ORIGINAL ARTICLE

COVID-19 autopsies of Istanbul

Murat Nihat Arslan[1] · Yalçın Büyük[2] · Nihan Ziyade[3] · Neval Elgörmüş[3] · Gözde Şirin[4] · İsmail Çoban[4] ·
Muhammed Emin Gökşen[4] · Taner Daş[5] · Arzu Akçay[6]

Abstract

Background/aims The aim of this study is to share autopsy findings of COVID-19-positive cases and autopsy algorithms for safely handling of suspicious bodies during this pandemic.

Methods COVID-19-positive cases of Istanbul Morgue Department were retrospectively analyzed. Sampling indications for PCR tests in suspicious deaths, macroscopic and microscopic findings obtained in cases with positive PCR tests were evaluated.

Results In the morgue department, 345(25.8%) of overall 1336 autopsy cases were tested for COVID-19. PCR test was found positive in 26 cases. Limited autopsy procedure was performed in 7 cases, while the cause of death was determined by external examination in the remaining 19 cases. Male-to-female ratio was found 3.3:1 and mean age was 60.0 ± 13.6 among all PCR-positive cases. Cause of death was determined as viral pneumonia in fully autopsied cases. Most common findings were sticky gelatinous fluid in cavities and firm and swollen lungs, varying degrees of consolidation. In microscopy, diffuse alveolar epithelial damage, type-II pneumocyte hyperplasia, hyaline membrane formation, fibrinous exudate, and fibrinous plaques in the alveoli were the most common findings.

Conclusions In COVID-19 autopsies, pulmonary findings were found to be prominent and the main pathology was pneumonia. Older age and findings of chronic diseases indicate that the cases were in the multirisk group in terms of COVID-19 mortality.

Keywords Autopsy · COVID-19 · Pulmonary findings · Risk factors

Introduction

Several coronaviruses (human coronaviruses) belonging to zoonotic human coronavirus family resulted in severe respiratory tract infections globally in recent times. Despite the fact that six types of human coronaviruses were defined, this family gained particular attention by Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) [1, 2].

✉ Murat Nihat Arslan
mnarslan@yahoo.com

Yalçın Büyük
doctorbuyuk@gmail.com

Nihan Ziyade
nihanziyade@gmail.com

Neval Elgörmüş
neyelgormus@yahoo.com

Gözde Şirin
gorucug@yahoo.com

İsmail Çoban
doktorcoban@gmail.com

Muhammed Emin Gökşen
emingoksen@gmail.com

Taner Daş
tanerdas@hotmail.com

Arzu Akçay
arzuakcay12@gmail.com

1   Istanbul Morgue Department, Council of Forensic Medicine, Istanbul, Turkey

2   Council of Forensic Medicine, Istanbul, Turkey

3   Postmortem Microbiology Laboratory, Istanbul Morgue Department, Council of Forensic Medicine, Istanbul, Turkey

4   Autopsy Unit, Istanbul Morgue Department, Council of Forensic Medicine, Istanbul, Turkey

5   Histopathology Unit, Istanbul Morgue Department, Council of Forensic Medicine, Istanbul, Turkey

6   Pathology Department, Gaziosmanpaşa Hospital Yeniyüzyıl University, Istanbul, Turkey

Coronavirus family is an enveloped, helical symmetry, positive-polar RNA virus family that causes disease in mammals and birds. In humans, they cause respiratory infections ranging from mild cold to pneumonia and rarely gastrointestinal complaints [3]. Although the history of coronaviruses began in the 1940s, the first human coronaviruses, HCoV229E and HCoV-OC43, were reported in the 1960s. After 2000s, HKU1, HCoV-NL63, SARS-CoV, and MERSCoV were shown as other members of coronavirus infecting humans [4]. The first four CoVs mentioned are universally constantly in circulation and are causative agents of about one-third of common colds in humans. SARSCoV and MERS-CoV differ from other coronaviruses in that they cause severe lung infections, and the fatality rates are 10% and 34%, respectively [5].

In December 2019, a group of patients with a new type of coronavirus was reported to be detected in Wuhan, China. International Committee of Taxonomy of Viruses (ICTV) named the new virus as SARS-CoV-2 [6].

After documented first cases in China, the infection has rapidly spread across the world and WHO declared "pandemic" on the 11th day of March. The first confirmed case of SARS-CoV-2 infection in Turkey was reported on March 10, 2020 and first mortality resulting from this infection on March 15. Though the infection has spread across the country after the report of first case, at the beginning, Istanbul was reported to have about two-thirds of total positive cases. Istanbul was one of the epicenters of the pandemic in Turkey.

