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Evaluation of postmortem pathological changes in the lung in SARS-CoV-2 RT-PCR positive cases

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This study was approved by the Turkish Ministry of Health, General Directorate of Health Services, Scientific Research Platform (Number: 2021-03-24T16_20_16, Date: 24.03.2021), and the Council of Forensic Medicine, Education and Scientific Research Commission (Number: 21589509/2021/333, Date: 30.03.2021).

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: The most common cause of death in COVID-19 is acute respiratory distress syndrome. Diffuse alveolar damage is the histological characteristic and counterpart of acute respiratory distress syndrome. Histopathological findings, accompanied by immunohistochemical findings, can provide valuable information in the pathogenesis of Covid-19. We aimed to investigate the histopathological findings by supporting our results with immunohistochemical staining in SARS-CoV-2 positive autopsies.
Methods: A total of 101 autopsy cases with positive postmortem SARS-CoV-2 rt-PCR tests between May 2020-May 2021 were investigated in this retrospective cohort study. Cases with negative postmortem swab samples on rt-PCR and those with severe autolysis were excluded from the study. Pathological changes in the lung were examined with hematoxylin and eosin-stained preparations. Immunohistochemical assay with pancytokeratin, TTF-1, IL-6, CD68, CD3, CD8, and antibodies against the SARS-CoV-2 nucleocapsid protein were also performed for further evaluation.
Results: Diffuse alveolar damage findings were present in 58 (61.7%) out of 94 cases in our study. Seventeen (18.1%) showed findings compatible with the exudative phase, 37 (39.3%) were in the proliferative phase, and 4 (4.3%) were in the fibrotic phase of diffuse alveolar damage. Pulmonary perivascular lymphocytic infiltrates contained more CD3 (+) T lymphocytes than CD8 (+) T lymphocytes, immunohistochemically.
Conclusion: The finding of more CD3 positive T lymphocytes than the CD8 positive T lymphocytes in the perivascular lymphocytic infiltrate correlates with the hypothesis of the direct destruction of CD8 (+) T lymphocytes or through impairment of cellular immunity by SARS-CoV-2 induced mediators. Detection of immunohistochemical staining with IL-6 in COVID-19 supports the cytokine storm mentioned in the previous studies and the role of IL-6 in cytokine storm in SARS-CoV-2 infection. The limited number of immunohistochemical studies on SARS-CoV-2 increases the importance of our study, which evaluates IL-6, CD3, and CD8 expressions at the tissue level. Autopsy research is important and contributes to the development of protective, diagnostic, and therapeutic modalities.

Keywords: Autopsy, SARS-CoV-2, COVID-19, IL-6, CD3, CD8

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Introduction

Coronaviruses are enveloped, non-segmented, positive single-stranded RNA viruses that predominantly cause upper respiratory tract infections, which led to severe acute respiratory syndrome (SARS) outbreak in 2003 and the Middle East respiratory syndrome (MERS) outbreak in 2012 [1]. Coronavirus infections caused by SARS and MERS arose from bats that infected civet cats and dromedary camels, respectively, as secondary hosts [1]. SARS-CoV, which caused severe acute respiratory syndrome (SARS) infection, and MERS-CoV, which caused the Middle East respiratory syndrome (MERS), are members of the beta coronavirus group, just like SARS-CoV-2, and have more than 79.6% similarity to the SARS-CoV in genetic sequence [2, 3]. SARS-CoV-2 was quite similar to a bat coronavirus formerly detected in Rhinolophus affinis from the Yunnan County of China with a genome sequence identity similarity of over 96% [3].

Most individuals who were infected with SARS-CoV-2 recovered from the disease; however, particularly the elder persons and those with comorbidities became heavily ill [4]. The mortality rate among patients with severe symptoms was high, despite intensive care, and ranged between 7-15% [4, 5]. The overall mortality rate in the world is about 2% [5]. As of August 17, 2021, there were 208 million confirmed cases and more than 4.3 million deaths from COVID-19 in a total of 222 countries in the world [5].

Diffuse alveolar damage, the histomorphological counterpart of acute respiratory distress syndrome (ARDS), is thought to be responsible for most of the deaths in persons infected with SARS-CoV-2. However, some cases with fatal outcomes result from cardiac damage, shock, pulmonary embolism, thrombosis, and associated stroke [4].

Autopsy examinations are particularly important and considered the gold standard to understanding the pathophysiology of COVID-19 [6]. However, most studies focused on the clinical and molecular aspects of SARS-CoV-2 [6]. At the very beginning of the pandemic, data from autopsies were limited, insufficient, and remained inadequate for a long time because of suggestions to suspend postmortem examinations in patients with suspected COVID-19 infection. Postmortem examinations were thought to carry a high risk for autopsy workers and required an autopsy unit with proper biosecurity accessories [4]. Subsequent studies added valuable information about the pathophysiology of the COVID-19 disease and helped to understand the course of the disease.

