



Interesting Images Obliterative Endotheliitis Leading to Cystic Lung Necrosis in Severe COVID-19 during the First Wave of the Pandemic

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Abstract: In the early months of the outbreak (2020–2022), COVID-19 was responsible for acute respiratory distress syndrome (ARDS) and an exceptional number of intensive care unit (ICU) admissions. Weaning difficulties from invasive mechanical ventilation (IMV) and many deaths related to COVID-19 were associated with persistent pulmonary hyperinflammation leading to pulmonary fibrosis and sometimes, in the first wave of the pandemic and before the use of dexamethasone was introduced, pulmonary cystic necrosis. A 72-year-old man hospitalized with severe COVID-19 required IMV and died on day 31 of refractory ARDS. Postmortem examination of the lungs found obliterative endotheliitis proximal to pulmonary cystic necrosis. The presence of SARS-CoV-2 envelope and complement/lectin (MASP-2) deposits near the endotheliitis lesions suggested that the virus acted directly on vascular involvement by a complement-mediated mechanism. Together with classic features of ARDS (epithelial lesions and diffuse alveolar damage), endothelial involvement with endotheliitis was the hallmark of severe COVID-19. Corticosteroids and complement blockade were sometimes beneficial for treating severe COVID-19, perhaps by preventing microvascular damage.

Keywords: COVID-19; ARDS; endotheliitis; CT scan; immunohistochemistry; autopsy

1. Introduction

COVID-19 was an extraordinary pandemic in terms of the number of patients treated in intensive care units (ICUs) for ARDS. Due to immune imbalance, patients with severe COVID-19 developed hyperinflammatory pulmonary syndrome resulting in severe hypoxemia and required invasive mechanical ventilation (IMV) [1]. In developed countries and before the emergence of the SARS-CoV-2 Omicron variant, ICU-related deaths were often linked to persistent inflammation (persistent ARDS under IMV) and lung fibroproliferation [2]. During the first epidemic wave, a large number of patients presented with persistent inflammation leading to pulmonary fibrosis and sometimes to pulmonary cystic necrosis [3]. Pulmonary histopathological findings were initially dominated by classic features of ARDS consisting of epithelial lesions with diffuse alveolar damage (DAD) [3–5]. Lesions more specific to COVID-19 were later described, consisting of microvascular involvement [6] and endothelial damage [7–9]. Microvascular inflammation and thrombosis (small arteries, arterioles, capillaries, venules, and small veins) appeared to characterize disease severity and to be the hallmark of COVID-19-related ARDS [3]. We report a case of severe and fatal COVID-19 with persistent ARDS leading to pulmonary involvement



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). with cystic necrosis. It may have been related to underlying endotheliitis related to viral envelope and complement deposits.

2. Case Presentation

A 72-year-old man was hospitalized with severe COVID-19. On day 8, his respiratory condition worsened, and he required IMV and four prone positioning sessions. On admission, a first chest computed tomography (CT) scan confirmed typical COVID-19 lesions with peripheral ground-glass opacities. A second CT scan was performed in the ICU for persistent ARDS four weeks later (Figure 1A) and showed cystic lung disease with peripheral necrosis. The patient died of refractory ARDS on day 31.



Figure 1. (**A**) CT scan four weeks after ICU admission; the lung section shows severe fibrosis (*) and pseudo-cysts (arrow). (**B**) Microscopic appearance of necrosis and fibrosis, hematoxylin–eosin–saffron

stain, original magnification \times 10. (C) Microscopic appearance of vascular lesions, hematoxylineosin–saffron stain: acute endotheliitis with cytoplasmic vacuolization (large arrow) and cell detachment (small arrow), original magnification \times 120. (D) Microscopic appearance of vascular lesions, hematoxylin–eosin–saffron stain, recanalized thrombosis, \times 70. (E) Immunostaining with anti-SARS-CoV-2 envelope antibody showing positive endothelial cells, suggesting COVID-19 endothelial injury, \times 200. (F) Immunostaining with anti-MASP2 showing patchy deposition indicating obliterating endarteritis suggesting overactivation of the complement system, \times 200. (G) Immunostaining with anti-C5B9 showing patchy deposition in the intima suggesting overactivation of the complement system, \times 250. (H) Immunostaining with anti-C4d showing patchy C4d deposition in the vascular intima of lung capillaries, \times 200.

