# COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Lviv and Lviv Region

[1]Yuliia Kuzyk , [1]Olena Gavrilyuk , [2]Marta Semko ,
[2]Borys Ribun, [3]Anna Arefyeva

[1]Danylo Halytsky Lviv National Medical University
[2]Lviv Regional Office for Autopsy
[3] Lviv Railway Clinical Hospital

**Introduction.** Worldwide, more than 182 million cases of COVID-19 and more than 3.9 million deaths have been confirmed since the virus was first identified. Advanced age and some co-morbid conditions, such as diabetes and cardiovascular diseases, are considered risk factors for the adverse course of the discussed pathology.  In recent years, several reports have been published about the results of the pathological examination of patients with COVID-19. Most often, in fatal cases, diffuse alveolar damage is described, which is characterized by intraalveolar edema, the appearance of "hyaline" membranes and the proliferation of pneumocytes and fibroblasts. However, the nature of the damage caused by SARS-CoV-2 remains unclear. The study of pathomorphological changes in severe fatal cases of COVID-19 is important for a better understanding of pathogenetic mechanisms of the development of pulmonary complications and the development of new effective methods of antiviral treatment.

**The aim** of the current study is to evaluate the gross and microscopic findings in COVID-19 patients' autopsy to investigate the clinicopathologic basis for adverse outcomes with a fatal course of the disease.

**Methods.** A retrospective analysis of 1036 consecutive autopsies associated with COVID-19 in 2020 was conducted based on Lviv Regional Office for Autopsy and Lviv Railway Clinical Hospital. The diagnosis of COVID-19 was confirmed by clinical signs of viral pneumonia, nasopharyngeal smear analysis, and radiological changes. A statistical study was performed with IBM SPSS Statistics 24.0.

**Results.** The majority (72.4%) were elderly (60+) males (54.1±1.5%) and females (45.9±1.5%), with an age range from 19 to 93 years (mean age 66.9 ± 0.4 years). All examined patients had pneumonia, which was detected during a clinical examination with CT diagnosis and confirmed at autopsy. The acute exudative phase of pneumonia was diagnosed in 18.5±1.2% of cases, proliferative phase – in 18.5±1.2%, and fibrotic phase – in 5.9±0.7%. And in 53.5±1.5% of cases, signs of progressive fibrosis associated with exudative lesions prevailed. COVID-19 was the single original cause of death in 88.7±1.0% of cases. The following were identified in the lungs: typical virus-induced changes in epithelial cells of the trachea, bronchi, bronchioles and alveoli (100%, n=1036); different phases of diffuse alveolar damage in the majority of cases (96.5±0.6%); manifestations of innate immunity were described; pathological changes in the microvasculature (large vessel thrombi were detected in 37.9±1.5%).

**Conclusion**. Our study results prove the importance of pathological examination of tissues during autopsies to determine the pathophysiological mechanisms and underlying causes of death of patients with COVID-19.

**Keywords:** COVID-19, SARS-CoV-2, diffuse alveolar damage, vessel thrombi, elderly males.

**ORCID IDs**
Yuliia Kuzyk:
    https://orcid.org/0000-0002-5235-5861
Olena Gavrilyuk:
    https://orcid.org/0000-0003-5280-5561
Marta Semko:
    https://orcid.org/0000-0001-8972-9364
Borys Ribun:
    https://orcid.org/0000-0001-8305-3180
Anna Arefyeva:
    https://orcid.org/0000-0001-5741-6187

# Патологія легень при COVID-19: багатоінституційна когорта розтинів Львова та Львівської області

[1] Юлія Кузик, [1]Олена Гаврилюк, [2]Марта Семко, [2]Борис Рібун, [3]Анна Ареф'єва

[1] *Кафедра патологічної анатомії та судової медицини, Львівський національний медичний університет імені Данила Галицького Міністерства охорони здоров'я України, Львів, Україна*
[2] *Комунальний заклад Львівської обласної ради «Львівське обласне патологоанатомічне бюро»*
[3] *Львівська клінічна лікарня на залізничному транспорті філії "Центр охорони здоров'я" АТ "Укрзалізниця"*

**Вступ.** Відомо, що причиною COVID-19 є раніше невідомий коронавірус SARS-CoV-2. У всьому світі з моменту первинного виявлення вірусу було підтверджено більше 182 мільйонів випадків COVID-19 та більше 3,9 мільйона смертельних випадків. Факторами ризику несприятливого перебігу обговорюваної патології вважають похилий вік та деякі коморбідні стани, такі, як цукровий діабет та серцево-судинні захворювання. За останні роки було опубліковано декілька повідомлень про результати патологоанатомічного дослідження пацієнтів з COVID-19. Найчастіше при летальних випадках описують дифузне альвеолярне пошкодження, яке характеризується інтраальвеолярним набряком, появою «гіалінових» мембран та проліферацією пневмоцитів і фібробластів. Тим не менше, характер пошкодження, спричиненого SARS-CoV-2 залишається досі незрозумілим. Дослідження патоморфологічних змін при важких летальних випадках COVID-19 є важливим для кращого розуміння патогенетичних механізмів розвитку легеневих ускладнень та розробки нових ефективних методів противірусного лікування.

**Метою** дослідження є оцінка макроскопічних і мікроскопічних результатів розтину трупів пацієнтів з COVID-19  для дослідження клініко-патологічної основи несприятливих наслідків захворювання із летальним перебігом.

**Матеріали та методи.** Це ретроспективне дослідження 1036 розтинів COVID-19, проведених у Львівському обласному патологоанатомічного бюро та Львівської залізничної клінічної лікарні у 2020 році. Діагноз COVID-19 підтверджено тестуванням ПЛР з носоглотки, рентгенологічні особливості вірусної пневмонії та клінічні симптоми. Статистичне дослідження проводилось за допомогою IBM SPSS Statistics 24.0.