In this comprehensive study, 101 patients who died due to COVID-19 disease and were autopsied at the Istanbul Forensic Medicine Institute, morgue department, were studied. SARS-CoV-2 positivity was detected with real-time reverse-transcriptase-polymerase-chain-reaction (rt-PCR) assay from pharyngeal/tracheal swabs or lung tissues. These cases were analyzed for their histopathological pulmonary findings. We aimed to compare the count of CD3 and CD8-positive perivascular T lymphocytes to assess their role in the pathogenesis of SARS-CoV-2 infection, and to investigate the tissue-level expression of IL-6 immunohistochemically, which has been reported to increase in bronchoalveolar fluid and plasma [7-9]. Also, the frequency and presence of megakaryocytes in the lung in COVID-19 infection were evaluated microscopically, and their relationship with acute lung injury and thrombosis was discussed. Our findings were evaluated in light of the current literature to reveal the pathological mechanisms of COVID-19. We think that the pulmonary findings in our autopsies will contribute to the literature since they were performed on a large series in one center and focused on morphological and immunohistochemical findings in the lung, which is the main tissue of acute injury in COVID-19.

Materials and methods

This study was authorized and approved by the Turkish Republic, Ministry of Health, General Directory of Health Services Scientific Research Platform ("2021-03-24T16_20_16"), and the Council of Forensic Medicine, Education and Scientific Research Commission ("21589509/2021/333-30.03.2021").

This retrospective cohort study was conducted on rt-PCR confirmed COVID-19 autopsies and the findings of 101 autopsy cases with positive postmortem SARS-CoV-2 rt-PCR assays performed at the Istanbul Council of Forensic Medicine between May 2020 and May 2021 were evaluated. Those with negative rt-PCR postmortem swab samples and 7 cases with severe autolysis were excluded from the study.

Autopsy technique and tissue sampling

Autopsy procedures were altered, and the autopsy technique was modified in the Morgue Department during the COVID-19 pandemic. All COVID-19 autopsy cases were examined by opening three cavities in the body as a rule. Organs other than the heart and central nervous system were not removed and investigated in their normal localization in the body. Tissue samples were obtained from the organs (heart, lung, liver, kidney, central nervous system) for histopathological examination. In addition to nasopharyngeal swabs, tracheal and lung swab samples were also taken from suspicious cases for COVID-19 infection. All cases were reviewed for macroscopic autopsy information. Age, gender, height, weight, and hospitalization information were noted if available.

Real-Time PCR

Postmortem SARS-CoV-2 rt-PCR assay, which is a nucleic acid amplification method that detects the viral RNA of COVID-19 was studied in the nasopharyngeal swab, deep tracheal swab, lung swabs, and paraffin blocks of tissues taken from the lungs of suspected cases. Nucleic acids were extracted on the QIASymphony (Qiagen / Germany) device using the QIAsymphony DSP Virus / Pathogen Midi kit. RealStar® SARS-CoV-2 RT-PCR Kit RUO (Altona Diagnostics, Hamburg, Germany) was used in the rt-PCR method and amplified in the Rotor-Gene (Qiagen / Germany) device according to the manufacturer's guide.

The nucleic acids were extracted from paraffin-embedded lung tissues which were fixated with 10% formalin solution. Sections of each lung block were cut by a rotary microtome device. Deparaffinization was performed with ethyl alcohol and xylene, after which the samples were incubated for

24 hours by adding proteinase K and ATL buffer solution [10-12].

Histopathological investigation

Histopathological changes detected in the lung were investigated in hematoxylin and eosin-stained sections with a "Nikon Eclipse Ni" research light microscope. In this study, demographic data such as age, gender, body mass index (BMI), event information, causes of death, and histopathological findings (alveolar edema, interstitial edema, interstitial inflammation, perivascular lymphocytic inflammation, the presence of megakaryocytes, acute bronchopneumonia, the presence of hyaline membrane, fibrin thrombus in the vascular lumen, and fibrin in the alveolar lumens, type II pneumocyte hyperplasia, alveolar hemorrhage, squamous metaplasia, and the presence of multinuclear cells in the alveolar lumen and infarct) were analyzed.

