


Histopathological findings in fatal COVID-19 severe acute respiratory syndrome: preliminary experience from a series of 10 Spanish patients

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ABSTRACT

In December 2019, an outbreak of severe acute respiratory syndrome associated to SARS-CoV2 was reported in Wuhan, China. To date, little is known on histopathological findings in patients infected with the new SARS-CoV2. Lung histopathology shows features of acute and organising diffuse alveolar damage. Subtle cellular inflammatory infiltrate has been found in line with the cytokine storm theory. Medium-size vessel thrombi were frequent, but capillary thrombi were not present. Despite the elevation of biochemical markers of cardiac injury, little histopathological damage could be confirmed. Viral RNA from paraffin sections was detected at least in one organ in 90% patients.

INTRODUCTION

Novel coronavirus-associated disease (COVID-19) was first detected in Spain on 31 January 2020, with more than 204 178 cases subsequently identified in 3 months.¹ Severe COVID-19 is associated with high circulating levels of inflammatory cytokines akin to a cytokine release syndrome that frequently results in respiratory failure. To date, scant histopathological information of infected patients is available. Few descriptions of histopathological findings have mainly reported pneumonitis and diffuse alveolar damage (DAD).^{2–5} To advance in the knowledge of COVID-19-associated tissue damage is important to understand the mechanisms of damage caused by SARS-COV-2.

METHODS

Postmortem multiorgan biopsies in 10 patients who died with SARS COV-2 infection were performed after oral authorisation of a first-degree relative. Biopsies were obtained without ultrasound guidance with the patient's corpse still on the hospital bed. See online supplementary files for a detailed description of methods.

RESULTS

Clinical characteristics are summarised in online supplementary table 1. Chest CT findings and images are shown in figure 1 and online supplementary table 1. Pathological characteristics are summarised in online supplementary table 2.

The size of lung biopsies ranged as follows: width (4.0–9.3 cm); height (1–50.5 cm) and depth (0.5–2 cm). Figure 2 shows the major findings of lung (figure 2A–C) and heart samples (figure 2D,E). All of our cases showed histopathological features of DAD in different stages. In four cases, medium-size vessel thrombi were remarkable (figure 2C). Capillary thrombi were not present in any case. In addition, mild chronic interstitial inflammation appeared in 6 out of 10 cases. Vascular smooth muscle hyperplasia was present in five cases.

Heart biopsies had no signs of inflammation (figure 2D) except in one case (figure 2E). Liver, kidney and small intestine biopsies showed no major pathological issues.

We performed RT-PCR for SARS-CoV2 in all organs. Nine patients had at least one organ with significant amount of SARS-CoV2 RNA, being most prevalent in lung (eight positive samples), followed by myocardium (seven positive samples).

See online supplementary files for a detailed description of the results.

DISCUSSION

In this report, we describe the histopathology of lung damage in COVID-19 with DAD in all lung samples, associated with medium size arterial thrombosis in four cases, and the presence of viral RNA in all organs. Remarkably, there was no major damage in the heart, liver, kidneys or small bowel.

All the patients in our series were older than 65 years and had, at least, one prior comorbidity. Additionally, acute medical complications are also relevant in terms of risk of death. All our patients also developed respiratory and biochemical cardiac derangement, in accordance with other reports.

DAD is a non-specific response of the lung to a multitude of injurious agents, and is characterised by oedema in the exudative stage and endothelial and alveolar lining cell injury with hyaline membrane formation that can progress to interstitial fibrosis during the organising phase. All of our cases showed histopathological features of DAD in different stages, with hyaline membranes, type II pneumocyte hyperplasia, hypertrophy and reactive atypia. This finding is consistent with the histological characteristics reported in other publications of COVID-19^{2–4} and with what has been described for

