Slovenian Medical Journal

Pathological lung changes in COVID-19 deceased patients

Patološke spremembe v pljučih pri umrlih bolnikih s covidom-19

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Key words:
COVID-19; lungs; pathology; autopsy

Ključne besede:
covid-19; pljuča; patologija; obdukcija

Received: 27. 4. 2020
Accepted: 25. 6. 2020

Abstract

Background: COVID-19 disease causes diffuse alveolar damage associated with a high mortality rate. The aim of the analysis of deceased patients with SARS-CoV-2 virus infection is to assess histopathological changes in the lungs as the key target organ in this infection.

Methods: We performed partial autopsies on patients with COVID-19 treated at the University Hospital of Respiratory and Allergic Diseases Golnik. We assessed various histopathological changes in the lungs of deceased patients with COVID-19 disease. We performed immunohistochemical stainings to prove the presence of SARS-CoV-2 virus in lung samples.

Results: The main histopathological finding was diffuse alveolar damage with hyaline membrane formation, interstitial and alveolar oedema and fibrinous exudation in the alveoli. Interstitial inflammatory infiltration was mild to moderate. In some patients, alveolar damage was partly organized, but without the presence of fibrosis. We found cytopathic changes of the alveolar epithelium consistent with viral infection in all patients. We found the presence of virus in lung samples of all patients.

Conclusion: COVID-19 disease affects the lungs, causing diffuse alveolar damage, which can lead to death. Autopsy still plays an important role in modern medicine, giving a contribution to the understanding of new diseases.

Izvleček

Izhodišče: Bolezen covid-19 povzroča difuzno alveolno okvaro, povezano z visoko stopnjo umrljivosti. Namen analize umrlih bolnikov, okuženih z virusom SARS-CoV-2, je oceniti histopatološke spremembe v pljučih kot glavnem tarčnem organu pri tej okužbi.

Metode: Opravili smo delne obdukcije bolnikov s covidom-19, ki so se zdravili na Univerzitetni kliniki za pljučne bolezni in alergijo Golnik. Ocenjevali smo izraženost različnih histopatoloških sprememb v pljučih umrlih. Z imunohistokemično metodo smo dokazovali prisotnost virusa SARS-CoV-2 v vzorcih pljuč.

Rezultati: V pljučih so izstopali znaki akutne alveolne okvare s tvorbo hialinih membran, intersticijskim in alveolnim edemom ter fibrinsko eksudacijo v alveolih. Spremljajoča vnetna reakcija je bila blago do zmerno izražena. Pri nekaterih bolnikih je bila alveolna okvara že v fazi organizacije, vendar brez prisotne fibroze. Pri vseh bolnikih smo našli citopatsko spremenjen alveolni epitel kot znak virusne okužbe celic. V vzorcih pljuč vseh bolnikov smo dokazali prisotnost virusa.

Zaključek: Bolezen covid-19 prizadene pljuča in povzroči difuzno alveolno okvaro, zaradi katere bolniki lahko umrejo. Obdukcija se je izkazala za pomembno tudi v svetu zaradi raziskovanja nove bolezni.

Cite as/Citirajte kot: Vlačić G, Kovačević M, Kern I. Pathological lung changes in COVID-19 deceased patients. Zdrav Vestn. 2020;89(11–12):603–13.

DOI: https://doi.org/10.6016/ZdravVestn.3097

1 Introduction

The COVID-19 disease pandemic, which started in December 2019 in China and spread around the world in a few months, presents a major challenge for healthcare systems globally.

Following the declaration of the COVID-19 disease epidemic in Slovenia, the University Clinic of Respiratory and Allergic Diseases Golnik (UCG) started diagnosing and treating COVID-19 patients.

COVID-19 disease affects the respiratory tract and leads to respiratory distress with a high mortality rate in a small number of patients. Most studies published during the pandemic deal with the microbiology, epidemiology, clinical presentation and treatment of the disease. Pathology publications are scarce, making this disease still insufficiently explored from a pathological point of view. The biggest question remains whether patients with COVID-19 disease die from or with the disease (1).

