

**Title:**

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

**List of authors**

Weiren Luo,<sup>1#\*</sup> Hong Yu,<sup>1#</sup> Jizhou Gou,<sup>1</sup> Xiaoxing Li,<sup>1</sup> Yan Sun,<sup>1</sup> Jinxiu Li,<sup>1</sup> Lei Liu<sup>1\*</sup>

**Full affiliations of all authors**

1 Department of Pathology, Department of Critical Care Medicine, National Clinical Research Center for Infectious Diseases, The Second Affiliated Hospital of Southern University of Science and Technology, Shenzhen Third People's Hospital, Shenzhen, People's Republic of China

# Contributed equally to this work

**Contact details of corresponding author**

\* Weiren Luo, Department of Pathology, The Second Affiliated Hospital of Southern University of Science and Technology, Shenzhen Third People's Hospital, National Clinical Research Center for Infectious Diseases, Shenzhen, China.

Email: [luoweiren@hotmail.com](mailto:luoweiren@hotmail.com)

\* Lei Liu, National Clinical Research Center for Infectious Diseases, The Second Affiliated Hospital of Southern University of Science and Technology, Shenzhen Third People's Hospital, Shenzhen, China.

Email: [liulei1961@hotmail.com](mailto:liulei1961@hotmail.com)

## **Abstract**

**Aim:** Novel coronavirus pneumonia ( COVID-19) have emerged as major global health threats since December, 2019. Up to now, the histopathology of critical patient with COVID-19 remains largely undisclosed.

**Methods:** We here performed lung organ dissection, and described the pathological changes of one COVID-19 critical patient by HE staining, immunohistochemistry and special staining including Masson staining, PAS staining and silver methenamin staining.

**Results:** The whole lung tissue displayed diffuse congestive appearance or partly haemorrhagic necrosis on gross examination. The haemorrhagic necrosis was prominently present in outer edge of the right lobe of the right lung. The cut surfaces of the lung displayed severe congestive and haemorrhagic changes. The main pathological lung changes showed bronchiolitis and alveolitis with proliferation, atrophy, desquamation and squamous metaplasia of epithelial cells. 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. 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 can also found several multinucleate giant cells and intracytoplasmic viral inclusion bodies. Masson staining indicated massive pulmonary interstitial fibrosis. Immunohistochemistry results showed positive for immunity cells including CD3, CD4, CD8, CD20, CD79a, CD5, CD38 and CD68.

**Conclusion:** We show clinical pathology of critical patient with COVID-19, which might provide a deep insight of the pathogenesis and the severity of this disease.

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

## **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.<sup>1,2</sup> Reports regarding epidemiological and clinical characteristics of COVID-19 is accumulating.<sup>3-5</sup> 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. Different from other types of COVID-19, critical patients tend to have worse outcome and high mortality.<sup>5</sup> However, up to date, its clinical pathology remains largely unknown. We here performed the lung organ dissection, and for the first time, describe the main pathological changes of critical patient with COVID-19.

## **Methods**

The 66-year-old patient had symptoms of high fever and cough when he came back to Shenzhen city from the Wuhan epidemic on January 4, 2020. He have no other basic diseases except 8-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. He was diagnosed as COVID-19 (critical type) and severe ARDS, and developed respiratory failure and was done with transplant. Informed consent was obtained.

Pathological tissues of the 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°C 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 silver methenamine kit (periodate, borax solution, silver nitrate hexamethylenetetramine powder).

### **Immunohistochemical staining**

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% H<sub>2</sub>O<sub>2</sub> to quench endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin (BSA). The slides were incubated with the primary antibodies including CD3, 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.

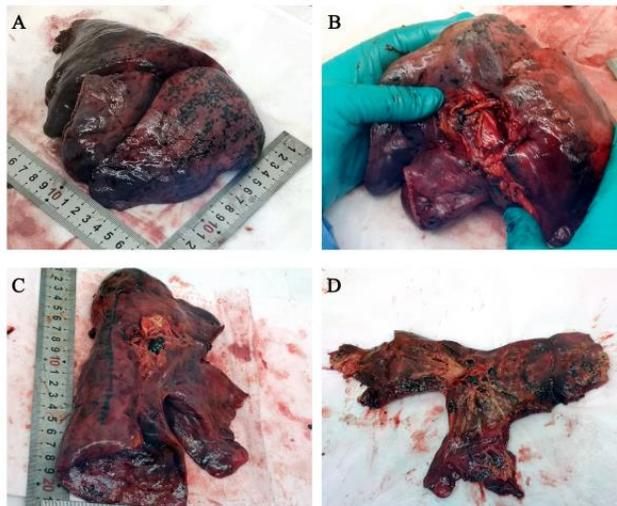
### **Special staining**

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. Immerse the sections into hexamine silver for 35 min (water bath 60 °C). The sections were

