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Morphopathological features induced by SARS-CoV-2 infection - a series of 57 autopsies

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Summary. Background. COVID-19 is a systemic disease with multiorgan damage, which requires a better understanding and deepening of histopathogenesis in order to improve treatment. Autopsy remains a gold standard to establish certain diagnoses and to integrate the morphological spectrum of lung lesions, explaining the cause of death, into a clinical context.

Methods and Results. The study included 57 autopsies performed during 2020-2021 associated with SARS-CoV-2 infection. Among the autopsies we performed, diffuse alveolar damage (DAD) was the most common pulmonary morphological change, 31.8% of them with acute or exudative phase and 33.3% with proliferative phase of DAD. Acute fibrous organizing pneumonia or organizing pneumonia with fibrous remodeling processes and pulmonary fibrosis were rarely observed. The most unfavorable outcome and death associated with SARS-CoV-2 infection was frequent in older men, with a high rate of comorbidities. Microscopically, SARS-CoV-2 presents many common aspects with MERS-CoV and SARS-CoV-1, such as alveolar hyaline membrane, desquamated alveolar cells, alveolar edema and alveolar and interstitial lymphocyte and monocytes infiltration.

Conclusions. Our study includes a large number of autopsies on patients with SARS-CoV-2 infection performed in Romania. COVID 19 associated pneumonia combines classical aspects of alveolar and interstitial pneumonia with some peculiarities. Autopsies are of major importance in understanding SARS-CoV-2 infection.

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Introduction

COVID-19 is a systemic disease with multiorgan damage, which requires a better understanding and deepening of histopathogenesis in order to improve treatment. On this line, autopsy remains the basic method both as a gold standard to establish certain diagnoses and also for research purposes to determine the mechanisms of the disease. Although the respiratory system and the immune system are mainly affected, extrapulmonary localizations have also been observed, especially in the elderly and those with dementia or other associated diseases such as hypertension, diabetes and renal failure. The etiological agent, SARS-CoV-2 virus, is an RNA-type virus that causes an immune response, with significant secretion of cytokines and chemokines, causing acute respiratory distress syndrome and multiple organ failure. This explains why infected patients have mild symptoms at the onset of the disease, with a sudden worsening during hospitalization, as a result of the "cytokine storm" in the body. Even if several studies have tried to explain the pathogenesis of the disease, however, certain pathophysiological mechanisms and morphological aspects are not fully elucidated. Although details of the cellular responses to this virus are not known, a probable course of events can be postulated based on past studies with SARS-CoV (Wu et and McGoogan, 2020; Deshmukh et al., 2021).

Autopsies also represent an important component of integrating the morphological spectrum of lesions, explaining the cause of death, into a clinical context and a core activity in the practical aspect of the pathology experience. The rate of autopsies has decreased significantly in recent years, due to the more complex legislation regarding human tissue examinations.

Autopsy, with its gross and microscopic examination of the tissue samplings still plays a critical role in the diagnosis and in uncovering the pathophysiology of new diseases, like COVID-19, and also in the control and prevention of infectious diseases.

The most frequent histopathological expression of the disease in the lungs is diffuse alveolar damage (DAD). It is represented by permanent damage of capillary endothelial cells and alveolar epithelial cells, with extravasation of protein-rich fluid into the interstitial and alveolar space, with hyaline membrane formation and, in some cases, with capillary thrombosis. This mechanism disturbs the surfactant stabilization, causing alveolar collapse and poor oxygenation and, as a consequence, a wide spectrum of histopathological lesions result in lung parenchyma of the patients with COVID 19 (Batah and Fabro, 2021).

Certainly, besides the usual questions that forensics are used to during autopsies, once the COVID-19 pandemic started, new questions and information about this infection and tanatogenesis emerged. Is the SARS-CoV-2 infection the cause of death? Is it just a circumstantial or favoring factor? Is it a special condition? Starting from these questions whose answers are defining legally, medically and scientifically, we analyzed 57 cases of SARS-CoV-2confirmed infections, autopsied in 2020 and 2021.

The main aim of the present study is to highlight our observations on the pathology of COVID-19 in lung tissue, focusing on the respiratory tract, and other parenchymal organs, based on complete autopsies in individuals who died in South Eastern Romania during the COVID-19 pandemic and were found to be positive for SARS-CoV-2. The second objective is to compare our results with other similar studies, reviewing the existing literature to date.

Materials and methods

The study included 57 autopsied patients in the Forensic Medical Service of the County Emergency Clinical Hospital "St. Apostle Andrei" Constanta, during 2020 and 2021, all of the cases being associated with SARS-CoV-2 infection, confirmed by RT-PCR through nasopharyngeal swab in their lifetime or through postmortem lung puncture. Since the Romanian authorities recommended not to autopsy SARS-CoV-2 patients except for legislative or research reasons, these forensic autopsies targeted patients with either confirmed or suspected SARS-CoV-2 infection within a certain legislation spectrum such as sudden death, violent death, foreign citizens deceased on Romanian territory, or complaints about medical care and death shortly after hospitalization. These situations offered the possibility to study pathological changes that occurred due to SARS-CoV2 infection. All the forensic autopsies, including tissue sampling (lung) for RT-PCR testing, were conducted according to the guidelines for the collection, handling, and analysis of clinical specimens that might contain SARS-CoV-2, initially based on previous recommendations for SARS-CoV-1 or MERS-CoV, released by Disease Control and Prevention (CDC) and the World Health Organization and adopted by the Romanian authorities (World Health Organization 2020).

Full thoracic and abdominal autopsies were performed in all cases, photos taken during autopsies and sampled fragments were removed from every pulmonary lobe, trachea, heart, brain, kidney, liver and spleen. The fragments were fixed in 10% formaldehyde solution, embedded in paraffin, sectioned at five microns and stained with hematoxylin-eosin in order to be microscopically analyzed. From the clinical point of view, parameters such as patient gender, age, personal and pathological history, smoking history, associated diseases, and symptoms such as cough, dyspnea, and ethnicity were considered.

The data obtained were statistically analyzed using IBM SPSS Statistic 26. Descriptive statistics were conducted with mean and standard deviation for continuous variables and frequency (percentage) for categorical variables.

Results

All of the 57 autopsies were performed by the Legal Medicine department on average two days after death. Regarding the studied group, different parameters were analyzed, both at pulmonary and extrapulmonary level and values are summarized in table I. The group consists of 38 males and 19 women patients with a median age of 67.98±14.19 years old (yo), but with different ranges between males and females (27-95 yo in males and 51-92 yo in females). All the cases had SARS-Cov2 infection confirmed by RT-PCR test.