Council of Forensic Medicine is an official organ of Ministry of Justice and provides expertise in medico-legal issues all over the country. Almost all Forensic autopsies are performed in the facilities of the council by its experienced specialists all over the country. Center Morgue Department (Istanbul) of CFM is well-developed and well-equipped facility having a special autopsy room that meets the standards recommended by the Centers for Disease, Control, and Prevention (CDC) for autopsies of COVID-19-positive patients [7]. Until establishment of "Airborne Infection Isolation Rooms" for autopsy facilities in other 5 provinces of the country, all COVID-19-positive autopsy cases were transferred to Istanbul for performing autopsies.

We aimed to share our autopsy findings of COVID-19-positive cases and algorithms developed for safely handling of suspicious bodies during this pandemic.

Materials and methods

This study was carried out with the approval of the Council of Forensic Medicine Scientific Committee and special permission of Ministry of Health.

Case selection and data collection

Autopsy records of Istanbul Morgue Department of the Council of Forensic Medicine during the COVID-19 pandemic (between February 2020 and June 2020) were retrospectively analyzed.

The algorithms of the Department for proper handling of bodies during pandemic were also evaluated together with the sampling indications for COVID-19 in suspicious deaths.

In PCR-positive cases, indications for autopsy or determination of cause of death without autopsy were discussed. In addition, macroscopic and microscopic findings and toxicological results obtained in cases with positive PCR test were evaluated.

Autopsy procedure and sample collection

During the COVID-19 pandemic, all autopsies were performed with a minimally invasive autopsy approach, and tracheal swab and lung swab samples were collected in addition to nasopharyngeal swab samples collected prior to autopsy. Histopathological samples were taken at autopsy, fixed in 10% formalin solution, and examined under light microscope after hematoxylin and eosin staining. Immunohistochemical investigations could not be performed due to lack of the supply of antibodies in the markets at the early stages of the pandemic. In addition, body fluid samples (blood, intraocular fluid, bile, and urine), liver and kidney tissue samples, and stomach contents were collected accordingly in suitable bodies and toxicological examination was performed.

In accordance with the law, all forensic autopsies were carried out by opening three cavities of the body. Unlike the standard autopsy technique, in minimally invasive autopsy technique, no organ or system was eviscerated except for the central nervous system and the heart, although it varies according to the case. Noneviscerated organs were evaluated by an in situ technique.

In cases considered as "possible COVID-19," a deep nasopharyngeal swab sample was collected prior to autopsy. During the autopsy, the tracheal swab sample was collected as shown in Fig. 1. Postmortem approach algorithm is shown in Fig. 2.

SARS-CoV-2 RNA real-time PCR

In order to determine SARS-CoV-2 in nasopharyngeal/tracheal swab samples and paraffin embedded lung tissue samples, real-time RT-PCR method, which is a nucleic acid amplification method that detects viral RNA, was applied. 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 by RT-PCR method and amplified in Rotor Gene (Qiagen/Germany) device in accordance with the manufacturer's recommendations.
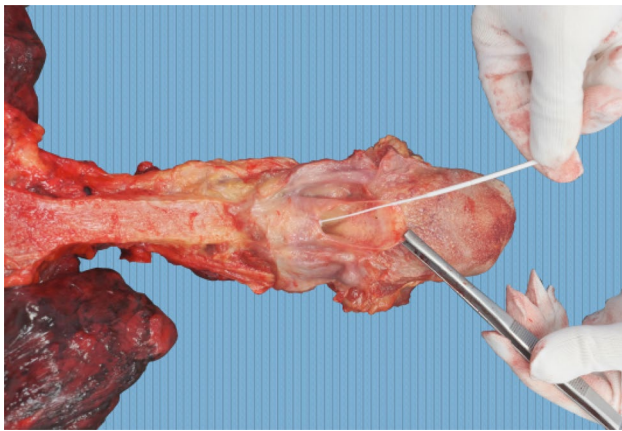
Fig. 1 Tracheal swab collection

In addition, nucleic acids were extracted from all paraffin-embedded lung tissues fixed with formalin. Three or four 10-mm-thick sections of each block were cut by a microtome. The xylene and ethyl alcohol deparaffinization procedure was applied to paraffin embedded lung tissues. It was then incubated for 24 h by adding 20 mL of proteinase K and 600 mL of ATL buffer solution [8].

Autopsy environment

Autopsy room of Istanbul Morgue Department has negative pressure ventilation system with Hygienic Air Handling

Units, performing 15 air changes per hour. Air in the autopsy room exhausted outside through a high efficiency particulate aerosol (HEPA) filter and also clean air flow supplied through a HEPA filter. Negative-pressure autopsy rooms were designed in accordance with the criteria defined by CDC as airborne infection isolation rooms (AIIRs) [7].

Results

In Turkey, the first case was reported on March 10, 2020, and the first COVID-19-related death occurred on March 15, 2020. The 2019-nCoV-RNA real-time PCR test was first started on February 22, 2020 in the Istanbul Morgue Department and continued to be applied as a routine examination on selected cases.

