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Title:

Clinical pathology of critical patient with novel coronavirus pneumonia (COVID-19)

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Summary

Background

Critical patients with novel coronavirus pneumonia (COVID-19) have worse outcome and high mortality. However, the histopathology of critical patient with COVID-19 remains undisclosed.

Methods

We performed the whole lung biopsy, and described the pathological changes of critical COVID-19 patient done with transplant by HE staining, immunohistochemistry and special staining observed under the microscopy.

Findings

The whole lungs displayed diffuse congestive appearance and partly haemorrhagic necrosis on gross examination. The haemorrhagic necrosis was prominently present in outer edge of the right lower lung. The cut surfaces of the lung displayed severe congestive and haemorrhagic changes. The main pathological changes showed massive pulmonary interstitial fibrosis, and partly hyaline degeneration, variable degrees of hemorrhagic pulmonary infarction. Small vessels hyperplasia, vessel wall thickening, lumen stenosis, occlusion and microthrombosis formation. Focal monocytes, lymphocytes and plasma cells infiltrating into pulmonary interstitium. Bronchiolitis and alveolitis with proliferation, atrophy, desquamation and squamous metaplasia of epithelial cells. Atrophy, vacuolar degeneration, proliferation, desquamation and squamous metaplasia in alveolar epithelial cells. Alveolar cavity congestion was prominent, and contained mucus, edema fluid, desquamated epithelial cells, and inflammatory cells. We also found several multinucleate giant cells and intracytoplasmic viral inclusion bodies. Special stains including Masson

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stain, sirius red staining, reticular fibers staining indicated massive pulmonary interstitial fibrosis. Immunohistochemistry showed positive for immunity cells including CD3, CD4, CD8, CD20, CD79a, CD5, CD38 and CD68.

Interpretation

We demonstrate the pathological findings of critical patient with COVID-19, which might provide a deep insight of the pathogenesis and severity of this disease.

Keywords Novel coronavirus pneumonia; COVID-19; SARS-CoV-2; Pathology; Critical patient

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Induction

The 2019 novel coronavirus pneumonia was officially named by the World Health Organization (WHO) as COVID-19. COVID-19 caused by SARS-CoV-2 was firstly emerged in December 2019, Wuhan city, and resulted in other cities including Shenzhen in China and other counties.[1,2] The world is experiencing a global viral epidemic of COVID-19. Reports regarding epidemiological and clinical characteristics of COVID-19 is accumulating.[3-5] Most of patients had fever and cough, and some patients developed acute respiratory failure, acute respiratory distress syndrome (ARDS), septic shock, and other severe complications. In comparison with other types of COVID-19, critical patients tend to have worse outcome and high mortality.[5] Currently, the diagnosed treatment of COVID-19 mainly rely on guideline consensus including contact history of epidemic area, laboratory tests and CT imaging examination.[6,7] There is no doubt that pathological examination is very important to elucidate the pathological changes, pathogenesis and the cause of death of COVID-19. However, up to now, its clinical pathology remains largely unknown. Different from lung puncture taken from the dead body of severe COVID-19 patient recently reported,[8] we dissected the whole lungs of surgical critical patient of COVID-19, therefore for the first time, described the main pathological changes of patient with critical type.

Materials and Methods

Patients and tissues

The over 60-year-old patient had symptoms of high fever and cough when the patient

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came back to Shenzhen city from the Wuhan epidemic on January 4, 2020. The patient have no other basic diseases except several-years hypertension but with good cardiac function. The brightness of the right and left lungs were increased and multiple shadows were observed by chest x-ray. This patient was diagnosed as COVID-19 (critical case) and severe ARDS, and later developed respiratory failure and was done with surgical operation. Surgical informed consent was obtained.

Pathological tissues of the whole lung organ were collected in P3 laboratory, fix all tissues with 4% neutral buffered formaldehyde (pH = 7.0) for 24h. Dehydrate the fixed tissues in an ethanol series (100%, 95%, 80% and 75%) for 1 min in each percentage, clear in xylene and then embed in paraffin wax. Finally, cut the slices and incubate for 40 min in 70℃ oven. Special histochemical stains, for example, Masson staining was used to detect pulmonary interstitial fibrosis, periodic acid Schiff (PAS) staining and silver methenamin staining were used to identify bacterial and fungal infections. Masson kit (iron hematoxylin, bright red acid fuchsin working fluid, aniline blue, phosphomolybdic acid). Periodic acid Schiff and sliver methenamine kit (periodate, borax solution, silver nitrate hexamethylenetetramine powder).