The majority of the deceased (65%) had preexisting conditions most frequently represented by diabetes, hypertension, heart failure, chronic active kidney disease and obesity. COVID-19 as a cause of death was considered in 42 cases of pneumonia and three cases with morpho-pathologically bronchopneumonia. Most of the patients (45 cases) were either home quarantined or hospitalized for severe respiratpry symptoms associated with SARS-CoV-2 infection, who died within 1 to 3 days from diagnosis or hospitalization. The other patients died either from severe trauma (eight cases) or due to various neoplasms (four cases), all with superimposed SARS-CoV2 infection (Table 1).

On gross examination, most of the cases showed severe lung pathology with aspects of interstitial pneumonia (42 cases), and bronchopneumonia (three cases). Mostly, the lungs were enlarged and heavy (Fig. 1A) with areas of hemorrhage (Fig. 1B).

The inflammatory lung injuries were accompanied by thrombi in septal vessels in three cases. Perihilar lymph nodes were often encountered (15 cases) as well as splenic changes such as enlarged spleen with hemorrhage and stasis coexisting in eight cases. Myocardial infarction (at least six hours old) was

associated and confirmed microscopically in three cases. In addition to the above-mentioned changes, no other remarkable lesions were observed besides organ degeneration in the context of the preexisting conditions (documented in 19 of the studied cases).

Microscopic evaluation was performed on samples taken during the autopsy of the 57 cases included in our study: lung (100%), brain (98.2%), liver (98.2%), heart (96.5%), kidney (96.5%), spleen (47.4%), lymph nodes (23.6%), pancreas (15.8%), prostate (8.8%) and bladder (7.0%).

Histopathological features of the lungs were evaluated on different anatomical areas: airways, alveoli and vascular bed. Diffuse alveolar damage (DAD) was the most common pulmonary morphological change observed in 45 cases, the vast majority of them being characterized by morphological features specific to the acute or exudative phase (31.8%) (Fig. 2A,B) and for the proliferative phase of DAD (33.3%) (Fig. 2C,D). Acute fibrinous organizing pneumonia (AFOP) or organizing pneumonia (OP) with fibrous remodeling processes and pulmonary fibrosis were observed in three cases, all of them being associated with DAD (Fig. 3A-E). The fibrotic phase of DAD was rarely encountered, being identified in five cases (Fig. 3F).

Intra-alveolar exudate with presence of a large number of macrophages was observed with a much higher frequency (73.3%) than a serous-fibrinous one (26.7%). Hyaline membranes were identified in 34 of the cases (59.6%). Alveolar hemorrhage was encountered in six cases (13.3%). The alveolar spaces were mostly filled with macrophages (43.9%), numerous de-squamated pneumocytes (75.4%) often with syncytial giant cell formation (31.6%) (Fig. 4A). Pneumocytes

Table 1. The main clinical and morphological changes in the lung parenchyma of patients with SARS-CoV-2 infection.

		M N=38 (%)	F N=19 (%)
Age (yo)	Range	27-92	32-89
	Mean ± SD	66.55±13.68	70.84±15.13
Diffuse alveolar damage	DAD -acute/exudative	12 (31.6%)	9 (47.4%)
	DAD proliferative	13 (34.2%)	6 (31.6%)
	DAD -chronic/fibrotic	4 (10.5%)	1 (5.3%)
	No	9 (23.7%)	3 (15.8%)
Intra-alveolar exudate	Serous	26 (68.4%)	15 (78.9%)
	Fibrinous	11 (28.9%)	3 (15.8%)
	No	1 (2.6%)	1 (5.3%)
Pulmonary edema	Yes	18 (47.4%)	10 (52.6%)
	No	20 (52.6%)	9 (47.4%)
Pulmonary congestion	Yes	29 (76.3%)	16 (84.2%)
	No	9 (23.7%)	3 (15.8%)
Intra-alveolar desquamative pneumocytes	Yes	31 (81.6%)	12 (63.2%)
	No	7 (18.4%)	7 (36.8%)
Cytopathic changes of pneumocyte II	Yes	9 (23.7%)	5 (26.3%)
	No	29 (76.3%)	14 (73.7%)
Intra-alveolar macrophages	Yes	15 (39.5%)	10 (52.6%)
	No	23 (60.5%)	9 (47.4%)
Intra-alveolar giant cells	Yes	11 (28.9%)	7 (36.8%)
	No	27 (71.1%)	12 (63.2%)
Intra-alveolar PMN	Yes	20 (52.6%)	12 (63.2%)
	No	18 (47.4%)	7 (36.8%)
Alveolar hyaline membranes	Yes	21 (55.3%)	13 (68.4%)
	No	17 (44.7%)	6 (31.6%)
Squamous metaplasia	Yes	1 (2.6%)	1 (5.3%)
	No	37 (97.4%)	18 (94.7%)
Interstitial changes	Acute inflammation	3 (7.9%)	0 (.0%)
	Chronic inflammation	24 (63.2%)	9 (47.4%)
	Acute and chronic inflammation	1 (2.6%)	3 (15.8%)
	No	8 (21.1%)	5 (26.3%)
Interstitial fibrosis	Yes	21 (55.3%)	13 (68.4%)
	No	17 (44.7%)	6 (31.6%)
Hemorrhage	Yes	15 (39.5%)	5 (26.3%)
	No	23 (60.5%)	14 (73.7%)
Thrombosis	Yes	16 (42.1%)	11 (57.9%)
	No	22 (57.9%)	8 (42.1%)

lining the alveolar spaces had associated cytopathic-type changes in 14 cases, characterized by nuclear atypia with hyperchromatic nuclei, intracytoplasmic basophilic inclusions and the formation of giant syncytial cells (Fig. 4B). Type 2 pneumocyte hyperplasia was also observed in some cases (Fig. 4B) and rarely with organized pneumonia, especially in forms with prolonged evolution of disease. Only two cases showed squamous bronchial or bronchiolar epithelial metaplasia (Fig. 4C).

The interstitial compartment was predominantly expanded by mild to moderate inflammatory infiltrate composed of lymphocytes (71% of the cases) (Fig. 4D), rarely associated with polymorphonuclear leukocytes (PMNs) (2%). Fibrosis was also observed either perivascular, peri-bronchial and interstitial fibrosis (19 cases) or only peri-bronchial and interstitial fibrosis (13 cases); only two cases were associated with peri-bronchial fibrosis.