decolorized by 0.1% gold chloride (100  $\mu$ l). Fix the tissues with 3% sodium thiosulfate for 1 min, replenish by the bouin's solution (37°C 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°C) for 30 s. Stain the specimens by Masson solution (100  $\mu$ l) for 30 min. Differentiate the sections by 1% phosphomolybdic acid (100 $\mu$ l). Remove phosphomolybdic acid and add the sections with 1% aniline blue (100  $\mu$ l). 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.

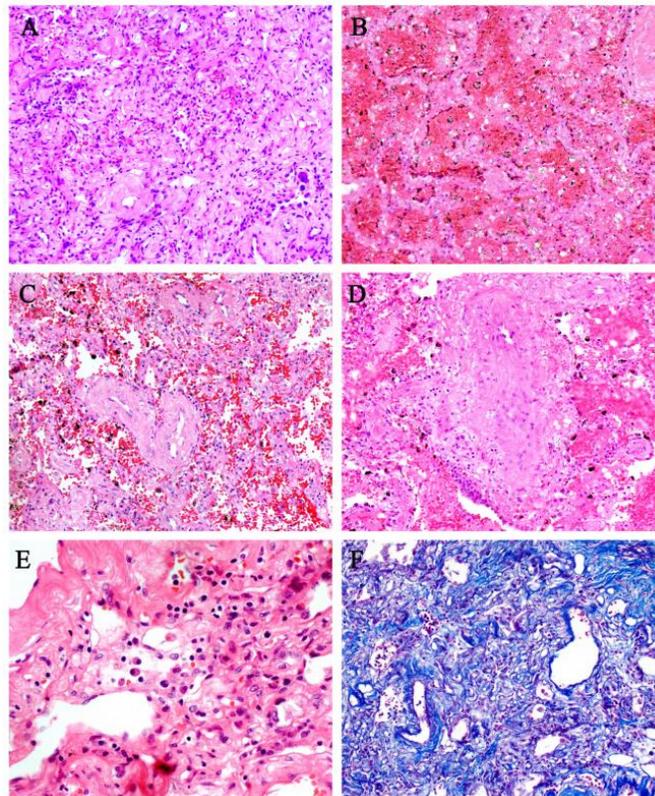
## Results

The surface of the whole lung was bronzing, and showed diffuse congestive appearance on gross examination (**Figure 1**). 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 were covered with mucinous and haemorrhagic exudation. The cut surfaces of the lung displayed severe congestive and haemorrhagic changes.



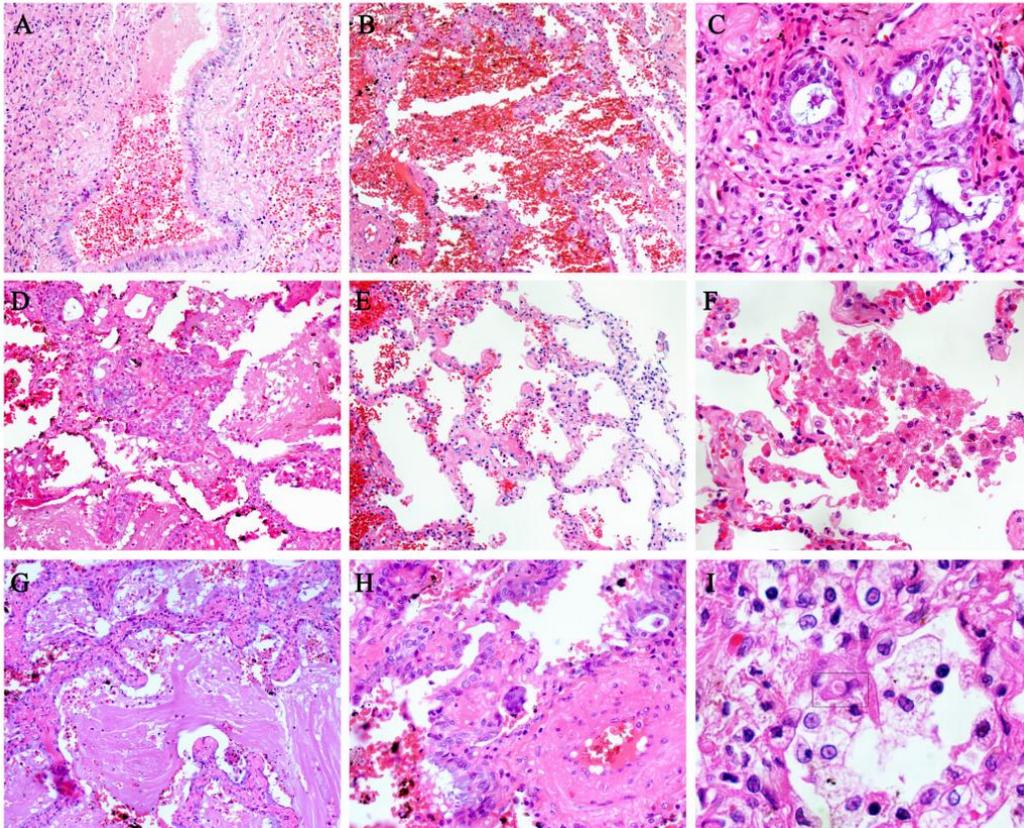
**Figure 1. Lung gross examination.** Gross morphology of the right lung (Panel A and B) and of the left lung (Panel C and D). Haemorrhagic necrosis is obvious in the outer edge of pulmonary right lobe.

