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CLINICAL AND HISTOPATHOLOGICAL FEATURES OF LUNG INJURY IN COVID-19 INFECTION

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ABSTRACT — The aim of the study was to evaluate the histopathological changes in the lungs of patients who died of a new coronavirus infection (COVID-19) in relation to the length of hospital stay.
We evaluated lung autopsy material, autopsy reports, and death summaries of 39 patients who died of COVID-19. The length of hospital stay ranged from a few hours to 25 days. At all stages of the disease, lung alterations (desquamation of bronchial and alveolar epithelium), circulatory disorders (alveolar edema and hemorrhages, congestion in small blood vessels, thrombosis), compensatory response (fibrosis) were identified.
The patients who died during the first week of hospitalization demonstrated predominant signs of circulatory disorders (alveolar edema, hyaline membranes, alveolar hemorrhages, congestion in small blood vessels). Fibrosis, usually not typical for the first week of acute respiratory distress syndrome, was detected in 46% of the deceased during the first week of hospitalization, which may be due to late hospitalization or patterns of fibrosis development in COVID-19. For those who died in the 2nd and 3rd weeks of hospitalization, the compensatory response and progression of fibrosis were noted. By the 3rd week, pulmonary fibrosis was detected in 91% of patients. Thrombotic complications (thrombosis, pulmonary artery thromboembolism) were observed in almost half of fatalities occurring during weeks 2–3. Hemorrhagic infarction was found in 43% (6 patients) who died during week 2 of hospitalization, three of them were diagnosed with pulmonary embolism, indicating progression of pulmonary vascular damage.

KEYWORDS — COVID-19, acute respiratory distress syndrome, histopathology in COVID-19, lungs.

INTRODUCTION

The new coronavirus infection (COVID-19) is a serious public health problem worldwide. As of February 2021, more than 2 million deaths from a new coronavirus infection [17] caused by SARS-CoV-2, an RNA-containing virus of the Coronaviridae family, Betacoronavirus genus, have been reported by the World Health Organization since the pandemic began.

Angiotensin-converting enzyme type II (ACE2) is known to be the main receptor for SARS-CoV-2 cell entry. However, based on the results of studies showing predominantly low levels of ACE2 expression on alveolar cells compared to the epithelium of the proximal tubules of the kidney, intestine, and testicular cells, alternative ways of virus entry into cells, through additional receptors and co-receptors other than ACE2, are discussed [5].

Bhatnagar J. et al. (2021) used in situ hybridization to detect SARS-CoV-2 ribonucleic acid (RNA) in alveolar cells, hyaline membranes, lung macrophages, airway epithelial cells, and endothelial cells and vascular walls of the brain stem, pia mater, lungs, heart, liver, kidneys, and pancreas [1]. Electron microscopy showed SARS-CoV-2 in type I and II alveolar cells, airway epithelium, enterocytes, and renal tubule epithelial cells. No viral particles were found in other organs, including heart, spleen and liver [2]. In a study by Martines B. et al. (2020) SARS-CoV-2 was detected only in alveolar cells, alveolar macrophages, but not in other organs (heart, liver, kidney, spleen, intestine) using immunohistochemical methods and electron microscopy of the post-mortem tissues [7].

Internal organ lesions in COVID-19 revealed by numerous histopathological studies are not specific [13]. To date, there are no publications proving a direct cytopathic effect of SARS-CoV-2 viral particles. Consequently, the mechanism of internal organ damage in COVID-19, whether it is due to a direct cytopathic effects of the virus or mediated by respiratory failure, remains unclear.

In the vast majority of COVID-19 fatalities, death occurs through respiratory-mediated mechanisms. According to the analysis of 2000 autopsies of COVID-19 victims performed in Moscow between March 20 and May 22, the immediate cause of death in 90% of cases was acute respiratory failure (clinically

diagnosed as acute respiratory distress syndrome) [18].

