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Case Report

COVID-19 beyond DAD: Persisting microcirculation thrombosis, hidden infections, and early pulmonary fibrosis as remaining challenges of the disease

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ABSTRACT

Background: Despite advances in the management of COVID-19, some events occurring in the course of disease still represent challenges to patient treatment.
Case presentation: Here we present a case of severe COVID-19 in which the patient received standard treatment including dexamethasone and prophylactic anticoagulation and died on the 24th day of disease. Autopsy showed exudative and proliferative DAD accompanied by remaining microcirculation thrombosis despite anticoagulation treatment, unperceived fungal infection, concomitantly with the presence of dense acellular fibrotic areas amidst organizing lung lesions.
Conclusions: Although improvements were achieved in COVID-19 therapeutics lung microcirculation coagulopathy, unperceived fungus infection and early developing alveolar fibrosis remain unsolved problems associated to the disease.

Introduction

Relevant progress has been made in the treatment of COVID-19 since the beginning of the pandemic. However, severe cases of this disease continue to result in high lethality due to events occurring in the course of the disease still representing challenges to patient treatment. The case reported below is a well-illustrated example of these challenging events: persisting microcirculation thrombosis, hidden infections, and early pulmonary fibrosis.

Case report

A 65 yr. old Brazilian man of African descent, retired mechanic, developed a dry cough that evolved to dyspnea after 4 days. Upon presentation, RT-PCR for SARS-CoV-2 was positive. As his symptoms progressed, he was admitted to the hospital on the 9th day post symptom onset (PSO) and began to receive Ceftriaxone, Azithromycin, Dexamethasone, and prophylactic anticoagulation with unfractionated heparin. He had a history of systemic arterial hypertension and diabetes mellitus, treated with Metformin, Losartan and Glibenclamide. He had quit smoking and drinking 10 years earlier. Disease progression prompted the need for invasive mechanical pulmonary ventilation support with high parameters. On the 10th PSO the patient was transferred to an intensive care unit at an infectious disease reference hospital. Upon admission, the patient was afebrile and SpO2 was 91%. White blood cell (WBC) count was 16,190, AST 564 U/L, ALT 285 U/L,

Abbreviations: ALT, alanina aminotransferase; AST, aspartato aminotransferase; DAD, diffuse alveolar damage; PSO, post symptom onset; SpO2, blood oxygen saturation; WBC, white blood cells.

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the prothrombin activity was 89%, serum creatinine 4.7 mg/dL, K 6.6 mmol/L with metabolic acidosis requiring hemodialysis. On the 16th PSO, a small volume of mucopurulent airway secretion was reported. Secretion amounts increased over the following days and WBC count rose to 35,140, with a left shift occurring on the 18th PSO. Serum albumin dropped to 2.4 g/dL. AST and ALT progressively dropped to 40 U/L and 64 U/L, respectively. Hemoculture (two samples), uroculture and rectal swab were all negative. Tracheal secretion culture revealed mixed flora. Culture of the central catheter tip grew MDR *Providencia stuartii*. Tracheostomy was performed on the 19th PSO. On the 20th PSO the patient became febrile (39 °C) and evolved with tachyarrhythmia, hemodynamic instability, leukocytosis with left shift, hyperthermia and worsening respiratory parameters that required reintubation. Piperacillin-Tazobactam and Meropenem were introduced. Chest X-ray showed ill-delimited irregular bilateral opacities, especially in the lower 2/3 of the lungs, with coalescence in the left perihilar region. On the 24 PSO, he developed shock and his respiratory parameters worsened. Although pronation was performed, the patient presented cardiac arrest and died during the procedure.

Autopsy

Diffuse alveolar damage (DAD) was observed in all quadrants of both lungs except the right upper lateral quadrant from which samples were not obtained. Acute exudative and chronic proliferative phases of DAD were concomitantly found, with a predominance of proliferative lesions (Fig. 1a). Hypertrophic-hyperplastic, atypic type 2 pneumocytes replaced the normal alveolar lining; some of these cells were shed into the alveolar lumen, mixing with the fibrinous hyaline membrane, which triggered reparative fibrosis (Fig. 1a–c). Rare type 2 pneumocytes containing vacuoles filled with viral particles were identified (Fig. 1c & d). Four different fibrosis patterns were observed: (1) Fibrous thickening the alveolar walls; (2) Fibrotic bands lining the alveolar septa; (3) Sessile paucicellular nodules projecting to alveolar lumen; (4) Complete filling of alveolar lumina (Fig. a and b). Recent fibrinous thrombi were found in small arteries of the superior medial quadrant of the left lung (Fig. 1e). Alveolar capillaries were packed with hyaline material containing red blood cells. Some of these capillaries contained fibrin bundles among red blood cells (Fig. 1e). The lateral inferior quadrant of the right lung showed a focus of bronchopneumonia with microabscesses. The medial inferior quadrant of the left lung was infected by fungus, presenting large hyaline hyphae with branches at a 45° angle or less (Fig. 1f). The hyphae formed large aggerates, infiltrated blood vessel walls and had invaded the alveolar septa, making the lung structures no longer evident. Areas of pulmonary infarction were observed in association with fungal infection.

