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PULMONARY PATHOLOGY OF THE NEW CORONAVIRUS DISEASE (COVID-19). THE PRELIMINARY ANALYSIS OF POST-MORTEM FINDINGS

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Background. *Currently, the patho- and morphogenesis of the new coronavirus infection (COVID-19) is being studied in depth. A comparative analysis of the morphological changes in the lungs of deceased patients is of importance, for various time periods after the onset of the first clinical symptoms. The clinical and morphological comparison should help to increase the qualified medical care for patients in the resuscitation profile and reduce the hospital mortality.* ***The aim*** *of the study was to formulate a working hypothesis for a conceptual scheme of clinical and morphological phases of development of the new coronavirus infection (COVID-19).* ***Methods.*** *An analysis of 80 fatal cases was carried out in the COVID-center of the Federal Research Clinical Center of FMBA of Russia. Along with the assessment of macro- and microscopic changes in the respiratory tract, additional histochemical van Gieson staining was applied and immunohistochemical studies were performed to assess the condition of the COVID-19-affected lungs.* ***Results.*** *The revealed features of diffuse alveolar damage in the case of the new coronavirus infection (COVID-19) made it possible to present a working hypothesis of the pathomorphogenesis of COVID-19 interstitial pneumonia. It proceeds through 3 phases: fulminant, persistent and fibrotic. Each phase is conditionally limited by certain time parameters and is characterized by certain morphological signs Dysregulatory activation of monocytic phagocytes, development of generalized microthrombosis, persistent signs of the exudative phase, pathological repair, progressive intraalveolar and interstitial fibrosis are the main links in the pathomorphogenesis of COVID-19 interstitial pneumonia. In response to the penetration of SARS-CoV-2, the T-cell immunity reactions prevail at the exudative and proliferative stages. At the fibrotic stage, the overall number of T-lymphocytes is drastically decreased, the cells of humoral immunity are not revealed. The CD8+ T-lymphocytes prevailing over CD4+ T-lymphocyte helpers is probably related to the autoimmune damage mechanisms.* ***Conclusions.*** *Damage to the lungs with the development of COVID-19 interstitial pneumonia is the main cause of the severe course of the disease and deaths. The revealed features of the pathomorphogenesis of the clinical and morphological phases of COVID-19 interstitial pneumonia will improve the quality of diagnosis and treatment of a new coronavirus infection (COVID-19).*

Keywords*: COVID-19, patho-morphogenesis, COVID-19 interstitial pneumonia, clinical and morphological phases.*

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BACKGROUND

The novel coronavirus infection (COVID-19) represents a potentially dangerous acute respiratory disease caused by the novel coronavirus (SARS-CoV-2), mainly with an aspiration transmission mechanism. In accordance with the sanitary legislation of the Russian Federation, the SARS-CoV-2 virus is assigned to the II pathogenicity group [1]. COVID-19 can occur not only in the form of a mild acute respiratory viral infection, but also in severe forms characterized by the clinical presentation of acute respiratory distress syndrome (ARDS) and multiple organ failure with high mortality [2, 3].

During the current period of the pandemic, scientific information about the etiology, epidemiology, pathogenesis, and morphological changes, as well as clinical aspects, treatment, and prevention of new coronavirus infection is being replenished [4–7].

The spread of COVID-19 poses a particular threat to decompensation of chronic diseases. Severe forms of COVID-19 are most often registered in patients with chronic obstructive pulmonary disease, obesity, di-

abetes mellitus, arterial hypertension, coronary heart disease, chronic kidney disease, and malignant neoplasms [8–11].

The pathogenesis of COVID-19 is under active investigation. The Russian and international literature statesthat the main receptor of cells to which the S-protein (Spike Protein) of the SARS-CoV-2 envelope binds is angiotensin-converting enzyme 2 (ACE2). Infection occurs with the participation of transmembrane protease serine 2 (TMPRSS2) which is required for activation of the S-protein [12–15].

The ACE2 receptor is found on cells of various organs (lungs, heart, kidneys, small intestine, etc.), it is also revealed on the immune system cells and the endothelial cells of arterial and venous vessels [16–19].

The main target of the SARS-CoV-2 virus is the respiratory tract. Alveolocytes of the types 1 and 2, vascular endothelial cells are damaged, which leads to disruption of functioning of the aero-hematic barrier and the surfactant alveolar complex [15, 16].

One of the most relevant pathogenetic concepts of COVID-19 is immune dysfunction (dysregulation) which is based on macrophage activation syndrome (MAS) [16, 20, 21].

Dysregulatory activation of monocytic phagocytes, noted in patients with severe forms of COVID-19, is possibly associated with a hyperimmune response that stimulates the monocytic-macrophage system of the lungs with a massive release of cytokines [16, 22, 23].

It has been noted that during the infectious process generalization, a high production of pro-inflammatory cytokines and chemokines occurs with the development of a “cytokine storm”. The severe course of COVID-19 is accompanied by the highest serum levels of interleukins (IL) 6, 8, 18, 1β, as well as tumor necrosis factor-alpha (TNFα). The risk of lethal outcome is associated with high serum IL6 levels [24, 25]. A hyperergic immune response underlies the development of ARDS and multiple organ failure in COVID-19 [22].

One of the supposed mechanisms of death of cells infected with SARS-CoV-2 is pyroptosis (a type of programmed necrotic cell death when, as a result of caspase 1 activation, the integrity of the plasma membrane is disrupted with the formation of pores and rapid release of the cell contents to the outside) [26, 27]. In the foci of inflammation (intraalveolar and in the interstitium), activated macrophages, in addition to producing inflammatory mediators, secrete growth factors that trigger the repair process and activate fibroblasts.

Activated neutrophils play an important role in the pathogenesis of early changes, reacting to any disorder of homeostasis in the respiratory organs. Activation of neutrophils contributes to damage to the endothelium, deterioration of the rheological properties of blood, activation of platelets and impaired microcirculation. The volume and degree of damage to the microvasculature correlates directly with the course and prognosis of the disease [20]. Activated neutrophils secrete a platelet activating factor, thereby causing the platelet aggregation and sequestration, the synthesis of platelet growth factor which stimulates sclerosis processes.

The system of mononuclear phagocytes of the lungs is comparable to the system of mononuclear phagocytes of the liver, and diffusely developing fibrosis of the lung tissue in the outcome of ARDS in COVID-19 in its course should be compared with certain forms of viral lesions of the liver parenchyma (for example, in viral hepatitis with an outcome as liver cirrhosis). The possibility of spread (persistence) of the SARS-CoV-2 coronavirus in the body due to infection of the immune system cells, more likely, macrophages, is not excluded [28].

Morphological signs of COVID-19 at the present stage are mainly reduced to the description of changes in the early (exudative) and late (proliferative) stages of ARDS. Also, damage to endothelial cells of the microvasculature with disorders in the blood coagulation system, the development of disseminated intravascular coagulation syndrome with multifocal microthrombosis and subsequent multiple organ dysfunction with a predominance of acute renal failure is verified [2, 3, 29].

Some experts believe that in relation to the definition of lung damage in COVID-19, the term “pneumonia” does not reflect at all the morphology, clinical and radiological signs of the pathological process registered when the lungs are affected by the SARS-CoV-2 virus. The term “viral lung injury” (viral pneumonitis, viral interstitiopathy) has been proposed for use [30]. A number of authors suggest the term “microvascular obstructive thromboinflammatory pulmonary syndrome” as a new name for severe COVID-19 [31].

The links of the pathogenesis and morphological aspects of the new coronavirus infection require further comprehensive study using contemporary research methods. The issues of correct registration of fatal cases and epidemiological safety during postmortem autopsy also remain relevant.

The study aimed to analyze the pathomorphogenesis of COVID-19 based on the autopsy studies performed with the formation of a working hypothesis of the conceptual scheme of the clinical and morphological phases of the disease development.

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METHODS

Study conditions

A comprehensive postmortem examination of autopsy material obtained after autopsy of 80 deceased patients at the Federal Clinical Research Centre of Federal Medical-Biological Agency of Russia with an underlying disease COVID-19 of a severe clinical course, confirmed by detection of SARS-CoV-2 RNA by the polymerase chain reaction method both during life and posthumously, was performed.