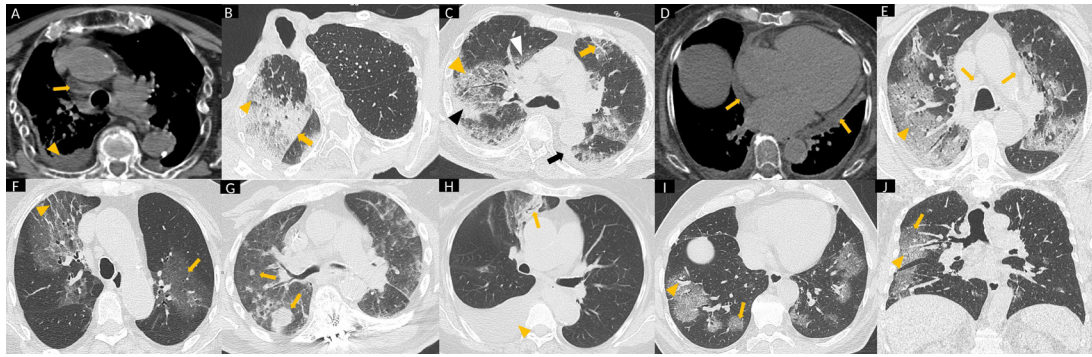


Figure 1 Chest CT images at time of admission showing COVID-19 presentations in 10 patients. (A) Case 1: bilateral PE (arrowhead) and right paratracheal lymphadenopathies (arrow) are observed. (B) Case 2: unilateral lung involvement. CPp (arrowhead) and CONS (arrow) are shown in the RUL. (C) Case 3: bilateral and patchy GGO (white arrowhead) with CONS (black arrowhead) and CPp (yellow arrowhead) are identified in upper lobes. Minimal bilateral PE (black arrow) and vascular ingurgitation (yellow arrow) are also observed. (D) Case 4: CT showing mild pericardial effusion (arrow) in a patient with cardiomegaly. (E) Case 5: predominant CPp (arrowhead) in upper lobes with mediastinal lymph node enlargement (arrows) is observed. (F) Case 6: axial CT images showed bilateral GGO (arrows) in upperlobes with bronchiectasis and peripheral fibrotic tracts (arrowhead). (G) Case 7: peripheral GGO, predominantly in the LUL, in a patient with lung metastases (arrows). (H) Case 8: GGO with CONS in the RUL. Air bronchogram (arrow) and right PE (arrowhead) are shown. (I) Case 9: predominant-GGO pattern with multilobar involvement and peripheral distribution is observed. Round-shaped opacities (arrow) withvascular ingurgitation (arrowhead) are identified. (J) Case 10: CT coronal reconstruction (lung parenchyma window) showing GGO (arrow) and CPP (arrowhead) in the right lung. CONS, consolidation; CPp, crazy paving pattern; GGO, ground-glass opacities, LUL, left upper lobe; PE, pleural effusion; RUL, right upper lobe.

respiratory syndromes produced by other coronaviruses.^{6,7}

When found, the inflammatory component in our biopsies was generally of a chronic type, mostly conformed by T lymphocytes, (CD3 positive). Signs of inflammation were absent (four cases), mild (four cases) or moderate (two cases). The four patients, who did not receive immunomodulatory drugs (corticosteroids and/or tocilizumab), showed similar histopathological findings than those who did received these drugs. The absence of cellular infiltrates is consistent with a cytokine release syndrome, in what appears to be the hyperinflammatory stage of COVID-19.^{2,8}

Small pulmonary artery thrombosis was evident in different stages of evolution (figure 2). According to the disseminated intravascular coagulation (DIC) standard of care, all our patients received prophylactic low-molecular-weight heparin. Despite heparin and in concordance with other studies, vascular thrombosis was highly prevalent. The precise mechanisms for the coagulopathy, whether a classic DIC, a pulmonary specific COVID-19 vasculopathy,⁹ or mediated by a diffuse endothelial damage via endothelitis,⁵ still requires further investigation.

Most patients had radiological features compatible with COVID-19, that is, diffuse, peripheral ground-glass opacities and air bronchogram, which has been associated with a worse prognosis. The only patient with unilateral, right sided, involvement had spent most of the time in a right lateral-decubitus position

for several years due to advanced Alzheimer disease.

Regarding the biochemical cardiac derangement frequently seen in infection by SARS-COV-2, it is remarkable that there was no clinically relevant right ventricle volume overload. On histopathological examination, myocardiocytes showed neither signs of inflammation or fibrosis, nor signs of pulmonary volume overload. Two cases showed myocardial hypertrophy, most likely not associated with COVID-19. We only found mild myocarditis in one patient, who happened to have the greatest elevation in troponin. This patient also had a follicular-cell lymphoma and received chronic immunosuppressive medication due to kidney transplantation. A previous study found that reversible, subclinical diastolic left ventricular impairment appears to be common in acute SARS-CoV-1 infection, even among patients without underlying cardiac disease,¹⁰ suggesting that left ventricular dysfunction in the acute phase might be attributable to the cytokine release syndrome. In the same report, an isolated post-mortem examination was performed, and no evidence of interstitial lymphocytic infiltrate or necrosis of myocardial cells was found.