Although the Slovenian guidelines for dealing with the COVID-19 epidemic do not recommend carrying out autopsies on patients who have died from COVID-19, we believed that due to the fairly unknown pathogenetic mechanisms and morphological characteristics of the disease, a thorough analysis of autopsy samples was needed (2). All available publications to date analyzed single cases or small series of patients (3,4). The first reports of histological changes in the lungs of deceased patients with COVID-19 disease were based on core-needle biopsies of the lung performed post mortem (4). The most extensive study to date analyzed autopsy samples of 21 patients who died with COVID-19 (5). Even less data is available about lung pathological changes in living patients with confirmed SARS-CoV-2 infection. In fact, the very first data on histopathological changes in the lungs of SARS-CoV-2-positive patients were from lung resections due to tumours, with SARS-CoV-2 infection confirmed afterwards (6). In the majority of cases, the leading pathological change is diffuse alveolar damage, in different phases of development, with subsequent cytopathic changes of the alveolar epithelium directly attributed to viral infection. Some authors describe frequent findings of pulmonary thromboembolism in patients who died from COVID-19 disease (7,8). Endothelial viral infection of lung blood vessels is also considered a possible pathogenetic mechanism of COVID-19 disease (5,9).

To analyze the histopathological characteristics of COVID-19 disease in the lungs, we examined autopsy lung samples taken from patients who died with confirmed SARS-CoV-2 infection. Autopsies, sampling, sample processing and analysis were performed in the UCG Cytology and Pathology Laboratory.

2 Materials and methods

Partial autopsies were performed on patients who died in UCG in the two months after the COVID-19 disease epidemic was declared in the Republic of Slovenia. SARS-CoV-2 infection was confirmed with real-time polymerase chain reaction and reverse transcription performed on nasopharyngeal swabs taken from subsequently hospitalized living patients. Autopsies were performed according to the legal regulations of the Republic of Slovenia. Autopsies were performed at least 24 hours after the patient's death. The entire right lung was removed together with left heart ventricle and right liver lobe samples. Secondary sampling was performed after 48 hours of fixation in 10% neutral buffered formalin. Five samples were systematically taken from every lobe of the right lung. The tissue samples were embedded in paraffin after 72 hours of fixation. Histological samples were routinely stained with haematoxylin-eosin (HE) and examined on a light microscope.

Within the histological examination, we assessed the presence of interstitial and alveolar oedema, interstitial inflammatory infiltrate, intra-alveolar fibrinous and inflammatory exudate, hyaline membranes, reactive changes of the alveolar epithelium, organizing inflammation and airway inflammation. Most of the morphological changes were assessed semiquantitatively with a 4-tier scale (- absence of changes; + mild/focal; ++ moderate; +++ severe). Inflammatory infiltration with neutrophils and lymphocytes was assessed qualitatively.

Special attention was given to changes of the alveolar epithelium, as they are a potential sign of the presence of the virus in the cells. This is the so-called cytopathic effect, which includes enlargement of cells and nuclei, foamy and/or coarsely vacuolized cytoplasm, multiple nuclei, ground glass nuclei and coarse chromatin. Other observed histopathological changes were noted separately.

Additional immunohistochemistry was performed on the automated stainer Benchmark XT (Roche, USA). Subtyping of immune cells in mononuclear cell infiltrates was performed using the following antibodies: CD20 (lymphocytes B), CD3 (lymphocytes T), CD4 (T helper cells), CD8 (T killer cells) and CD163 (macrophages). We used anti-surfactant protein A antibody to mark the alveolar epithelium. We assessed the virus's presence in different cells using rabbit antibody SARS-CoV-2 (2019-nCoV) anti-nuclear virus protein (clone 019, titer 1:500, Sino Biological, China). Dot-like and diffuse positive cytoplasmic staining were considered positive.

The study was approved by the Committee for Medical Ethics of the Republic of Slovenia on 7 May 2020 (decision no. 0120-201/2020/7).

3 Results

3.1 Patients

Seven patients (two female, five male) were included in the study. All patients were treated at UCG for confirmed SARS-CoV-2 infection, died during hospitalization and were taken to our lab for autopsy. The average age was 81.7 years (range 68 – 91). Average hospitalization time was 16 days (range 3 – 28). All patients had multiple chronic diseases. Cardiovascular disease was the most frequent, being present in six patients. Two patients had diabetes mellitus type 2, one of whom was insulin-dependent. Three patients had dementia, one paranoid schizophrenia, one parkinsonism and one epilepsy. Two patients had active malignant diseases. Two patients had chronic lung disease (asthma and

Table 1: Patient data.