Characteristics (gradation) of the studied groups

Considering the wide range of morphological changes detected in the lungs, which are based on diffuse alveolar damage, the study groups were graded in accordance with three stages of ARDS (exudative, proliferative, and fibrotic).

The study group 1 consisted of 12 cases of lethal outcome within 10 days from the onset of clinical signs of COVID-19, the group 2 included 28 cases (days 11 to 20), and the group 3 comprised 40 cases (days 21 to 45).

In all cases, postmortem autopsies were performed in compliance with temporary guidelines for the study of deceased patients with suspected new coronavirus infection [29].

Research methods

Histological, histochemical, and immunohistochemical methods were used to analyze the autopsy material. Fragments of the trachea, large bronchi, lungs, and internal organs were fixed in formalin for at least 72 h, and then embedded in paraffin. Serial paraffin sections were stained with hematoxylin and eosin, and picro-fuchsin according to van Gieson. Sections with a thickness of 3 μm were made from paraffin blocks, which were stained using conventional techniques.

In 13 cases, immunohistochemical studies were performed with antibodies to CD3, CD4, CD8, CD20, CD31 (PECAM-1), CD34, CD57, CD68, CD138, Cytokeratin 5 & 6, smooth muscle actin, surfactant-associated protein A (surfactant A), and collagen type IV. Dewaxing, rehydration, antigen retrieval, and staining were performed using a specialized automated BenchMark® ULTRA system (Ventana, USA).

Statistical analysis

The results of postmortem examination are presented in a preliminary form, as the collection and study of the material continues. Statistical processing will be performed later on a larger volume of cases.

RESULTS

Key research outcomes

Autopsy macroscopic and subsequent histological examination of the trachea and lungs of patients who died from the new coronavirus infection revealed morphological signs that distinguish it from other acute respiratory viral infections.

A special aspect of the macroscopic presentation of the trachea is the unevenness of hemorrhagic changes in the mucous membrane, which were often absent or minimally manifested in the proximal part and were moderately or sharply expressed in the distal part and main bronchi.

Histological examination of the distal trachea and bronchi of large and medium calibers revealed the processes of impaired blood circulation in the microvasculature vessels of the submucosal layer in the form of microangiopathy (erythrocytic sludge, stasis, forming blood clots, perivascular edema). Circulatory disorders developed synchronously with the processes of damage, desquamation, and focal basal cell hyperplasia of the respiratory epithelium (Fig. 1) with the formation of squamous metaplasia foci. Active metaplastic processes, possibly induced by a virus, aggravate the infectious process course, contributing to the spread of the pathogen, and disrupt mucociliary clearance, leading to a decrease in the epithelium barrier function.

In 12 cases of lethal outcome within 10 days from the onset of the disease, the lungs were macroscopically enlarged, heavy, of doughy or dense consistency, low-air, on a cut with large areas of a “lacquered” appearance, dark red (cherry) color (Fig. 2, A, B). There were areas of uneven color with alternating grayish, light purple foci, and pronounced edema. In 5 cases (death 7 to 10 days after the onset of the disease), sin-

Fig. 1. Basal cell hyperplasia of the tracheal epithelium. Staining with hematoxylin and eosin, × 100.

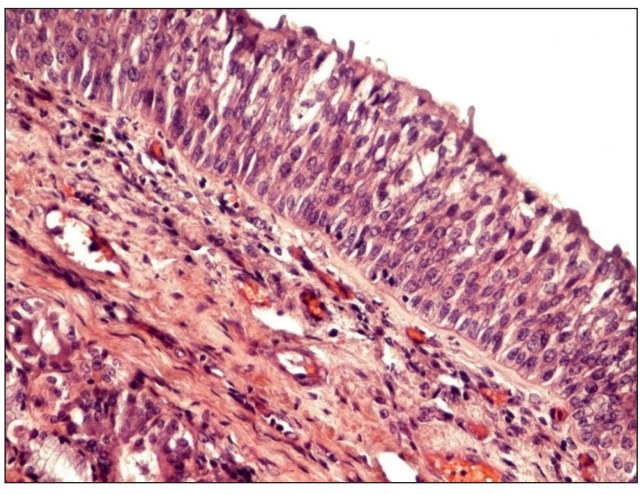
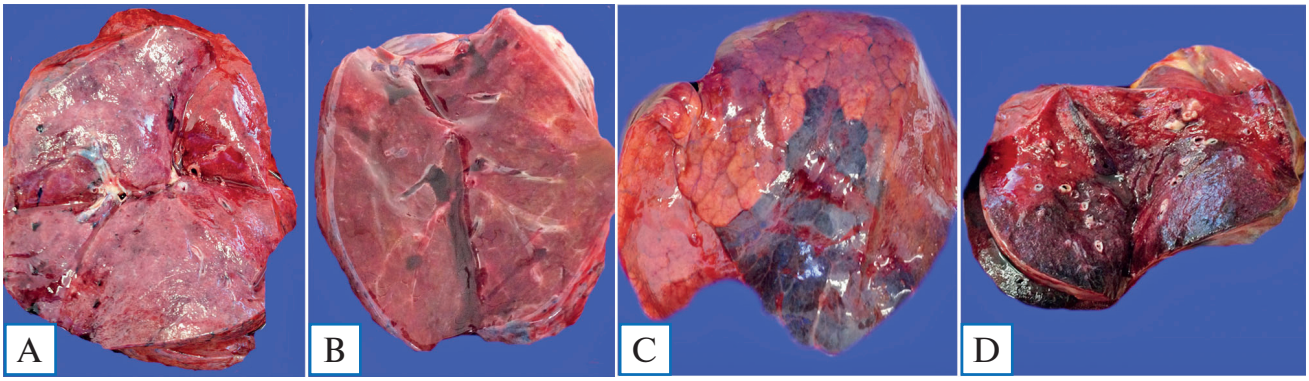


Fig. 2. Macroscopic presentation of the lungs in the fulminant phase of COVID-19 interstitial pneumonia, days 9–10 of illness.



Note. A, B — "lacquered" lungs in the section; C — area of hemorrhagic pulmonary infarction; D — hemorrhagic infarction of the lung in the section.

gle granular grayish-yellow foci were also registered, and areas of hemorrhagic infarctions of regular triangular shape were clearly identified (Fig. 2, C, D) with obstructive thrombi in segmental and subsegmental branches of the pulmonary arteries.

Histological examination in all cases revealed the main morphological signs of diffuse alveolar damage. Massive death of type 1 alveolocytes and synchronous damage to the capillary endothelium lead to exudation of fluid and protein molecules into the intraalveolar space, the development of pulmonary edema, represented by serous exudate, with additional precipitates and fibrin filaments in the lumens of the alveoli (Fig. 3, schematic representation 1). Along the contours of the alveolar passages, alveolar sacs, alveoli and part of the bronchioles, hyaline membranes are formed in the form of strip-like homogeneous eosinophilic masses (Fig. 3, schematic image 2). Dead type 1 alveolocytes start to be substituted compensatorily with the proliferating type 2 alveolocytes. Denudation occurs, which is "exposure" of the basement membranes of the aero -hematic barrier (Fig. 3, schematic representation 3–4) with the destruction of its "working zone".

In the alveoli, among the fragmented hyaline membranes, there are diffusely located cellular infiltrates from pulmonary macrophages (including the formation of polynuclear forms), polymorphonuclear leukocytes, and a few fallen leaves-like arranged lymphocytes. Polymorphonuclear leukocytes are localized mainly along the interalveolar septa, where signs of microcirculation disorders in the form of erythrocyte sludge, stasis, foci of extravasation and capillary dilatation are revealed (Fig. 4, A–C).

There are fibrin and erythrocyte-fibrin thrombi in the lumen of the branches of the pulmonary arteries. The endothelium in the zones of attachment of thrombotic masses is reactively altered, it has signs of intracellular edema, swelling, and enlarged nuclei. There is also edema of the subendothelial layer with hyperplasia of the medial layer muscle cells. The perivascular inflammatory cell reaction in all cases is poorly expressed, and represented by accumulations of lymphoid cells and macrophages. At the same time, postcapillary venulitis with diffuse lympholeucocytic infiltration is determined (Fig. 5, A–C).