**Результати.** Більшість померлих (72,4%) становили пацієнти похилого віку (після 60 років); 54.1±1,5% чоловіків та 45.9±1,5% жінок. Вік померлих коливався від 19 до 93 років (середній вік 66,9 ± 0,4 років). У всіх обстежених пацієнтів була пневмонія, виявлена під час клінічного обстеження з КТ-діагностикою та підтверджена на розтині. Гостра ексудативна фаза пневмонії була діагностована у 18,5±1,2% випадків, проліферативна фаза – у 18,5±1,2%, фібротична фаза – у 5,9±0,7% та в 53,5±1,5% випадків переважали ознаки прогресуючого фіброзу, пов›язаного з ексудативними ураженнями. COVID-19 був єдиною першопричиною смерті у 88,7±1,0% випадків. Виявлено основні структурні закономірності у легенях хворих на COVID-19: типові індуковані вірусом зміни епітеліальних клітин трахеї, бронхів, бронхіол та альвеол (100%, n = 1036); різні фази дифузного пошкодження альвеол у більшості ви-

Праці НТШ Медичні науки                    Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
2022, Том 69, № 2    ISSN 2708-8634 (print)              ISSN 2708-8642 (online)    2022, Vol. 69, 2
Оригінальні дослідження: клінічні науки                    Original research: Clinical sciences

падків (96,5±0,6%); були описані прояви вродженого імунітету; патологічні зміни мікросудин (тромби великих судин виявлені у 37,9±1,5%).

**Висновки.** Результати нашого дослідження підкреслили критичну важливість гістопатологічного дослідження тканин, особливо при проведенні клінічних розтинів, для з›ясування механізмів шляху та основних причин смерті у пацієнтів з COVID-19.

***Ключові слова:*** COVID-19, SARS-CoV-2, дифузне пошкодження альвеол, тромби судин, літні чоловіки.

**Introduction.** It is known, that COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and was first detected in December 2019 in Wuhan (China), after a series of pneumonia of unknown etiology [1, 2]. In March 2020, COVID-19 was declared a pandemic by the World Health Organization (WHO)  [3]. As of today, around 192 million COVID-19 cases have been confirmed worldwide with almost 4.1 million lethal cases [4].

In Ukraine, 2.2 million cases are reported now with 52.8 thousand deaths [4].

As Lax S. F. et al. [5], Phua J et al. [6], and Zhou F et al. [7] reported that risk factors for adverse outcomes and death in patients with COVID-19 were old age and some comorbidity (diabetes and cardiovascular disease). Rapid deterioration of the respiratory and hemodynamic conditions in some patients with discussed diseases was proven [6, 7].

During the pandemic period only, Lax S. F. et al. [5], Borczuk, A.C. et al. [8] and Samsonova M.V. et al. [9] reported about autopsy investigation in COVID-19 patients but the information is still limited. More data is available from the SARS-CoV-1 coronavirus epidemic that happened in 2003 when the autopsy showed diffuse alveolar damage (DAD), which included edema, hyaline membranes, pneumocytes and fibroblast proliferation [5, 10]. The most significant were pulmonary changes [10, 11]. The authors proposed morphological patterns for three phases of DAD. In the exudative phase, authors consider the main changes such as hyaline membranes, desquamation of pneumocytes, cellular or protein exudate, hemorrhage in the alveoli, and fibrinoid necrosis of small arteries. The phase of organization or proliferation is characterized by interstitial and intraalveolar proliferation of fibroblasts, lymphocytic inflammation, hyperplasia of type II pneumocytes, and fibrin deposition. The fibrous phase is characterized by dense collagen fibrosis and architectural remodeling. Nevertheless, organ damage features caused by SARS-CoV-2 are still known incompletely. The morphologic basis study of severe and fatal COVID-19 outcomes can be useful in discussing the development of pathology and new treatment approaches [5].

The aim of the current study is to evaluate the gross and microscopic findings in COVID-19 patients' autopsy to investigate the clinicopathologic basis for adverse outcomes with a fatal course of the disease.

**Methods.** This is a retrospective study of 1036 consecutive COVID-19 autopsies performed in Lviv Regional Office for Autopsy and Lviv Railway Clinical Hospital in 2020 and was conducted following the principles of the Declaration of Helsinki and approved by the local Medical Ethical Committee of three referral centers (Protocol No. 2 dated 02/26/2018 of the Bioethics Committee of Danylo Halytsky Lviv National Medical University). The diagnosis of COVID-19 was confirmed by clinical manifestations, nasopharyngeal swabbed secretion testing and viral pneumonia radiologic features. Complete autopsies were performed in 100% of cases. In 1036 cases, all lung lobes were samples, including central and peripheral areas. The tissue samples from the brain, kidneys, heart, liver, spleen, pancreas, thyroid glands, adrenals, small and large intestine were fixed in 10% buffered formalin for histopathologic examination. After fixation, the lungs were dissected systematically from the apex to the basis and multiple samples were processed for histopathologic examination (hematoxylin and eosin and MSB method modified by Zerbino-Lukasevich stain).

