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Histopathological model of COVID-19 pneumonia

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Patients with severe coronavirus disease 2019 (COVID-19) have respiratory failure with hypoxemia and acute bilateral pulmonary infiltrates, consistent with ARDS. We aimed to study histological and immunohistochemical changes in the lungs in patients with severe coronavirus infection. All cases with COVID-19 were presented with viral desquamative pneumonia at different stages. Stage I - the lungs had pronounced hyperemia, dilated capillaries in the septa and many macrophages in the alveoli. Stage II has been characterized by desquamation of alveolocytes and their viral transformation into giant mononuclear cells, as well as the formation of syncytial structures. In stage III, alveolar damage and capillary proliferation were diffuse. In stage IV, fibrosis and collagen formation begin, which is more pronounced in the periphery of the lobe and propagate to the center. The autopsies revealed a consistent pattern of alveolar pulmonary injury and identified four stages in the course of COVID-19 pneumonia.

*Key word*s: COVID-19 pneumonia, SARS-CoV-2, diffuse alveolar damage, histological stages.

Introduction

Severe acute respiratory distress syndrome-associated coronavirus-2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19), was initially identified in the Hubei province of China in December [13] and declared a pandemic by the World Health Organization in March 2020 [5]. Currently, 272,811,994 patients have fallen ill and 5,340,001 have died, which is 1.95% mortality.

SARS-CoV-2 is a coronavirus which utilizes angiotensin converting enzyme 2 (ACE2) as a source of cellular entry. ACE2 is expressed in lung alveolar cells, bronchial epithelium and vascular endothelial cells explaining why the respiratory tract and lung serve as a primary point of viral entry.

Radiologic evaluation of COVID-19 cases has similarly shown findings generally described as being more similar to organizing pneumonia, particularly in the earlier

phases of the disease. Much of the recognition of this pneumonia has been radiologic, described as ground glass nodules with progression to consolidation [1, 2, 4].

Typical features of Covid-19 pneumonia include hyaline membranes and alveolar cell 2 hyperplasia in 87% of patients. Alveolar pneumocytes frequently appeared atypical, enlarged, and sometimes multinucleated with syncytial features [10].

Autopsy reports of the lungs in COVID-19 to date have primarily shown diffuse alveolar damage (DAD) or acute lung injury.

We aimed to study histological and immunohistochemical changes in the lungs of patients deceased from Covid-19.

Material and Methods

Autopsy procedures

This is a prospective study of 15 consecutive COVID-19 autopsies performed in "Sv. Georgi "University Hospital, Plovdiv, Bulgaria. Specimen was taken from both lungs, from the central parts and the periphery. The study was conducted at the Morphological Center of the Medical University of Plovdiv. From each lung, 4 blocks were released for routine hematoxylin-eosin (HE) staining.

Genetic testing: Thirteen of the cases were diagnosed with a PCR (Polymerase Chain Reaction) test, one case was confirmed with an antibody (IgM/G) test and one with an rapid antigen test.

Histological examination

Autopsy material was fixed in 10% neutral buffered formalin and submitted for standard processing with haematoxylin and eosin staining.

Immunohistochemistry

Immunohistochemical staining was performed on formalin fixed, paraffin-embedded 5-μm sections following citrate pH 6.0 antigen retrieval, endogenous biotin, and peroxidase block. Dako's immunostainer is used. Immunohistochemically, both lungs were examined with CD34 for endothelial damage.

Results

Clinical Data

Out of 15 presented patients, 6 are women, 9 are men. SARS (Severe Acute Respiratory Syndrome) developed in 8 patients as a complication of COVID-19 pneumonia. Most patients developed the complication after 14 days of illness, 7 patients were on mechanical ventilation, 4 died in the emergency department, and the complication developed within hours. The earliest development of the syndrome is on day 7 of the onset of the disease. The clinical data, main disease and its complications, as well as the cause of death are summarized in **Table 1**.