With the development of hemorrhagic infarction, blood flows from the bronchial artery, which ruptures the capillaries and pours out into the lumen of the alveoli (Fig. 3, schematic image 5).

An immunohistochemical study of autopsy material of 3 patients who died within 10 days from the onset of the disease was performed. In all cases, a predominance of CD3+ T-lymphocytes over CD20+ B-lymphocytes was found; and CD4+ T-helpers prevailed over CD8+ T-suppressors (Fig. 6, A, B). CD20+ B-lymphocytes, CD57+ NK-cells, CD138+ plasma cells are few, located mainly in the form of rare perivascular and peribronchial clusters. Positive expression of the CD68 antigen confirmed the presence of a large number of pulmonary macrophages located mainly in the lumen of the alveoli (Fig. 6, C). High expression of CD31 (PECAM-1) was also documented in the endothelium of blood and lymphatic vessels, macrophages, granulocytes, while at this stage, no proliferation of blood and lymphatic vessels was detected (Fig. 6, D).

Evaluation of the expression of surfactant-associated protein A reveals its overproduction by proliferating type 2 alveolocytes and phagocytosis by pulmonary macrophages, which, filling the lumens of the alveoli, enter into the composition of the exudate consisting of dead type 1 alveocytes, lysed erythrocytes, and fibrin. An additional perfusion block occurs to the formed hy-

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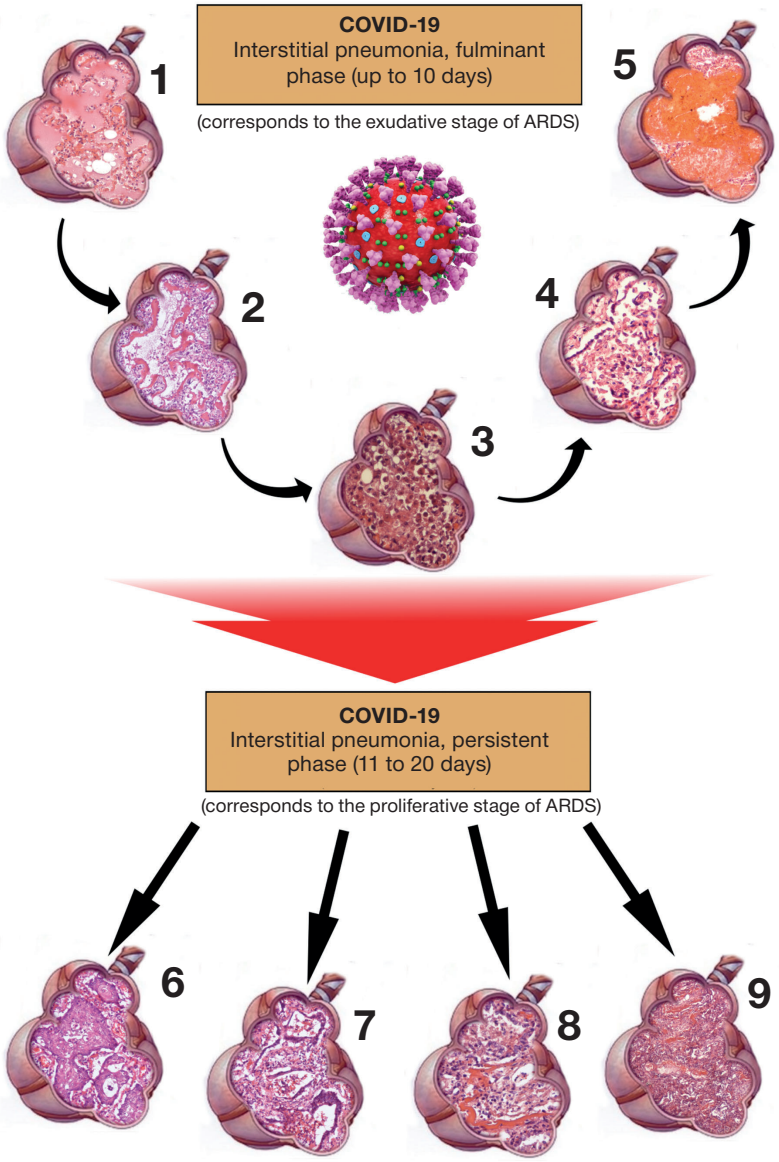
Fig. 3. Pathomorphological phases of COVID-19 interstitial pneumonia corresponding to exudative (1–5) and proliferative (6–9) stages of acute respiratory distress syndrome (designations 1–9 are given in the text) [Illustration by F.G. Zabozlaev].

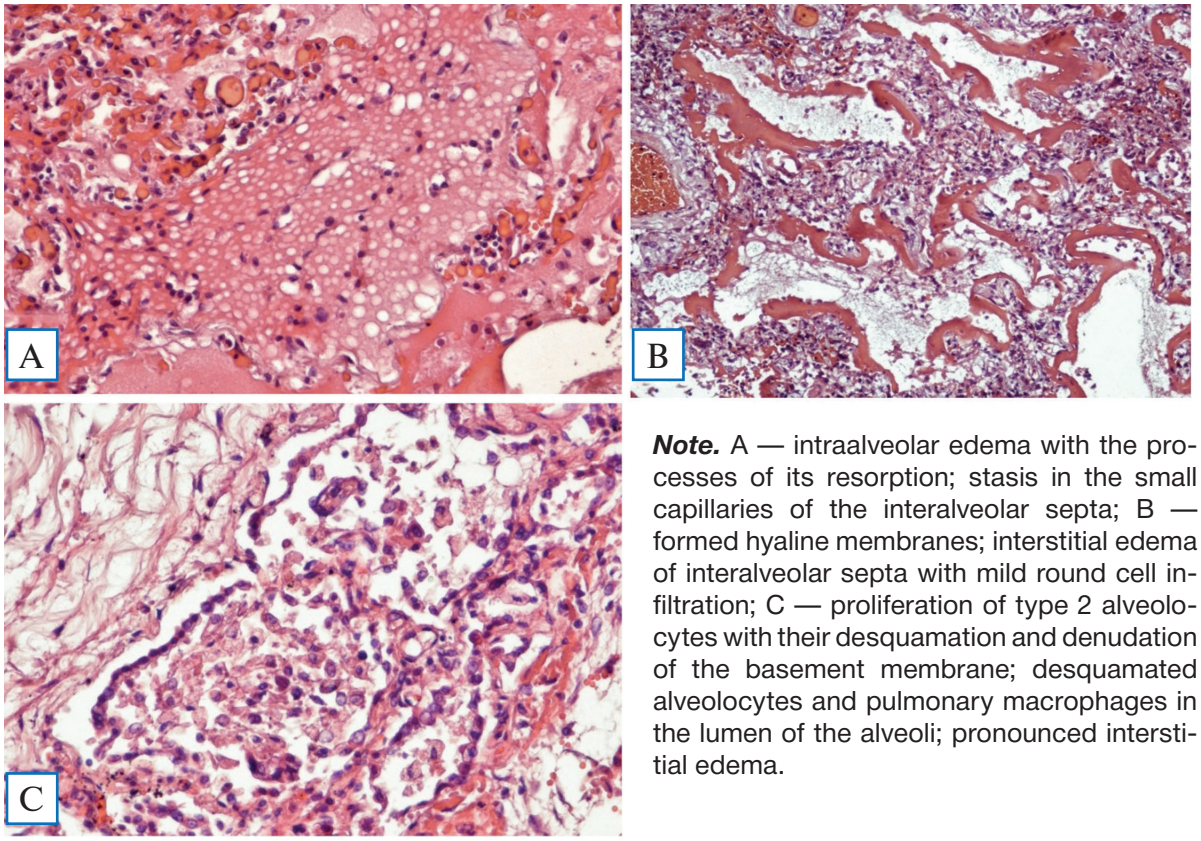


aline membranes, which prevents gas exchange in the alveoli.

Expression of type IV collagen was revealed in areas of deformed “bare” basement membranes of the aero -hematic barrier without accumulation of this marker in the interalveolar septa.