The liver presented focal sclerosing cholangitis with concentric fibrosis of a biliary duct, sinusoidal dilatation in zones 2 and 3 and mild (10%) hepatocellular steatosis.

The spleen showed low cell density in the red and white pulp. White

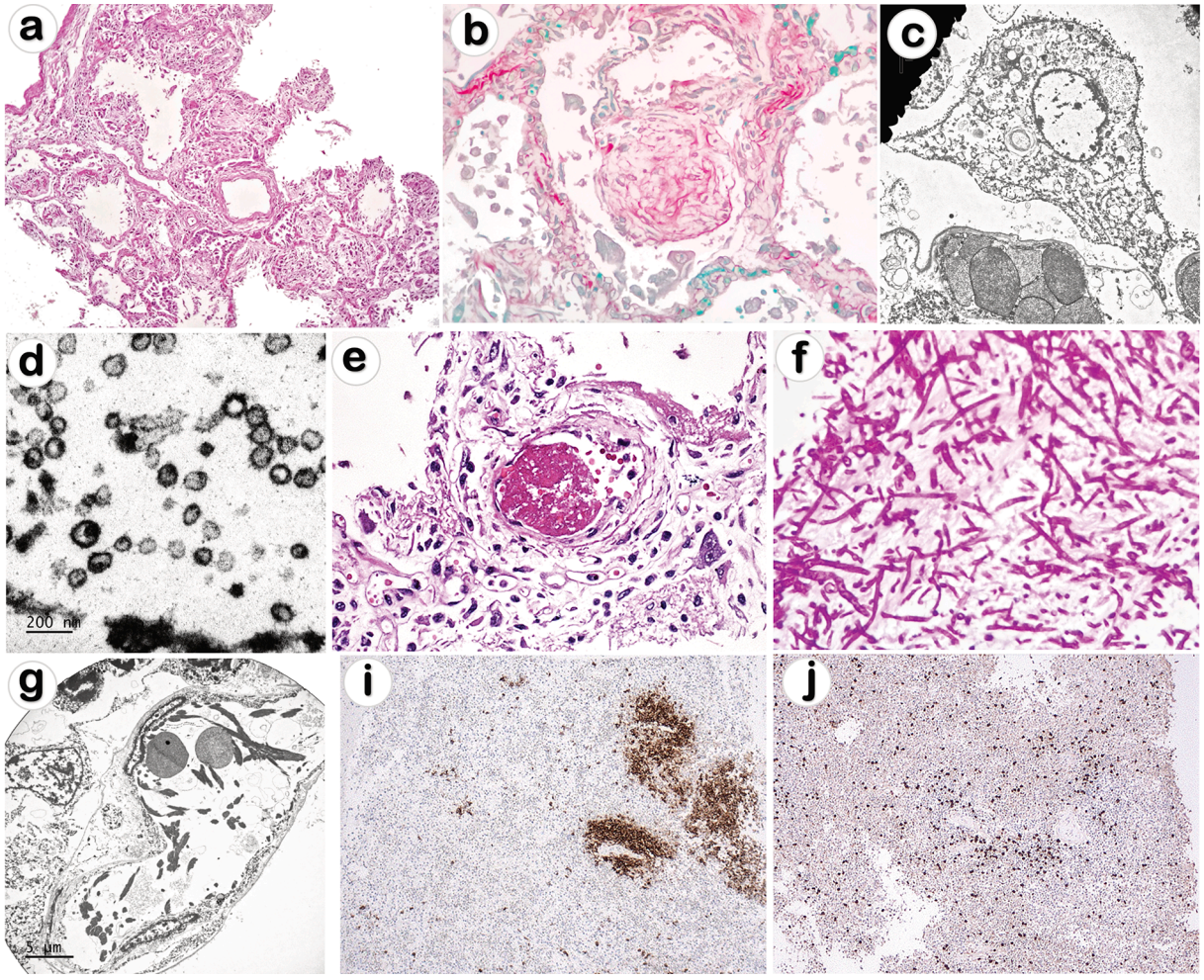

Fig. 1. Lung (**a**-**g**) and spleen (**i**-**j**) of a patient with COVID-19: **a** and **b**: panoramic view of lung with exudative and proliferative DAD (**a**) various patterns of lung fibrosis including intra-alveolar plugs containing acellular fibrosis with collagen bundles (**b**). **c**: type 2 pneumocyte with a vacuole containing viral particles (**d**). **e** and **g**: Microcirculation coagulopathy: thrombi in small artery (**e**) and fibrin bundles among erythrocytes in capillaries (**g**). **f**: Invasive fungus infection. **i** and **j**: Disruption of spleen structure with lymphoid follicle atrophy and low amounts of B (**i**) and T (**j**) cells.

pulp disruption was evidenced, with lymphoid follicle atrophy and low numbers of CD20 + B-cells, as well as low CD3 + T-cells in the periarteriolar lymphoid sheath. In addition, T lymphocytes were dispersed throughout the red pulp (Fig. 1I & j).