Acute respiratory distress syndrome (ARDS) is a common complication of critical illness developing due to noncardiogenic pulmonary edema as a result of damage (dystrophy, necrosis, apoptosis) of endothelium, alveolar epithelium, their basal membranes (including the air-blood barrier) and increased permeability of microcirculation in response to various aggressive factors [9].

The histological classification of ARDS [9] includes three stages: exudative, fibroproliferative, and fibrotic.

Samsonova M. et al. (2020) observed that in contrast to viral pneumonia caused by influenza A/H1N1 virus, there is no definite relationship between the duration of the disease and histopathological changes in the new coronavirus infection. According to authors, this can be explained by the subtle onset of the disease and an asymptomatic period in some patients [15].

Polak S. et al. (2020) performed a systematic review of data from 192 autopsies of COVID-19 patients, which revealed alveolar epithelial damage and pulmonary circulatory disturbances at all stages of the disease, and fibrosis at third week of disease [12].

To date, a number of issues concerning the pathogenesis of the new coronavirus infection remain unresolved. Mechanisms of internal organ damage in COVID-19 can be clarified by thorough histopathological studies, since pathological examination is a powerful tool for learning the patterns of abnormalities in any disease.

The aim of the study was to analyze morphological changes in the lungs of patients who died from COVID-19 in relation to the length of hospital stay.

MATERIAL AND METHODS

We analyzed lung autopsy material from 39 patients with laboratory-confirmed new coronavirus infection while alive, autopsy reports and death summaries. The study included fatal cases in which the new coronavirus infection (COVID-19) was the main cause of death.

The mean age of the deceased was 62 years (ranging from 22 to 94 years). 15% (6 patients) were young (25–44 years), 23% (9 patients) were middle-aged (44–60 years), 44% (17 patients) were elderly (60–75 years), 8% (3 patients) were senile (75–90 years), 10% (4 patients) were long-livers (over 90 years). The length of hospital stay averaged 10 days (ranging from a few hours to 25 days). The following death rate was observed: during the first week of hospital stay — 33% (13 patients), during the second week — 36% (14 people), and during the third week — 31% (12 people). The direct causes of death were respiratory failure in 92% of cases (36 patients), pulmonary embolism (PE) in 5% (2 patients), and disseminated intravascular coagulation (DIC) in 3% (1 person).

The organ samples for histological examination were taken during autopsy in accordance with the current legislation to verify the pathological diagnosis and to clarify the cause of death. In compliance with the current Guidelines of the Ministry of Health of the Russian Federation, the organ samples were fixed for 72 hours in a neutral 10% formalin solution [6]. Later, the sections were processed using standard paraffin-embedding technique. Histological sections of 4 μm thickness were made. Histological sections of lungs were stained with hematoxylin and eosin. Special histological staining with phosphotungstic acid-hematoxylin (PTAH) was used to detect fibrin. Weigert-Van Gieson staining was used to detect elastic fibers, connective tissue, and collagen.

Histological preparations were examined using a NikonEclipseNi-U microscope (Japan). Qualitative parameters are presented as frequencies and percentages.

RESULTS

Histological examination of samples from patients who died during the first week of hospitalization (13 persons) revealed hyaline membranes (Fig. 1) in 10 patients (77% of those who died during the first week of hospitalization), alveolar edema (Fig. 2) was observed in 7 persons (54%), alveolar hemorrhages were noted in 6 persons (46%), fibroplastic response and fibrosis were detected in 3 (23%) and 6 (46%) persons, respectively. Small vessel congestion was found in 10 patients (77%), thromboembolism of pulmonary artery branches was seen in 2 patients (15%). Bronchial and alveolar epithelial desquamation was revealed in 6 (46%) and 5 (38%) patients, respectively. Metaplasia of alveolar epithelium was observed in 2 cases (15%). Purulent pneumonia was detected in 3 persons (23%).