Patient	Days of hospitalization	Age	Sex	Comorbidities
1	3	91	F	HF, AF, AH
2	22	81	M	Dementia, immobility, aspiration pneumonia
3	21	80	M	St post biliary pancreatitis and sepsis, AF, HF, parkinsonism, COPD
4	8	90	F	HF, DM, dementia, asthma, st. post CVI
5	28	68	M	Small cell lung carcinoma (cT3N3M1b), AH, DM, schizophrenia, sepsis
6	12	74	M	Dementia, HLP, epilepsy, AH, immobility
7	19	88	M	Prostate cancer, AH, HLP, fibrothorax

Abbreviations: F – female, M – male, HF – heart failure, AF – atrial fibrillation, AH – arterial hypertension, COPD – chronic obstructive pulmonary disease, DM – diabetes mellitus, CVI – cerebrovascular insult, HLP - hyperlipidaemia

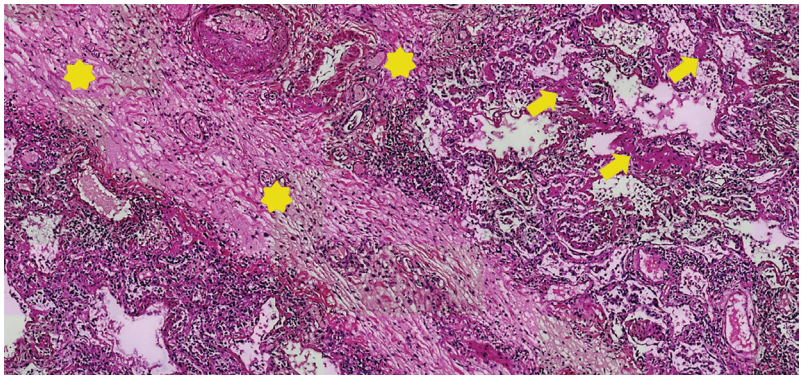

Figure 1: Interstitial oedema (marked with asterisks) and hyaline membranes (marked with arrows) (HE, 4x).

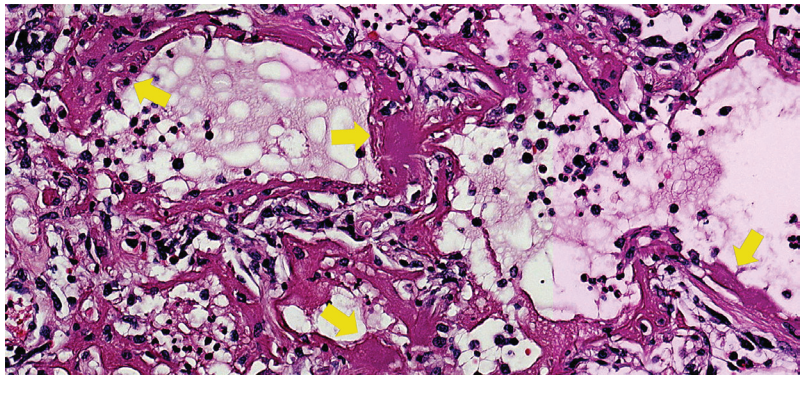

Figure 2: Hyaline membranes (marked with arrows) (HE, 20x).

chronic obstructive lung disease). Two patients were previously treated for acute conditions (biliary pancreatitis with sepsis, and sepsis of unknown origin, respectively). One patient was clinically diagnosed with aspiration pneumonia as a complication of COVID-19 treatment. Clinical data are shown in Table 1.

3.2 Histopathological changes in the lungs

We found interstitial oedema in six patients (Figure 1); only one patient did not have any signs of interstitial oedema. All patients had congestion of lung blood vessels and alveolar oedema. Interstitial inflammatory infiltration was found in all patients. We graded it as mild to moderate, and in all patients, it consisted of lymphocytes. Distribution of lymphocytes T and B was equal. There was an equal proportion of T helper and killer cells. An increased number of interstitial and alveolar macrophages was observed in all patients. Severe, moderate and mild

hyaline membrane formation and fibrinous exudate in the alveoli were found in four, one and one patient, respectively (Figures 1 and 2). We did not find hyaline membrane formation and fibrinous exudate in the alveoli in the lungs of one patient with small cell carcinoma.