About 4% of the cases showed interstitial hemorrhage. In two cases, it was also noticed morphological changes suggestive for complement associated microvascular injury with fibrinoid necrosis of the vessel wall and red cell extravasation (Fig. 5A). Thrombosis (a material composed of fibrin, red blood cells and leukocytes) was identified in 24 cases (53.3%), both in large vessels and in small vessels. Thrombosis of small vessels was the most predominant feature (94.74%) and only three cases had been associated with thrombosis of large vessels (5.26%). In the lungs, microthrombi made up of fibrin and platelets, were observed in proportion of 85% of cases, mostly in the capillaries of the alveolar septa (Fig. 5B), but also in medium (Fig. 5C) and large vessels (Fig. 5D). Perivasculitis was noticed in four cases (8.9%) (Fig. 3C).

In 60% of cases included in the present study other histological lesions were associated, including infections such as aspergillosis (one case), tuberculosis (one case), HIV (one case), tumors (one case, malignant), aspiration pneumonia (two cases), and lung abscess (one case).

The autopsies we conducted revealed no specific COVID-19 changes in the other organs. The most common cardiac pathological lesions observed were diffuse myocardial sclerosis (95%), myocardial infarction (7.5%), left ventricle hypertrophy (58%), cardiac lipomatosis (74%), aortic and systemic atheromatosis (25.5%), subacute myocarditis (5.5%), giant cell myocarditis (1.8%) and lymphocytic myocarditis (1.8%). Other organs also showed preexisting or trauma related conditions such as cerebral atrophy (25%), stroke (12.5%), thrombosis (16.1%), fat liver (51.8%) and/or liver congestion, nephro-angiosclerosis (74.5%), congestion and chronic interstitial nephritis (18.2%), splenic and lymph nodes reactive changes. A limited number of cases presented history of neoplasia (colon, prostate).

Discussion

Our study includes a large number of patients (57 cases) and is one of the most important research projects performed on autopsy, as only the report of Edler et al. (2020), which included 80 cases, is larger (Edler et al., 2020). Other similar international autopsy studies have published their observations on a smaller number of cases, such as those published by Italian group on 38 patients (Carsana et al., 2020), or by Swiss or German groups that included 21 and 12 COVID-19 patients respectively (Menter et al., 2020; Wichmann et al.,

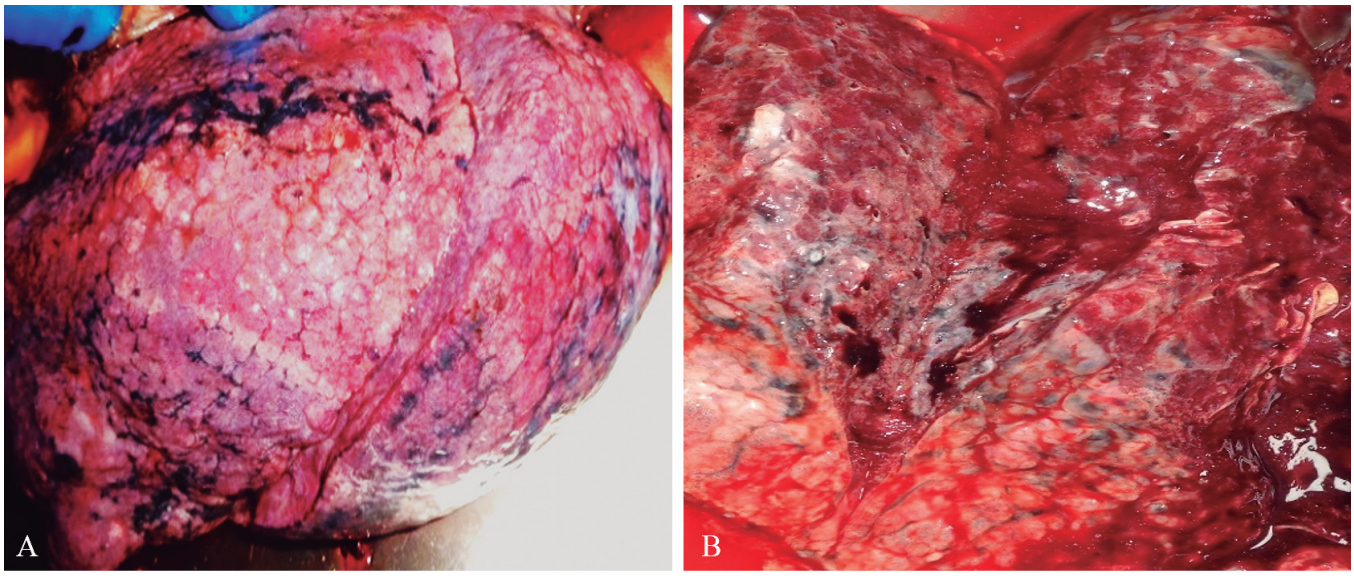

Fig. 1. Gross aspect of lung with SARS-CoV-2 infection. **A.** heavy and congested lungs, with slightly raised areas delimited by hyperemic bands on the pleural surface. **B.** on cutting section, the lung is characterized by extensive thrombosis and areas of hyperemia alternating with fibrosis.

2020). There are also many studies limited to a very small number of cases included in their reports.

Data published in the literature so far revealed that the most unfavorable outcome and eventually death associated with SARS-CoV-2 infection is more frequent in older men, with a high rate of comorbid conditions. Also, in the present study, men are mostly affected (38 out of 57 cases) with a median age of 68 years and with one or more associated comorbidities such as cardiac or renal disease.

Morphopathological features of lung parenchyma on patients with COVID-19 described in the literature showed similarities with changes described previously in SARS-CoV-1 and MERS-CoV (Zangrillo et al., 2020; Venter and Ritcher, 2020) but some differences had also been identified. (Menter et al., 2020; Wichmann et al., 2020; Satturwar et al., 2021). Gross examination of the lungs with COVID-19 revealed a diffuse or focal inferior lobe consolidation, increased weight and volume, often bilateral and sometimes associated with edema. Although there are a few cases described with pleurisy, pleural effusion is still uncommon in COVID-19 (Xu et al., 2020). Our study shows a preferential involvement of the inferior lobes, with evolution towards bilateral localization, features also described by other authors in dynamics (Venter and Richter, 2020; Zangrillo et al.,