Between February 22, 2020 and June 9, 2020, 345 of 1336 autopsy cases were tested for COVID-19. A total of 348 cases were tested, three of which being tested on lung tissue samples collected at autopsy. In 26 (7.5%) cases, 2019-nCoV-RNA real-time PCR test was found positive. Limited autopsy procedure was performed in 7 cases with positive test results, and in the remaining 19 cases, the cause of death was determined by a detailed external examination together with the data obtained from death scene examination and medical records.

Male-to-female ratio was found 3.3:1 among the PCR test–positive cases. Most common age group was 41–70 age group. Mean age was 60.0 ± 13.6 among PCR-positive cases. Demographic features are shown in Table 1.
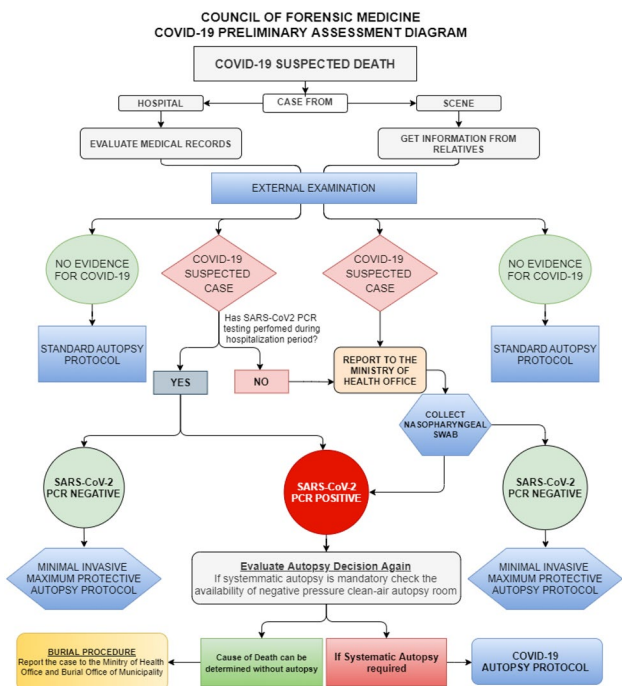

Fig. 2 COVID-19 preliminary assessment diagram of Council of Forensic Medicine

Table 1 Demographic features of cases

SARS-CoV-2-RNA real-time PCR	Positive	Negative	Total
Sex			
Male	20	254	274
Female	6	68	74
Total	26	322	348
Age groups			
Infant	0	6	6
11–20 years	0	8	8
21–30 years	0	28	28
31–40 years	1	47	48
41–50 years	6	65	71
51–60 years	6	76	82
61–70 years	6	50	56
71–80 years	5	28	33
81–90 years	1	12	13
91–100 years	1	2	3
Mean age			
Male	58.2 ± 10.2	51.1 ± 17.3	
Female	67.6 ± 23.1	49.3 ± 20.2	
All	60.0 ± 13.6	50.7 ± 17.9	

Cause of death was determined as viral pneumonia (COVID-19) in 14 cases, blunt trauma in 4 cases (three fall from height and one road transportation injury), and hanging in one case among the cases in which cause of death was determined without autopsy (Table 2). In all autopsied cases, cause of death was determined to be viral pneumonia (COVID-19) (Table 3).

In one case, since the PCR test was negative in the nasopharyngeal swab taken during the examination of the body at the time of investigation by prosecutor prior to the arrival of the body to the autopsy unit, no other PCR test was performed before the autopsy. However, due to the suspicious

appearance in the lungs at autopsy, the PCR test was performed on paraffin embedded lung tissue and was found positive. In all other cases, positive results were obtained in PCR tests performed before autopsy, and autopsies were initiated by taking precautions against known risks. All test results are shown in Table 4.

At autopsy, pleural adhesions were observed except for two cases. There was an accumulation of sticky gelatinous fluid in the chest cavity, plastered on the visceral pleura in two of the cases (Fig. 3). In all cases, the lungs filled the chest cavity and were swollen and tight. Two cases had unilateral and five cases had bilateral firm lungs. There was no