In patients who died during the second week of hospitalization (14 persons), pulmonary fibrosis was detected in 12 cases (86%), hyaline membranes in 3 cases (21%), alveolar edema was observed in 8 cases (57%), alveolar hemorrhages were noted in 8 persons (57%). Small vessel congestion was observed in 11 persons (79%), thromboembolism of pulmonary artery branches was seen in 3 persons (21%), pulmonary vascular thrombosis (Fig. 3, 4) was found in 4 persons (29%). Bronchial and alveolar epithelium desquamation was detected in 3 (21%) and 2 (14%) cases, respectively. Hemorrhagic pulmonary infarction was detected in 6 patients (43%). Purulent pneumonia was found in 3 persons (21%).

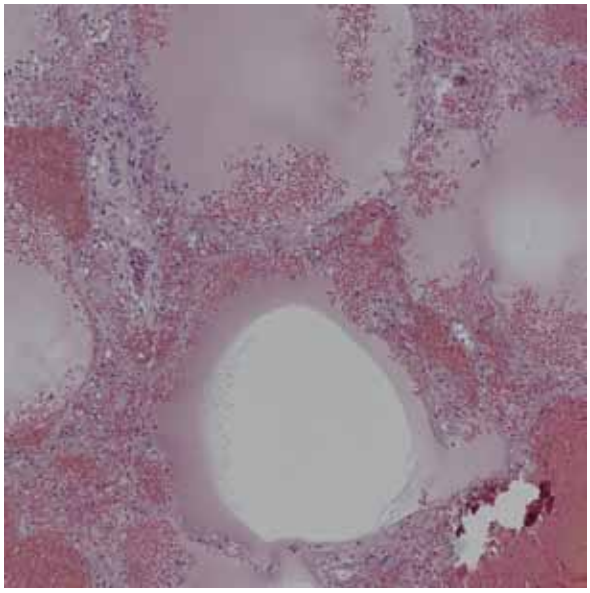

Fig. 1. *Hyaline membranes, alveolar edema. H&E staining,×10*

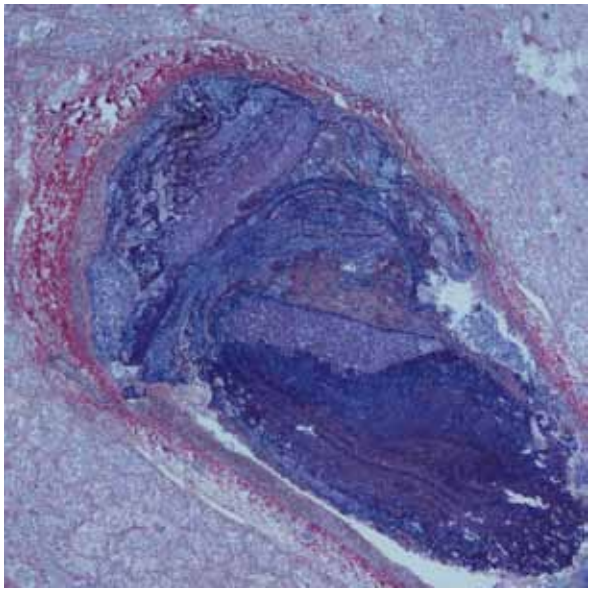

Fig.3. *Thrombus in the pulmonary artery. Phosphotungstic acid-hematoxylin (PTAH) staining, ×4*

