post-acute phases of this infection. Because detection of SARS-CoV-2 RNA is more sensitive and faster than virus isolation, most guidelines have used viral real-time PCR results as a marker for infectious coronavirus.

Detectable SARS-CoV-2 RNA in respiratory specimens persisted 4–6 months (median of 20 days) after disease onset in survivors.<sup>3,4</sup> However, long-term SARS-CoV-2 shedding was reported in some individuals after their initial positive test. Usually, affected individuals eventually cleared the SARS-CoV-2 infection from the upper respiratory tract after developing neutralising antibody, but the virus was still able to replicate in epithelial cells in the lower respiratory tract, suggesting that it could cause pneumonia or other persistent interstitial lung changes.<sup>5</sup>

Even though only one patient had persistent positivity for COVID-19 longer than one month by RNA-test of nasopharyngeal swab, all patients had areas of interstitial opacities in the lungs during imaging examination up to 6 months after disease onset. Additionally, all eight patients did not show a good immune response to the SARS-CoV-2 virus via nucleocapsid

IgG. A previous study showed that patients with long COVID-19 illness exhibited lower levels of neutralising antibodies than patients with shorter durations of this infection.<sup>6</sup>

When an immune response is weak or absent, the virus will multiply and massive destruction of the affected tissues will occur, especially in the lungs. The damaged cells induce an intensive response that is primarily mediated by pro-inflammatory cells and because of this, lung inflammation is the leading cause of life-threatening respiratory disorders at the severe stage of COVID-19 or persistent interstitial lung changes in moderate COVID-19 cases.<sup>7</sup> Cytokine release syndrome seems to play an important role in patients with moderate to severe conditions, as demonstrated by the high levels of inflammatory cytokines IL-1 and TNF in the lungs of COVID-19 patients. These cytokines are strong inducers of T-lymphocytes and influx of T-cells from the circulation into the site of viral infection.<sup>8</sup>

Lymphocytopenia is often seen in COVID-19 patients in relation to increasing severity of disease. A clinical study also demonstrated that inflammatory cytokines can be used as predictors for COVID-19 severity and survival.<sup>9</sup> These data correlate with our histopathological examination of the transbronchial biopsies that revealed the dominant infiltration of the lung tissue by CD8-positive T-cells. The virus could be eradicated by CD8-positive T-cells, but in some cases, the very intense activity of these cells induces diffuse alveolar damage that far exceeds the harm that would have been caused by viral replication and induces lethal tissue destruction or irreversible changes like fibrosis.<sup>10</sup> This has been supported by other findings, which revealed a relation between virus-specific CD8-positive T-cells and lethal SARS

diseases in infected mice<sup>11</sup> and documented interstitial mononuclear inflammatory infiltrates in heart tissue in fatal cases of COVID-19.<sup>7</sup>

Notably, we did not find any viral cytopathic effects at the time of lung biopsies. Potentially this can be explained by clearing the virus from the lungs at this time. This was consistent with immunohistochemistry tests for the SARS-CoV-2 nucleocapsid protein performed on lung biopsies, which were negative in all patients. Only one patient (Case 7) had positive nasopharyngeal swab RNA-test for SARS CoV-2 at the time of the biopsy. We hypothesise that could be reinfection of the upper respiratory tract without new acute involvement of the lungs.

Early cytokine measurements and histological evaluation of the intensity of inflammatory cell infiltration in lung tissue can be reliable predictors of outcome and may help determine which patients are likely to develop respiratory failure or a long-term COVID-19-related pulmonary complication. Once moderate to severe lung damage occurs, efforts should be made to disrupt the underlying pathomorphological events. The lung biopsy may provide substantial insights relevant for patients with ongoing and eliminated SARS-CoV-2 infection by recognising acute and chronic histopathological changes in the lungs. This can open the possibility of initiating appropriate treatment to prevent prolonged proinflammatory responses and potentially further progression of pulmonary fibrosis.<sup>12</sup>

We have described a novel lung injury pattern associated with SARS-CoV-2 pneumonitis which manifests as a marked interstitial CD8-positive T-cell lymphocytic infiltration. Our findings provide a better understanding of the changes in the lungs due to SARS-CoV-2 infection. Ideally, this information will contribute to the improvement of clinical outcome in COVID-19 patients.

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