Table 2 Cases in which the causes of death were determined without autopsy

Gender Age	Clinical findings	Co-morbidity	Previous COVID-19 diagnosis	Incident	PCR test at hospital after death	Cause of death
Male 59	Cough, dyspnea, vomiting	Hepatitis B	No	Felt sick and taken to the hospital	Yes, positive	Viral pneumonia (COVID-19)
Female 84	Weakness and dyspnea 18 days ago	No known chronic disease	Positive	Felt sick and died at home	No	Viral pneumonia (COVID-19)
Male 58	No	No	No	Car crash	No	Blunt trauma
Male 45	Cough	Hypertension	No	Felt sick and taken to the hospital	No	Viral pneumonia (COVID-19)
Male 62	Not known	No known chronic disease	No	Felt sick and taken to the hospital	No	Viral pneumonia (COVID-19)
Male 61	Not known	No known chronic disease	No	Fall from height	No	Blunt trauma
Male 53	Not known	Diabetes	No	Found dead at home	No	Viral pneumonia (COVID-19)
Female 40	Chest pain	No known chronic disease	No	Felt sick and died at home	Yes, positive	Viral pneumonia (COVID-19)
Male 74	Not known	No known chronic disease (mental retardation)	No	Found dead at home (decomposition)	No	Viral pneumonia (COVID-19)
Male 71	Not known	Dementia	No	Found dead at nursing home	No	Viral pneumonia (COVID-19)
Male 45	Fever, cough	No known chronic disease	No	Found dead at home	No	Viral pneumonia (COVID-19)
Male 71	Weakness, cough	Chronic Bronchitis	No	Found dead at home	No	Viral pneumonia (COVID-19)
Male 49	Fever	Chronic Pulmonary disease	No	Fall from height	No	Blunt trauma
Male 64	No	No known chronic disease	Positive	Hanging	No	Hanging
Male 73	CT findings	Chronic heart and liver diseases, diabetes	Test negative	Felt sick and taken to the hospital	No	Viral pneumonia (COVID-19)
Female 72	Flu-like symptoms	Hypertension, bedridden	No	Felt sick and died at home	Yes, positive	Viral pneumonia (COVID-19)
Male 43	Fever, cough, dyspnea	Not known	No	Felt sick and died at home	No	Viral pneumonia (COVID-19)
Female 94	CT findings	Bedridden	Not known	Felt sick and died at home	No	Viral pneumonia (COVID-19)
Male 55	Hospitalized for COVID-19	Not known	Yes	Fall from height	No	Blunt trauma

Table 3 PCR test–positive autopsied cases

No.	Gender Age	Clinical findings	Co-morbidity	PCR during hospitalization	COVID-19 diagnosis	Incident	Duration of the disease	Cause of death
1	Male 68	No data	Hypertension, Diabetes	No	No	Felt sick and taken to the hospital, death in the hospital	No data	Viral infection (COVID-19)
2	Male 63	Hypotension, X-ray findings	Hypertension	Yes, positive	Yes	Prisoner—died in hospital	Death occurred 4 days after the onset of symptoms	Viral infection (COVID-19)
3	Male 67	CT findings	Rheumatic disease	Yes, negative	No	Found dead	No data	Viral infection (COVID-19)
4	Female 48	Dyspnea, weakness	No data	No	No	Felt sick and taken to the hospital, death in the hospital	Death occurred 7 days after the onset of symptoms	Viral infection (COVID-19)
5	Male 43	ICU admission—dead at ICU	Nonspecific pulmonary diseases	Yes, positive	Yes	Prisoner—died in hospital	Death occurred 11 days after the onset of symptoms	Viral infection (COVID-19)
6	Male 53	Pulmonary symptoms (lung cancer also)	Lung cancer	Yes, positive	Yes	Malpractice claim—lung cancer false operation claim	Death occurred 5 days after the onset of symptoms	Viral infection (COVID-19)
7	Male 53	ICU patient due to renal failure—uremic encephalopathy	Renal failure (hemodialysis patient)	Yes, negative (3 days ago)	No	Died in airplane (coming to Turkey for renal transplantation)	No data	Viral infection (COVID-19)

Table 4 2019-nCoV-RNA real-time PCR tests after death

No.	PCR test before prosecutor examination	PCR test before autopsy	PCR test during autopsy	PCR test to paraffin embedded tissue
1	Yes, negative	No	No	Yes, positive
2	Yes, positive	Yes, positive	Yes, positive	Yes, positive
3	No	Yes, positive	Yes, positive	Yes, negative
4	No	Yes, positive	Yes, positive	Yes, positive
5	No	Yes, positive	Yes, positive	Yes, positive
6	No	Yes, positive	Yes, positive	No
7	No	Yes, positive	Yes, positive	No

sign of major thromboembolism in the pulmonary truncus. In all cases, areas of consolidation of varying degrees were noted in lung sections.

At autopsy, samples were taken from the lungs, one from each lobe, and embedded in paraffin blocks. Routine histopathological examination on hematoxylin and eosin staining procedure was performed and examined under a light microscope. Regardless of the duration of the disease, patchy or diffuse interstitial lymphocytic infiltration of viral pneumonia and diffuse alveolar epithelial damage (DAD) in various stages was detected in lung tissues of all 7 cases. Prominent hyaline membrane formation, type II pneumocyte hyperplasia, fibrinous exudate, or fibrinous plaques in the alveoli were seen as the components of DAD (Fig. 4). Fibrin thrombi were seen in the vascular lumens in three of the cases. Two cases were found to have squamous metaplasia in lung samples.