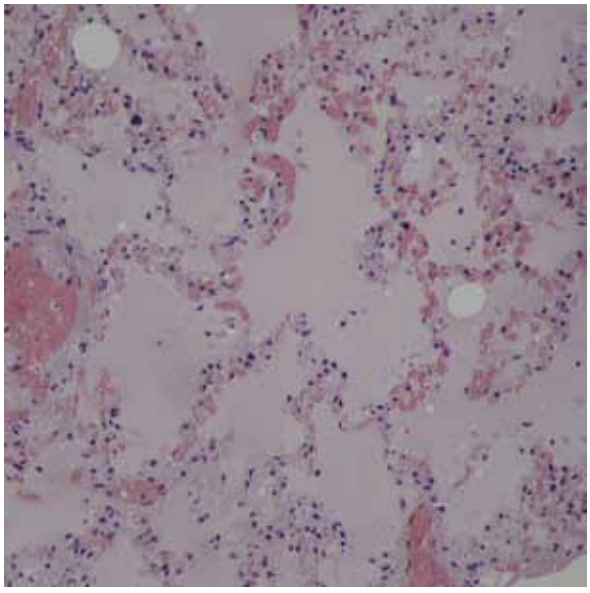

Figure 2. *Alveolar edema. H&E staining,×20*

In patients who died during the third week of hospitalization (12 persons), pulmonary fibrosis (Fig. 5) was detected in 11 cases (91%), hyaline membranes in 2 cases (16%), alveolar edema was observed in 1 case (8%), alveolar hemorrhages were noted in 8 patients (67%). Small vessel congestion was observed in 5 patients (42%), pulmonary vascular thrombosis - in 4 persons (33%), DIC syndrome was recorded in 1 person (8%). Desquamation of bronchial and alveolar epithelium was detected in 5 (42%) and 4 (33%) patients, respectively. Metaplasia of alveolar epithelium was observed in 3 patients (25%). Purulent pneumonia was seen in 4 cases (25%).

CONCLUSION

Thus, at all stages of the disease, lung alteration (desquamation of bronchial and alveolar epithelium), circulatory disorders (alveolar edema and hemorrhages, congestion in small blood vessels, thrombosis), compensatory response (fibrosis) were found.

In patients who died during the first week of hospitalization, circulatory disorders (alveolar edema, hyaline membranes, alveolar hemorrhages, microcirculatory system congestion) prevail. It is noteworthy that fibrosis, usually not typical for the first week of ARDS, was detected in 46% of the patients, which could be due to late hospitalization or specific pattern of fibrosis development in COVID-19.

Those who died in the 2nd and 3rd weeks of hospitalization showed predominant compensatory response and progression of fibrosis. By the 3rd week, pulmonary fibrosis was detected in 91% of patients.

Thrombotic complications (thrombosis, pulmonary thromboembolism) were noted in almost half of patients who died in weeks 2–3. Despite the large number of studies of hemostasis in COVID-19, the mechanisms of thrombosis are not fully understood.

Coagulation disorders have been noted in many viral diseases, including coronavirus infection, Ebola and Dengue fever [3, 11, 14]. Coronavirus infection is assumed to induce endothelial dysfunction caus-