SARS-CoV-2 RNA was identified in the trachea and lungs to detect the protein-coding RNA of

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
Original research: Clinical sciences

the virus. For this, the FTD™ SARS-CoV-2 molecular diagnostic test system was used, which is designed for the qualitative detection of RNA specific for SARS-CoV-2 coronavirus, with the detection of target genes ORF1ab and N, in clinical samples (nasopharyngeal and oropharyngeal swabs). The test system confirmed the etiology of the disease. The diagnosis was made on the day of hospitalization when patients already had clinical signs of pneumonia. Microbiologic studies were not performed because we used archived materials from routine autopsies which did not include additional histochemical or other methods.
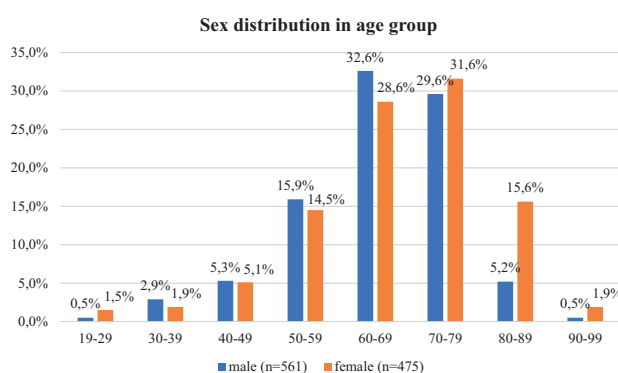


Figure 1. The number of male and female patients in different age groups of our autopsy cohort (n=1036)

Histological evaluation was performed and selected. We used morphological patterns that have already been described. According to them, all samples were divided into three phases of DAD: exudative, proliferative and fibrous [5, 10, 11].

Statistical analysis was performed with StatPlus for Macintosh. Sample parameters given in the tables were denoted as follows: quantitative data are expressed as an M ± SD (where M is the mean, SD-standard dimension and n is the size of the analyzed subgroup).

**Results.** The majority (72.4%) in our autopsy cohort were elderly (aged 60+, Fig.1) males
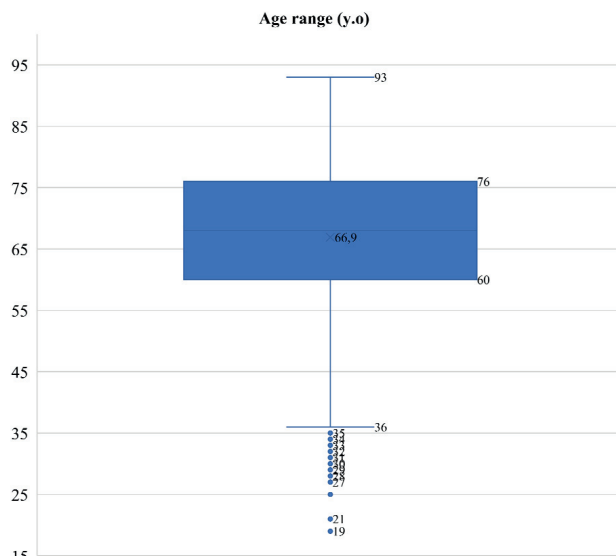


Figure 2. The patients' age range in our autopsy cohort (n=1036)

(54.1±1.5%) and females (45.9±1.5%). The age of observed patients varied from 19 to 93 years with a mean age of 66.9 ± 12.2 years (fig.2).

The length of COVID-19-related hospital stays (LoS) among our autopsy cohort ranged from 20 min to 2 months (mean LoS 9.2 ± 2.5 days), in 7 cases (0.7±0.3%) patients died at home. In the majority of cases (52.4%), before death, patients stayed at the hospital for less than one week (Table 1).

COVID-19 was diagnosed during the hospital stay in 67.9±1.4% of cases (n=704) mostly by PCR using a nasopharyngeal swab (84.1±1.1%, fig. 3).

One comorbidity pathology was identified in 494 patients (47.7±1.6%) and the most common were hypertension (17.5±1.2%) and diabetes (11.3±1.0%), or their combination (3.8±0.6%). The frequency of comorbidity pathologies is represented in Table 2.

*Table 1.*

**COVID-19-related length of hospital stay distribution among surveyed patients (n=1029)**

| COVID-19-related length of hospital stay | n | % |
|---|---|---|
| <24 hours | 71 | 6.7 |
| 2-7 days | 469 | 45.7 |
| 8-14 days | 286 | 27.8 |
| >14 days | 203 | 19.8 |

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
Original research: Clinical sciences

*Table 2.*

**The frequency of comorbidity pathologies in our autopsy cohort (n=494)**

| Comorbidity pathologies | n | % |
|---|---|---|
| Hypertension | 256 | 24.7 |
| Diabetes mellitus | 147 | 14.2 |
| Obesity | 5 | 0.5 |
| Leukemia | 4 | 0.4 |
| Chronic alcohol intoxication | 4 | 0.4 |
| Rheumatoid arthritis | 3 | 0.3 |
| Asthma | 2 | 0.2 |
| Myelodysplastic syndrome | 2 | 0.2 |
| Thrombosis | 1 | 0.1 |
| Other | 31 | 3.0 |

**COVID-19 detection methods in our autopsy cohort (n=1036)**



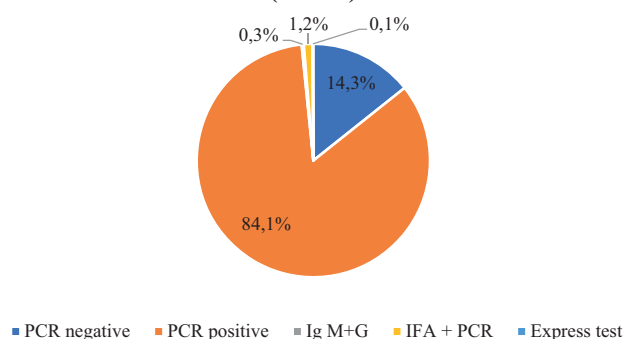■ PCR negative  ■ PCR positive  ■ Ig M+G  ■ IFA + PCR  ■ Express test

Figure 3. COVID-19 detection methods in our autopsy cohort (n=1036)

All examined patients (n = 1036) had pneumonia, which was detected during a clinical examination with CT diagnosis and confirmed at autopsy.  Acute exudative and proliferative phases of pneumonia were diagnosed in the same number of cases (18.5±1.2% and 18.5±1.2%). The fibrotic phase was identified in 5.9±0.7% of cases. 53.5±1.5% of surveyed patients had atypic pneumonia. And in most cases, a mixed picture prevailed with signs of progressive fibrosis associated with exudative lesions (53.5±1.5%).