Symptoms of acute phase DAD were detected in patients with short disease duration, while in the patient with a disease duration of 11 days, findings of organized phase DAD

and presence of intra-alveolar necrotic material due to viral cytopathic effect were detected (Fig. 5). The findings were consistent with acute lung injury in the acute phase depending on the duration of the disease. Autopsy findings are summarized in Tables 5 and 6.

Discussion

COVID-19 is a viral acute respiratory infection reported to affect many systems in the body [9]. After the epidemic was declared as a pandemic, academic studies were published by many centers around the world and information was shared. Publications sharing the clinical hypotheses and treatment recommendations regarding the pathogenesis of the disease, diagnostic criteria of the disease from a propedeutics perspective, personal protective measures in terms of biosafety, and findings of autopsy in terms of public health protective measures and forensic medicine have taken their places in the literature.

Although the necessity of autopsy for the diagnosis of SARS-CoV 2 is discussed by some authors [10], in autopsy studies, useful information was presented in revealing the
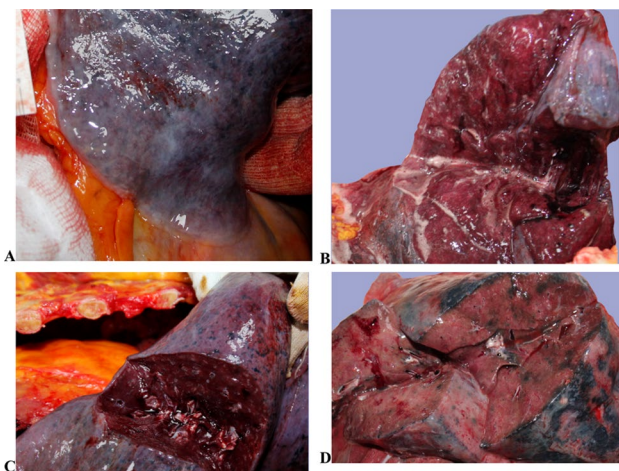

Fig. 3 In situ macroscopical lung findings. **A** Sticky gelatinous fluid on the contours of the lungs. **B** Excessive edema. **C** Dilated vessels and consolidation on the cut surfaces. **D** Consolidation, red hepatization
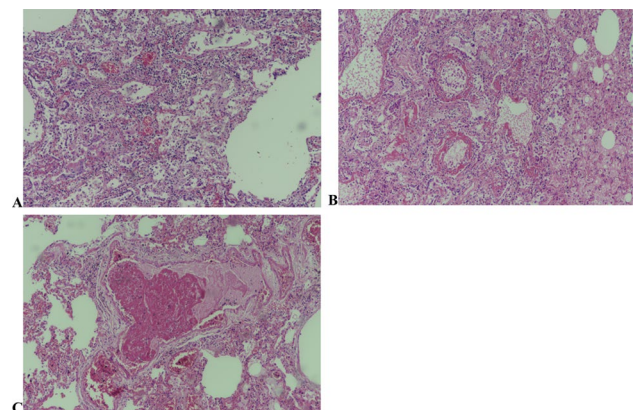

Fig. 4 **A** Type II pneumocyte hyperplasia with reactive cytologic atypia. **B** Alveoli wall covered with hyaline membranes and type II pneumocytes. **C** Fibrin thrombi in vessel lumens
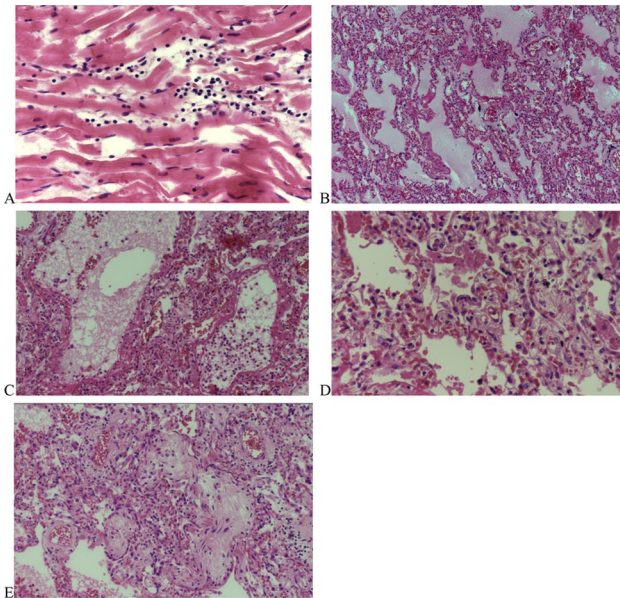
Fig. 5 Case #2. **A** Focal lymphocytic infiltration of myocardium, without myocyte necrosis (H&E, × 400). **B** DAD with hyaline membrane formation, paucicellular interstitial lymphocytic infiltration, edema and congestion, no microvascular thrombosis (H&E, × 200). **C** Granulocytic infiltration of focal bronchopneumonia associated with DAD (H&E, × 400). **D** Prominent proliferation of type II pneumocytes, subacute phase of DAD (H&E, × 400). **E** Fibrotic plugs in airspaces, subacute phase of DAD (H&E, × 400)

pathological findings of the disease and discussing the pathophysiology of the disease. In the commentary they wrote, Pomara et al. defended the lack of autopsy data of the disease and the necessity of postmortem sampling and autopsy in revealing the pathophysiology [11]. Moreover, as Edler et al. stated, in order to keep the statistical data of COVID-19 deaths accurate, appropriate cases should be autopsied [12].