As showed in **Figure 2**, histopathological findings showed extensive pulmonary interstitial fibrosis with partly hyaline degeneration, and pulmonary hemorrhagic infarct. Small vessels showed hyperplasia, vessel wall thickening, and lumen stenosis and occlusion. Interstitial infiltration of inflammatory cells including lymphocytes, plasma cells and mononuclear cells. On the other side, pulmonary interstitial fibrosis was confirmed by Masson staining, and no other bacterial and fungal infections were detected by special staining.



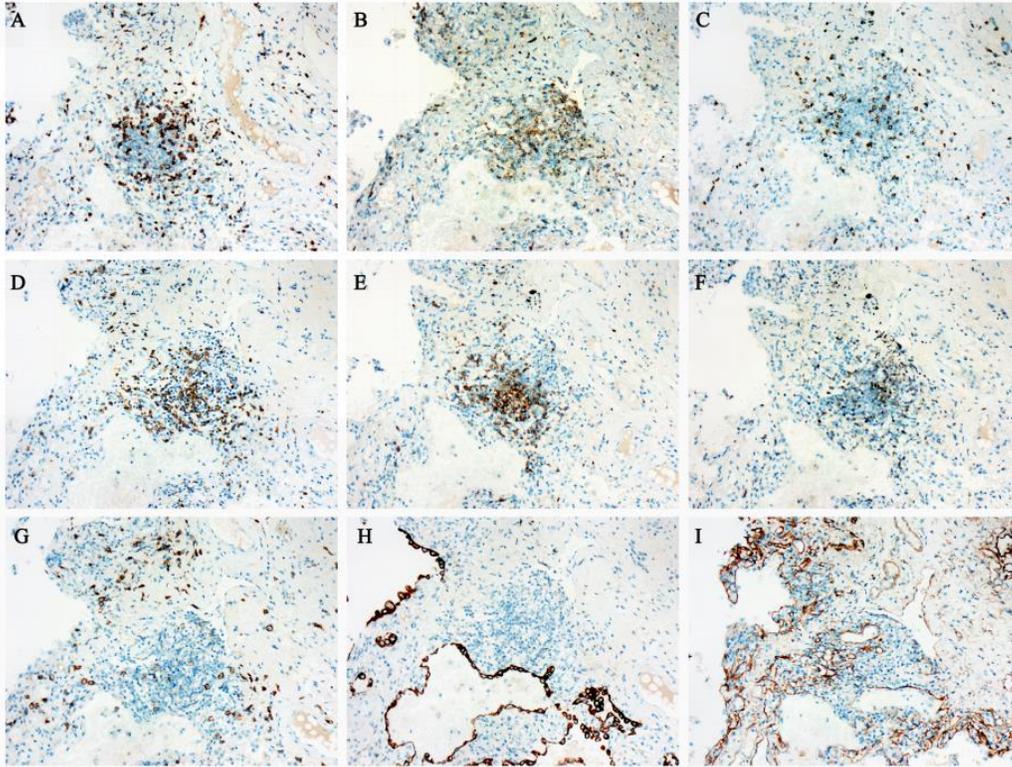
**Figure 2. Pulmonary interstitial histopathology associated with critical patient** **A:** Massive pulmonary interstitial fibrosis. **B:** Pulmonary hemorrhagic infarct. **C:** Vascular wall thickening and lumen stenosis. **D:** Bronchiolitis obliterans are surrounded by inflammatory cells. **E:** Interstitial plasma cells infiltrating. **F:** Lung interstitial fibrosis by Masson stain.