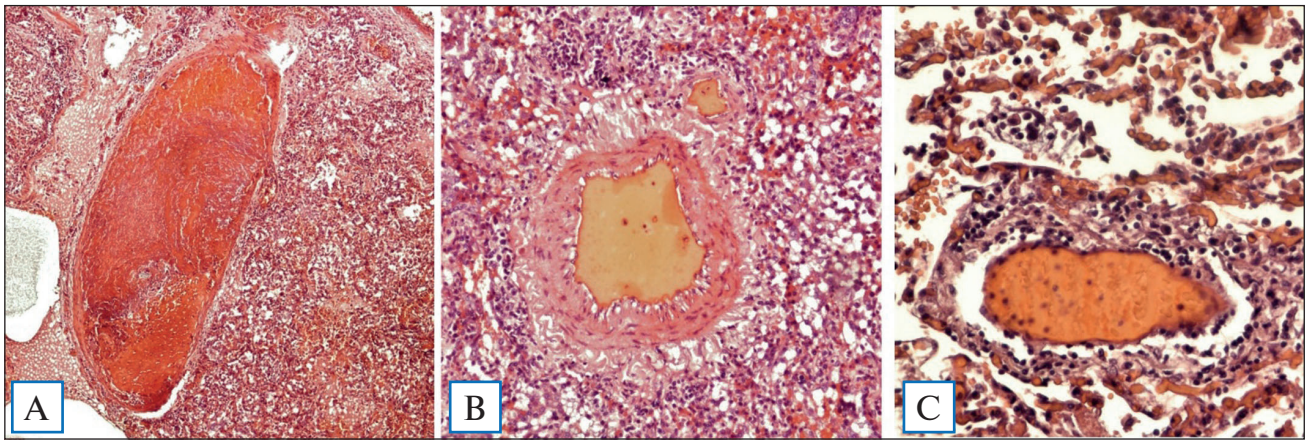
At a macroscopic examination, the lungs of 28 deceased patients of the group 2 (days 11–20) were enlarged, of a denser consistency, with low air content or airless. The section showed “mosaic” segments in the form of pronounced hyperemia, widespread confluent and focal hemorrhages, in combination with yellowish-pink and grayish-whitish areas. In 19 cases, hemorrhagic infarctions (with diffuse localization in the segments of the lungs) were revealed in the presence of obstructing whitish and dark red thrombi in the branches of the pulmonary arteries. In general, the morphological presentation of the lungs in the section resembled porphyric granite, which enabled to call them figuratively “porphyric lungs” (Fig. 7, A). There were also hemorrhages also in the visceral and parietal pleura. Along with the above-described macroscopic signs, persistent areas of mild or moderate edema, and “lacquered” zones were found with great constancy in individual segments. In 16 cases, there were granular-shaped foci due to a secondary bacterial infection. In the subpleural regions, whitish areas of fibrosis with the formation of a grayish reticular pattern of various lengths and topography were verified.

Fig. 4. Microscopic presentation of alveoli in the fulminant phase of COVID-19 interstitial pneumonia. Staining with hematoxylin and eosin, × 100



Note. A — intraalveolar edema with the processes of its resorption; stasis in the small capillaries of the interalveolar septa; B — formed hyaline membranes; interstitial edema of interalveolar septa with mild round cell infiltration; C — proliferation of type 2 alveolocytes with their desquamation and denudation of the basement membrane; desquamated alveolocytes and pulmonary macrophages in the lumen of the alveoli; pronounced interstitial edema.

Fig. 5. Pathomorphological changes in the vessels of the lungs in the fulminant phase of COVID-19 interstitial pneumonia. Hematoxylin and eosin staining.



Note. A — fibrin-erythrocyte thrombus in the small branch of the pulmonary artery, × 40; B — edema of the subendothelial layer with hyperplasia of muscle cells of the medial layer; perivascular inflammatory cell response, × 100; C — postcapillary venulitis with diffuse lymphoid infiltration, × 200.

In all cases, histological examination reveals persistence of morphological signs of the exudative stage of diffuse alveolar damage with the presence of intraalveolar and interstitial edema and the formation of newly formed hyaline membranes. At the same time, there is an ingrowth of granulation tissue into the respiratory bronchioles, alveolar passages, and alveoli (Fig. 3, schematic representation 8–9; Fig. 7 B). The areas of structural rearrangement of the lung parenchyma architectonics, namely the zones of atelectasis and dystelectasis with compensatory dilatation of the adjacent alveoli and terminal bronchioles, are determined simultaneously. The lumens of most of the alveoli are slit-like. In the lumens of the alveoli, macrophages are detected,

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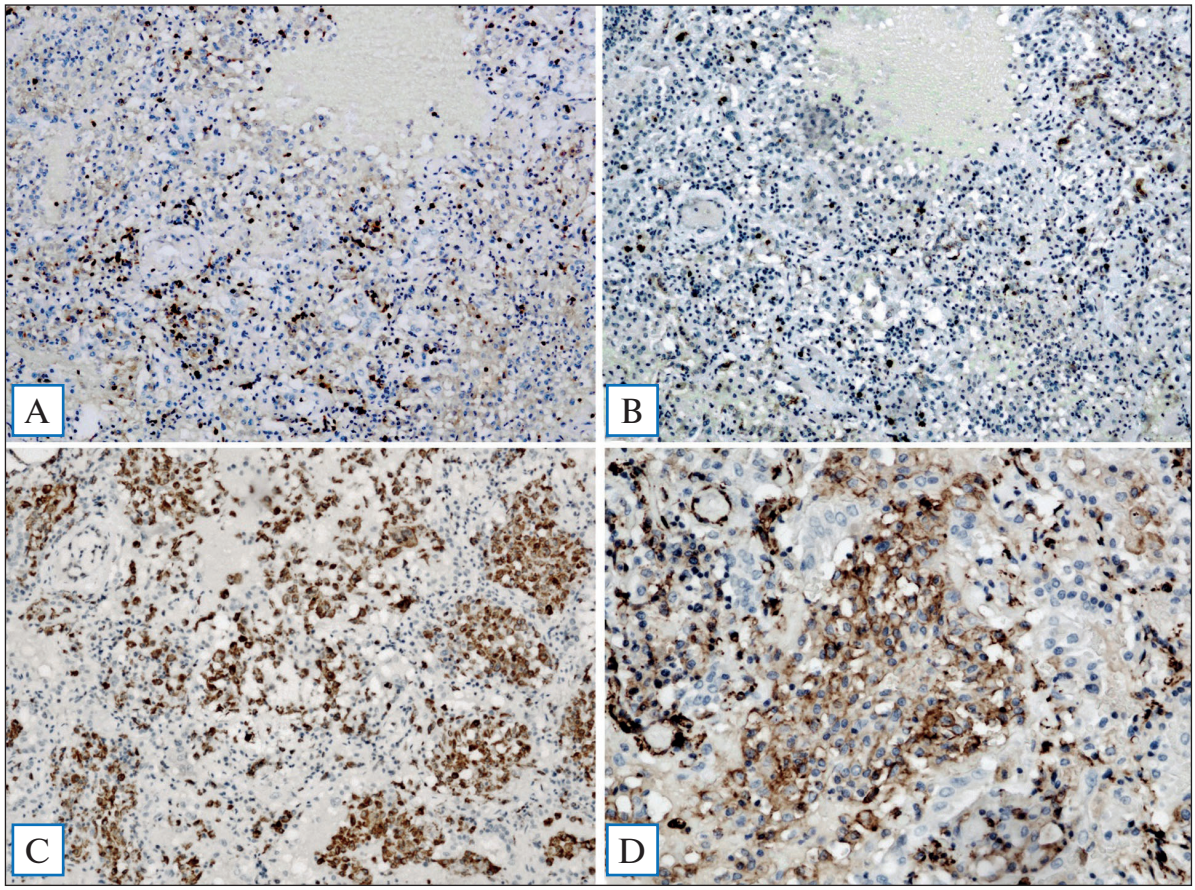
Fig. 6. Immunohistochemical analysis of lung changes in the fulminant phase of COVID-19 interstitial pneumonia. Hematoxylin and eosin staining.