In autopsy cases and clinical case series, bilateral ground-glass appearance, and alveolar opacities on thoracic computed tomography, typical ARDS findings such as diffuse alveolar damage, pulmonary edema, formation of hyaline membranes, type II pneumocyte hyperplasia, lymphadenopathy, pleural effusion, and microthrombi in small vessels are frequently reported respiratory system findings in histopathological examination. In contrast to SARS-CoV infection, computed tomography has also detected pleural effusion, lymphadenopathy, and cystic changes [9, 13–18].

In the study of Wichmann et al., postmortem computerized tomography was performed to the cases and demonstrated reticular infiltrations and bilateral consolidating infiltrates [19]. In the review of Lai et al., it was reported that bilateral lung involvement was prominent in terms of findings detected by imaging methods in SARS-CoV-2 pneumonia, and the most common finding in computed tomography examination was the ground-glass appearance [20].

Computed tomography findings were not directly evaluated as specific for COVID-19 or as diagnostic alone [16]. It has been shown that cardiomegaly, epicarditis/pericarditis, and myocarditis are prominent findings in the cardiovascular system, which is the other system frequently involved. Clinical findings of cardiovascular system involvement have also been reported as acute myocardial damage, arrhythmias, ST segment changes, and troponin elevation. In addition to the lung and heart, liver, brainstem, and kidney involvements have also been reported [9].

General views on pathogenesis are that COVID-19 infection targets the respiratory system, causing pneumonia and cardiovascular complications. Although clinical findings include fever, dry cough, rhinorrhea, sore throat, weakness, headache, loss of smell, hypoxemia, dyspnea, hemoptysis, diarrhea, lymphopenia, and increase in plasma proinflammatory cytokines [9, 15, 18, 21, 22], acute respiratory distress syndrome, cardiac pathologies, and thromboembolism have been blamed for the severe clinical course [9, 15]. In the COVID-19 case series presented with central nervous system complications (stroke) published by Avula et al., they reported that hypercoagulability and microthrombi formation caused by the disease may be responsible for the pathogenesis. In addition, it has been discussed that the virus directly invades the central nervous system and that hypoxia may also be effective [23]. Although the name of the COVID-19 epidemic was defined as SARS-CoV-2 (severe acute respiratory syndrome), as in previous coronavirus outbreaks, it was argued that the virus did not only affect the respiratory system but also could have central nervous system involvement. Nervous system findings such as headache, dizziness, confusion, disturbances in consciousness, ataxia, acute cerebrovascular disease, and loss of smell have been reported. Seizures have also been reported in some publications. Nervous system involvement is defined as a poor prognosis criterion for COVID-19 [24, 25].

Thromboembolism is frequently emphasized in studies discussing the pathogenesis in various clinical presentations [13, 23, 26]. Lodigiani et al. reported that thromboembolic complications developed in 7.7% of their cases in their study in which they investigated the frequency of venous thromboembolism in COVID-19 patients hospitalized in a university hospital in Italy [27]. Increased d-dimer level and fibrin degradation products shown in deceased cases indicate coagulation activity resulting in thromboembolism [9, 26]. In addition to the increase in coagulation activity, it is argued that virus-induced endotheliitis in endothelial cells may also play a role in thrombotic events in organs [9] which are probably shown as one of the main targets of the virus [26].

Similar to SARS-CoV, ACE2 (angiotensin converting enzyme) receptors in the lung have been defined as functional receptors for SARS-CoV-2 [2, 28]. SARS-CoV-2 virus binds directly to the ACE2 receptor, which is a cellular