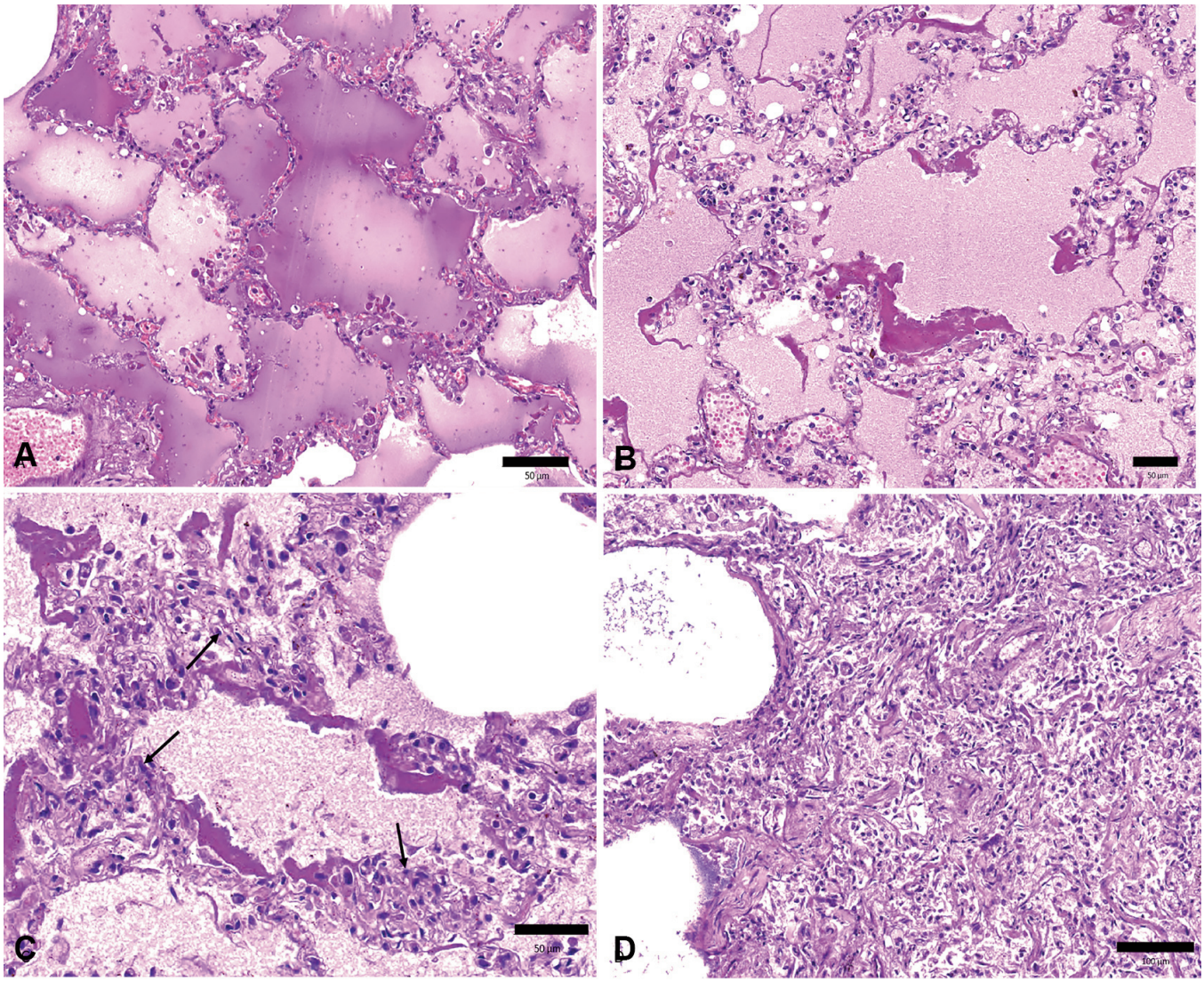

Fig. 2. Morpho-pathological features of DAD. **A.** early- exudative phase DAD characterized by extensive edema and congestion of the lung. **B.** exudative phase of DAD with additional frequently thick hyaline membranes lining alveolar spaces. **C.** early organizing / proliferative phase of DAD with scattered foci of spindle cell proliferation (black arrow), mild thickening of alveolar walls, collapse of some alveoli and prominent hyaline membranes. **D.** organizing / proliferative phase of DAD with large areas of fibroblast proliferation into the septa, which are thicker than normal, and frequently collapsed alveoli. (Col HE). Scale bars: A-C, 50 μm; D, 100 μm.