Note. A — expression of CD4 by T-lymphocyte helpers, × 100; B — expression of CD8 by T-lymphocyte suppressors, × 100; C — expression of CD68, × 100; D — expression of CD31 (PECAM-1), × 200.

some of which have foamy cytoplasm, karyomegaly, and form multinuclear structures, cells of desquamated alveolar epithelium merging into polykaryons, lymphoid cells, and polymorphonuclear leukocytes.

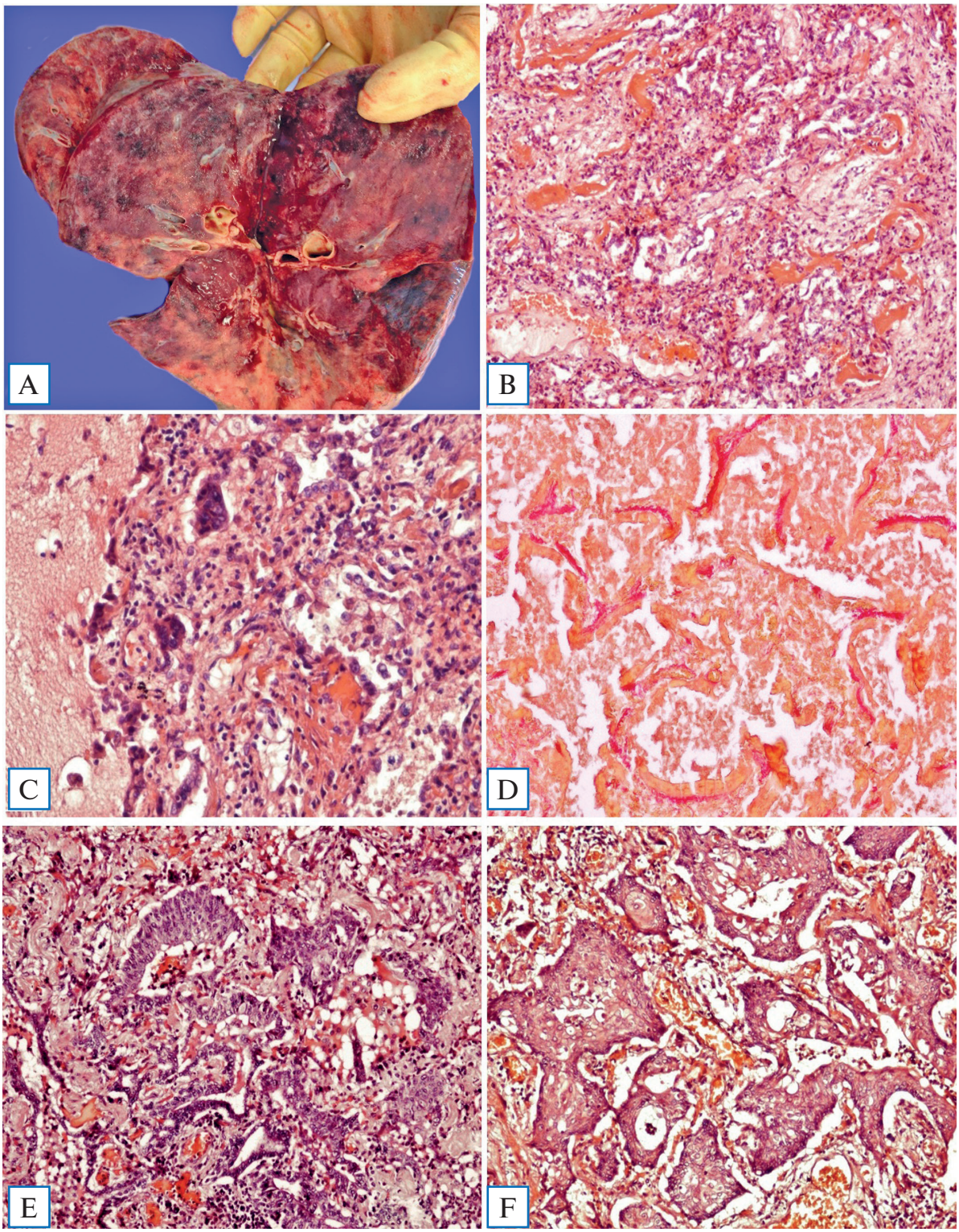
In the interstitium, there are numerous foci of vascular proliferation, and areas of granulation tissue. In all cases, the interalveolar septa are deformed and thickened due to the proliferation of collagen fibers and inflammatory cell infiltration represented by macrophages, lymphoid cells, and polymorph-nuclear leukocytes. Mild or moderate perivascular fibrosis was revealed (Fig. 7, C, D).

Hyperplasia of bronchiolar epithelium with areas of squamous metaplasia and reactive dysplasia is noted (Fig. 3, schematic representations 6–7). There is also a displacement of the boundaries of the bronchoalveor transition towards the alveolar passages with bronchiolization of the alveolar epithelium. In presence of proliferative changes, there is a massive desquamation of the alveolar and bronchiolar epithelium (Fig. 7, E, F).

In cases of accession of a secondary bacterial infection, in the pulmonary parenchyma, there are foci of acute inflammation around the bronchi or bronchioles in size from acinus to segment. And there is inflammatory infiltration of the walls of the bronchioles with the accumulation of predominantly purulent and mixed exudate in the lumens of the alveoli, bronchioles, and bronchi.

An immunohistochemical study of autopsy material of 5 patients who died within 11–20 days from the onset of the disease was performed. In all cases, CD3+ T-lymphocytes were predominant over CD20+ B-lymphocytes, while in 2 cases of the proliferative phase, there was prevalence of cytotoxic CD8+ T-suppressors over CD4+ T-helpers (Fig. 8, A, B), and in 3 cases, the ratio of the two types T-lymphocytes were equal. The count of CD20+ B-lymphocytes, CD57+ NK-cells was significantly reduced in comparison with the exudative phase. Plasma cells were not detected (negative reaction to CD138). Expression of the CD68 antigen was detected in all cases in a large number of functionally active macrophages located mainly in the lumens of the alveoli, less in the interstitium of the interalveolar septa (Fig. 8, C). CD31 (PECAM-1) is expressed in en-

Fig. 7. Gross and microscopic presentation of the lungs in the persistent phase of COVID-19 interstitial pneumonia, day 18 of illness. Staining with hematoxylin and eosin, × 100 (B, C, E, F); staining according to Van Gieson, × 100 (D)



Note. A — "porphyric" lung; B — areas of deformed alveolar parenchyma with hyaline membranes, ingrowth of granulation tissue into respiratory bronchioles, alveolar passages and alveoli; C — intraalveolar edema, macrophages and desquamated alveolocytes with the formation of multinuclear structures, monocytes; D — mild/moderate perivascular fibrosis; E, F — hyperplasia of the alveolar and bronchiolar epithelium with areas of squamous metaplasia and reactive dysplasia.