COVID-19 was a single original cause of death in 88.7±1.0% of surveyed cases; in 3.0±0.5% (n=31) – the first underlying pathology in combination with acute coronary syndrome. Except COVID-19, the most frequent causes of death in our autopsy cohort were stroke (2.3±0.5%, n=24), diabetes (1.3±0.3%, n=13) and cancer (0.6±0.2%, n=6). Acute respiratory distress syndrome (ARDS) was diagnosed in 36 patients (3.5±0.6%), pulmonary embolism in 148 patients (14.3±1.1%), systemic thrombosis in 11 patients (1.0±0.3%), *Clostridioides difficile* infection in 2 patients (0.2%).

Clinicopathological diagnostic discrepancies were documented in 22 patients (2.1±0.4%) – cardiovascular pathology (n=5), stroke (n=2), renal diseases (n=3), leukemia (n=2), chronic obstructive pulmonary disease (n=2), alcohol-related liver disease (n=1), meningitis (n=1), hepatic cirrhosis (n=1), HIV (n=1), peptic ulcer disease (n=1), other (n= 3) were mentioned as the main clinical diagnoses.

In 15.8±1.1% of cases, associated bacterial or fungal pneumonia was detected. Sepsis was diagnosed in 4 cases (0.4±0.2%).

According to autopsy results, the most pronounced changes were found in the lungs. Macroscopic evaluation results demonstrated edematous and congested lungs with patchy involvement and diffuse consolidation area. The lungs were enlarged, airless, diffusely compacted, dark red with a brownish tint on the cut and a pink fluid flowing down from the cut surface (figure 4).

Microscopic evaluation of lung airways, alveoli, and the vascular bed was performed. Tracheitis of varying severity (from catarrhal-hemorrhagic to fibrinous-necrotic, mostly desquamative) was diagnosed in 91.0±0.9% of cases (n=943).

The four main structural patterns in COVID-19 patients' lungs were:
• Typical virus-induced changes in epithelial

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
Original research: Clinical sciences
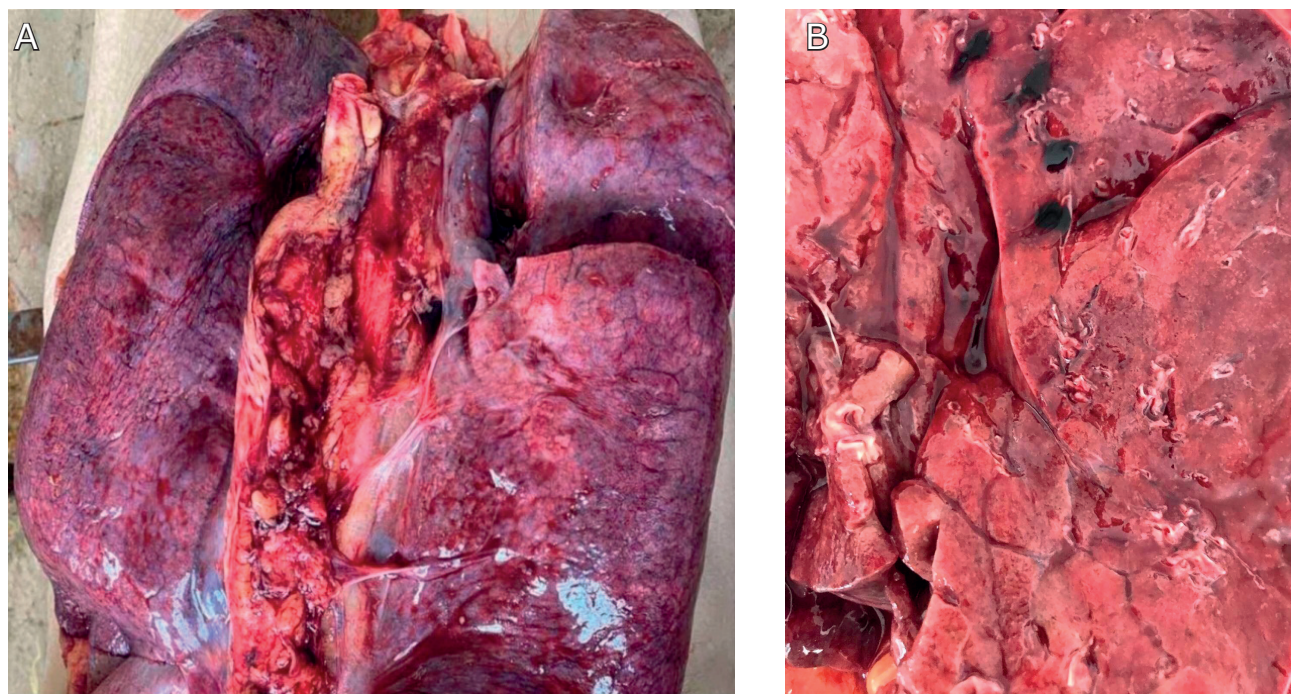
Figure 4. The gross examination of lungs during COVID: A – Enlarged lungs of the COVID-19 patient; B – Lung cut of the COVID-19 patient: a section of lung tissue with edematous fluid and redness on the surface
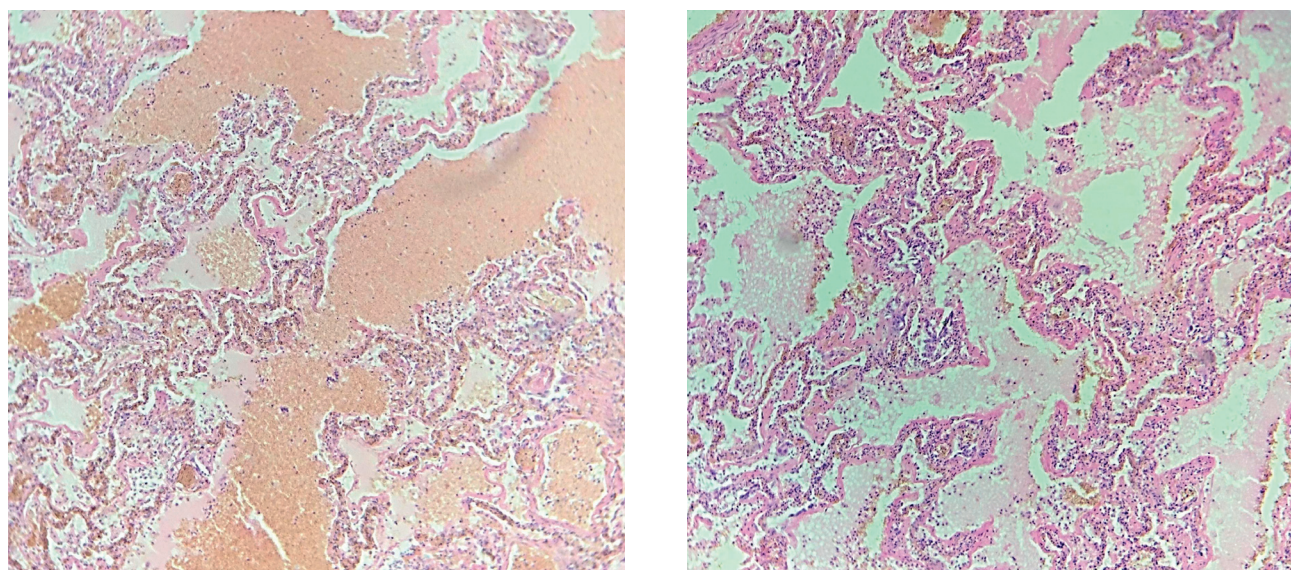