Immunohistochemical staining

BenchMark Ultra, Roche's fully automatic immunohistochemical slide staining system was used for the immunohistochemical procedures. The presence of SARS-CoV-2 was also revealed by antibodies against the SARS-CoV-2 nucleocapsid protein (E8R1L): A monoclonal mouse mAb (Cell signaling Technology, Danvers, Massachusetts, USA). Positive staining was defined as cytoplasmic and membranous staining. Additional immunohistochemical staining for Pancytokeratin (PanCK) (Dako FLEX Monoclonal Mouse Anti-Human Cytokeratin, Clone AE1/AE3, Ready to use), TTF-1 (Dako FLEX Monoclonal Mouse Anti-Thyroid Transcription Factor, Clone 8G7G3/1, Ready to use), IL-6 (Santa Cruz Biotechnology, Inc, Monoclonal Mouse antibody, dilution 1/100), CD68 (Dako FLEX Monoclonal Mouse Anti-Human CD-68, Clone KP1, Ready to use), CD3 (Dako FLEX Polyclonal Anti-Human CD-3, Ready to use), CD8 (Dako FLEX Monoclonal Mouse Anti-Human CD-8, Clone C8/144B, Ready to use) were performed on the lung tissues for further evaluation. PanCK and TTF-1 were used to differentiate the cells of pneumocyte origin from the megakaryocytes, CD68, for differentiation of histiocytes from megakaryocytes, IL-6, to investigate its role in the cytokine storm, and CD8 and CD3i for the assessment and comparison of the amount of CD3 and CD8 positive T cells in perivascular lymphocytic infiltrates.

Statistical analysis

The program used for statistical analysis was SPSS (Statistical Package for the Social Sciences) 2012 version 21. Descriptive statistical methods (standard deviation, average, median, frequency, minimum, maximum, ratio), and the Chi-Square test were used to compare the data. A *P*-value of <0.05 was considered significant.

Results

Of the 101 cases examined, 79.2% were male (n = 80) and 20.8% were female (n = 21). Their mean age was 56.1 (18.9) (range 7-98) years, mean weight, 82.7 (20.7) kilograms, mean height, 169.6 (10.2) cm, and mean body mass index, 28.8 (7.2) kg/m^2. The mean ages of the males and females were 55.7 (18.7) years and 58.10 (20.3) years, respectively, their mean BMIs, 28 (6.3) and 31.8 (9.6) kg/m2, respectively, their mean weights, 83.2 (19.2) and 80.8 (26.2) kg, respectively, and their mean heights, 172.4 (9.3) and 158.9 (5.3) cm, respectively. BMI was remarkably higher in females than in males (P<0.05). Postmortem SARS-CoV-2 rt-PCR assay was positive for SARS-CoV-2 in all cases. RT-PCR samples were taken from the nasopharyngeal swab, deep tracheal swab and, lung swabs during the autopsy, and paraffin blocks sections from cases histopathologically suspected for COVID-19 during the routine pathological investigation.

Seven out of 101 cases were excluded from the histopathological examination due to severe autolysis findings, which distorts morphology. Diffuse alveolar damage (DAD) findings were present in 58 (61.7%) out of 94 cases included in our study (Table 1). Seventeen cases (18.1%) with DAD findings were in the exudative phase, 37 (39.3%) were in the proliferative phase, and 4 (4.3%) were in the fibrotic phase. The histopathological findings are summarized in Table 2. Forty-three (45.7%) cases were hospitalized, 48 (58.1%) were not, and in 3 (3.2%) cases, there was no information about hospitalization. The time between the onset of the disease and death could not be determined precisely due to limitations in obtaining clinical information.

Table 1: Histopathological stages of diffuse alveolar damage of COVID-19 cases

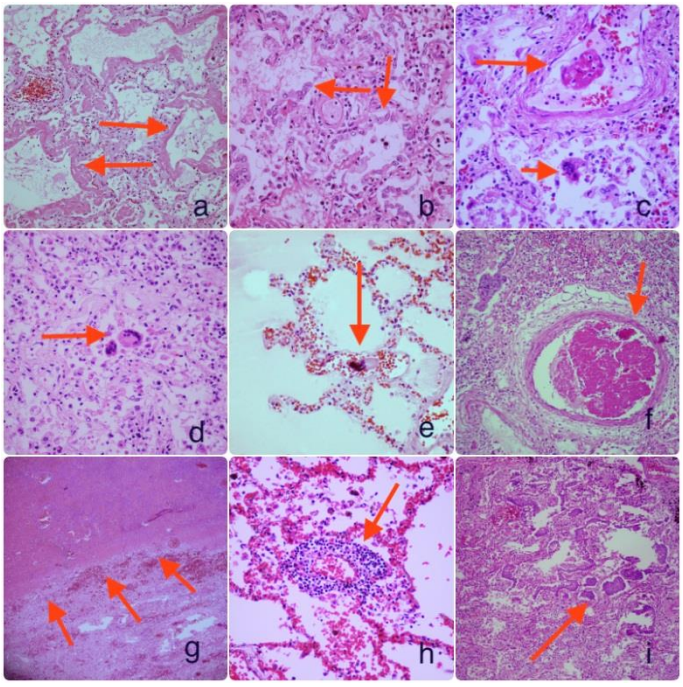
Diffuse Alveolar Damage	n	%
Absent	36	38.3%
Exudative stage	17	18.1%
Proliferative stage	37	39.3%
Fibrotic stage	4	4.3%
Total	94	100