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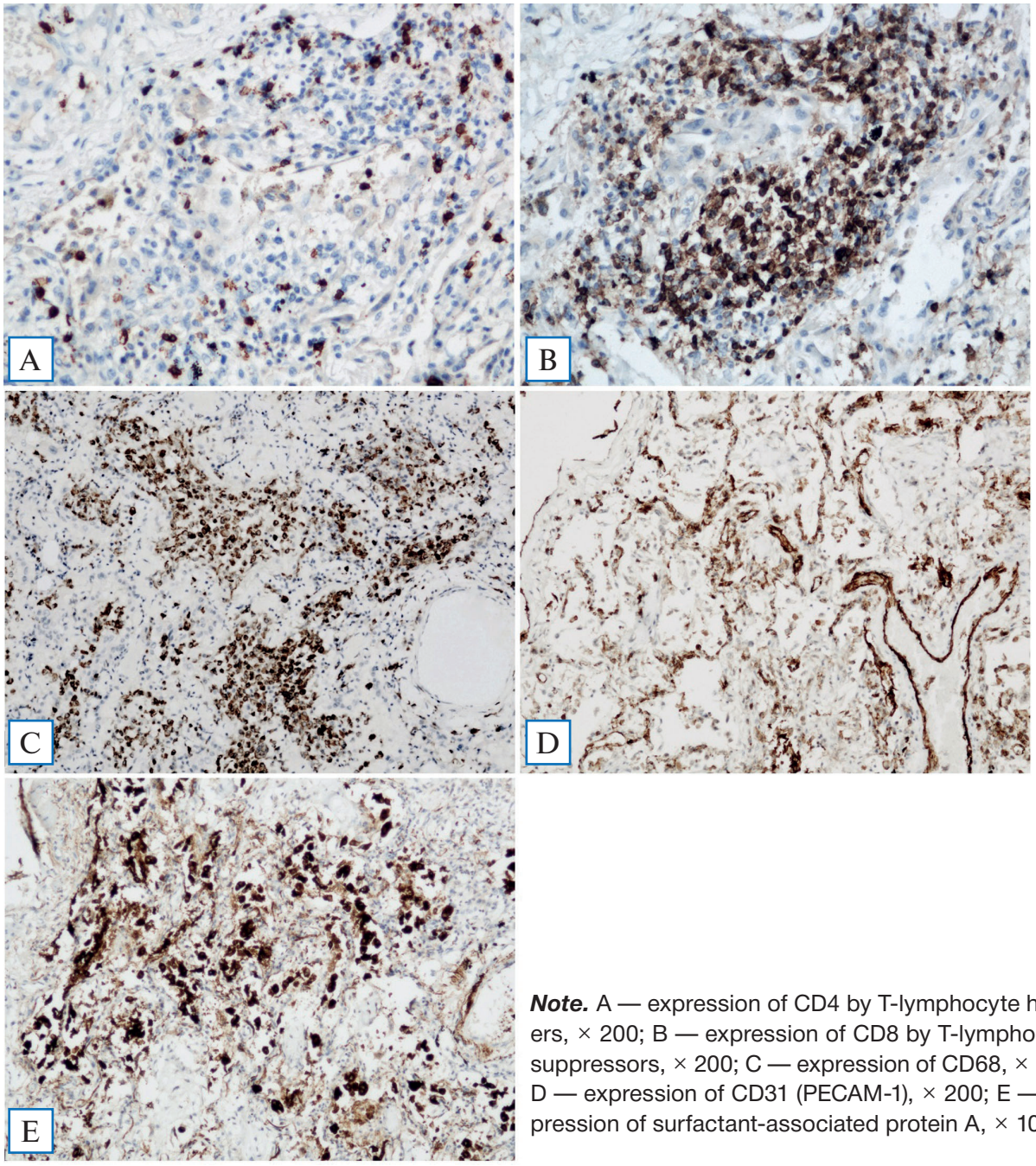
dothelial cells of blood vessels, including granulation tissue, small lymphatic vessels, and also predominantly in macrophages (Fig. 8, D). An immunohistochemical study with CD31 and CD34 revealed signs of focal damage to the endothelium. A positive reaction to surfactant-associated protein A was revealed, mainly in the areas of localization of alveolar macrophages (Fig. 8, E).

In 40 patients who died within 21–45 days from the onset of the disease, the lungs were enlarged and airless. On the visceral pleura, there were multiple hemorrhages, and less often an adhesive process. On the section, the lung parenchyma was mottled, with a clear gradation of stages of diffuse alveolar damage, in some cases with "staged" changes. Mainly in the upper parts of the lungs, there was the alternation of zones of sharp plethora, focal or massive hemorrhages, hemorrhagic infarctions, combined synchronously with extensive areas of fibrosis, starting mainly in the subpleural regions and occupying several segments in the middle and lower parts of both lungs. In 14 cases, there were signs of an associated secondary bacterial infection. The

Fig. 8. Immunohistochemical analysis of lung changes in the persistent phase of COVID-19 interstitial pneumonia. Hematoxylin and eosin staining.

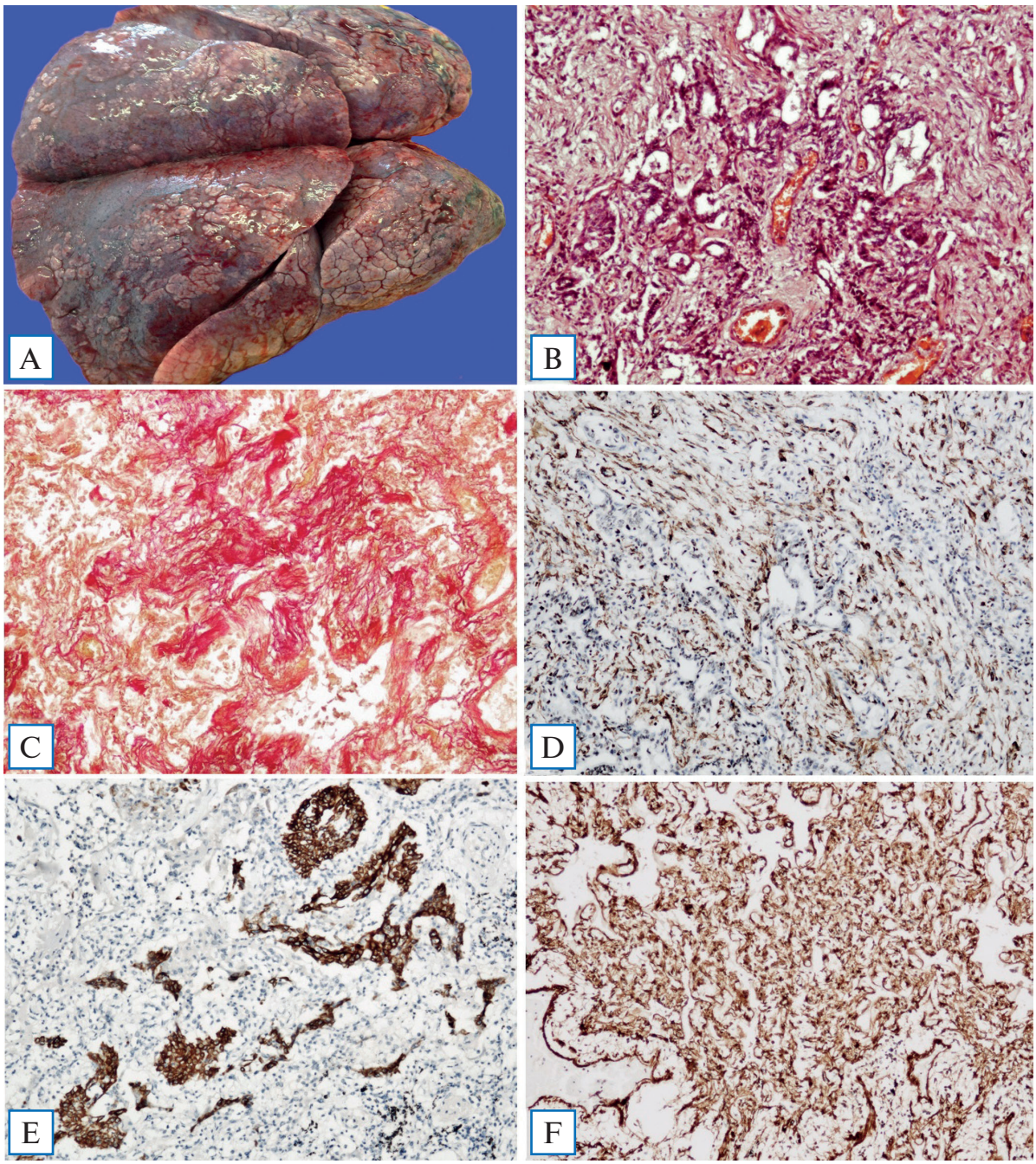


Note. A — expression of CD4 by T-lymphocyte helpers, × 200; B — expression of CD8 by T-lymphocyte suppressors, × 200; C — expression of CD68, × 100; D — expression of CD31 (PECAM-1), × 200; E — expression of surfactant-associated protein A, × 100.

lung parenchyma in the areas of fibrosis is dense, of "rubber" consistency; with superinfection, it is of small and large lobular structure. The visceral pleura of the lung adjacent to the areas of fibrosis with an undulating surface resembled visually a capsule of a cirrhotic liver (Fig. 9, A).