Immunohistochemical staining

The procedure of IHC was done as previously described.[9] In brief, paraffin-embedded sections were deparaffinized in xylenes for 20 min and rehydrated in an ethanol gradient. The sections were submerged into EDTA buffer and boiled for 2 mins with high-pressure for antigenic retrieval. After natural cooling, the slides were treated with 3% H2O2 to quench endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin (BSA). The slides were incubated with the primary antibodies including CD3,

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CD20, CD79a, CD4, CD8, CD5, CD68, CD38, CD31, TTF1, CK5/6, CK7, CK19, SMA, F VIII and Collagen IV (working dilution, Zymed, San Francisco, CA) overnight at 4 °C. The sections were reacted with the biotinylated secondary antibody (Zymed, San Francisco, CA) and visualized with 3,3′-diaminobenzidine (DAB) under the microscopy.

Special staining

Special staining includes Masson staining, sirius red staining, reticular fibers staining and PAS staining. According to Masson staining, the tissues were fixed immediately in 10% formalin after dissection, paraffin-embed and section at 1.5 μm thickness. The slices were deparaffinized in dimethylbenzene, oxidize the slices with 1% periodate for 30 min and rinse with distilled water. Fix the tissues with 3% sodium thiosulfate for 1 min, replenish by the bouin's solution (37℃ water bath for 4 h) and wash the sections by distilled water for 5 min. The sections was dyed by Mayer hematoxylin and put into hot water (45℃) for 30s. Stain the specimens by Masson solution (100 μl) for 30 min. Differentiate the sections by 1% phosphomolybdic acid (100μl). Remove phosphomolybdic acid and add the sections with 1% aniline blue (100 μl). Rinse the sections with distilled water, add 1% acetic acid. Dehydrate the sections lastly by 95% and 100% alcohol (10 s and 1 min, respectively) and seal.

Results

As shown in figure 1, the surface of the whole lung was rufous, and showed diffuse congestive appearance on gross examination. Most of them had punctate hemorrhage and partly haemorrhagic necrosis. Of note, the haemorrhagic necrosis was mainly present in outer edge of the right lower lobe, middle lobe and upper lobe of the lung. The bronchi

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were covered with mucinous and haemorrhagic exudation. The cut surfaces of the lung displayed severe congestive and haemorrhagic changes.

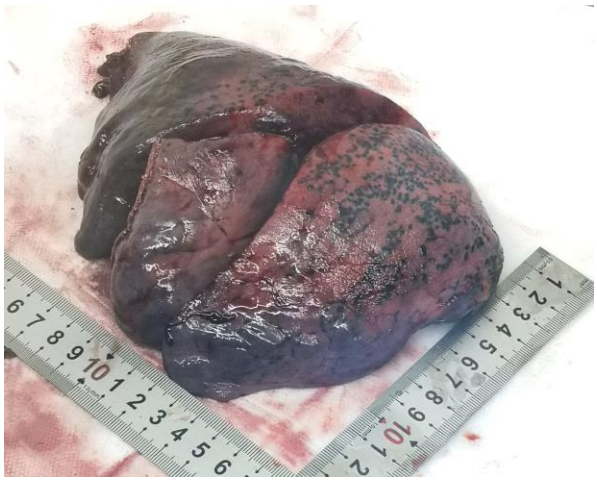

Figure 1. Gross morphology of the right lung. Haemorrhagic necrosis is obvious in the outer edge of pulmonary right lobe.

Histopathological findings showed the main pulmonary pathological patterns was that extensive pulmonary interstitial fibrosis with partly hyaline degeneration, and pulmonary hemorrhagic infarct (figure 2A-C). Small vessels showed severe congestion, vessel wall thickening, and lumen stenosis and occlusion (figure 2D-E). Microthrombosis formations were present in the lumen (figure 2F-G). Focal interstitial infiltration of inflammatory cells including lymphocytes, plasma cells, macrophage and mononuclear cells (figure 2H-I).