Table 2: Histopathological findings in the lung

Histopathological Findings	n (%)
Hyaline Membrane	50 (53.2%)
Type II pneumocyte hyperplasia	41 (43.6%)
Alveolar edema	85 (90.4%)
Interstitial oedema	80 (85.1%)
Fibrin thrombus	49 (52.1%)
Perivascular Inflammation	63 (67%)
Alveolar fibrin	44 (46.8%)
Squamous metaplasia	33 (35.1%)
Alveolar hemorrhage	36 (38.3%)
Multinuclear cells	33 (35.1%)
Acute bronchopneumonia	60 (63.8%)
Interstitial inflammation	73 (77.7%)
Infarction	4 (4.3%)
Megakaryocytes	63 (67%)

There was alveolar edema in 85 (90.4%), interstitial edema in 80 (85.1%), interstitial inflammation in 73 (77.7%), perivascular lymphocytic inflammation (Figure 1h) and megakaryocytes in 63 (67%) (Figure 1e), and acute bronchopneumonia in 60 (63.8%). There were hyaline membranes in the alveolar walls in 50 (53.2%) (Figure 1a), fibrin thrombus in the vascular lumen in 49 (52.1%) (Figure 1c -long arrow- and 1f), fibrin in the alveolar lumens in 44 (46.8%), type II pneumocyte hyperplasia in 41 (43.6%) (Figure 1b), alveolar hemorrhage in 36 (38.3%), squamous metaplasia (Figure 1i) and multinuclear cells in alveolar lumens in 33 (35.1%) (Figure 1c -short arrow- and Figure 1d), and infarction (Figure 1g) in 4 (4.3%) cases.

Figure 1: Histopathological findings of the lung in COVID-19 cases (Hematoxylin and eosin stain -H&E-)



a: Exudative pattern with a hyaline membrane (arrows) x200. b: Proliferative phase of diffuse alveolar damage with type II pneumocyte hyperplasia (arrows)x400. c: Intra-alveolar multinuclear giant cell (short arrow), and at the top, a fibrin thrombus in the vascular lumen (long arrow) x400. d: Intra-alveolar multinuclear giant cell (arrow)x400. e: Megakaryocyte in the vascular lumen (arrow)x400 f: Fibrin thrombus in the vascular lumen (arrow) and associated squamous metaplasia x200. g: Pulmonary infarction (arrows)x200. h: Perivascular lymphocytic cuffing (arrow)x400. i: Squamous metaplasia (arrow)x200.

Hyaline membrane, a typical feature of DAD and especially the histological hallmark of the exudative phase, was found in 50 (53.2%) cases. Type II pneumocyte hyperplasia was present in 43 (45.7%). Exudative, proliferative, or fibrotic stage of DAD was not detected in the lungs of 36 cases. The histopathological pulmonary findings found in these cases, which do not have signs of diffuse alveolar damage, are shown in Table 3.

Table 3: Histopathological pulmonary findings of SARS-CoV-2 rt-PCR (+) and diffuse alveolar damage (-) 36 autopsy cases

Histopathological Findings	n (%)
Alveolar edema	29 (80.6%)
Megakaryocytes	18 (50.0%)
Interstitial edema	24 (66.7%)
Perivascular Inflammation	24 (66.7%)
Interstitial inflammation	23 (63.9%)
Alveolar hemorrhage	10 (27.8%)
Fibrin thrombus	9 (25.0%)
Acute bronchopneumonia	6 (16.7%)
Multinuclear cells	4 (11.1%)
Alveolar fibrin	3 (8.3%)
Squamous metaplasia	1 (2.8%)
Infarction	1 (2.8%)

COVID-19: Coronavirus disease of 2019, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, rt-PCR: reverse-transcriptase-polymerase-chain-reaction

Immunohistochemical staining revealed that pulmonary perivascular lymphocytic infiltrate contained more CD3 (+) T lymphocytes than CD8 (+) T lymphocytes. Cytoplasmic and membranous immunohistochemical staining of Interleukin-6 (IL-6) were found in the lung. In addition, CD68, Pancytokeratin (PanCK) and, Thyroid Transcription Factor-1 (TTF-1) were applied to the lung tissue for differentiation of histiocytes and epithelial cells from megakaryocytes immunohistochemically.

Discussion

Histopathological findings in most organs in COVID-19 infection are quite similar to those seen in SARS and MERS infections [13]. In the studies related to SARS-CoV-2, the lung, heart, and vascular systems are the most affected by the infection. Among these organs, histopathological findings are most common and severe in the lungs [13]. Pulmonary histopathological findings of COVID-19 cases were at different phases of diffuse alveolar damage, with associated type II pneumocyte hyperplasia, pneumocyte desquamation, intra-alveolar hemorrhage, fibroblast plugs, squamous metaplasia, interstitial thickening, patchy chronic inflammation, presence of megakaryocytes, hemophagocytosis, and vascular damage [4]. The histopathological findings obtained in our study are consistent with the findings of the previous studies.