There was alveolitis with atrophy, proliferation, desquamation and various changes of squamous metaplasia of alveolar epithelial cells (mainly type II), as listed in **Figure 3**. The remaining pulmonary alveoli showed thickened septum, necrosis and desquamation of alveolar epithelial cells. In addition, massive fibrinous exudate, multinucleate giant cells and intracytoplasmic viral inclusion bodies were observed. Necrotizing bronchiolitis manifested necrosis of bronchiolar wall and epithelial cells were present in the lumen.



**Figure 3. Pulmonary alveoli changes of COVID-19.** **A:** Necrotizing bronchiolitis, necrotic bronchial epithelial cells are present in the lumen. **B:** Atrophy of alveolar epithelial cells. **C:** Squamous metaplasia of bronchiole epithelial cells **D:** Squamous metaplasia of alveolar cells. **E:** Thickened 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).

Immunohistological findings showed positive for immunologic cells including CD3, CD4, CD8, CD20, CD79a, CD5 and CD38. (**Figure 4**). 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, CK19, SMA, F VIII and Collagen IV also exhibited positive (Some data not shown).



**Figure 4. Immunohistological findings in severe COVID-19.** The positive expressions of CD3 (A), CD4 (B), CD8 (C), CD20 (D), CD79a (E), CD5 (F), CD38 (G), CK7 (H) and collagen IV (I).

## Discussion

Novel coronavirus pneumonia caused by SARS-CoV-2 has an worldwide outbreak since it firstly emerged in December 2019, Wuhan city, China.<sup>3,6</sup> As far as we know, no pathological findings of critical patient with COVID-19 have been described. 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.<sup>7-9</sup> Actually, in this study, our results also showed the major patterns including alveolar edema with hemorrhage and bronchiolitis and alveolitis accompanied with inflammatory injury of epithelial cells. Of note, we observed that extensive pulmonary interstitial fibrosis, vascular wall thickening, lumen stenosis and occlusion occurred generally under the microscopy. These major changes might contribute to critical patients developing severe respiratory failure. On the other side, gross detection found that haemorrhagic necrosis existed predominantly in outer edge of the right lung lobe. This observation brings us two hints as below: 1) It could be one of the main causes of severe patient death. 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.<sup>10</sup> 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. Additionally, immunologic cells focally infiltrated into lung interstitium. These results might explain why critical patient had acute lung dysfunction and ground-glass shadow detection.

In summary, for the first time, we demonstrate the pulmonary histopathology of

COVID-19 critical patient, which might provide a better knowledge of the pathogenesis of this disease.

### **Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed

### **Acknowledgments**

We thank Shenzhen Institute of Hepatology for collaboration and providing the P3 laboratory. We thank Marge limited company for providing ultrasonic rapid tissue dehydrator. This work was supported by National Natural Science Foundation of China (81872202); Natural Science Foundation of Guangdong Province (2018A030313778); and Guangdong Provincial Key Laboratory of Cell Microenvironment and Disease Research (2017B030301018).

## References

1. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020. pii: S0140-6736(20)30211-7.
2. Wu JT, Leung K, Leung GM. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. *Lancet*. 2020. pii: S0140-6736(20)30260-9.
3. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020. doi: 10.1056/NEJMoa2001017
4. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N Engl J Med*. 2020. doi: 10.1056/NEJMoa2001316
5. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*. 2020. doi: 10.1001/jama.2020.1585.
6. Holshue ML, DeBolt C, Lindquist S, et al. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med*. 2020. doi: 10.1056/NEJMoa2001191.
7. Ding Y, He L, Zhang Q, et al. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. *J Pathol*. 2004;203(2):622-30.
8. Ding Y, Wang H, Shen H, et al. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. *J Pathol*. 2003;200(3):282-9.

9. Nicholls JM1, Poon LL, Lee KC, et al. Lung pathology of fatal severe acute respiratory syndrome. *Lancet*. 361(9371):1773-8.
10. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020. doi: 10.1038/s41586-020-2012-7.