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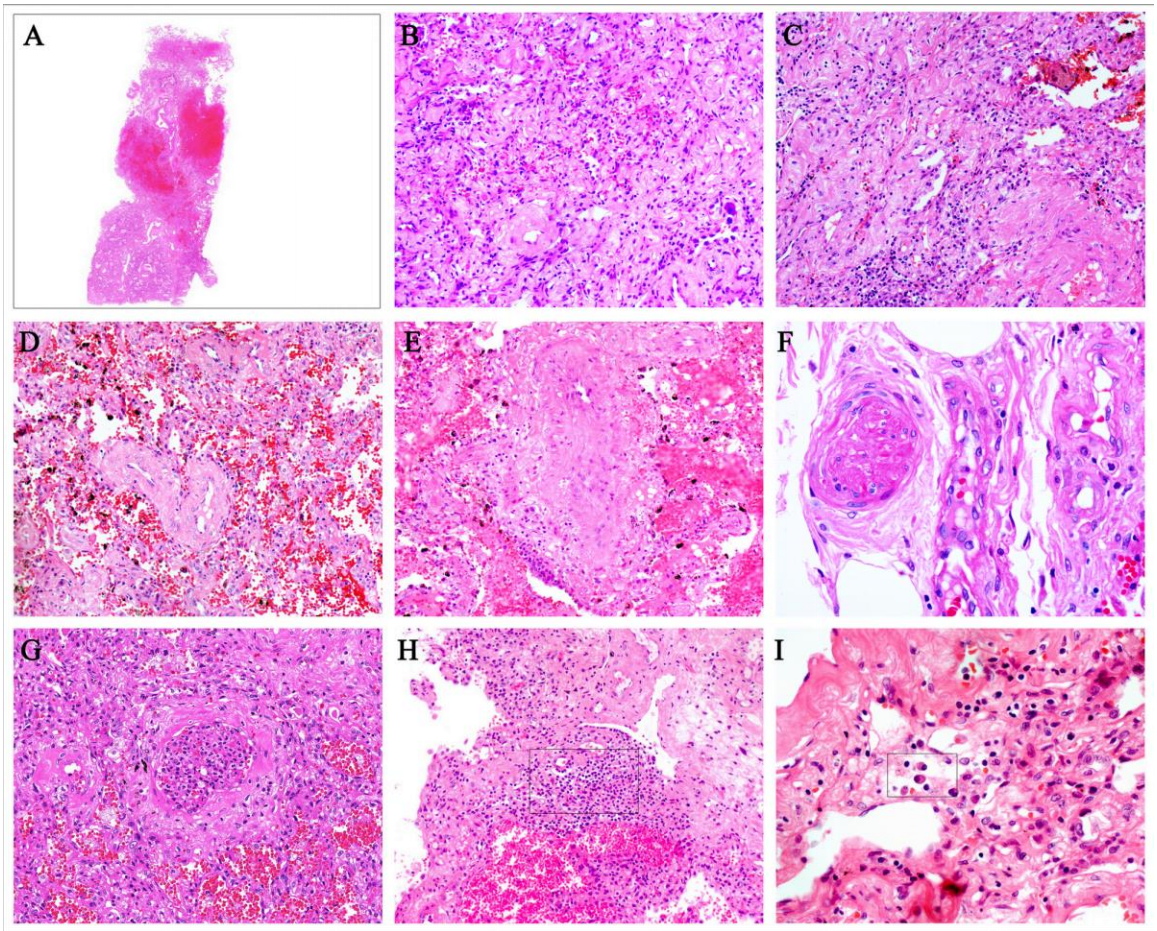

Figure 2. Pulmonary interstitial histopathological changes. (A) The whole slide imaging (WSI) by HE staining. Extensive pulmonary hemorrhagic changes and focal hemorrhagic Infarction (B) Massive pulmonary interstitial fibrosis. (C) Pulmonary interstitial fibrosis accompanied with partly hyaline degeneration. (D) Vascular wall thickening, lumen stenosis and hemorrhagic changes. (E) Boangiitis obliterans are surround by inflammatory cells. (F and G) Microthrombosis formation. (H) Focal inflammatory cells in the interstitium (square indicates). (I) Interstitial plasma cells infiltrating (square indicates).