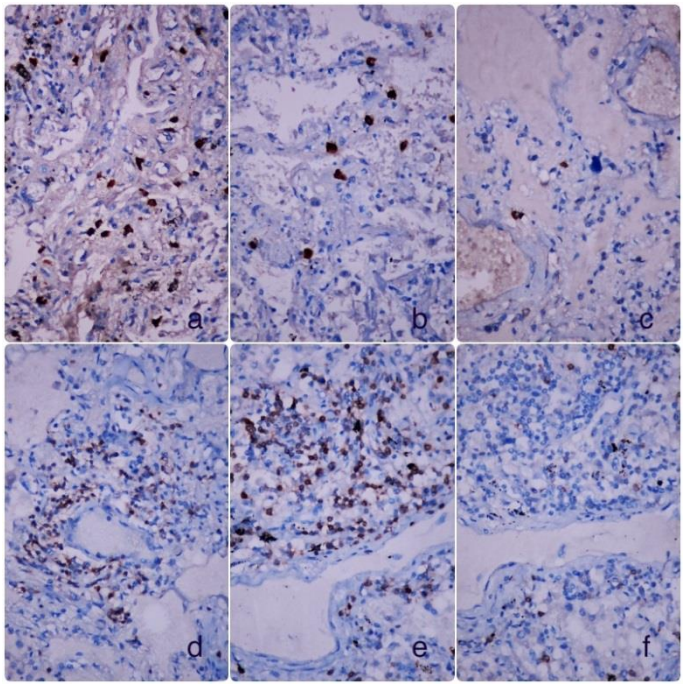
Normally, viruses enter the host cells and manipulate their cellular process to replicate and spread to the other cells [14]. Angiotensin-converting enzyme-2 (ACE2) is a membrane protein that is thought to be the host cell receptor for SARS-CoV-2 [14, 15]. It has been shown that COVID-19 patients have remarkably more ACE2-positive cells in their lungs [16]. Changes in the amount of ACE2-positive endothelial cell count play a crucial role in morphological changes seen in the vascular endothelial cells in COVID -19 patients [16].

Histopathologically, diffuse alveolar damage (DAD) is the morphologic equivalent of ARDS [17]. The lung responds quite similarly to most injuries regardless of the cause. The microscopic appearance depends on the time between the offending agent and the time of death, also on the extent and severity of the damage [17]. Histopathological findings of diffuse alveolar damage (DAD) found in COVID-19 are indistinguishable from DAD which develops due to other causes. The exudative (acute) phase of DAD is evident during the first week of the onset of disease and is characterized by inflammatory cell-mediated alveolar damage with alveolar edema and/or bleeding, congestion in capillary vessels, and hyaline membranes with or without microvascular thrombus. Hyaline membranes are the main morphologic characteristic of the exudative phase of diffuse alveolar damage and are most remarkable between 3-7 days after the onset of disease [17]. The subacute proliferative (organized) phase of DAD begins at the end of the first week and morphologically shows type II pneumocyte hyperplasia at the alveolar surface with associated alveolar wall thickening, myofibroblast proliferation, and reactive pneumocytes [4, 17]. Type II pneumocyte hyperplasia, which is characterized by prominent cytological reparative atypia such as cytomegaly, nucleomegaly, prominent nucleolus, clearing of nuclear chromatin, multinucleation, and squamous metaplasia, can be explained as a secondary response to the damage to the lung tissue caused by the virus or other offending agents [18]. The fibrotic (chronic) phase shows signs of honeycomb lung with fibrosis of the alveolar cavities, interstitium, and associated squamous metaplasia [4]. DAD does not always progress to the fibrotic (chronic) phase, sometimes it heals entirely without any sequelae. In studies, SARS-CoV-2 deaths were mostly detected at the exudative phase of diffuse alveolar damage compared to SARS-CoV-1 deaths, which indicates that the lungs in COVID-19 patients deteriorated faster, were more severely damaged, and caused earlier death [4]. However, in our study, 37 (39.3%) COVID-19 cases were in the proliferative phase and 17 (18.1%) were in the exudative phase of diffuse alveolar damage. The proliferative phase of diffuse alveolar damage indicates that these deceased persons were infected with the SARS-CoV-2 virus for more than 1 week.

More decedents at the proliferative phase than the exudative phase found in our study may be related to earlier diagnosis and hospitalization. We believe that the filiation process and starting treatment at an early stage of infection, which are strictly performed during the pandemic in our country, might extend survival. In addition, some of the cases that we investigated in the study were those that were hospitalized and received treatment. This explains the high number of cases in the proliferative stage in our study. Hereby, it is important to determine infected cases and start medical treatment as soon as possible to extend survival time.