Table 5 Gross autopsy findings

No.	Gender Age	Height/weight (BMI)	Thorax cavity	Pleural effusion	Heart weight	Pulmonary truncus	Lung consistency	Lung surfaces	Lung slices	Other
1	Male 68	179 cm/130 kg (40.6)	Pleural adhesions	No	490 g	No thrombi	Bilateral firm	Anthracotic pigmentation Fibrous pleural thickening	Edema Consolidation	No specific finding
2	Male 63	179 cm/94 kg (29.3)	No finding	No	512 g	No thrombi	Firm left lung	Subpleural petechiae Covered with gelatinous sticky fluid	Edema Consolidation Vessel dilatation	Polycystic kidneys
3	Male 67	167 cm/80 kg (28.7)	Pleural adhesions	Small amount Serous fluid	357 g	No thrombi	Bilateral firm	Anthracotic pigmentation	Edema Consolidation	Sub-acute subdural hematoma
4	Female 48	158 cm/73 kg (29.2)	Pleural adhesions	No	374 g	No thrombi	Firm right lung	Anthracotic pigmentation	Edema Consolidation	Trachea filled with foamy edema fluid
5	Male 43	175 cm/93 kg (30.4)	Left side pleural adhesions	480 mL serous fluid at left side	300 g	No thrombi	Bilateral firm	Covered with gelatinous sticky fluid	Edema Consolidation	Trachea mucosa covered with mucoid infectious fluid
6	Male 53	180 cm/98 kg (30.2)	Pleural adhesions	No	483 g	No thrombi	Bilateral firm	Excessive pleural adhesions	Edema Consolidation (Hepatization)	Operation scars on the right lung Space occupying lesion on right lung (filled with hematoma) Submucosal hemorrhages on trachea
7	Male 53	179 cm/84 kg (26.2)	No finding	400 mL purulent effusion at right side	338 g	No thrombi	Bilateral firm	Fibrous pleural thickening	Edema Consolidation	Trachea mucosa covered with foamy mucoid infectious fluid

BMI body mass index

Table 6 Histopathologic findings (H&E staining)

No.	Gender Age	Heart	Pulmonary						Other
			DAD	Hyaline membranes	Type II pneumocyte hyperplasia	Pneumonia	Thrombi	Other	
1	Male 68	Moderate perivascular and interstitial fibrosis	+	+	+	Lobular	Fibrin thrombi in large and medium calibrated vessels	Squamous metaplasia	Liver: moderate steatosis Kidney: chronic pyelonephritis CNS: hyperemia
2	Male 63	Mild perivascular fibrosis Hypertrophy Focal lymphocytic infiltration without myocytes necrosis	+	+	+	Interstitial	-	Focal bronchopneumonia excessive edema Fibrotic plugs in airspaces	None
3	Male 67	Focal myocardial scarring Perivascular and interstitial fibrosis Hypertrophy	+	+	+	Interstitial	-	Excessive edema, Patchy sparse inflammation	Liver: grade II steatosis Duramater: organizing hematoma (1 to 3 months)
4	Female 48	Edema, congestion	+	+	+	Interstitial	Fibrin thrombi in pulmonary vessels	Edema	Thyroid: nodular hyperplasia
5	Male 43	Focal scarring on papillary muscle	+	+	+	Interstitial	Fibrin thrombi in pulmonary vessels	Squamous metaplasia Viral cytopathic effect (necrotic material in alveoli)	Liver: mild steatosis Olfactory tract: severe corpora amylacea Deposits
6	Male 53	Mild perivascular fibrosis	+	+	+	Interstitial	-	Pleuritis with purulent fibrin	Liver: mild steatosis
7	Male 53	Granulation tissue Myocardial scarring	+	+	+	Lobular	-	-	Liver: Von Meyenburg complex (biliary microhamartoma) Kidney: chronic pyelonephritis, nodular glomerulosclerosis

DAD diffuse alveolar damage, *CNS* central nervous system

receptor, and it is thought to enter the cell via this receptor [13, 15, 24]. ACE2 receptor is an ectoenzyme attached to the plasma membrane and is found in many tissues such as the lower respiratory tract, heart, kidney, and gastrointestinal tract [2]. It is thought that it acts as ACE2 as a result of binding to the receptor, causing an increase in angiotensin II protein and the release of reactive oxygen derivatives accordingly. It has been reported that hypoxia and complement activity were caused by the inability to restrict blood flow to damaged vessels in the lung, and the influence of coagulation pathways plays an important role in the pathogenesis [13]. It is claimed that hypercoagulopathy and changes in coagulation pathways are associated with inflammatory response [26, 29].

In the autopsies of Istanbul, the cases were evaluated by the sampling criteria recommended by the Ministry of Health and modified by the Morgue Department in terms of death cases. Only three cases had a positive PCR test during hospitalization. This has shown the importance of correctly determining the sampling criteria. The SARS-CoV-2-RNA real-time PCR test was performed on nasopharyngeal swabs obtained at the Morgue Department before autopsy in two cases who had suspicious CT findings during the hospitalization period but were reported to be negative for the PCR test, and positive results were obtained. It is thought that many factors, from sampling methods to test methods, as well as the ability of the test to detect positive cases, may play a role. While evaluating the indications for sampling, as well as the standard criteria of the Department, anamnesis questioning the clinical findings, suspicious contacts, and symptoms is of great importance.

Macroscopically and microscopically lung pathologies were prominent in Istanbul autopsies. Regardless of the duration of the disease, it was observed that there was a viscous firmness in the lungs in all cases, and the lungs were covered with sticky gelatinous material in two cases. Consolidation was observed in lung sections in all cases.