Table. 1. The clinical data, main disease and its complications and the cause of death in Covid19

№	Age	Gender	Day of symptoms	Cause of death
1.	55	f	21	**SARS-Cov2**. ARV. DAD, *Generalised thrombosis*, *Brain infarction*.
2.	63	f	21	COVID19. ARV. DAD, *Generalised thrombosis:* Pulmonary thrombosis.Thrombosis in small cerebral arteries. Abacterial thrombotic endocarditis of the mitral and aortic valves. Infarct in the brainstem. Infarcts in the spleen and kidneys
3.	56	f	14	*COVID19 DAD**. Pulmonary thromboembolism***. Phlegmon in the area of the operative wound with antibiotic therapy. Ulcerative-necrotic, fibrinous-purulent colitis with local pelvic fibrinous-purulent peritonitis.
4.	62	m	7	COVID19 – Billateral fibrinous-purulent pneumonia. ***SARS-COV2 Pulmonary thromboembolism*** with acute right heart dilatation.
5.	56	m	8	***COVID19 ARV. DAD, stage III***. Exacerbated chronic total heart failure. Complete dilated hypertrophy of the heart.
6.	69	m	30	COVID19 DAD, ***SARS-COV2. Brain infarction.*** Died in the ED.
7.	37	m	14	***SARS-Cov2***. COVID19 DAD. Treatment in another hospital. Died in the ED.
8.	81	f	18	COVID19 - DAD. ARV. Bilateral pleural effusions. *Generalised thrombosis*: Thrombotic complications: splenic infarction, thrombotic infarction of the right kidney, thrombosis of the left coronary artery, thrombosis of cerebral arteries.
9.	70	m	7	***SARS-Cov2***. COVID19 DAD. Left coronary artery thrombosis. Acute anterior myocardial infarction. Died in the ED.
10.	61	m	14	***SARS-Cov2***. ARV. COVID19 DAD. Pulmonary thrombosis.
11.	88	m	10	***SARS-Cov2***. COVID19 DAD.
12.	51	f	9	***SARS-Cov2***. ARV. COVID19 DAD.
13.	75	m	14	***SARS-Cov2***. ARV. COVID19 DAD.
14.	86	f	13	***SARS-Cov2***. COVID19 DAD. Died in the ED.
15.	66	m	14	***SARS-Cov2***. COVID19 DAD.

Legend: DAD – Diffuse Alveolar Damage; ARV – Assisted Respiratory Ventilation; ED – Emergency Department

Pulmonary findings

Lung tissue was obtained from autopsies with a postmortem interval ranging from 1 to 16 days during October 2020 and November, 2021.

Macroscopically, all of the cases demonstrated diffuse and severe pulmonary changes. The lungs were enlarged bilaterally, heavy (600-700 g), with greatly reduced elasticity and increased density. Their cut surface were homogeneous, with a brownish-reddish color and scattered, firm nodular areas.

Microscopically, we have identified four stages in the development of COVID-19 pneumonia. Stage I – the lungs have pronounced hyperemia, dilated capillaries in the septa and many macrophages in the alveoli.

Stage II is characterized by desquamation of alveolocytes and their viral transformation into giant mononuclear cells (cytopathic effect), as well as the formation of syncytial structures **(Fig. 1A)** and diffuse alveolar damage (DAD) (**Fig. 1B**). The basement membranes are exposed. The inflammatory component is mild, with T lymphocytes and many macrophages. B lymphocytes are totally effaced. Multiple thrombosis in small and medium-sized vessels also occurs at this stage. The capillaries of the septa begin to proliferate.

In stage III, capillary proliferation is diffuse with budding formation. There is fresh granulation tissue in the alveoli, which proliferates diffusely and completely obliterates the alveolar spaces **(Fig. 1C).** CD34 marks endothelial cell proliferation (**Fig. 1D**).

In stage IV, fibrosis and collagen formation begin, which are more pronounced in the periphery of the lobe and propagate to the center (**Fig. 1E**). In SARS-Cov2 cases, the lung parenchyma is saturated with blood, and the basement membranes and capillaries are torn (**Fig. 1F**).