The main limitation of our study is the tissue sample size due to the postmortem biopsy technique used. However, autopsies in COVID-19 patients were only allowed under strict biosecurity regulations.

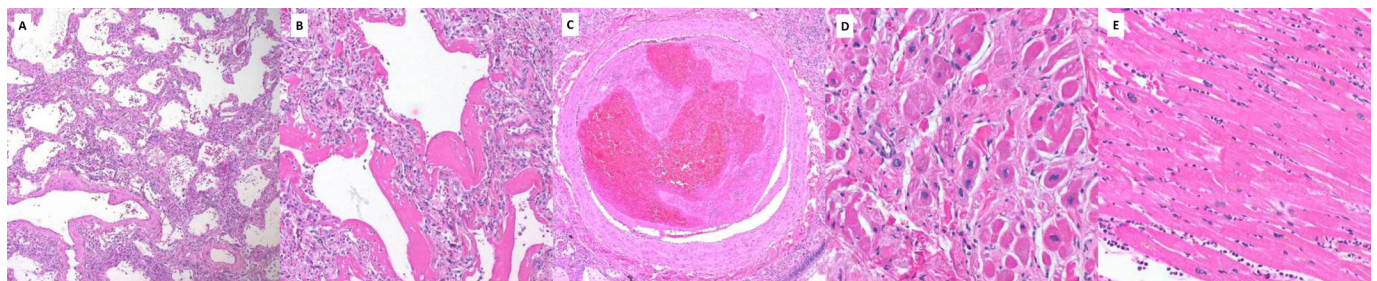


Figure 2 (A) Thickening of alveolar wall with few inflammatory cells. Insert: CD3 positive lymphocytes. (B) Well-developed hyaline membranes. (C) Thrombus in medium size vessels of lung. (D) Moderate myocardial hypertrophy and fibrosis. (E) Slight interstitial inflammatory infiltrates in myocardial tissue of patient #8.

To our knowledge, this is the first report where RT-PCR for SARS-CoV2 has been tested in all organ samples from each patient. It is remarkable that 9 out of the 10 patients had at least one organ with significant amount of SARS-CoV2 RNA, being most prevalent in lung tissue.

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Contributors BR has sampled all postmortem biopsy and written the manuscript. LG-T, AA, LA and CEDA have equally contributed on histopathological interpretation. JJZ and JAQ have reviewed the manuscript and done the english editing. AE radiology interpretation and images selection. MFL and FCT microbiology samples. JLDP, GE and CJI attended the patients and helped with postmortem biopsies. MFL and MDL designed the study and reviewed the manuscript.

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REFERENCES

- 1 Johns Hopkins Coronavirus Resource Center. Mortality analyses. Available: <https://coronavirus.jhu.edu/data/mortality>
- 2 Xu Z, Shi L, Wang Y, *et al.* Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020;8:420–2.
- 3 Barton LM, Duval EJ, Stroberg E, *et al.* COVID-19 autopsies, Oklahoma, USA. *Am J Clin Pathol* 2020;153:725–33.
- 4 Bradley BT, Maioli H, Johnston R, *et al.* Histopathology and ultrastructural findings of fatal COVID-19 infections. *medRxiv*. 2020.
- 5 Varga Z, Flammer AJ, Steiger P, *et al.* Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020;395:1417–8.
- 6 Nicholls JM, Poon LLM, Lee KC, *et al.* Lung pathology of fatal severe acute respiratory syndrome. *Lancet* 2003;361:1773–8.
- 7 Alsaad KO, Hajeer AH, Al Balwi M, *et al.* Histopathology of Middle East respiratory syndrome coronavirus (MERS-CoV) infection - clinicopathological and ultrastructural study. *Histopathology* 2018;72:516–24.
- 8 Shi Y, Wang Y, Shao C, *et al.* COVID-19 infection: the perspectives on immune responses. *Cell Death Differ* 2020;27:1451–4.
- 9 Fogarty H, Townsend L, Ni Cheallaigh C, *et al.* COVID19 coagulopathy in Caucasian patients. *Br J Haematol* 2020;189:1044–9.
- 10 Li SS-lung, Cheng C-wah, Fu C-lai, Li SSL CCW, CL F, Chan YH, *et al.* Left ventricular performance in patients with severe acute respiratory syndrome: a 30-day echocardiographic follow-up study. *Circulation* 2003;108:1798–803.