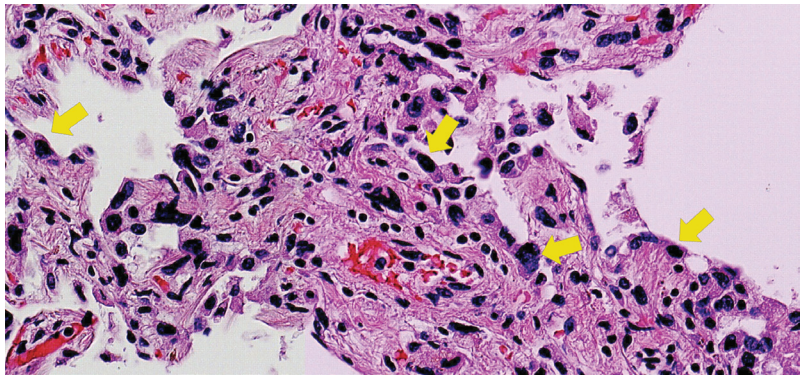

Figure 3: Severe reactive changes (marked with arrows) of alveolar epithelial cells (HE, 40x).

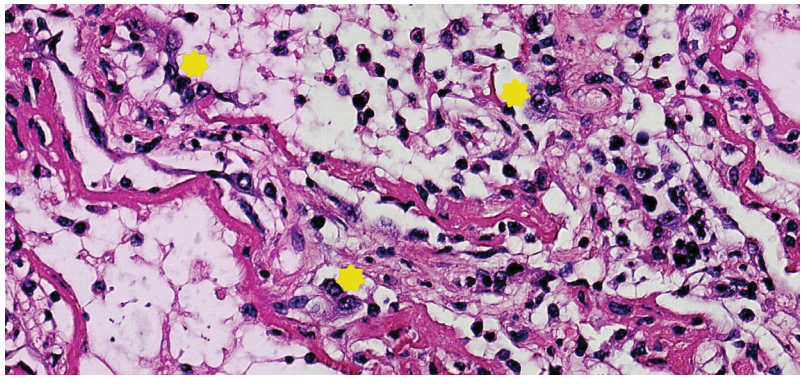

Figure 4: Severe cytopathic changes (marked with asterisks) of alveolar epithelial cells (HE, 40x).

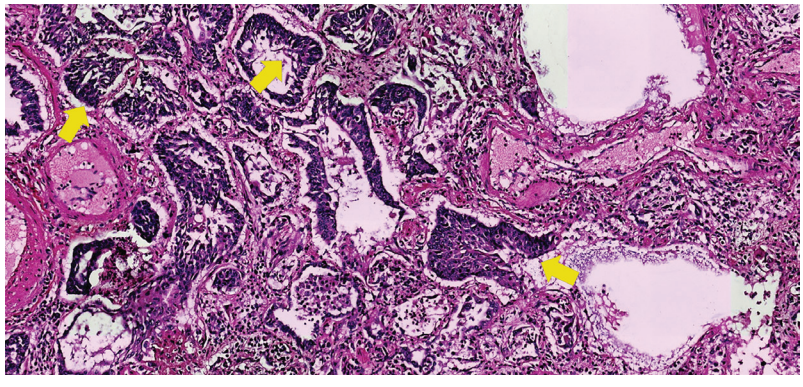

Figure 5: Squamous cell metaplasia in the alveoli (marked with arrows) (HE, 4x).

Reactive changes of the alveolar epithelium with marked cytopathic changes were found in six patients (Figures 3 and 4). The alveolar epithelium was mostly exfoliated, with a marked proliferation of type 2 pneumocytes. Severe, moderate and mild alveolar exudative inflammation was present in one, three and three patients, respectively. Qualitatively, the exudate consisted mostly of neutrophils in the severe inflammation case, mixed cellularity in moderate and lymphocytes-macrophages combination in mild cases. Moderate signs of organizing inflammation (interstitial and alveolar fibroplasia) were present in three patients. Severe, moderate and mild airway inflammation was present in one, four and two patients, respectively. Qualitatively, we observed the presence of neutrophils in patients with severe and moderate airway inflammation. Lymphocytes were present in one patient with moderate airway inflammation. Both patients with mild airway inflammation had lymphocytic inflammatory infiltrate. Squamous metaplasia was present in all patients, with predominant peribronchial distribution (Figure 5). We observed foreign material with associated foreign body granulomatous reaction in the small airways of three patients, which was considered a reliable sign of aspiration pneumonia. We found thromboemboli in the small branches of the pulmonary artery in three patients. One patient had signs of intra-alveolar haemorrhage, with erythrocyte extravasation and abundant siderophages in the alveoli. All semi-quantitative histology assessment results are shown in Table 2.