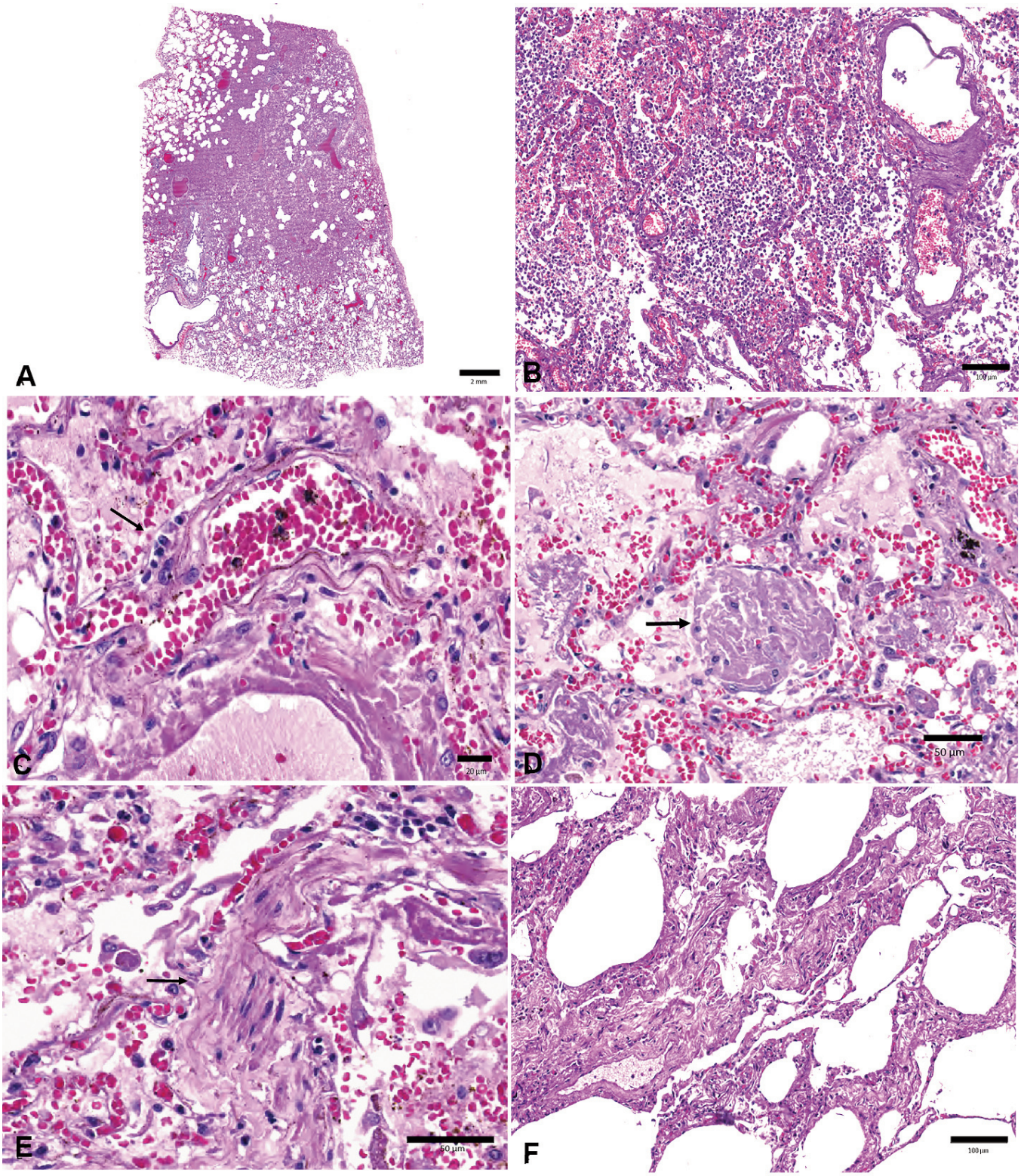

Fig. 3. Morpho-pathological features of DAD. **A.** Organizing pneumonia pattern of DAD, with consolidation area. **B.** congestion and intense intra-alveolar inflammation, mostly with neutrophils, suggestive of acute pneumonia. **C.** Organizing pneumonia pattern of DAD with perivasculitis (black arrow), congestion and edema within the septa. **D.** Organizing pneumonia pattern of DAD with alveolar spaces filled with fibroblast plugs (black arrow), desquamated pneumocytes, red blood cell extravasation and a mixed infiltrate of inflammatory cells (lymphocytes, neutrophils, macrophages). **E.** Organizing pneumonia pattern of DAD with fibroblast proliferation (black arrow) within the septa. **F.** Chronic / fibrotic phase of DAD with moderate fibrosis of alveolar septa. (Col HE). Scale bars: A, 2 mm; B, F, 100 μm; C, 20 μm; D, E, 50 μm.

2020). From the microscopical point of view, SARS-CoV-2 presents many similar aspects with MERS-CoV and SARS-CoV-1, among which were noticed: alveolar hyaline membrane, desquamation of alveolar epithelial cells, alveolar edema and alveolar and interstitial lymphocyte and monocyte infiltration (Wichmann et al., 2020; Xu et al., 2020). The predominant morphological feature in the lungs of deceased patients with COVID-19 infection is diffuse alveolar damage (DAD) (Carsana et al., 2020; Menter et al., 2020; Wichmann et al., 2020). DAD is the histologic finding observed in patients with acute respiratory distress syndrome regardless of the etiology, no specific morphological change being identified for SARS-CoV-2 infection (Li et al., 2020). These changes occur due to both epithelial and endothelial injury after an initial insult that leads to increased permeability of the alveolar-capillary barrier with consequent edema, release of proinflammatory and repair chemical mediators, impairment of the metabolism of surfactant and abnormalities in the coagulation pathways. The interplay of these mechanisms varies depending on the causative agent, but the ultimate morphology of DAD has similar features (Bradley et al., 2020; Carsana et al., 2020; Fox et al.,