The histological examination revealed structural disorganization of the lung parenchyma with a change in

Fig. 9. Gross and microscopic presentation, immunohistochemical analysis of lung changes in the fibrotic phase of COVID-19 interstitial pneumonia, day 36 of illness. Staining with hematoxylin and eosin, × 100 (B, D–F); van Gieson's staining, × 100 (C).



Note. A — gross specimen of the lungs: the visceral pleura of the lung with a small tuberous surface, adjacent to the areas of fibrosis, resembles visually a capsule of a cirrhotic liver; B — proliferative, hyperplastic, and metaplastic changes in the bronchial and alveolar epithelium with the formation of adenomatous structures, foci of squamous metaplasia with foci of keratinization and reactive dysplasia; C — diffuse fibrosis of the pulmonary parenchyma in the fibrotic phase of COVID-19 interstitial pneumonia; D — expression of smooth muscle actin of proliferating myofibroblasts; E — expression of cytokeratin 5 & 6 in foci of squamous metaplasia; F — expression of type IV collagen.

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normal histoarchitectonics due to rapidly progressing fibrosis. Alveoli were predominantly collapsed (atelectasis and dystelectasis) with single slit-like lumens (Fig. 10, schematic representation 1–3). Interalveolar septa are sharply thickened due to the deposition of collagen fibers with foci of proliferation of fibroblasts and myofibroblasts, reduction of the capillary bed, diffuse infiltration with macrophages, polymorphonuclear leukocytes and few lymphocytes. The epithelial lining of deformed interalveolar septa is represented by proliferating type 2 alveolocytes, with signs of pronounced reactive and dysregenerative changes.

There are proliferative, hyperplastic, and metaplastic changes in the bronchial epithelium with the formation of adenomatous structures, foci of squamous metaplasia with foci of keratinization and reactive dysplasia. In zones of adenomatous hyperplasia, there is proliferation of metaplastic bronchiolar epithelium into the lumens of the alveolar passages (Fig. 9, B). Van Gieson staining revealed diffuse pulmonary fibrosis at this stage (Fig. 9, C).

An immunohistochemical study of autopsy material of 5 patients who died within 21–45 days from the onset of the disease was performed. In all cases of the fibrotic phase, the prevalence of CD8+ T-suppressors over CD4+ T-helpers was revealed, while the total number of T-lymphocytes was significantly reduced. No CD20+ B-lymphocytes, CD57+ NK-cells were detected.

A positive reaction to the smooth muscle actin of proliferating myofibroblasts and myofibroblastic foci (Fig. 9, D) was noted. In areas of squamous metaplasia, there was a positive reaction to cytokeratin 5 & 6 (Fig. 9, E).

A pronounced expression of type IV collagen is determined along the course of thickened basement membranes, including the vascular bed, bronchial tree, in the interstitium of thickened and sharply deformed interalveolar septa (Fig. 9, F). In some areas, a more intense reaction was noted around the foci of squamous metaplasia.

DISCUSSION

Our preliminary experience of postmortem examination of deceased patients with severe forms of polysegmental, subtotal, and total pneumonia caused by the novel coronavirus (SARS-CoV-2), combined with extensive interdisciplinary information, enables to present a working hypothesis of the pathomorphogenesis of coronavirus (COVID-19) interstitial pneumonia (hereinafter COVID-19-interstitial pneumonia).

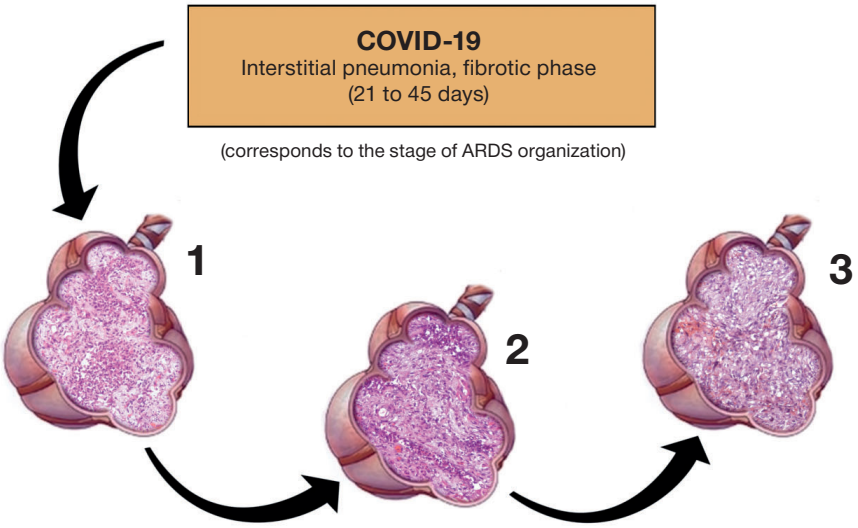
Acute respiratory distress syndrome in COVID-19-interstitial pneumonia differs from the classical pattern of diffuse alveolar injury, modified in 2016 [32]. According to this scheme, diffuse alveolar damage includes 2 stages with a total duration of about 14 days (Fig. 11).

In our opinion, ARDS in COVID-19 has 3 stages, which accompany the development of the corresponding clinical and morphological phases of COVID-19-interstitial pneumonia:

1) exudative stage with the development of the fulminant phase of COVID-19-interstitial pneumonia;

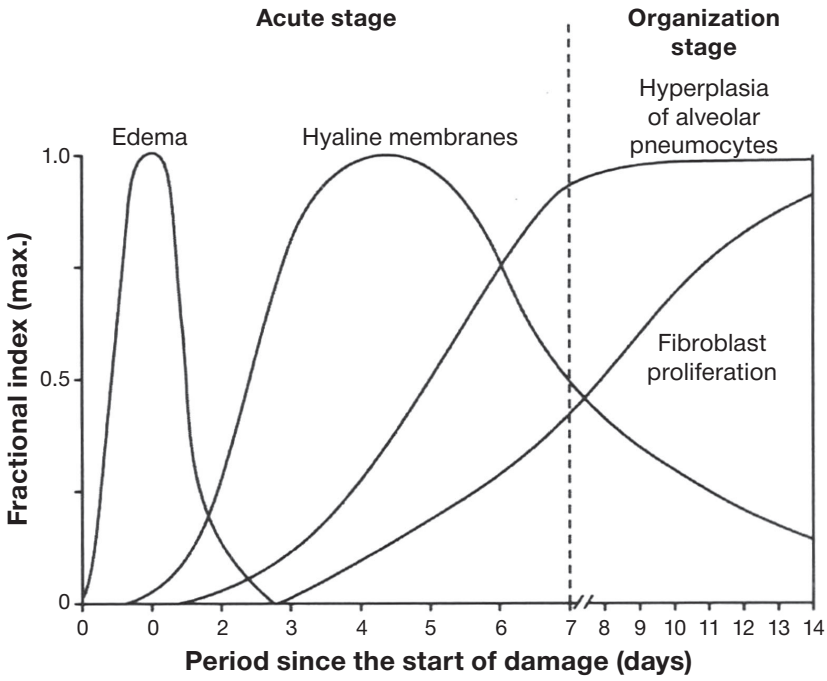
2) proliferative stage with the development of a persistent phase of COVID-19-interstitial pneumonia;

Fig. 10. Pathomorphology of the fibrotic phase of COVID-19 interstitial pneumonia.



Note. ARDS — acute respiratory distress syndrome [Illustration by F.G. Zabozlaev].

Fig. 11. Stages of acute respiratory distress syndrome according to Anna-Luise A. Katzenstein, 2016 [32].