A distinctive feature of COVID-19 is "cytokine storm syndrome", in which an increase in proinflammatory cytokines leads to endothelial cell destruction and the resultant increase in vascular permeability. This leads to fluid collection in the alveoli, causing hypoxia and, in critically ill cases, acute respiratory distress syndrome [19-21]. The pathogenesis of COVID-19 involves an inflammatory response involving a group of mediators, including IL-6. IL-6 is a proinflammatory cytokine that has pleiotropic functions which include autoimmunity, inflammation, and acute phase response [22, 23]. IL-6 alters the host defense through various immune-stimulating mechanisms such as altering antigen-dependent B lymphocyte differentiation, increasing IgG formation by B lymphocytes, and managing monocytes and their differentiation to macrophages [23]. IL-6 also activates complements and triggers coagulation [24]. Also, the level of serum SARS-CoV-2 nucleic acid is tightly related to exceedingly elevated levels of IL-6 [23]. In our study, cytoplasmic and membranous staining and the expression of IL-6 in the lung were detected in rt-PCR positive SARS-CoV-2 decedents (Figure 2a, 2b, 2c). However, while most previous studies investigated IL-6 in the bronchoalveolar lavage aspirate and plasma, in our study, this was also confirmed at the lung tissue level. Detection of immunohistochemical expression of IL-6 in the lung tissue in COVID-19 cases supports the cytokine storm mentioned in previous studies and the role of IL-6 in cytokine storm in SARS-CoV-2 infection. IL-6 was pointed out as an indicator of lung damage and may indicate morbidity and mortality. [7, 8]. The role of IL-6 in SARS-CoV-2 infection should be carefully evaluated to understand whether it is a pathogenic or protective effect. Further studies may be useful for therapeutic purposes, particularly for patients who are critically ill and resistant to supportive care.

Figure 2: a,b,c: Interleukin-6 (IL-6) immunohistochemistry in SARS-CoV-2 infected lung tissue, cytoplasmic and membranous staining x400, d,e: Higher count of CD3 (+) T lymphocytes at the site of perivascular lymphocytic cuffing in the lungs x400, CD3. f: Lower count of CD8 (+) T lymphocytes at the site of perivascular lymphocytic cuffing in the lungs x400, CD8.

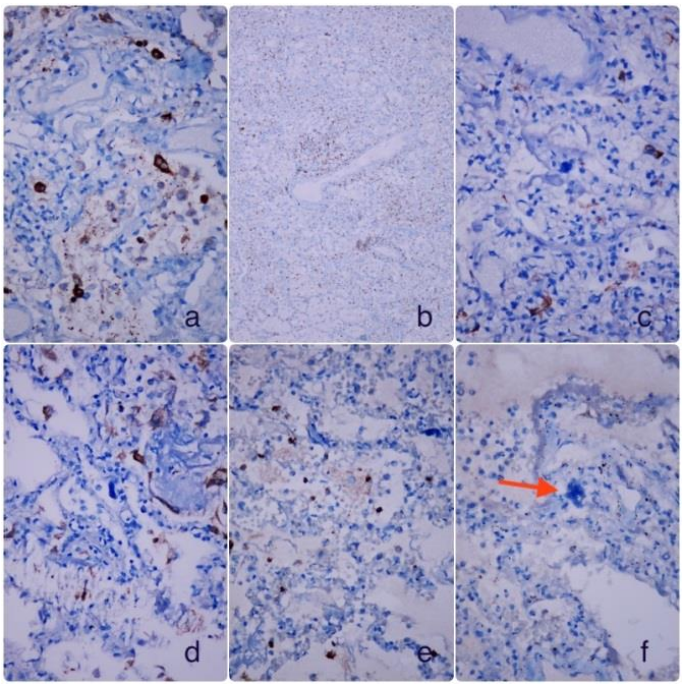


Lymphocytes and proinflammatory mediators such as IL-6 have a role in the antiviral activity, which aims to clear the virus [25]. Generally, viral infections stimulate T lymphocytes, which leads to rapid increases in CD8 (+) T lymphocytes. Lymphocytes were found mainly in the pulmonary perivascular areas and detected in 63 (67%) of 94 cases we evaluated. In our study, CD3 (+) T lymphocyte immunohistochemical staining was significantly higher than CD8 (+) T lymphocyte staining in the pulmonary perivascular areas, indicating a predominance of CD3 (+) T lymphocytes over CD8 (+) T lymphocytes in the perivascular lymphocytic infiltrate, in line with another study [26]. This finding supports the hypothesis of Huang JL et al. [27], who stated that the SARS-CoV-2 virus directly or indirectly destructs the CD8 (+) T lymphocytes through impairment of cellular immunity or SARS-CoV-2 induced-mediators, but the mechanism is not clear yet. However, an immunohistochemical study by Frisoni P et al. [24] reported that CD8 (+) T cells and CD20 (+) B cells were mainly found in the lung. They showed that the reason for this was that none of the patients in their study had received any treatment that would activate the immune system, for they died in an out-of-hospital setting [24]. In our study, 45.7% (n=43) of the cases were hospitalized. The immunohistochemical staining patterns of CD3 and CD8 T lymphocytes in the pulmonary perivascular areas are demonstrated in Figures 2d, 2e, and 2f.