Using immunohistochemistry, we confirmed the presence of SARS-CoV-2 in the lungs of all patients. A positive reaction was mostly observed in the alveolar epithelium, macrophages and endothelium of small blood vessels (Figure 6).

Table 2: Histopathological changes in the lungs. The number of patients with appropriate assessment of intensity of the changes is noted.

	No. changes	Mild changes (+)	Moderate changes (++)	Severe changes (+++)
Interstitial oedema	0	1	2	4
Interstitial inflammation	0	3	4	0
Alveolar inflammation	0	3	3	1
Airway inflammation	0	2	4	1
Hyaline membranes, fibrinous exudate	1	1	1	4
Reactive alveolar epithelium	1	1	3	2
Organizing inflammation	4	0	3	0

4 Discussion

The main pathological change observed in the lungs of autopsied patients who died of viral SARS-Cov-2 infection was the presence of hyaline membranes and fibrinous exudate in the alveoli, the hallmarks of acute lung injury. All deceased patients treated at UCG for COVID-19 disease were elderly and had multiple comorbidities. Viral infection was confirmed in the alveolar epithelium and macrophages. Compared to the published results of Chinese studies performed with post mortem core-needle biopsy of the lung (4), our study has the advantage of systematic sampling and examination of the entire lung, which excludes possible sampling errors. We focused on histopathological changes in the lungs, which was the first goal of this analysis, albeit we took samples from other organs as well.

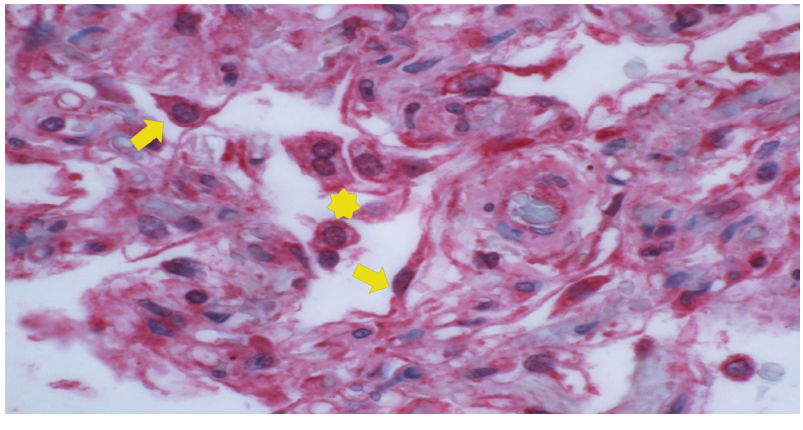

Figure 6: Immunohistochemical confirmation of SARS-CoV-2 virus presence in alveolar epithelial cells (marked with arrows) and macrophages (marked with asterisks)(40x).

The cause of death in almost all of the patients who died from COVID-19 in our analysis was diffuse alveolar damage. We found hyaline membranes and fibrinous exudate in the alveolar spaces and some other signs of the exudative phase of diffuse alveolar damage, such as senescence and reactive changes of the alveolar epithelium, first described in early reports from Chinese authors (4,10). In the lung samples of some patients, we found signs of early organization of diffuse alveolar damage with mild thickening of the alveolar septa due to interstitial fibroplasia and foci of intra-alveolar fibroplasia. We did not find signs of advanced fibrosis in any of our cases. We observed foci of alveolar squamous metaplasia without cell atypia in all our cases which, combined with marked organization of inflammation, confirms the presence of the proliferative phase of diffuse alveolar damage, as demonstrated in other large series of patients (5,7). The extent of the inflammatory reaction in the lung parenchyma was surprisingly small, showing mild to moderate interstitial lymphatic infiltration composed of B and T lymphocytes, the proliferation of macrophages in the alveoli and interstitium and mild to moderate granulocytic exudate in the alveoli. Lymphatic infiltration is most probably caused by the viral infection itself (4,6). Some patients had bronchopneumonic exudative inflammatory