There was necrotizing bronchiolitis manifested necrosis of bronchiolar wall and epithelial cells were present in the lumen. Alveolitis with atrophy, proliferation, desquamation and various changes of squamous metaplasia of alveolar epithelial cells were observed (mainly type Ⅱ) (figure 3A-D). The remaining pulmonary alveoli showed thickened

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septum, necrosis and desquamation of alveolar epithelial cells (figure 3E-F). In addition, massive fibrinous exudate, multinucleate giant cells and intracytoplasmic viral inclusion bodies were observed (figure 3G-I).

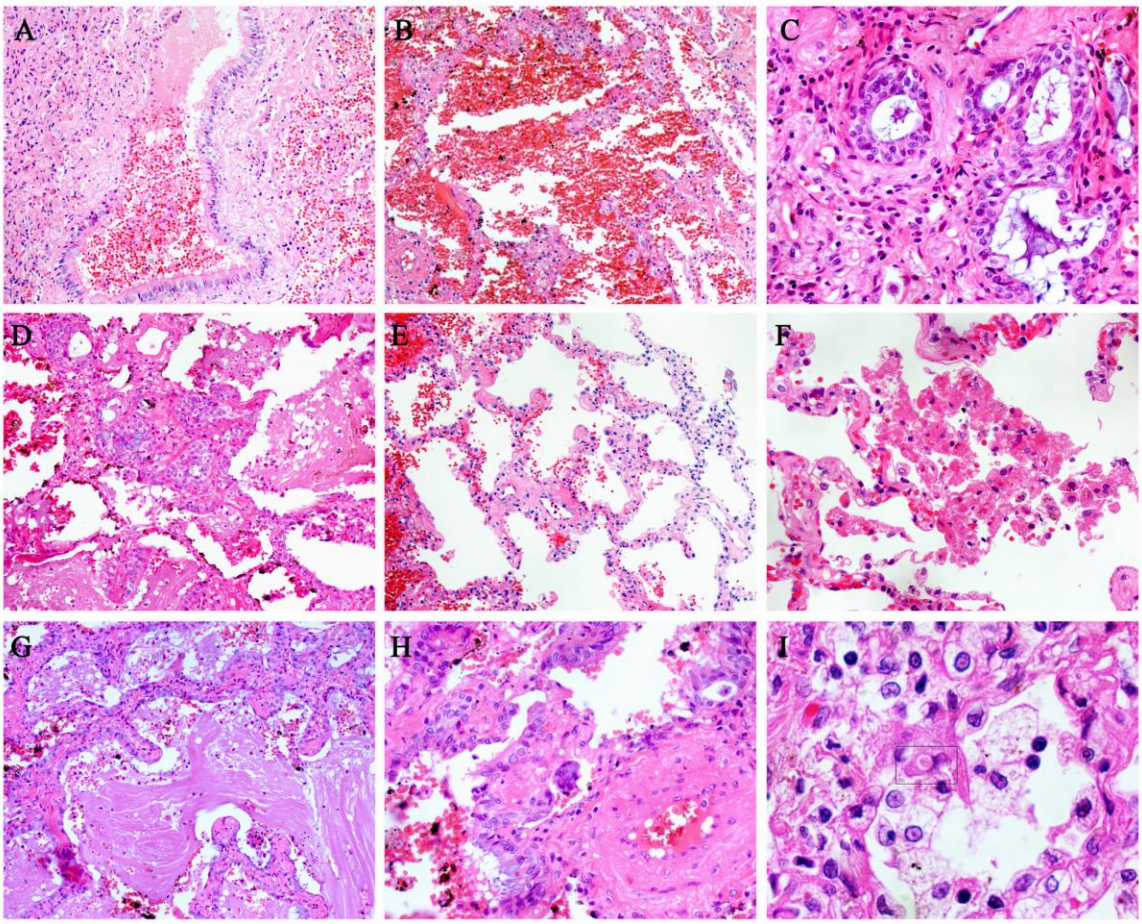

Figure 3. Pulmonary alveoli changes. (A) Necrotizing bronchiolitis, necrotic bronchial epithelial cells are present in the lumen. (B) Atrophy of alveolar epithelial cells and diffuse alveolar hemorrhage. (C) Squamous metaplasia of bronchiole epithelial cells (D) Squamous metaplasia of alveolar cells. (E) Widened alveolar septum. (F) Necrosis and desquamation of alveolar epithelial cells. (G) Inflammatory cells and massive fibrinous exudate in the lumen. (H) Multinucleate giant cell. (I) Intracytoplasmic viral inclusion body in alveolar epithelial cell (square frame indicates).