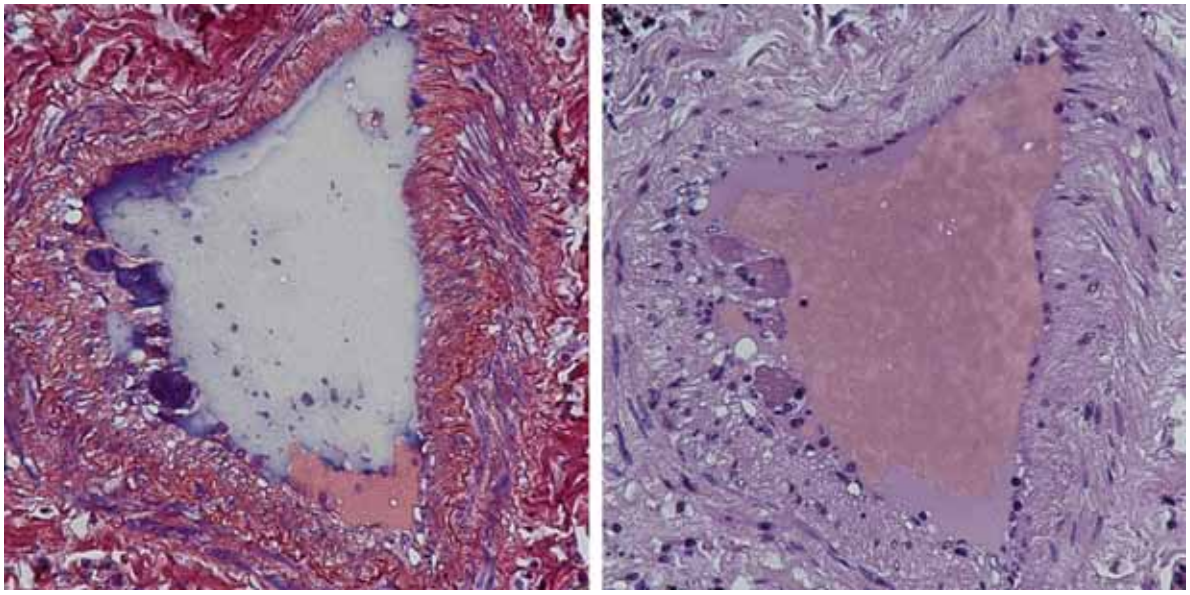

Fig. 4. *Mural thrombus in the pulmonary artery. PTAH staining, ×40 (on the left); H&E staining, ×40 (on the right)*

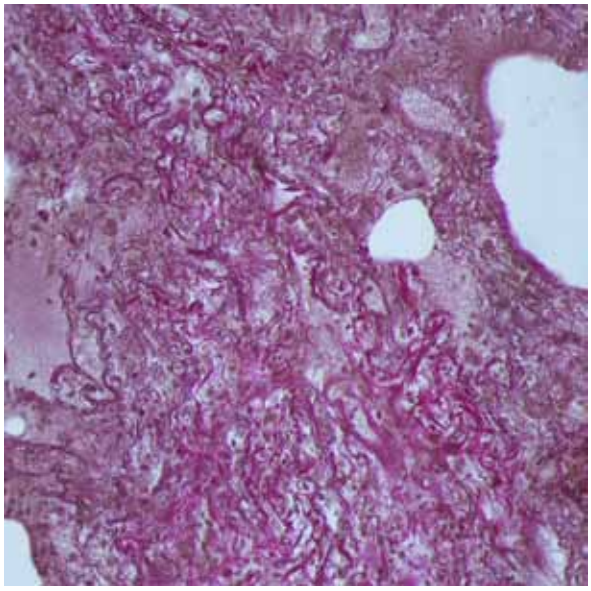

Fig. 5. *Lung fibrosis. Elastic fibers appear purple-red to brown in color. Collagen stained various shades of red. Weigert-Van Gieson staining, ×20*

ing abnormal coagulation. Thrombosis could also have underlying autoimmune mechanisms [8, 14, 19]. Varga Z. et al. (2020) suggest vasculitis and leukocytic infiltration of pulmonary vascular walls as a cause of endothelial damage [16].

Samsonova M. et al. (2020) having analyzed autopsy material of 123 patients who had died of COVID-19, revealed infiltration of pulmonary vascular wall by single lymphocytes only in 8.13% of patients, which is not typical for vasculitis [14]. In our study, no signs of vasculitis were found in any observation.

Notably, according to the results of our study, most deceased patients (86% of cases) with pulmonary thrombi were on mechanical ventilation. Consequently, the role of ventilator-associated complications in the pathogenesis of lung damage in COVID-19 cannot be excluded [10]. According to the experimental data, mechanical lung ventilation is accompanied by significant histological abnormalities in the lungs, including pulmonary vascular thrombosis [4].

Hemorrhagic lung infarction, detected in 43% (6 people) who died in the second week of hospitalization, of which three were diagnosed with PE, indicates the progression of pulmonary vascular system damage.

To date, many issues of pathogenesis of new coronavirus infection and its complications remain open. The ongoing pandemic and the requirements to improve the treatment strategy in severe COVID-19 necessitate further research in order to better understand the pathogenesis of this infection.

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