Figure. 5. Diffuse alveolar damage, exudative phase: A and B – total hemorrhagic pneumonia with edema, intraalveolar hemorrhage, multiple hyaline membranes and severe vascular plethora. Hematoxylin and eosin stain ×60

cells of the trachea, bronchi, bronchioles and alveoli (100%, n=1036).
• Different phases of diffuse alveolar damage in the majority of cases (96.5±0.6%).

In the early exudative phase, intraalveolar edema, hyaline membranes, lining the contours of the alveoli, and desquamation of the bronchiolar and alveolar epithelium were detected. Alveolocytes with altered shape, enlarged nuclei, coarse-grained chromatin and distinct nucleoli were found in the walls and lumens of the alveoli. Interalveolar septa capillaries and pulmonary arteries and vein branch plethora

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
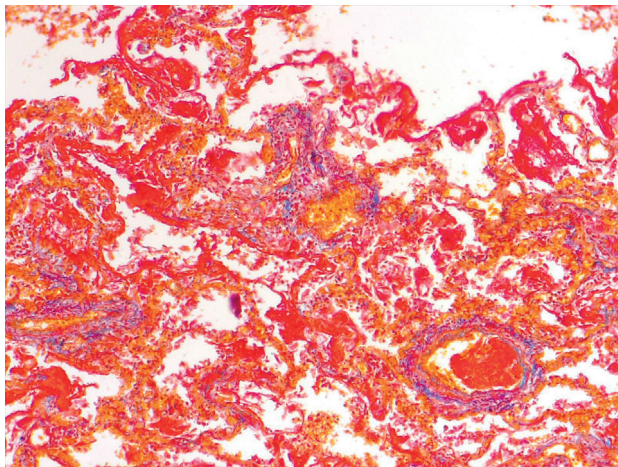Original research: Clinical sciences

Figure 6.  Diffuse alveolar damage, exudative phase: total hemorrhagic pneumonia with multiple hyaline membranes in the lumens of the alveoli and "mature" fibrin clots in the lumens of blood vessels. MSB method modified by Zerbino-Lukasevich stain x 10
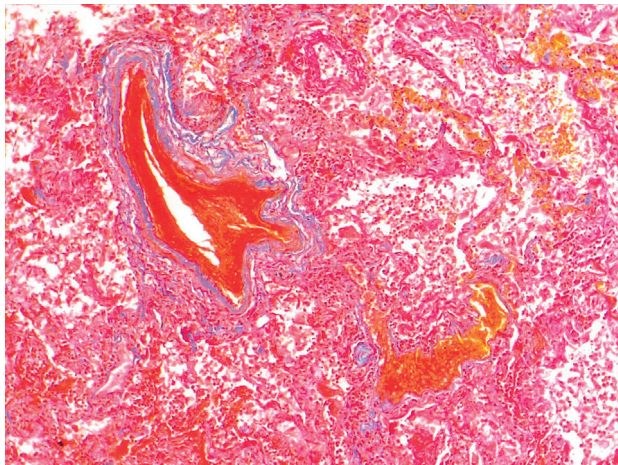


Figure. 7. Diffuse alveolar damage, organizing phase: total hemorrhagic pneumonia with multifocal intra-alveolar deposition of "mature" fibrin, interstitial and intra-alveolar proliferation of fibroblasts, growths of immature connective tissue and intra-alveolar proliferation of fibroblasts. MSB method modified by Zerbino-Lukasevich stain x 10

with erythrocyte sludge, fibrin or organic descending blood clots, and foci of perivascular blood effusions were found in the exudative phase (Fig.5, 6). Intra-alveolar erythrocyte accumulation from small parts to almost complete lumen filling was detected in almost all cases (94.4±0.7%). Siderophages and/or hemosiderin deposition (perivascular and in the walls of the pulmonary artery branches) were also identified. Multifocal intraalveolar fibrin deposit interstitial and intra-alveolar prolifer-

ation of fibroblasts we found at the organizing phase (fig.7).
• Manifestations of congenital immunity were described.