On the other side, pulmonary interstitial fibrosis, as well as thickening of the vessel wall and fibrinous exudate were displayed by Masson staining (figure 4A-E). Enlarged and ruptured alveolar septum, massive pulmonary hemorrhage in alveolar cavity were found

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(figure 4F). In addition, extensive pulmonary interstitial fibrosis was also confirmed by other special stains including sirius red staining (figure 4G), reticular fibers staining (figure 4H) and PAS staining (figure 4I). No other bacterial and fungal infections were detected by special staining.

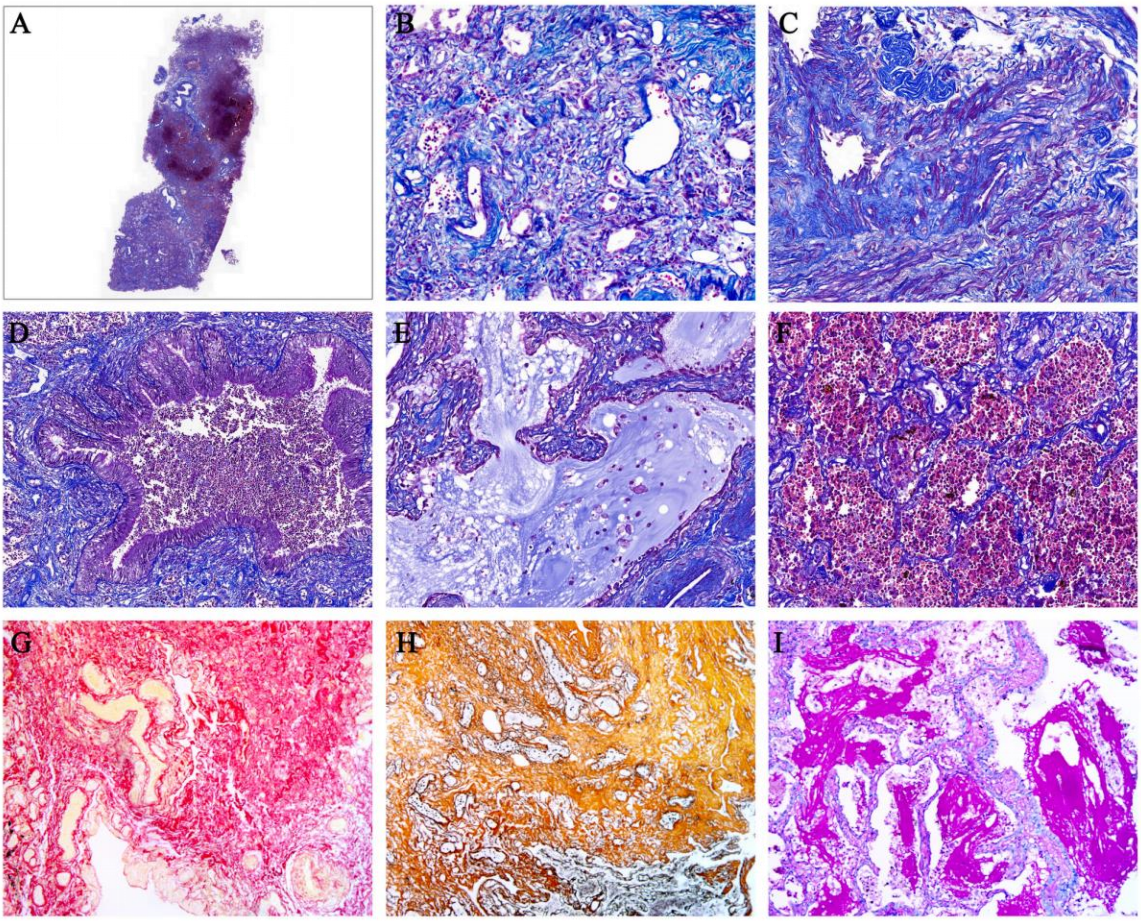

Figure 4. Pulmonary changes by special stain. (A) The whole slide imaging (WSI) by Masson staining. (B) Interstitial fibrosis by Masson stain. (C) The thickening of the vessel wall by Masson staining. (D) Fibrotic walls of dilated bronchioles by Masson staining. Desquamation of epithelial cells and inflammatory cells including macrophages in the lumen. (E) Massive fibrinous exudate in the bronchiole lumen. (F) Enlarged alveolar septum, and partly ruptured septum. Massive pulmonary hemorrhage in alveolar cavity. (G) Interstitial fibrosis by sirius red staining. (H) Interstitial fibrosis by reticular fibers staining. (I) Fibrinous transudation by PAS staining.