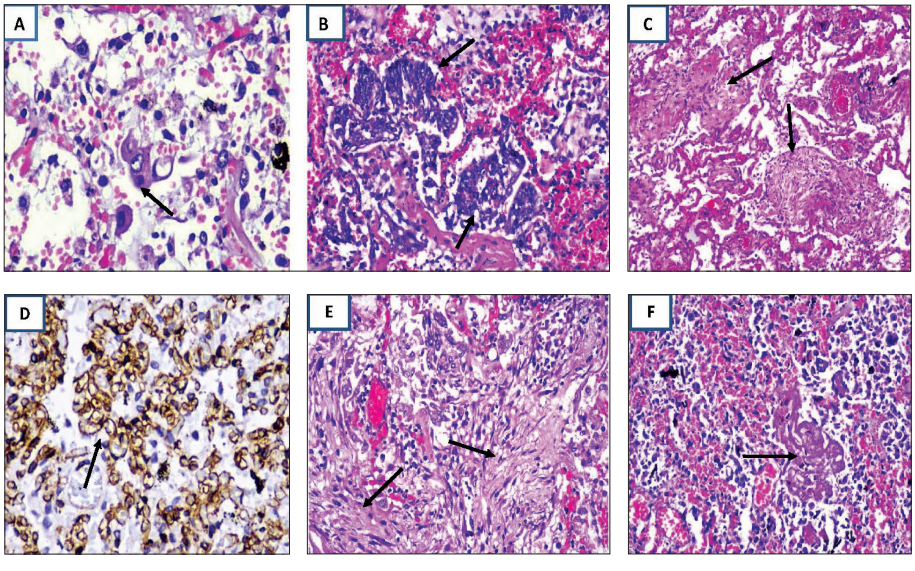

Fig.1. A) Cytopathic viral effect in alveolar pneumocytes type II with giant transformed cells, (HE, x20). **B)** Formation of syncytial epithelial structures and diffuse hemorrhages in SARS-Cov2, (HE, x20). **C)** Fresh granulation tissue in the alveoli partly or completely obliterates the alveolar spaces, formation of "fibrin balls" (HE, x20). **D)** Proliferation and budding of young capillaries, (CD34x10). **E)** Fibrous changes in organizing pneumonia, (HE, x4). **F)** Diffuse hemorrhages and intra-alveolar fibrin in SARS-Cov2 (HE, x20).

Discussion

Autopsy reports of the lungs in COVID-19 to date have primarily shown diffuse alveolar damage (DAD) or acute lung injury. The pulmonary autopsy findings are primarily limited to case reports or single center cohort studies. More recent findings have reported a wider spectrum of histological lesions involving both the epithelial and vascular components in lung and other organs. This has led to an increased awareness of the intrinsic complexity of the disease [1-7].

On average, patients with COVID 19 develop diffuse, bilateral, viral desquamative pneumonia on day 14 from the onset of the disease. *SARS-Cov2* and thromboses are observed on day 7 at the earliest. Most of the studies demonstrate venous thromboembolism and microthrombi in arterioles and venules [8,10].

In presented study patients developed SARS-Cov2 accompanied by respiratory syndrome and rapid lethal outcome in 60%. DAD is the predominant histopathologic pattern identified in lung pathology from patients with COVID-19. DAD is caused by "endothelial and alveolar lining cell injury which leads to fluid and cellular exudation," culminating in physical disruption of the blood-air barrier [7,11]. According to literature data DAD in Covid 19 is divided into three histopathological phases that generally correlate with the time from pulmonary injury:1) Acute (exudative) phase, 2) Subacute (organizing) phase, and 3) Chronic (fibrotic) phase [12]. However, in our study, we divided phase 2 (organizing) in two stages - the stage 2 of capillary proliferation and stage 3 – the formation of fresh granulation tissue. The fourth stage is the formation of collagen fibers and fibrous tissue.

According to the Hariri's study acute phase of DAD occurs within 1 week of the initial injury and is characterized by intra-alveolar hyaline membranes, edema, and alveolar wall thickening without significant inflammation. Vascular thrombosis and microthrombosis are frequently observed in DAD, even in the absence of a systemic hypercoagulable state, and they are thought to result from local inflammation [8].