In all cases of COVID-19 pneumonia, edema and scant mononuclear infiltration within the interstitium alveolar septa were identified. Alveolar spaces contain macrophages, granulocytes. Significant accumulation of intraalveolar neutrophils, with multiple, large foci of micro- and macroabscess formation were interpreted as signs of superimposed bacterial/ fungal infection.
• Pathological changes in the microvasculature.

Analysis of laboratory data showed that all these patients had deviations in markers of coagulation, anticoagulant, fibrinolytic systems and indicators of systemic inflammation. But the exact analysis of these anomalies was impossible due to differences in the lists of clinical examinations and the absence of some data. During histology examination, focally or diffusely, thrombi of the large vessels were identified in 37.9±1.5% of surveyed lungs. Microthrombi containing fibrin and platelets were found in arteries <1.0 mm in diameter and in alveolar capillaries. In 39.6±1.5% of cases with diffuse microthrombi, it was also detected in a larger vessel. And 157 patients had diffused large vessel thrombi, 68.0±1.4% also had microthrombi. The described changes are probably the result of impaired hemostasis in DIS syndrome, which naturally involves microvessels. In addition, necrosis found in visceral organs appears to be a consequence of thrombosis. At the same time, in 12 cases with the addition of bacterial pneumonia, inflammatory lymphocytic perivascular and transmural infiltration of small vessels was detected in combination with thrombosis, which can be considered a manifestation of vasculitis.

Tubular necrosis was found in the kidneys in 43.0±1.5% of cases, sometimes with an accumulation of eosinophilic homogeneous masses in the lumens. In 0.3±0.2% of observations, myocardial necrosis was identified. The brain was characterized by changes in membranes and tissues. There were vascular space expansions with vasculitis and blood clots, as

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
Original research: Clinical sciences

well as small diffuse areas of neuroglia rarefaction. Severe edema was detected in the membranes. Signs of tissue structure focal damage in other organs were observed up to parenchymal cell necrosis, as well as changes associated with comorbid chronic diseases previously present in surveyed patients.

**Discussion and Conclusion**. It is known that COVID-19 can progress to severe acute respiratory syndrome with pneumonia and ARDS [12].

ICD coding of COVID-19 includes U07.1 – COVID-19 confirmed by laboratory testing; U07.2 – suspicious for COVID-19 with inconclusive laboratory testing; RA01.0 – COVID-19 (definite); RA01.1 – COVID-19 (suspected or probable).

Elderly men predominated among our autopsy cohort, which is consistent with global data. It has been proven that the male gender and advanced age (> 65 years) are the main risk factors for disease progression [13] and SARS [14]. The results of the meta-analysis [14] show that patients with COVID-19 are mostly adults aged 19 to 76 years (mean age 59.0 ± 9.6 years), with a higher share of men and women being reported. Our patient cohort was older (up to 93 years) with a mean age of 66.9 ± 1.2 years and 54.1% men. Similar data are presented by Borczuk A.C et al. [8], where the autopsy cohort consisted of 69.0% men with a mean age of 73 years (range, 30 to 96 years).

To our best knowledge, there are only a few large reports on COVID-19 autopsy cohorts been published over the past year: one is from the USA and Italy (n=68) [8] and two from Russia (n=700 and n=543) [9, 15]. Our study represented the largest (n=1036) autopsy cohort of COVID-19 patients.

Different levels of COVID-19 severity have been identified, and hospital care can range from general to oxygen support and intensive care units, where patients can even be intubated, and the length of stay is highly dependent on these criteria [15]. Rees E.M. et al. [15] with a systematic review revealed that estimates for LoS among patients who died in the hospital were generally shorter (Me 4-21 days) than those for patients who were discharged alive

(Me 4–53 days). Those data are comparable with the LoS indicator of our autopsy cohort.

It was proved that cardiovascular disease, such as arterial hypertension or diabetic angiopathy, and chronic lung pathology prevalence were significantly higher ($p < 0.00001$) in critical patients compared to non-critical ones [13]. In our study, almost half of the patients (47.7%) had comorbidity with the most common hypertension (17.5%) and diabetes (11.3%). The percentage of mentioned pathologies in Borczuk A.C et al. [8] cohort was higher and reached 65.0% (hypertension) and 45.0% (diabetes); in Rybakova MG et al. [16] study these indicators were 87.0% and 35.7%.

The discussed pathology may progress to severe ARDS and its main pathological phenotypes include pneumonia and DAD. All patients (100%) of the present study were clinically diagnosed with pneumonia, which was confirmed after the later morphological investigation. It is known that up to 40% of hospitalized patients developed ARDS [17, 18]. In our autopsy cohort, 22.0% had ARDS and in 3.5% of cases, it was fatal. Pathophysiological changes in ARDS were manifested by acute and diffuse inflammatory lesions of the alveolar-capillary barrier, which were caused by increased vascular permeability in combination with a decrease in the pliability and size of aerated lung tissue. This resulted in a significant reduction in gas exchange and the development of hypoxemia [11, 19]. Such changes lead to severe lung damage with a sharp decrease in gas exchange, which requires invasive ventilation and/or extracorporeal membrane oxygenation. In almost all cases of SARS-CoV-2, manifestations of DAD have been described, which involve endothelial and alveolar-capillary epithelial cells accumulating protein-rich fluid in the interstitial and alveolar space, eventually culminating in the hyaline membrane formation and thrombosis of small vessels and capillaries. This development compromises the stabilization of surfactants due to progressive alveolar collapse and increasing deterioration of oxygenation [17]. Despite research and advances in SARS-CoV-2, pathomechanisms responsible for initiating SARS secondary to viral infection have not been fully elucidated.

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
Original research: Clinical sciences

We found that COVID-19 was the single original cause of death in 88.7% of surveyed cases while Rybakova MG et al. [16] reported only 43.0%.