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Immunohistological findings showed positive for immunologic cells including CD3 (figure 5A), CD4 (figure 5B), CD8 (figure 5C), CD20 (figure 5D), CD79a (figure 5E), CD5 (figure 5F) and CD38 (figure 5G). Notably, we found that the positive expressions of immunologic cells were present focally in lung interstitium and near blood vessels. In addition, CD31, TTF1, CK5/6, CK7 (figure 5H), CK19, SMA, F VIII and Collagen IV (figure 5I) also exhibited positive (some data not shown). The HE image of these serial immunohistological sections as shown in figure 2H.

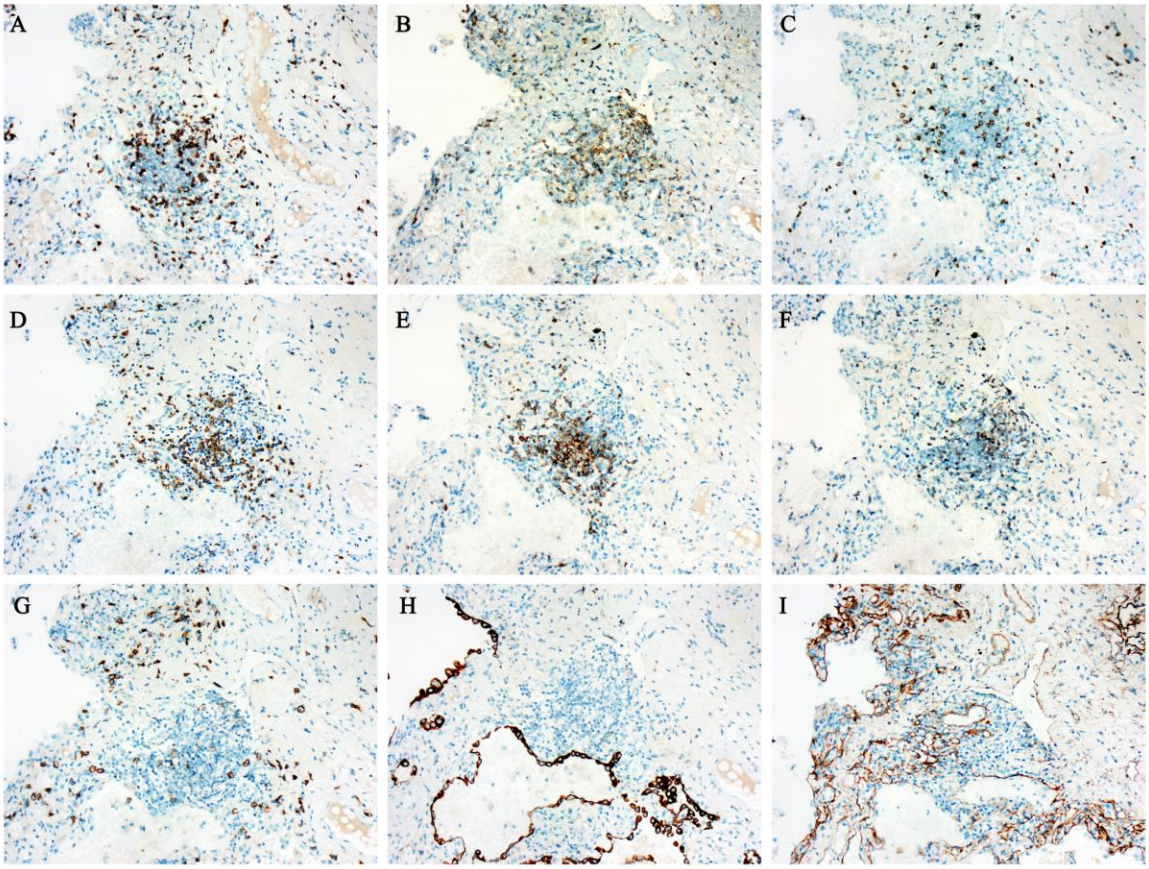

Figure 5. Immunohistological results in severe COVID-19. Serial sections showed the positive expressions of CD3 (A), CD4 (B), CD8 (C), CD20 (D), CD79a (E), CD5 (F), CD38 (G), CK7 (H) and collagen IV (I).