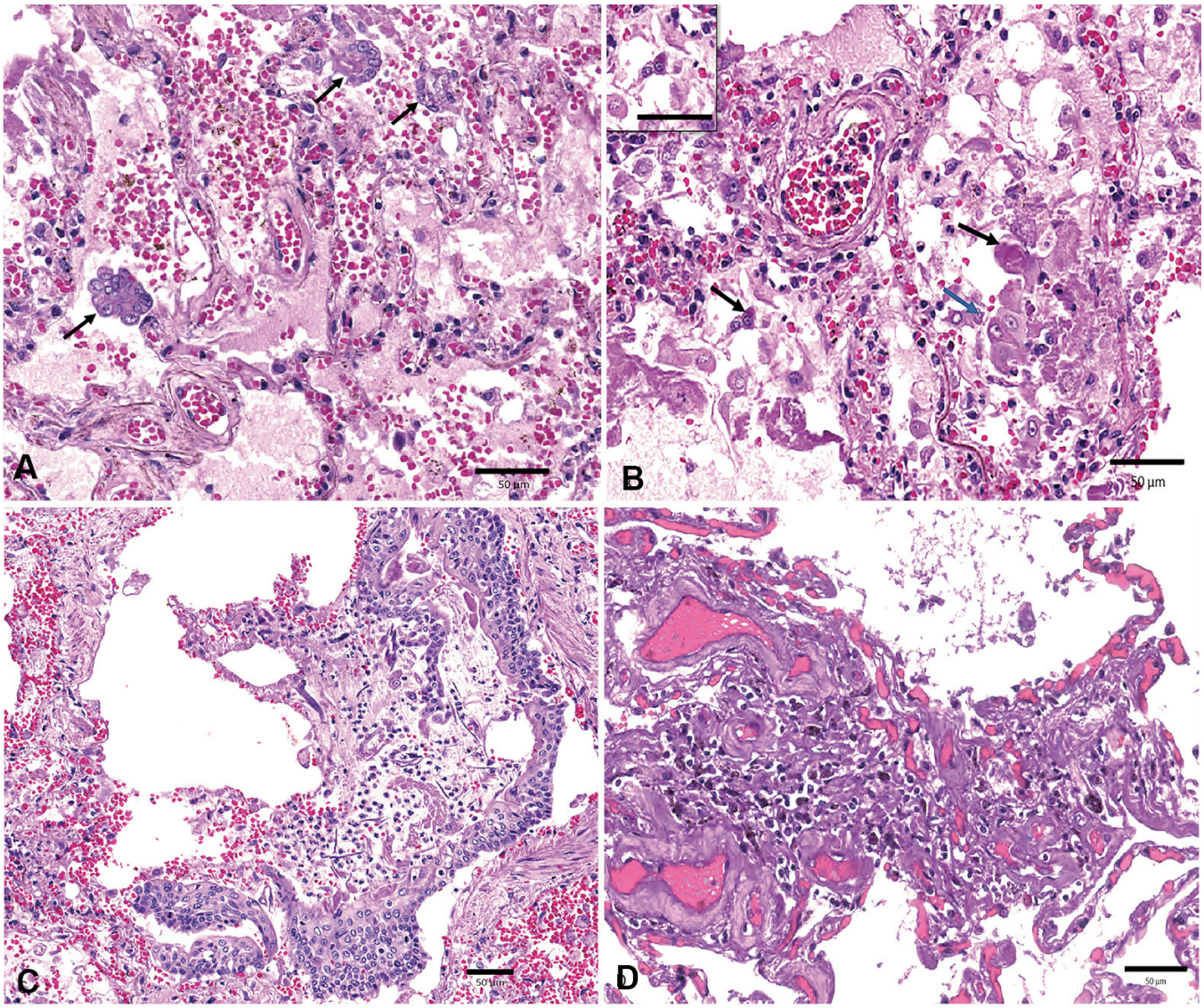

Fig. 4. Morphological features identified in the lung tissue with SARS-CoV-2 infection. **A.** alveolar spaces with scattered multinucleated giant cells (black arrow), extravasated red blood cells and mild patchy lymphocytes within the septa. **B.** rare viral cytopathic-like changes with intracytoplasmic inclusion (black arrow) and hyperplasia of type II pneumocytes (blue arrow); in case: viral cytopathic-like changes. **C.** squamous metaplasia of the bronchial epithelium. **D.** septal thickening by moderate interstitial mononuclear infiltrate with lymphocytes and scattered hemosiderin-laden macrophages. (Col HE). Scale bars: 50 μm, Insert in B, 100 μm.

2020; Lacy et al., 2020). Our study also highlights that the lungs by far suffer the most important damage from COVID-19 with predomination of DAD in different stages of evolution such as: acute/exudative phase; proliferative/organizing phase (early organizing phase or organizing pneumonia-like); both phases (exudative and proliferative); chronic/fibrotic phase.

The acute phase of DAD, during the first 10 days of viral infection, is mainly characterized by edema and/or hemorrhage in the alveolar spaces which sometimes are lined focally by hyaline membranes, alveolar septa with vascular congestion, increased cellularity of alveolar wall and sometimes perivasculitis (Satturwar et al., 2021). In the present study, acute/exudative DAD was the most frequent morphological change (21 cases) from which early exudative phases of DAD were mostly observed (13 cases) consistent with other reports like Xu et al. (2020) or Fox et al. (2020). They both reported similar morphological changes of lung autopsy in patients with SARS-CoV-2 which also corresponded to the exudative phase of DAD: cellular exudates, desquamation of alveolar epithelial cells with pneumocytes inside alveolar spaces and rarely viral cytopathic-like changes (large nuclei, prominent nucleoli and amphophilic granular cytoplasm) (Fox et al., 2020; Xu et al., 2020;). Konopka et al. (2020) described changes specific for the first evolutive stage of DAD with interstitial edema and hyaline membranes but in

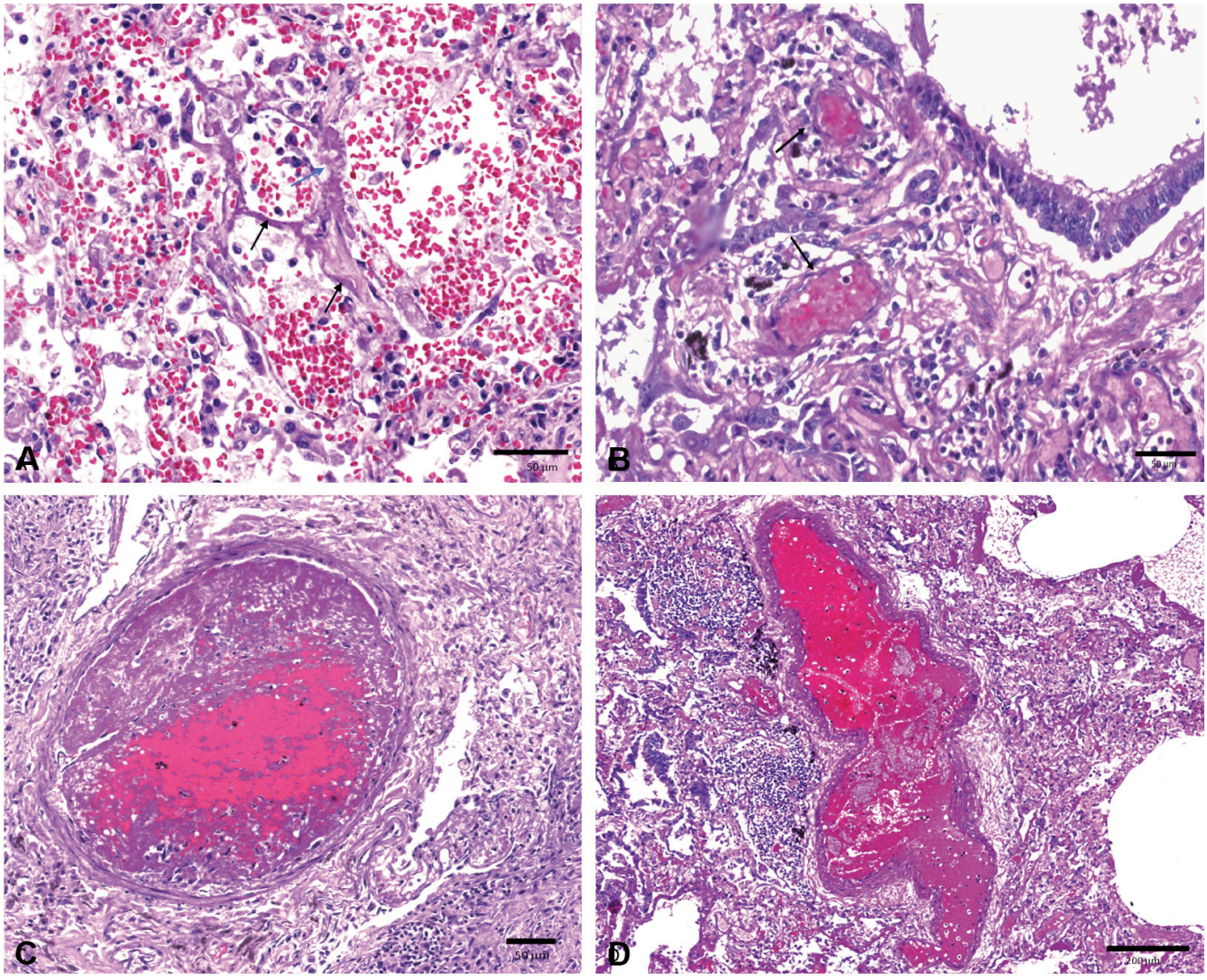

Fig. 5. Vascular changes identified in lungs of patients with SARS-CoV-2 infection. **A.** morpho-pathological changes consistent with complement associated microvascular injury characterized by capillary injury with red blood cell extravasation and luminal (black arrow) and mural (blue arrow) fibrinoid necrosis. **B.** numerous hyaline thrombi (black arrow) of micro-vessels. **C.** early organizing thrombus of a medium size vessel. **D.** fibrin thrombus in a large vessel. (Col HE). Scale bars: A-C, 50 μm; D, 200 μm.

addition their study highlighted the presence of focal pneumocyte hyperplasia and fibrinous airspace exudate with neutrophils, and the report of Barton et al. (2020) presented a case with focal alveolar/interstitial edema hyaline membranes and without interstitial organization or T-lymphocytes infiltration (Konopka et al., 2020). All these case presentations proved that the first phase of DAD can have different aspects depending not only on the period of infection but also on the patient's immune status.