It is known that SARS-CoV-2 enter human organisms through angiotensin-converting enzyme-2 (ACE-2) receptors [18]. ACE-2 is presented in alveolar epithelium, hepatocytes, renal tubular epithelium, biliary epithelium, and enterocytes, respectively the pathological findings in patients with COVID-19 were most significant in the lungs, but were also found in other organs and systems, such as the liver, kidneys, and the lymphatic system [18].

A major acute pathologic finding like in other studies [5, 8, 9, 11, 16] was bilateral DAD, which included lung edema, hyaline membranes, pneumocytes and fibroblast proliferation. In 87% of patients in Borczuk A.C et al. [8] study and 96.5% of cases in our cohort, typical features of DAD were found, at least focally. The most unexpected pathological finding was thrombotic occlusion of pulmonary arteries, which was identified both by rough examination and microscopy. This important finding is a consequence of parenchymal pulmonary hemorrhage and hemorrhagic infarction.

The combination of alveolar and vascular events underlies the rapid, sometimes unpredictable clinical deterioration observed in severe COVID-19, which was also found in our autopsy series.

In previous investigations, a high incidence of venous thrombosis and pulmonary embolism was reported as the death cause in 30.0% of cases [19, 20]. In the study by Wichmann et al. [20], pulmonary microthrombosis was documented in all cases (100%, n=12), and in our cohort, pulmonary embolism was fatal in 14.3% of cases when large vessel thrombi also had microthrombi focally or diffusely in 39.6% and 68.0%. Similar data were reported in the study by Borczuk A.C et al. [8]. The mechanisms of pulmonary artery thrombosis and coagulopathy in patients with COVID-19 remain not fully studied. Lax S. F. et al. [5] consider that there was a causal relationship between inflammatory and reparative processes associated with DAD, as thrombus is often found in small pulmonary arteries, most likely due to

endothelial damage. Endothelial damage may also be associated with direct viral infection of endothelial cells expressing ACE-2 receptors, or with a host reaction, as recently suggested [5, 22]. According to the latest data, D-dimer levels higher than 2500 μg/L become a selection criterion for CT angiography to identify segmental or subsegmental pulmonary artery thrombosis [23]. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS) was proposed as a new terminology for severe pulmonary COVID-19 [5, 24]. However, this term does not describe the clinically important sign of pulmonary artery lesions of the medium and larger size found in the current study. At the same time, the issue of damage to small and medium-sized arteries in COVID-19 is being discussed. Damage to small vessels and capillaries is a manifestation of DIS syndrome, characterized by the formation of obstructing microthrombi and ischemia of visceral organs with the development of necrosis. This fully corresponds to the clinical picture of multiple organ failure in severe cases of the disease. But this explanation does not provide an answer to the cause of damage to medium-sized vessels. This can be explained by the development of vasculitis and endotheliitis, which are described in individual cases [8, 22]. It should be noted that among our cases, there were 12 cases of lymphocytic inflammation in the wall of small arteries, which can be interpreted as a manifestation of coronavirus vasculitis.

Kidneys and liver chronic changes could contribute to fatal outcomes in COVID-19 patients [5]. According to the literature, it is known that acute renal tubular injury associated with hypoxia is the cause of end-stage renal failure and is not caused by viruses due to the high expression of ACE-2 receptors in the tubular epithelium. [5, 25].  And in our cohort, epithelial necrosis was found in kidneys in 43.0% of cases, sometimes with an accumulation of eosinophilic homogeneous masses in the lumens, which correlates with the findings of Borczuk A.C et al. [8].

Acute hepatocyte necrosis, as well as cholestasis, described in patients with COVID-19, should be more associated with hypoxia or sepsis (due to central venous thrombosis and systemic inflammation), and not with the di-

Праці НТШ Медичні науки
2022, Том 69, № 2    ISSN 2708-8634 (print)
Оригінальні дослідження: клінічні науки

Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
ISSN 2708-8642 (online)    2022, Vol. 69, 2
Original research: Clinical sciences

rect viral effect of SARS-CoV-2 [5, 26]. Liver damage as part of the systemic inflammatory response syndrome and sepsis (which was also observed in our patients) may have independent prognostic value due to changes in the secretion of acute phase reagents, cytokines or other mediators of inflammation and coagulation factors and may subsequently contribute to subsegmental and segmental pulmonary thrombosis [27].

Frequent lymphocyte depletion in the lymph nodes and spleen reported with SARS-1 coincides with lymphopenia caused, at least in part, by increased secretion of endogenous cortisol through the hypothalamic-pituitary-adrenal axis associated with hyperplasia

over hyperplasia [5]. Spleen stroma changes were documented in 28.8% of cases with the emptying of the lymphoid tissue T- and B-dependent zones, which was also reported in another study [9].

In conclusion, the conducted study of autopsies of patients in Lviv and Lviv Region corresponds to general world trends regarding clinical and pathomorphological changes in COVID-19. We have confirmed that the target for SARS-CoV-2 is lung damage with the development of DAD and subsegmental and segmental thrombosis of pulmonary arteries, which become lethal. Our histological findings in patients with COVID-19 indicate diffuse alveolar lung damage in the acute exudative,

proliferative, and fibrotic phases of the disease and include 3 main forms of damage: epithelial, vascular, and fibrotic. The combination of these pathomorphological mechanisms may explain the rapid clinical deterioration of the severe form of COVID-19. Severe pulmonary changes described in combination with disseminated thrombosis (a manifestation of DIS syndrome) lead to multiple organ failure involving kidneys, liver, and spleen. The question of the mechanisms of damage to the vascular bed in COVID-19 remains open because thrombotic microangiopathy in DIS syndrome does not explain all the morphological findings. Therefore, our further research will be devoted to the study of vasculitis as a key type of damage to the vascular wall and the leading mechanism of the development of thrombotic complications in COVID-19.