In acute stage, our study correlates with the changes found by other authors such as focal hyperplasia of type II pneumocytes, some of which showed mild cytologic atypia. Some enlarged pneumocytes showed abundant cytoplasm with a ground-glass appearance, and prominent eosinophilic nucleoli. The main histological findings in the lungs in this cases include: 1) serous exudation, 2) infiltration of lymphocytes around blood vessels and in the alveolar septa, 3) aggregation of multinucleated giant cells within alveolar spaces, 4) hyperplasia of type II pneumocytes, 5) intracytoplasmic viral-like inclusion bodies. Our results completely coincide with the findings in the lungs described by other authors [6].

The subacute phase of DAD occurs approximately 1 week after the initial pulmonary injury and is characterized by microscopic organization of the fibrin followed by fibroblast migration and secretion of young "loose" collagen. Hyaline membranes become slowly incorporated into organizing fibrotic tissue, which begins to appear in airspaces, alveolar ducts, and alveolar walls. Reactive atypical changes in type II pneumocytes and squamous metaplasia may be present. In this research, we divided phase 2 (organizing), in the stage of capillary proliferation and stage 3 - the formation of fresh granulation tissue, as the two stages are well separated and follow a certain sequence in all studied cases.

Most findings, from single institution case series, have also described that first stage of DAD will ultimately resolve, whereas others evolve into a chronic fibrotic phase (weeks to months after the initial injury) with progressive architectural remodeling and interstitial fibrosis. In the extreme, these changes may resemble usual interstitial pneumonitis, the histopathological correlate of idiopathic pulmonary fibrosis [3,7,8,12].

In this research, the major pulmonary findings were diffuse alveolar damage in the acute or organising phases. Histological examination revealed focal pulmonary microthrombi in 13 patients. The major histopathological observation in most series of patients who died with COVID-19 was diffuse alveolar damage-type lung injury in the acute or organizing phases (86%). Lung tissue showed pulmonary oedema, prominent reactive type 2 pneumocytes, intra-alveolar fibrin, and hyaline membranes.

Stage IV, AFOP (acute fibrinous and organizing pneumonia) is characterized by formation of "fibrin balls" within the alveolar spaces, with organization resulting from fibroblast migration and secretion of young collagen within fibrin aggregates. OP (organizing pneumonia) can be seen in isolation or in combination with DAD or AFOP and is characterized by intraluminal tufts of plump fibroblasts and young/immature collagen tissue within alveolar ducts and distal airspaces [9]. Most of the published COVID-19 autopsy case series describe the acute phase of DAD as the prominent acute lung injury pattern. However, features of organizing fibrosis were reported on histopathologic examination in 52% of the COVID-19 autopsy cases. In most cases, organizing fibrosis was described as either focal or in the setting of mixed acute and organizing phases of DAD [6].

Severe respiratory syndrome develops most commonly in the second and third stage. We detected its earliest occurrence on day 7 of the disease up to day 21. Patients on respiratory ventilation (RV) developed the syndrome later and those on home therapy – earlier. Patients 1, 2, 7 and 10 with *SARS-Cov2* were treated in hospital. Three patients passed away in the Emergency Department due to severe respiratory syndrome. These patients were on home treatment and the respiratory insufficiency developed within hours.

The deceased cases described at this stage, usually at 7-10 days after the onset of symptoms, are characteristic for patients who have developed respiratory distress syndrome. The early fatal development of *SARS-Cov2* was observed in delta variants of Covid19, in patients with many concomitant diseases and obesity. It is noteworthy that patients with the Delta variant of Covid19 develop generalized thrombosis (patients 1-10). While in patients with the Omicron variant, these complications are absent (patients 11-15). Possible explanations are: 1) the mild course of the disease 2) the preventive use of anticoagulants since the diagnosis of Covid19 infection.

Conclusion

This study identified four stages in the course of COVID-19 pneumonia. Pneumonia lasts between two and three weeks. Severe cases manifested by subsequent proliferation of connective tissue and fibrosis, usually located in the subpleural areas of the lungs. We believe that the detailed study and categorization of the stages in the course of COVID-19 pneumonia, as well as the determination of the time interval of each stage, will support the therapeutic approaches in each of the stages.

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