changes, which were limited to the airways and surrounding lung parenchyma and were most probably caused by aspiration or bacterial superinfection. Other authors also reported aspiration bronchopneumonia in a number of cases (11). Almost half of the patients in the Swiss study died of bronchopneumonia caused by bacterial superinfection (5). Among other pathological changes observed in our study, it is worth mentioning the congestion of blood vessels, alveolar oedema and, in one patient, marked alveolar haemorrhage. We found lung thromboembolism in three out of seven cases. Blood clots were found in small-calibre lung arteries, possibly due to advanced coagulopathy seen in severe COVID-19 disease (7). Two studies reported more frequent findings of lung thromboembolism. The German series reports lung thromboembolism in one third of patients, which is comparable to our results (7). Microthrombosis was found in all 12 patients, which is similar to the Swiss series (5). In the published study conducted by Austrian colleagues, which reported thrombosis of the pulmonary arteries in all 11 patients, we found the presented photomicrographs of blood clots unconvincing, as the changes presented there most probably show post mortem clotting (8). They propose that in-life forming of blood clots is directly connected with the viral infection of endothelial cells, which present on their surface receptors for SARS-CoV-2 (9). The histopathological changes found in the lungs of patients who died with COVID-19 disease are not pathognomonic for SARS-CoV-2 viral infection. Equal changes were described in the lung samples of patients infected with other coronaviruses (SARS-CoV and MERS-CoV) and also with other viral infections. Ackermann et al. compared the histopathological changes in the lungs of patients who died of COVID-19 disease to those who died of influenza (H1N1). They found signs of diffuse alveolar damage and mild interstitial inflammatory infiltration in both, while endothelial changes and microthromboses were more frequent in COVID-19 disease (12). All findings in the lungs of patients who died with COVID-19 reported to date, including in our analysis, represent an advanced, severe course of infection (Table 3). Among the first published cases of patients infected with SARS-CoV-2 are two patients who had lung cancer surgery (6). Resected lung samples of both patients showed changes consistent with early acute lung injury, with alveolar oedema, fibrinous exudate, and mild inflammatory infiltrate, but without the formation of hyaline membranes. One of the patients in our analysis, who was previously diagnosed and treated for disseminated small cell lung carcinoma, also showed mild histopathological changes in the lung; thus, we concluded that he died of his primary malignant disease, with concomitant COVID-19 disease.

All patients who died with COVID-19 disease had in-life infection with the SARS-CoV-2 virus, confirmed with a nasopharyngeal swab. We confirmed the viral SARS-CoV-2 infection with the immunohistochemical technique and marked cytopathic changes of the alveolar epithelium in all our patients. For immunohistochemical confirmation of SARS-CoV-2 infection, we used antibody against antigen on the nuclear protein of the virus, similarly as in two other studies, which together included three patients (14,15). Confirmation of the presence of the virus in formalin-fixed paraffin-embedded tissue is also possible with in situ hybridization techniques (16). Most publications used real-time polymerase chain reaction with reverse transcription to confirm viral infection

Table 3: Review of published histopathological changes in the lungs in the literature available in English.

Author	Number of patients	Living patient/post mortem	Sampling method	Main findings
Tian S et al (4)	4	Post mortem	Core-needle biopsy	HM, pneumocyte type 2 hyperplasia, OP
Barton LM et al (11)	2	Post mortem	Autopsy	DAD, bronchopneumonia, aspiration, chronic bronchitis, oedema
Tian S et al (6)	2	Living patient	Lobectomy	Oedema, pneumocyte type 2 hyperplasia, interstitial inflammation, multinucleated giant cells
Xu Z et al (10)	1	Post mortem	Autopsy	DAD, interstitial inflammation, reactive pneumocytes type 2, cytopathic effect, aspiration
Zeng Z et al (12)	1	Alive patient	Lobectomy	Exudative inflammation, multinucleated giant cells, pneumocyte type 2 hyperplasia, cytopathic effect
Lacy JM et al (20)	1	Post mortem	Autopsy	HM, interstitial inflammation, pneumocyte type 2 hyperplasia, alveolar haemorrhage
Wichmann D et al (7)	12	Post mortem	Autopsy	DAD, pneumocyte type 2 hyperplasia, organization, bronchopneumonia
Lax S et al (8)	11	Post mortem	Autopsy	DAD, pulmonary thromboembolism, oedema, pneumocyte type 2 hyperplasia
Menter T et al (5)	21	Post mortem	Autopsy	DAD, bronchopneumonia, pulmonary thromboembolism, alveolar haemorrhage
Konopka KE et al (3)	1	Post mortem	Autopsy	DAD, interstitial inflammation, pneumocyte type 2 hyperplasia
Adachi T et al (14)	1	Post mortem	Autopsy	DAD, organization, squamous cell metaplasia, pneumocyte type 2 hyperplasia
Yao et al (24)	3	Post mortem	Minimally invasive autopsy	HM, lung fibrosis, interstitial inflammation
Golnik	7	Post mortem	Partial autopsy	HM, pneumocyte type 2 hyperplasia, interstitial oedema, squamous cell metaplasia, organization, bronchopneumonia