Postmortem examination of the lung showed large areas of dense subpleural fibrosis in cystic lesions and abscesses. Light microscopic examination of formalin-fixed paraffin sections of the lung showed collagen fibrosis, ultimately leading to almost complete destruction of the pulmonary parenchyma and pulmonary infarction (Figure 1B). Within the fibrosis, mononuclear inflammation was identified with a predominance of TCD8+ lymphocytes and IgG4-positive plasma cells. These lesions were associated with intense vascular damage corresponding to several stages of endotheliitis: acute endotheliitis with endothelial thickening, cytoplasmic vacuolization and cell detachment in small- to medium-sized pulmonary arteries (Figure 1C), and obliterating endotheliitis with intimal proliferation leading to luminal obliteration and thrombosis with fibrin thrombi or images of recanalized thrombosis (Figure 1D). CD3⁺CD8⁺ and CD3+CD4⁺ T-cells were observed in the intima. Immunohistochemistry (IHC) for anti-SARS-CoV-2 envelope showed positive endothelial cells. IHC with anti-MASP2 and anti-C5B9 was positive with patchy deposition indicating obliterating endotheliitis in the intima (Figure 1F,G). These vascular lesions were associated with pulmonary necrosis with the appearance of ghost cells. Patchy C4d deposition was observed in the vascular intima of the lung capillaries (Figure 1H). Real-time RT-PCR was negative on a lung sample. Transmission electron microscopy of lung, kidney, and heart samples was not contributive.

3. Discussion

During the first two years of the pandemic, the primary clinical presentation of severe COVID-19 infection was ARDS related to hyperinflammatory pulmonary syndrome [10,11]. IL-6, IL-1, and complement hyperactivation were then at the heart of the inflammatory storm [12]. Corticosteroids [13], IL-6 [14], or complement blockade [15] were effective to some extent in treating the most severe patients. Hyperinflammation was associated with a prothrombotic state related to immunothrombosis around the endothelium [16]. Endothelial inflammation, or endotheliitis, has been shown to be one of the hallmarks of severe COVID-19 [3,7,8].

SARS-CoV-2 first infects the upper airway tract epithelium after binding to the ACE2 receptor of type II pneumocytes [17]. Among the first immune effectors, the complement system is activated on the lectin and alternative pathways. On the lectin complement pathway, MASP-2 recognizes the SARS-CoV-2 N protein [18] and triggers the cascade. In some patients, SARS-CoV-2 crosses the epithelial barrier to reach the endothelial system [8], overactivating the innate immune system and thrombosis to cause endothelial damage.

We confirmed that endothelial involvement may characterize the most severe forms of COVID-19. In the lungs, obliterative endotheliitis may lead to subpleural cystic necrosis. Endotheliitis activated coagulation [19], promoting endothelial and microvascular thrombosis. In such lesions, the presence of complement deposits (C5b-9 and MASP2) suggests that overactivation of the complement system may be involved in endotheliitis [20,21]. C5a anaphylatoxin may also play a role in the attraction of myeloid cells to the site of inflammation [22] and promote thrombosis due to its close connection to coagulation pathways and the formation of neutrophil extracellular traps (NETs) [23]. Therefore, C5a blockade may be an effective therapeutic option [15]. IL-6 may also promote lung inflammation

and fibrosis, activating IgG4-positive plasma cells or CD4⁺Th17 T cells [24]. Lung necrosis has previously been described in multicentric Castleman disease, an IL-6 associated disease [25]. Our observations may be useful in guiding anti-inflammatory therapies to prevent pulmonary fibrosis or necrosis secondary to persistent ARDS.