Megakaryocytes are normally found in the lung and partially act in platelet homeostasis [28]. Megakaryocytes and their platelet production have attracted attention in recent decades [28]. Many studies showed that these platelets are not characteristic but play role in acute lung injury, which may be related to the activation of the coagulation cascade [28-30]. However, some studies indicate that their number also increases in the lung in diffuse alveolar damage, shock, sepsis, and burns [28-32]. The SARS-CoV-2 infection causes the release of megakaryocytes which may enter the capillaries and embolize to

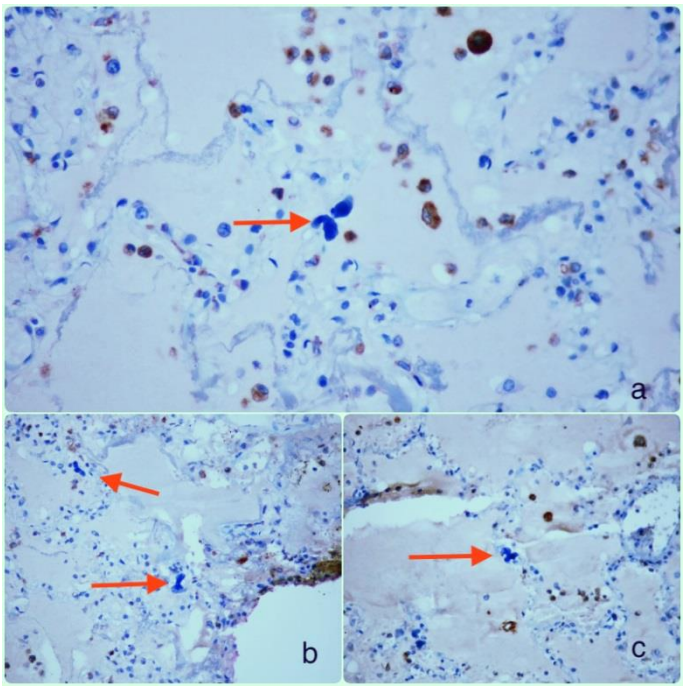
the other organs [33]. If the peripheral demand of platelet increases, the number of megakaryocytes rises in the bone marrow. It was thought that SARS-CoV-2 could drastically modify the human coagulation system as well as trigger fibrin thrombus formation in the distal pulmonary arteries by increasing megakaryocytes [33]. Interstitial megakaryocytes are common, especially in patients with macroscopic pulmonary embolism [26, 34]. These findings show us that COVID-19 infection, together with severe acute lung damage, affects the coagulation system, causing it to turn into a fatal infection by promoting coagulation. Borzcuk et al. [15] reported the presence of platelets and/or fibrin microthrombi in 84% of SARS-CoV-2 cases. In our study, fibrin thrombus was detected in 49 (52.1%) cases and the presence of megakaryocytes, in 63 (67%) cases. Activation of the coagulation cascade and triggering of fibrin thrombus formation in COVID-19, which benefits from preventive anticoagulant and antiplatelet treatment, may play role in mortality. SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb recognizes endogenous levels of total SARS-CoV-2 nucleocapsid protein and does not react with nucleocapsid proteins from SARS and MERS coronaviruses. In our study, SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb was used in some cases, and cytoplasmic and membranous staining was observed in the infected cells (Figure 3a, 3b, 3c, 3d, 3e, 3f). Megakaryocytes were not stained with SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb (Figure 3f), CD68 (Figure 4a, 4b, 4c), Pancytokeratin (Figure 5a, 5b, 5c), and TTF-1 (Figure 5d, 5e, 5f). CD68, Pancytokeratin (PanCK), and Thyroid Transcription Factor-1 (TTF-1) immunohistochemical staining were used for differentiation of histiocytes and epithelial cells from megakaryocytes, considering that it may be of pneumocyte origin due to the viral cytopathic effect.