## References

1. Kiss S, Gede N, Hegyi P, Nemet D, Foldi M, Dembrovszky F et al. Early changes in laboratory parameters are predictors of mortality and ICU admission in patients with COVID-19: a systematic review and meta-analysis. Med Microbiol Immunol. 2021; 210: 33-47. https://doi.org/10.1007/s00430-020-00696-w
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J et al.  A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020; 382(8):727-733. https://doi: 10.1056/NEJMoa2001017
3. WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-11-March-2020.
4. https://www.worldometers.info/coronavirus/. Accessed on June 12, 2021
5. Lax SF, Skok K,  Zechner P, Kessler HH, Kaufman N, Koelblinger C et al.  Pulmonary Arterial Thrombosis in COVID-19 with Fatal Outcome. Results From a Prospective, Single-Center, Clinicopathologic Case Series. Annals of Internal Medicine. 2020;173 (5): 350-361. doi:10.7326/M20-2566
6.  Phua J, Weng L, Ling L, Egi M, Lim C-M, Divatia J-M et al. Asian Critical Care Clinical Trials Group. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir Med. 2020; 8:506-517. https://doi.org/10.1016/ S2213-2600(20)30161-2
7. Zhou F, Yu T, Du R, Fau G, Liu Yi, Liu Z et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395:1054-1062. https://doi.org/10.1016/ S0140-6736(20)30566-3
8. Borczuk AC, Salvatore SP, Seshan SV, Patel SS, Bussel JM, Mostyka M et al. COVID-19 pulmonary pathology: a multi-institutional autopsy cohort from Italy and New York City. Modern Pathology 2020; 33:2156-2168. https://doi.org/10.1038/s41379-020-00661-1
9. Samsonova M, Mikhaleva LM, Zaratyanz OV, Varyasin VV, Bykanova AV, Mishnev OD,  et al. Lung pathology with COVID-19 in Moscow. Russian Journal of Archive of Pathology. 2020; 82 (4): 32-40. https://doi.org/10.17116/patol20208204132
10. Nicholls JM,  Poon LL,  Lee KC, Ng WF, Lai ST, Leung CY, Chu CM et al. Lung pathology of fatal severe acute respiratory syndrome. Lancet. 2003; 361:1773-1778. DOI: 10.1016/s0140-6736(03)13413-7

Праці НТШ Медичні науки        Proc Shevchenko Sci Soc Med Sci    www.mspsss.org.ua
2022, Том 69, № 2    ISSN 2708-8634 (print)       ISSN 2708-8642 (online)     2022, Vol. 69, 2
Оригінальні дослідження: клінічні науки           Original research: Clinical sciences

11. Batah SS, Fabro AT Pulmonary pathology of ARDS in COVID-19: A pathological review for clinicians. Respiratory Medicine.2021; 176:106-239. DOI: 10.1016/j.rmed.2020.106239

12. Yoshikawa A, Munkhdelger J, Bychkov A. COVID-19. PathologyOutlines.com website. https://www.pathologyoutlines.com/topic/lungnontumorcovid.html. Accessed April 13th, 2021.

13. Zheng Z, Peng F, Xu B, Zhaoa J, Liuc H, Peng J et al. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. J Infect. 2020; 81(2):e16-e25. DOI: 10.1016/j.jinf.2020.04.021.

14. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020; 180(7):934-943. doi:10.1001/jamainternmed.2020.0994

15. Rees EM, Nightingale ES, Jafari Ya, Waterlow NR, Clifford S, Pearson CAB et al. COVID-19 length of hospital stay: a systematic review and data synthesis. BMC Medicine. 2020; 18:270. https://doi.org/10.1186/s12916-020-01726-3

16. Rybakova MG, Karev VE, Kuznetsova IA. Anatomical pathology of novel coronavirus (COVID-19) infection. First impression. Russian Journal of Archive of Pathology. 2020; 82(5):5-15. https://doi.org/10.17116/patol2020820515

17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020; 323(11):1061-1069. doi:10.1001/jama.2020.1585

18. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020; 181:271-280.e8. DOI: 10.1016/j.cell.2020.02.052

19. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao S-Yu. Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. J Thorac Oncol. 2020; 15:700-704. DOI: 10.1016/j.jtho.2020.02.010

20. Wichmann D, Sperhake JP, Lütgehetmann M, Steurer S, Edler C, Heimemann A et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Ann Intern Med. 2020; 173(4):268-277. doi:10.7326/M20-2003

21. Bösmüller H, Matter M, Fend F, Tzankov A. The pulmonary pathology of COVID-19. Virchows Archiv. 2021; 478:137-150. https://doi.org/10.1007/s00428-021-03053-1

22. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020; 395:1417-1418. https://doi.org/10.1016/ S0140-6736(20)30937-5

23. Leonard-Lorant I, Delabranche X, Severac F, Helms J, Pauzet C, Collange O et al. Acute pulmonary embolism in COVID-19 patients on CT angiography and relationship to D-dimer levels. Radiology. 2020; 20:15-61. https://doi.org/10.1148/radiol.2020201561

24. Ciceri F, Beretta L, Scandroglio AM, Colombo S, Landoni G, Ruggeri A et al. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. Crit Care Resusc. 2020; 15;22(2):95-97.

25. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin-converting enzyme. FEBS Lett. 2002; 532:107-10. DOI: 10.1016/s0014-5793(02)03640-2

26. Horvatits T, Drolz A, Trauner M, Fuhrmann V. Liver injury and failure in critical illness. Hepatology. 2019;70:2204-2215. DOI: 10.1002/hep.30824

27. Sun J, Aghemo A, Forner A, Valenti L. COVID-19 and liver disease. Liver Int. 2020; 40(6):1278-1281. https://doi.org/10.1111/liv.14470