The main morphological feature of the organizing phase of DAD, the second one, is represented by fibroblastic or/and myo-fibroblastic proliferation, firstly occurring in the septa. Lacy et al. (2020) published a case of a sudden death due to COVID-19 infection in which the authors identified hyaline membranes, mild mononuclear infiltrates, desquamating pneumocyte hyperplasia with focal multinucleated cells, acute alveolar hemorrhage focally present, alveolar fibrin, but without any evidence of viral inclusions, specific cytopathic changes, granulomas or fibroblast proliferation (Lacy et al., 2020). Other changes occur in the alveolar septa which are lined by reactive/reparative type II pneumocyte hyperplasia with or without desquamations, squamous metaplasia and multinucleated giant cells (Wichmann et al., 2020; Xu et al., 2020). If this process is prolonged it can lead to acute fibrinous organizing pneumonia (AFOP) or organizing pneumonia (OP) with fibrous remodeling processes and pulmonary fibrosis, the final phase (Wichmann et al., 2020; Xu et al., 2020). In the research of Copin et al. (2020), there has been reported a series of six cases of postmortem biopsies with changes specific for AFOP without another acute lung injury (Copin et al., 2020). In another series of 20 autopsies, Hwang et al. (2005), state that AFOP and squamous metaplasia were diagnosed in cases with a chronic and long duration of the disease (Hwang et al., 2005). We also noticed in our study group three cases with morphological changes suggestive for AFOP (acute fibrinous and organizing pneumonia) but these were associated with DAD changes.

Thrombosis of the pulmonary small arteries can be also identified in the proliferative phase due to neutrophil extracellular trap signal. Wichmann et al. (2020) also stated the presence of deep venous thrombosis within small lung arteries in 58% of their cases (seven patients) (Wichmann et al., 2020) and also, Ackermann et al. (2020) describes thrombi within pulmonary arteries of 1-2 mm diameter in 57% of COVID-19 patients (Ackermann et al., 2020), similar to our research, in which we report a high frequency (53.3%) of cases with arteriolar thrombi. We noticed that both large and small vessels were associated with the presence of fibrino-haematic and leukocyte debris material, but with small vessel predomination (94.74%). Also, in a small number of cases (four cases) we reported associated perivasculitis. The study carried out by Lax et al. (2020) highlighted that thrombosis of small and mid-sized pulmonary arteries was found to occur along with DAD in all cases, although they received prophylactic anticoagulant therapy (Lax et al., 2020). This finding is also consistent with the present study in which all cases with fibrin thrombi identified in the small and medium lung arterioles have been associated with DAD. The authors suggested that a combination of both lesions (DAD and thrombosis) could cause the rapid clinical deterioration in severe COVID-19, and that early anticoagulant cure is mandatory (Lax et al., 2020)

Similar conclusions about DAD and signs of thrombosis or microangiopathy in the small vessels and capillaries of the lungs have been reported in the African American community with COVID-19 (Fox et al., 2020). In a study of Carsana et al. (2020), fibrin thrombi in the small arterial vessels (<1 mm diameter) were observed in 87% of cases, around half of which had involvement of more than 25% of the lung tissue, accompanied by elevated high levels of D-dimers (Carsana et al., 2020; Grasseli et al., 2020). These aspects might explain the severe hypoxemia that characterizes ARDS in patients with COVID-19 and the good response to anticoagulant therapy (Carsana et al., 2020; Grasseli et al., 2020). Capillary microthrombosis in areas of DAD is associated with diffuse endothelial damage. This feature, although not pathognomonic, was observed in 47.4% of the lung specimens of our study. There are clinical studies which postulate that severe cases of COVID-19 can be associated with a hypercoagulable status, at high risk for thrombosis and D-Dimer values greater than 1µg/mL, thus with an unfavourable outcome for the patients (Zhou et al., 2020).

Our study noticed the presence of fibrino-haematic and leukocyte debris material in 53.3% of cases, both in large and small vessels, but with small vessel predomination (94.74%). Also, in a small number of cases (four cases), associated perivasculitis was reported. The study carried out by Lax et al. 2020 highlighted that thrombosis of small and mid-sized pulmonary arteries was found to occur along with DAD in all cases, although they received prophylactic anticoagulant therapy (Lax et al., 2020). The authors suggested that a combination of both lesions (DAD and thrombosis) could cause the rapid clinical deterioration in severe COVID-19, and that early anticoagulant cure is mandatory (Lax et al., 2020). This finding is also consistent with our study which observed that fibrin thrombi in the small and medium lung arterioles were associated with DAD in all cases.

Morphological features suggestive for viral cytopathic-like changes are: damaged or atypical enlarged pneumocytes with large nuclei, prominent nucleoli, amphophile granular cytoplasm and type II pneumocyte hyperplasia with focal sloughing of the pneumocytes, hyaline membrane formation, intra-alveolar hemorrhage and intra-alveolar neutrophil infiltration (Bradley et al., 2020; Xu et al., 2020). In the present retrospective study, pneumocytes with viral cytopathic effect were identified in 12 cases (26.7%),

macrophages were identified intra-alveolar in 23 cases (51.1%) and multinucleate giant cells of the syncytial type were observed in 16 cases (35.6%).