Figure 3: SARS-CoV-2 nucleocapsid protein (E8R1L) Mouse mAb immunoreactivity



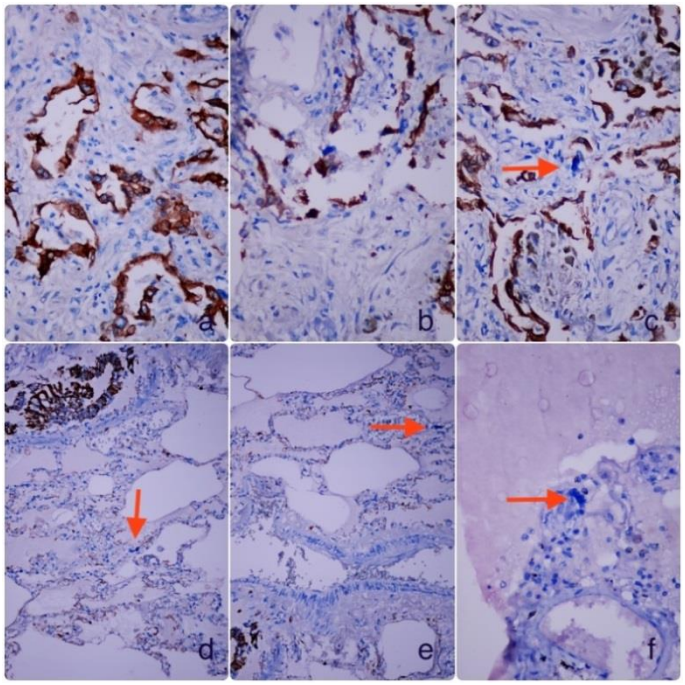
a,c,d,e: Antibodies against SARS-CoV-2 nucleocapsid protein were used to detect the infected cells. x400. b: Antibodies against SARS-CoV-2 nucleocapsid protein were used to detect the infected cells. x200. f: No staining was observed in megakaryocytes (Arrow) x 400.

Figure 4: CD68 immunohistochemical staining



a,b,c: CD68 staining was not observed in the megakaryocytes (arrows)x 400.

Figure 5: A: Pancytokeratin (PanCK) (a,b,c) and TTF-1 (d,e,f) immunohistochemical staining



a,b: Pancytokeratin (PanCK) immunoreactivity of Type II pneumocytes x400. c: Pancytokeratin (PanCK) immunoreactivity was not detected in the megakaryocytes (arrow)x400. d,e,f: TTF-1 immunoreactivity was not detected in the megakaryocytes (arrows)x400.)

Das et al. [35] stated that the presence of multinuclear syncytial cells and smudge cells in the light microscopic examination may give some clues to the viral etiologic agent, but without characteristic intranuclear or intracytoplasmic inclusions, the etiologic agent could not be assessed clearly. In addition, various histopathological findings in viral infections were evaluated and interstitial inflammation was reported as the most common among these morphologic findings. Although interstitial inflammation has no directive and predictive effect on the etiology of the viral agents, it is the most common finding in the presence of viral infection [35]. In our study, no intranuclear or intracytoplasmic inclusions were detected in 94 SARS-CoV-2 positive cases by light microscopy, in line with the current studies [35-38]. In addition, SARS-CoV-2-specific

histopathological, viral cytopathic changes, which differ from other viral infections that cause acute lung injury, were not observed in our study.

Limitations

Although the histopathological findings of the lungs were evaluated in a large series in this study, that we could not reach clinical information, such as concomitant diseases and the duration of SARS-CoV-2 positivity, limited our comprehensive evaluation. The lack of information on the period between the onset of the disease and death may also be considered a study restriction. Since our study was conducted in forensic autopsies, although all our cases had positive rt-PCR assay results, the causes of death in some cases were due to forensic causes. In addition, due to the delays in the supply of immunohistochemical markers during the pandemic, SARS-CoV-2 Nucleocapsid Protein (E8R1L) Mouse mAB, Pancytokeratin (PanCK), TTF-1, IL-6, CD68, CD3, and CD8 immunohistochemical assays could be performed in a limited number of cases. Lastly, an electron microscopic evaluation could not be performed for further investigation of the ultrastructural level.

Conclusion

Our study is important for the evaluation and verification of histopathological findings in the lungs of cases with positive SARS-CoV-2 rt-PCR tests. Our findings reveal that DAD findings are the predominant pathology in the lung in autopsies; however, in some cases, only minor findings were seen in the lung without associated DAD findings. Perivascular lymphocytic infiltrates in the lung contained more CD3 positive T lymphocytes than CD8 positive T lymphocytes, which correlates with the hypothesis of direct destruction of CD8 (+) T lymphocytes or through impairment of cellular immunity by SARS-CoV-2 related mediators.

Immunohistochemical staining with IL-6 in the lung in COVID-19 cases supports the role of IL-6 in cytokine storm and may require further investigation.

The number of megakaryocytes is increased in the lungs, which correlates with reports which mention that SARS-CoV-2 infection results in megakaryocyte release and promotes coagulation and vascular thrombosis, as in most other causes of acute lung injury. Also, no intranuclear or intracytoplasmic inclusions were seen in any SARS-CoV-2 rt-PCR positive cases by light microscopy.

Autopsies are highly important in understanding the pathogenesis of SARS-CoV-2 infection and contribute to the development of diagnostic and therapeutic modalities for infected patients. We thought that the pulmonary findings we describe will contribute to helping better perceive the SARS-CoV-2 pathogenesis and could be of guidance to the development of new treatment strategies and modalities.

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