Intercostal skeletal muscle presented multiple foci of macrophage infiltrate, skeletal muscular cell lysis with vacuolization, nuclear proliferation and internalization. The myocardium presented slight hypertrophy.

Discussion

The present autopsy revealed some highly relevant pathological alterations associated with COVID-19 following the introduction of dexamethasone and prophylactic anticoagulation therapy, which have greatly improved disease treatment during the course of the pandemic [1]. These new protocols have resulted in most lesions associated with severe disease being restricted to the lungs. However, some relevant events that may contribute to disease lethality and post-COVID-19 sequelae are extremely challenging in the context of clinical diagnosis and have not received due attention to date. These are illustrated as follows in the case presented herein: (1) opportunistic bacterial and undetected fungal infection have arisen in part due to prolonged stays in intensive care units, the need for antibiotic therapy, mechanical ventilation and the use of other invasive devices, as well as immunosuppression and spleen compartments disruption caused by the disease. Although bacterial infections can be perceived by clinical and laboratory signs, aggressive invasive fungal infections present a diagnostic challenge in patients with COVID-19 [2]. This is not only restricted to the diagnosis of the fungal infection *per se,* but also the characterization of the fungal agent. Although *Aspergillus fumigatus* is the species most commonly involved in these events, *Fusarium*, *Mucorales*, *Scedosporium* are all agents that cause similar infections and cannot always be easily distinguished from *Aspergillus* using only conventional light microscopy [3]. Unfortunately, the material obtained from the histological sections for PCR generated a very weak band not allowing sequencing. (2) The persistence of microcirculatory thrombotic events despite the use of prophylactic anticoagulation. Recent studies have not shown significant advantages resulting from therapeutic anticoagulation in patients with severe COVID-19 [4]. A better understanding of the pathways linking inflammation and coagulation may help to devise new strategies for controlling microvascular coagulation [5]. (3) Fast-developing lung fibrosis creating nonfunctioning alveolar spaces, which may permanently impair pulmonary function and become secondary infection-prone sites. Early antifibrotic treatment may be a relevant consideration for these patients [6]. Although minimally invasive autopsies, not always allow a clear distinction of the lung lobes we consider that the samples from the lower quadrants may contain samples from the medial and lower lobe of the right lung and from the lower and distal regions of the upper lobe of the left lung.

Although an altered transaminases profile may indicate the presence of hepatic lesions, only mild changes were observed in the liver and the sclerosing cholangitis was focal and may correspond to nonspecific changes. No clinical signs of hepatic dysfunction were observed and a complete set of tests for hepatic disease was not performed. Elevated levels of AST, higher than ALT levels, may also be attributable to pulmonary and muscular damage. Indeed, skeletal muscle damage was present in several muscle samples collected from this patient. The small decrease in serum albumin levels, in the context of minor liver alterations, may have resulted from dilution due to intravenous fluid infusion.

Finally, although kidney samples were not obtained in this autopsy, due to technical problems, acute kidney injury is commonly associated with severe COVID-19. Two main histological changes associated with this event are acute tubular necrosis and epithelial tubular cell degeneration such as loss of epithelial brush border and tubular cell tumefaction and vacuolization [7].

Conclusion

Although improvements were achieved in COVID-19 therapeutics, lung remaining microcirculation coagulopathy despite the use of preventive anticoagulation, unperceived fugus infection and early developing alveolar fibrosis are challenges associated to the disease. Our findings call attention to these lesions possibly implicated in death or post-COVID-19 sequelae and may be overlooked or complicate clinical management.

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CRediT authorship contribution statement

Lilian V.S. Carvalho: Data curation, Formal analysis. **Cassianada Silva Souza:** Investigation. **Jonathan L.M. Fontes:** Methodology, Investigation. **Lara Cardoso:** Formal analysis. **Milton Salomar:** Investigation. **Amaro Nunes Duarte-Neto:** Formal analysis. **Claudio Figueira:** Investigation. **Reginaldo Brito:** Methodology, Investigation. **Bianca Mesquita:** Methodology, Investigation. **Luiz A.R. de Freitas:** Conceptualization, Formal analysis, Writing – review & editing. **Geraldo G.S. Oliveira:** Conceptualization, Investigation, Formal analysis, Writing – review & editing. **Washington L.C. dos-Santos:** Conceptualization, Funding aquisition, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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