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Discussion

Novel coronavirus pneumonia caused by SARS-CoV-2 has an worldwide outbreak since it firstly emerged in December 2019, Wuhan city, China.[3,10] As far as we know, no pathological findings of the critical type of COVID-19 patient have been described. Recently, Wang FS group performed needle samples taken from one severe case of COVID-19 when the patient died from sudden cardiac arrest. They found that the left lung had pulmonary oedema with hyaline membrane formation indicating early-phase ARDS.[8] In this study, the surgical patient was diagnosed as critical case with COVID-19, and developed respiratory failure. In contrast, hyaline membrane formation was not detected, indicative of severe-phase ARDS and consistency with clinical diagnosis.

It was reported that the virus homology was over 85% between novel coronavirus pneumonia and severe acute respiratory syndrome (SARS). It means that the pathological changes of COVID-19 might be similar to SARS patient. With regard to the pulmonary pathology of SARS, Ding and other research groups had found the pulmonary changes including localized haemorrhage and necrosis, pulmonary alveolitis and bronchitis, desquamation of alveolar epithelial cells.[11-13] Actually, our results also showed the general pulmonary damages including alveolar edema with hemorrhage and bronchiolitis and alveolitis accompanied with inflammatory injury of epithelial cells. In the present study, one of the main pathological changes of critical COVID-19 was diffuse pulmonary interstitial fibrosis, suggestive of changes in late-stage disease. In addition, vascular wall thickening, lumen stenosis and occlusion occurred frequently under the microscopy, which might explain why some critical patients have pulmonary hypertension in later stage. However, the cause about vascular wall thickening and lumen stenosis need to be

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further investigated. Microthrombosis formation was also detected. These major changes might explain why late stage of critical patients develop severe hypoxaemia and respiratory failure. On the other side, gross detection found that haemorrhagic necrosis existed predominantly in outer edge of the right lower lung lobe. This observation brings us two following hints: 1) It could be one of the main causes of fatal death about critical patients. 2) The main lesions of COVID-19 might firstly originate from here.

Recent study indicates that SARS-CoV-2 has the same cell entry receptor ACE2 as SARS-CoV.[14,15] Generally, ACE2 protein is expressed in alveolar cells, bronchial epithelium and vascular endothelium, therefore SARS-CoV-2 protein binds to ACE2 would result in acute lung injury and pulmonary edema. We observed abundant pulmonary edema and hemorrhage, desquamated bronchial and alveolar epithelial cells.

On the other side, cytokine storm links to an excessively exaggerated immune response, and uncontrolled proinflammatory responses, which causes severe organ diseases including lung damages.[16-18] Several representative cytokines have been identified including IL-1β, IL-18, TNF-α, IL-6, IL-8 and IL-10, which are produced and regulated by various immunological cells including CD4 T cells and CD8 T cells.[19] Interestingly, we observed that lymphocytes including CD3 T cells 4 T cells and CD8 T cells , monocytes and plasma cells infiltrating into pulmonary interstitium, and these various types of inflammatory cells were confirmed by immunohistological method. It should be noted that local haemorrhagic necrosis occurred preferentially in outer edge of the right lower lung on gross detection. We suggest that the cytokine storm released by these inflammatory cells including CD4 and CD8 T cells could eventually lead to hemorrhagic necrosis, and ultimately result in severe and even fatal respiratory dysfunction of patients. More rigorous studies including experimental tests are needed to prove this prediction.

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In summary, we demonstrate the whole pulmonary biopsy histopathology of COVID-19 critical patient, which might provide a better knowledge of the main pathological change of this disease, and help clinicians to take timely and effective treatment measures.

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Disclosure of Potential Conflicts of Interest

As to the images in this academic case report, no reproduction in public without permission. No other potential conflicts of interest were disclosed.

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