4. Conclusions

Endotheliitis is the hallmark of severe COVID-19 and may have led, in the first wave of the pandemic, to downstream pulmonary cystic necrosis. The massive use of corticosteroids and other immunosuppressive therapies for these critically ill patients may explain the disappearance of such lesions in the months that followed.

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Informed Consent Statement: Patient consent was waived due to his death. Informed consent was obtained from family.

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References

- Blanco-Melo, D.; Nilsson-Payant, B.E.; Liu, W.C.; Uhl, S.; Hoagland, D.; Møller, R.; Jordan, T.X.; Oishi, K.; Panis, M.; Sachs, D.; et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* 2020, *181*, 1036–1045.e9.
- Märkl, B.; Dintner, S.; Schaller, T.; Sipos, E.; Kling, E.; Miller, S.; López, F.F.; Grochowski, P.; Reitsam, N.; Waidhauser, J.; et al. Fatal cases after Omicron BA.1 and BA.2 infection: Results of an autopsy study. *Int. J. Infect. Dis.* 2022, 128, 51–57.
- Copin, M.C.; Parmentier, E.; Duburcq, T.; Poissy, J.; Mathieu, D.; Lille COVID-19 ICU and Anatomopathology Group. Time to consider histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. *Intensive Care Med.* 2020, 46, 1124–1126.
- Schaller, T.; Hirschbühl, K.; Burkhardt, K.; Braun, G.; Trepel, M.; Märkl, B.; Claus, R. Postmortem Examination of Patients With COVID-19. JAMA 2020, 323, 2518–2520. [CrossRef] [PubMed]
- Zhang, H.; Zhou, P.; Wei, Y.; Yue, H.; Wang, Y.; Hu, M.; Zhang, S.; Cao, T.; Yang, C.; Li, M.; et al. Histopathologic Changes and SARS-CoV-2 Immunostaining in the Lung of a Patient With COVID-19. *Ann. Intern. Med.* 2020, 172, 629–632. [CrossRef] [PubMed]
- 6. Wadowski, P.P.; Panzer, B.; Józkowicz, A.; Kopp, C.W.; Gremmel, T.; Panzer, S.; Koppensteiner, R. Microvascular Thrombosis as a Critical Factor in Severe COVID-19. *Int. J. Mol. Sci.* 2023, 24, 2492.
- Varga, Z.; Flammer, A.J.; Steiger, P.; Haberecker, M.; Andermatt, R.; Zinkernagel, A.S.; Mehra, M.R.; Schuepbach, R.A.; Ruschitzka, F.; Moch, H. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020, 395, 1417–1418.
- 8. Ackermann, M.; Verleden, S.E.; Kuehnel, M.; Haverich, A.; Welte, T.; Laenger, F.; Vanstapel, A.; Werlein, C.; Stark, H.; Tzankov, A.; et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in COVID-19. *N. Engl. J. Med.* **2020**, *383*, 120–128.
- 9. Magro, C.; Mulvey, J.J.; Berlin, D.; Nuovo, G.; Salvatore, S.; Harp, J.; Baxter-Stoltzfus, A.; Laurence, J. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl. Res. J. Lab. Clin. Med.* **2020**, *220*, 1–13.
- 10. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020, *395*, 497–506.