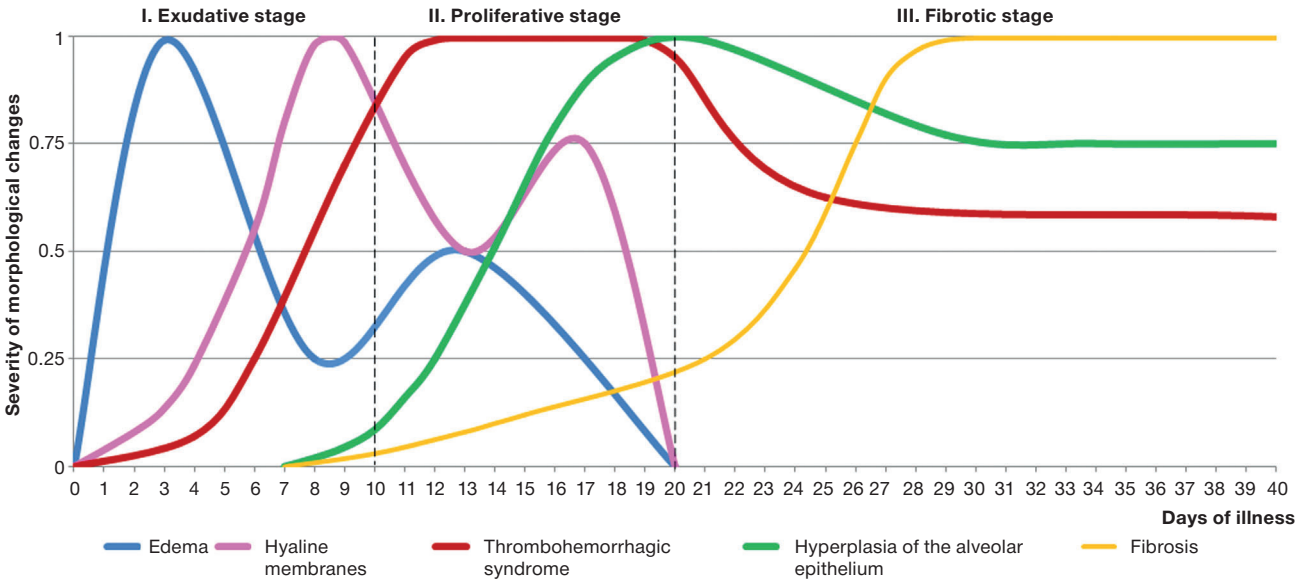


3) fibrotic stage with the development of the fibrotic phase of COVID-19-interstitial pneumonia.

Each stage corresponds to a certain time of the disease development and is represented by characteristic macro- and microscopic signs (Fig. 12).

The exudative stage corresponds to the acute phase of diffuse alveolar damage with the development of widespread pulmonary edema and the subsequent formation of hyaline membranes (Fig. 3); its duration takes up to 10 days from the onset of clinical symptoms of the disease. A distinctive characteristic of the exudative stage is dysregulatory activation of monocytic phagocytes, possibly associated with a hyperimmune response that stimulates the monocytic-macrophage system of the lungs with the subsequent development of microthrombosis in the pulmonary vessels, and in severe forms, it is associated with generalized microthrombosis with damage to the vessels of the heart, kidneys, brain, upper and lower limbs. In case of lethal outcomes during the exudative stage, we considered

Fig. 12. Stages of acute respiratory distress syndrome corresponding to the phases of COVID-19-interstitial pneumonia development.



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it necessary to indicate the fulminant course of the corresponding fulminant phase of COVID-19 interstitial pneumonia.

A special aspect of the proliferative stage, which is up to 20 days from the onset of clinical symptoms of the disease, is a wide range of morphological manifestations, a constant combination of persistent signs of the exudative stage in the form of newly emerging foci of intraalveolar edema and hyaline membranes with hyperplastic, reactive, and dysregenerative changes, initial signs of fibrosis development. These morphological changes suggest an atypical course of ARDS with a new coronavirus infection (COVID-19). In this case, the immediate causes of death are most often associated with thromboembolic complications and a secondary (bacterial) infection.

The postmortem examination of the lungs in the fibrotic stage (death within days 21 to 45 or more from the onset of the disease) showed subtotal, but more often total, lesion of the parenchyma with the development of diffuse intra-alveolar and interstitial fibrosis with almost complete absence of functionally viable lung tissue. It is noteworthy that interstitial fibrosis, which develops for a long time and accompanies the course of common interstitial pneumonia, as well as the fibrous variant of nonspecific interstitial pneumonia, in the fibrotic phase of the progressive severe course of COVID-19 interstitial pneumonia, is formed within just 1.5–2 months. We also previously noted this symptom in acute interstitial pneumonia (Hamman-Rich syndrome).

In cases of survival of patients with the fibrotic phase of COVID-19-interstitial pneumonia, qualified follow-up is required, since their severe disability can be predicted, which requires constant respiratory support and consideration of lung transplantation due to the high probability of pneumonocirrhosis and neoplastic aberration.

Immunohistochemical studies performed in a small volume indicate preliminary that in response to the infiltration of the SARS-CoV-2 virus, the reactions of T-cell immunity prevail, which is more pronounced in the exudative stage with a further decrease. The prevalence of CD8+ T-lymphocytes-suppressors over CD4+ T-lymphocyte helpers in persistent and fibrotic phases of coronavirus (COVID-19) interstitial pneumonia can be considered as a sign of a probable autoimmune lesion.

The weak manifestation of humoral immunity reactions in the fulminant phase of coronavirus (COVID-19) interstitial pneumonia, as well as the absence of CD20+ B-lymphocytes and plasma cells in the fibrotic stage, require further study and clinical and morphological comparisons.

Lung damage with the development of COVID-19 interstitial pneumonia is the main cause of severe disease and lethal outcomes. Severe inflammatory infiltration of the lung tissue by proinflammatory macrophages, generalized damage to the microvasculature and larger vessels with the development of thromboembolic complications, progressive fibrosis of the lung parenchyma, and secondary bacterial infection are predictors of a poor prognosis.

CONCLUSIONS

1. Lung damage in a new coronavirus infection (COVID-19) is based on the development of ARDS (diffuse alveolar damage) with an atypical course, causing COVID-19-interstitial pneumonia with synchronous damage to the respiratory tract and microvasculature.

2. Morphological signs of the fulminant phase of the progressive severe course of COVID-19 interstitial pneumonia, leading to a rapid lethal outcome (up to 10 days), correspond to the exudative stage of ARDS in combination with a monocytic-macrophage hyperimmune reaction and the development of obstructive thrombo-inflammatory processes in the microvasculature of the lungs or are of generalized nature.

3. Morphological signs of the persistent phase of the progressive severe course of COVID-19-interstitial pneumonia resulting in lethal outcome (up to 20 days) correspond to the proliferative stage of ARDS. At this stage, there is a persistence of changes in the exudative stage in combination with a monocytic-macrophage hyperimmune reaction, the development of generalized obstructive thrombo-inflammatory processes not only in the microvasculature, but also in larger vessels, as well as widespread thrombosis and thromboembolic complications.

4. Morphological signs of the fibrotic phase of the progressive severe course of COVID-19-interstitial pneumonia, resulting in lethal outcome (21 to 45 days), correspond to the fibrotic stage of ARDS with dysregeneratory metaplastic and dysplastic changes, a multiplicative sharply forced effect of fibrosis and fibrotic remodeling of the lung parenchyma.

5. Immunohistochemical studies, performed in a small volume, indicate preliminary that with COVID-19 interstitial pneumonia, T-cell immunity reactions predominate. A sharp decrease in the total number of T-lymphocytes, the absence of CD20+ B-lymphocytes and CD57+ NK-cells in the fibrotic stage is an

indicator of progressive suppression of immunological reactivity, while the increased expression of CD68 and CD31 (PECAM-1) in macrophages indicates a poor prognosis.

6. Positive immunohistochemical reactions to smooth muscle actin of proliferating myofibroblasts and myofibroblastic foci, to cytokeratin 5 & 6 areas of squamous cell metaplasia, as well as diffuse expression of type IV collagen in the localization area of both intraalveolar and interstitial fibrosis confirm the processes of pathological repair and fibrotic remodeling of the pulmonary parenchyma in the fibrotic phase of COVID-19-interstitial pneumonia.

ADDITIONAL INFORMATION

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AUTHOR CONTRIBUTIONS

F.G. Zabozlaev wrote the article, arranged the methodological support and general guidance; E.V. Kravchenko performed proofreading of the article, prepared the illustrative material, processed additional research methods; A.R. Gallyamova analyzed the literature, processed the morphological material; N.N. Letunovsky processed the immunohistochemical studies. All authors made a significant contribution to the preparation of the article, read and approved the final version before its publication.

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