Abbreviations: HM – hyaline membranes, OP – organizing pneumonia, DAD – Diffuse alveolar damage

(4,5,7,15). Due to the rapid degradation of RNA post mortem, the above-mentioned method can be problematic in samples obtained during autopsy, although it should be considered in the case of autopsy of patients who died of respiratory failure of unknown aetiology. This method could also be useful in incidental findings of acute lung injury in tissue samples from living patients. The presence of the virus was also confirmed with electron microscopy, showing viral particles in cytoplasmic vesicles bound to the cell membrane and floating free in the alveolar spaces (5,9,17). Cytopathic changes of the alveolar epithelium observed in histological samples of the lung included cell enlargement, foamy and coarsely granular cytoplasm, enlarged nuclei, multiple nuclei, ground

glass change of nuclei and coarse chromatin. Other authors described similar changes (17). We could expect to see the cytopathic changes of alveolar epithelial cells listed above in different types of samples obtained from living patients with COVID-19 disease, i.e. in bronchoalveolar lavage. Due to their similarity to tumour cells, these could pose a diagnostic challenge for a pathologist. We are familiar with cytopathic changes in different viral infections of the lung (adenovirus, respiratory syncytial virus, influenza virus, parainfluenza virus, cytomegalovirus, herpes simplex virus, measles virus). For some of them, we can identify relatively specific cytomorphological changes, while for the vast majority of them, there is a considerable overlap of changes and confirmation of the presence of the virus in the sample is mandatory. We found similar changes in the lung samples from our patients who died with COVID-19. There is an interesting publication of changes found in bronchoalveolar lavage obtained from a patient put on extracorporeal membrane oxygenation (18). A markedly increased level of activated plasma cells was observed between macrophages in lymphocytes, which was discordant with histopathological changes known to date.

In the time of the COVID-19 disease pandemic, autopsy has regained its fundamental purpose in exploring this new, previously unknown disease. According to recommendations published in the Republic of Slovenia for handling patients who have died from COVID-19 disease, autopsy was not recommended. Post-mortem contagiousness of patients who died with COVID-19 disease is not known, although the virus is included in the high-risk group of viruses, similar to SARS-CoV, MERS-CoV, HIV, rabies virus, and hepatitis B, C and D viruses (19). Because there were no published data on histopathological changes in the lung caused by the SARS-CoV-2 virus at the time the epidemic was declared, we decided to perform autopsies on patients who died of COVID-19 in UCG. To prevent excessive risk of infection of employees, we performed partial autopsy with in situ sampling of the lungs, liver and heart. We performed the autopsies according to the protocol based on international recommendations for performing autopsies of patients infected with SARS-CoV-2 (20-23). Only two persons were present at the autopsy, the pathologist and autopsy assistant, both using personal protective equipment. To avoid the formation of aerosol, we did not use water and an oscillatory saw. Subatmospheric air pressure and appropriate ventilation with more than 15 cycles of air change per hour were provided.

During the COVID-19 epidemic, we faced a challenge in successfully protecting the staff in pathology laboratories who manage fresh, non-fixed cytological and histological samples from the airway of living patients. Since we did not have specific guidelines for that situation in the Republic of Slovenia, we successfully implemented international guidelines, which included the use of personal protective equipment (FFP2 masks, goggles or glasses, nitrile gloves) when handling and processing non-fixed cytological samples, especially with centrifugation (24). By following the guidelines, we did not record any cases of infection among our laboratory personnel. We adjusted our work in the autopsy room and the laboratory to the possibility of unrecognized SARS-CoV-2 virus infection.

5 Conclusion

The lungs are the main target organ in severe COVID-19 disease, presenting as diffuse alveolar damage. The clinical and pathological characteristics of deceased patients in this analysis confirm the data previously known from the literature and contribute to understanding the pathogenesis of SARS-CoV-2 infection. We proved the importance of performing autopsies on patients who died from previously unknown diseases in modern times.

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