- Bradley, B.T.; Maioli, H.; Johnston, R.; Chaudhry, I.; Fink, S.L.; Xu, H.; Najafian, B.; Deutsch, G.; Lacy, J.M.; Williams, T.; et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: A case series. *Lancet* 2020, 396, 320–332. [CrossRef]
- Holter, J.C.; Pischke, S.E.; de Boer, E.; Lind, A.; Jenum, S.; Holten, A.R.; Tonby, K.; Barratt-Due, A.; Sokolova, M.; Schjalm, C.; et al. Systemic complement activation is associated with respiratory failure in COVID-19 hospitalized patients. *Proc. Natl. Acad. Sci.* USA 2020, 117, 25018–25025. [PubMed]
- 13. RECOVERY Collaborative Group. Dexamethasone in Hospitalized Patients with COVID-19. *N. Engl. J. Med.* **2021**, *384*, 693–704. [CrossRef] [PubMed]
- 14. REMAP-CAP Investigators. Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19. N. Engl. J. Med. 2021, 384, 1491–1502.
- Vlaar, A.P.; Witzenrath, M.; van Paassen, P.; Heunks, L.M.; Mourvillier, B.; de Bruin, S.; Lim, E.H.; Brouwer, M.C.; Tuinman, P.R.; Saraiva, J.F.; et al. Anti-C5a antibody (vilobelimab) therapy for critically ill, invasively mechanically ventilated patients with COVID-19 (PANAMO): A multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Respir. Med.* 2022, 10, 1137–1146. [PubMed]
- Wichmann, D.; Sperhake, J.P.; Lütgehetmann, M.; Steurer, S.; Edler, C.; Heinemann, A.; Heinrich, F.; Mushumba, H.; Kniep, I.; Schröder, A.S.; et al. Autopsy Findings and Venous Thromboembolism in Patients With COVID-19: A Prospective Cohort Study. *Ann. Intern. Med.* 2020, 173, 268–277. [PubMed]
- Zhang, H.; Rostami, M.R.; Leopold, P.L.; Mezey, J.G.; O'Beirne, S.L.; Strulovici-Barel, Y.; Crystal, R.G. Expression of the SARS-CoV-2 ACE2 Receptor in the Human Airway Epithelium. *Am. J. Respir. Crit. Care Med.* 2020, 202, 219–229. [CrossRef] [PubMed]
- Gao, T.; Zhu, L.; Liu, H.; Zhang, X.; Wang, T.; Fu, Y.; Li, H.; Dong, Q.; Hu, Y.; Zhang, Z.; et al. Highly pathogenic coronavirus N protein aggravates inflammation by MASP-2-mediated lectin complement pathway overactivation. *Signal Transduct. Target. Ther.* 2022, *7*, 318.
- Coenen, D.M.; Mastenbroek, T.G.; Cosemans, J.M.E.M. Platelet interaction with activated endothelium: Mechanistic insights from microfluidics. *Blood* 2017, 130, 2819–2828.
- Xie, C.B.; Jane-Wit, D.; Pober, J.S. Complement Membrane Attack Complex: New Roles, Mechanisms of Action, and Therapeutic Targets. Am. J. Pathol. 2020, 190, 1138–1150.
- Leung, L.L.K.; Morser, J. Carboxypeptidase B2 and carboxypeptidase N in the crosstalk between coagulation, thrombosis, inflammation, and innate immunity. J. Thromb. Haemost. 2018, 16, 1474–1486.
- 22. Carvelli, J.; Demaria, O.; Vély, F.; Batista, L.; Chouaki Benmansour, N.; Fares, J.; Carpentier, S.; Thibult, M.L.; Morel, A.; Remark, R.; et al. Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis. *Nature* **2020**, *588*, 146–150. [PubMed]
- Bonaventura, A.; Vecchié, A.; Dagna, L.; Martinod, K.; Dixon, D.L.; Van Tassell, B.W.; Dentali, F.; Montecucco, F.; Massberg, S.; Levi, M.; et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat. Rev. Immunol.* 2021, 21, 319–329. [PubMed]
- 24. Peng, M.; Shi, J.; Feng, R. Multicentric Castleman Disease as a Rare Cause of Diffuse Lung Cysts. *Am. J. Respir. Crit. Care Med.* **2020**, 201, 1292–1293. [CrossRef]
- 25. Pillai, S. T and B lymphocytes in fibrosis and systemic sclerosis. Curr. Opin. Rheumatol. 2019, 31, 576–581. [PubMed]

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