Interstitial inflammatory infiltrate is the main lesion of lung pathology, being represented by a wide range of changes, such as chronic non-specific inflammatory infiltrates in 71.1% of cases, hemorrhagic infiltrates in 4.4%, acute inflammatory infiltrates in 2.2 % and mixed infiltrate in 4.4%. Pulmonary stasis was associated in 82.2% of cases. Anthracosis was identified in 18 cases (40%). These features were also focally associated with patterns of interstitial pneumonia (presence of inflammatory lymphomonocytic infiltrate along the slightly thickened interalveolar septa), organizing pneumonia (alveolar loose plugs of fibroblastic tissue), and acute fibrinous organizing pneumonia (some alveolar spaces containing granulocytes and fibrin, with the formation of balloon structures) (Carsana et al., 2020). Although many of the patients had rapid progression and death within 1-3 weeks from hospital admission, a subset had a longer time since the debut of symptoms and thus longer evolution. These patients presented with higher degrees of fibrosis. Some studies reported longer disease course with organizing phase of DAD with progression to fibrosis. In one case, serial testing for virus showed disappearance at 23 days, and autopsy at 45 days revealed lung fibrosis. Although fibrosis is a feature observed with longer duration of illness, it is unclear which patients will develop fibrosis (Schwensen et al., 2020; Borczuk, 2021).

Although clinicians describe mucous plugging and airway obstruction in the patient care setting (Yao et al., 2020), these were not often encountered in autopsy series, with one exception in which these findings were found with suppuration (Carsana et al., 2020). An inflammatory, often neutrophil-rich tracheitis and tracheobronchitis is described in 47% of cases in one series; of those cases, the majority were never intubated, excluding airway trauma from intubation as a cause (Bradley et al., 2020; Borczuk, 2021). Chronic airway inflammation was also reported in other autopsy series (Carsana et al., 2020; Borczuk et al., 2021), as well as our study that shows the presence of chronic inflammatory damage in tracheas in five cases [8.7%]. These results may be caused by the extent of large airway sampling.

Besides the specific viral changes such as DAD with or without microthrombi, we encountered an abscess associated in one case and two cases with an early bacterial pneumonia. One patient was diagnosed with acute organized pneumonia and one patient with lobar pneumonia (red hepatization stage). As an associated infectious pathology, invasive pulmonary aspergillosis was diagnosed in a single case of a patient infected with SARS-CoV-2 and pulmonary tuberculosis in the miliary form in a single case. Carsana et al. (2020) described four patients (11%) with bacterial abscesses (one or two abscesses per lung, <5 mm in diameter), and one (3%) with a single fungal abscess (<7 mm in diameter), which were considered to have formed after hospital admission (Carsana et al., 2020). In other papers (case reports and case series) there was evidence of consolidation of abundant intra-alveolar neutrophilic infiltration, consistent with bronchopneumonia of a superimposed bacterial infection during the hospitalization in up to 55% of cases (Lax et al., 2020; Menter et al., 2020; Wichmann et al., 2020).

Extrapulmonary histopathology in COVID 19 autopsies

Our study showed many nonspecific changes for SARS CoV2 infection in other organs than the lung, almost exclusively consisting of preexisting conditions, like chronic heart disease, old myocardial infarction, atheromatosis, cerebral atrophy, fatty liver, chronic renal disease or various neoplasia. Some cases presented acute lesions like acute myocardial infarction, myocarditis or stroke that can be considered as the effect of SARS-CoV2 infection.

The SARS-CoV-2 infection-associated cardiovascular malfunction has been reported by some lines of clinical studies (Afewerky, 2020; Chen et al., 2020; Li et al., 2020; Shi et al., 2020). The findings of these studies suggest the causality of the SARS-CoV-2 to the myocardium and blood vessels injury as the highest risk of mortality in COVID-19 patients, next to pulmonary injury. Analysis of SARS-CoV-2 infection-associated death cases, including an observational study where 36.5% of them showed acute myocardial injury and 50% substantiate cardiovascular dysfunction, establish cardiovascular disease as one of the major aggravating medical conditions in SARS-CoV-2 pathology (Afewerky, 2020). Moreover, other clinical studies have also reported coagulation aberrations in severe and critical COVID-19 patients (Chen et al., 2020; Li et al., 2020; Shi et al., 2020) that may lead to deep venous thrombosis and disseminated intravascular coagulation. However, whether the cardiac injury in COVID-19 patients is the direct or indirect pathogenic consequence of the SARS-CoV-2 infection still remains unclear.

The clinical spectrum of COVID-19 has a large series of symptoms, including digestive (such as nausea, vomiting or diarrhea), tegumentary (in case of intravascular disseminated coagulation or caused by prolonged use of hygienic products), osteo-muscular (muscle pain, arthritis), all with nonspecific histopathologic presentation (Afewerky, 2020).

Conclusions

The lung injury observed in autopsy cases with SARS CoV2 infection is dominated by diffuse alveolar damage (DAD) with different evolution patterns (exudative, proliferative, fibrotic) and by a hypercoagulable status reflected by frequent and extensive thrombosis, mainly on the small and medium pulmonary arteries, which represent an important cause of morbidity and mortality. Other morphological aspects

associated with COVID-19 infection are cytopathic changes of pneumocytes, hyperplasia and/or desquamation of type II pneumocytes, multinucleated giant cells, squamous metaplasia, and organizing pneumonia. In a few cases alterations specific for complement associated microvascular injury were also noticed, reinforcing the hypothesis that COVID-19 is a disease mainly of the vessels. During disease evolution, a patients may develop complications like broncho-pneumonia, organizing pneumonia or acute fibrinous organizing pneumonia, which worsen the patient's health. In our opinion, COVID 19 associated pneumonia combines classical aspects of alveolar and interstitial pneumonia with some peculiarities represented by the presence of predominant hyaline alveolar membrane, extensive thrombosis and by unsystematic fibrosis.

Even though our study includes the largest number of autopsies on patients with SARS-CoV-2 infection performed and reported in the south-eastern region of Romania, the number is still small compared to the tens of thousands of people who have died from COVID-19 so far. It requires further investigations to be able to extract the most valuable morphological information for patients to benefit from the most appropriate therapy. However, our report strengthens previous observations related to different stages of the disease, because the death of the patients included in this research had occurred not only due to COVID-19 infection. All these morphological changes are important indicators of etiology and pathogeny and thus may impact the therapeutic approach. From this point of view, we can conclude that autopsies are of major importance in understanding SARS-CoV-2 infection and thus they open the way to a better management of the disease.

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Author Contributions. MD: microscopic examination, study design, original draft preparation writing-review and editing of revision. ME: microscopic examination, literature search, data collection, original draft preparation, writing-review and editing of revision. AAN: microscopic examination; literature search, study design, data collection; original draft preparation, writing-review and editing of revision. GIB: microscopic examination, literature search, data collection, statistical analysis and data interpretation, original draft preparation, writing-review and editing of revision. LSNC: performed autopsies, forensic diagnosis, data collection; original draft preparation. SD: microscopic examination and final microscopic diagnosis, data collection. MFP: performed autopsies, forensic diagnosis, and data collection.

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Data Availability Statement. The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethics and general data production regulation.

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