COVID-19 has often been defined as a systemic infection involving the pulmonary and cardiovascular system. It has been reported that inflammatory mechanisms and immune system play a role in this involvement [9, 30]. Cytokine storm syndrome with immune system irregularities is thought to exacerbate interstitial pneumonia resulting in diffuse alveolar damage and cause viral sepsis with hypercoagulability, thus creating a vicious cycle [31].

Pleurisy, pericarditis, diffuse or patchy lung consolidation, congestion, and pulmonary edema have been reported among the gross lung findings. It has been reported that there may be an increase in weight in the lungs or that the weight may be within normal limits [18, 19, 32–34]. Hypercoagulability, pulmonary thrombotic microangiopathy, deep vein thrombosis, and pulmonary thromboemboli, which have been reported as having important role in pathogenesis, were

the most frequently reported findings in COVID-19 related deaths. While pulmonary intravascular coagulation occurs due to the inflammatory response, it has also been reported that dilatation develops in the right heart cavities due to pulmonary hypertension arising from infected alveolar ACE2 cells [29]. Fibrotic phase is rarely observed in cases with short disease periods in terms of pulmonary findings [33].

Barton et al. reported patchy and sparse interstitial inflammation of the lung in one of the Oklahoma cases. A similar patchy sparse inflammation was detected in one of Istanbul's COVID-19 autopsies (Fig. 6c). In the same case, a negative result was obtained from the PCR test performed on paraffin embedded lung tissue. Although bronchopneumonia is accepted as a finding of secondary infection, which is one of the criteria for poor prognosis, a focal bronchopneumonia focus was found in one of the Istanbul cases similarly. As in the New Orleans cases reported by Fox et al., no finding indicating secondary pulmonary infection was found in the other 6 cases in Istanbul [34].

Considering the pathological findings of Istanbul autopsies, although the time from the onset of symptoms to death is variable, findings indicating that the cases are in different stages of ARDS have been identified. Although diffuse alveolar damage was detected in all cases, type II pneumocyte hyperplasia was accompanied by squamous metaplasia in only two cases. There was no information about the duration of the disease in one of these two cases; the other died after 11 days of the disease. Also, in this case, the presence of necrotic material in the alveoli, which indicates viral cytopathic effect, was evaluated as a sign of the subacute phase of ARDS. However, in a case known as the disease period of 4 days, fibrotic plugs were observed, indicating subacute diffuse alveolar damage period. Although it is thought that the presence of bronchopneumonia, which is considered as a secondary infection in the same case, may be effective in
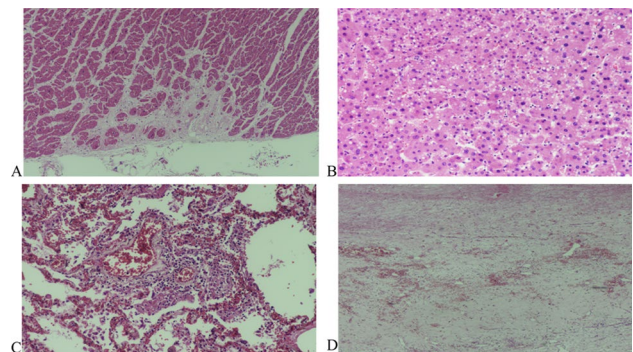

Fig. 6 Case #3. **A** Focal area of myocardial scar (H&E, × 200). **B** Steatosis of Hepatocytes (H&E, × 200). **C** Patchy lymphocytic interstitial infiltration and fibrinous exudate in the alveoli. This image was taken from one of the few areas where inflammation was obvious. In other areas, infiltration was sparse or absent (H&E, × 400). **D** Organizing subdural hemorrhage (H&E, × 200)

the emergence of this finding, it is thought that the staging of lung findings may be influenced by other personal risk factors, regardless of the duration of the disease.

When evaluated in terms of thromboembolism, which is an important criterion in poor prognosis and mortality, no thrombus was observed in the pulmonary truncus and main pulmonary vessels in Istanbul autopsies, but fibrin thrombi or fibrinous deposits were observed in histopathological examination. There was no evidence of major pulmonary thromboses like the cases reported by Grimes et al. [35]. Fox et al. similarly reported that there was no thrombus in the main pulmonary vessels in their cases [34]. Edler et al. reported that 40% of the cases had deep vein thrombosis and 9 cases had pulmonary thromboembolism in their studies on 80 cases autopsied in Germany with positive COVID-19 tests. Considering that the vast majority of these cases were hospitalized or cared for in a nursing home, it was thought that immobilization during the illness could also be effective in the formation of thromboembolism [12]. Pulmonary microthromboembolism was not also detected in any of the autopsy cases of Istanbul. This finding has shown that in COVID-19 cases of Istanbul, thromboembolism involving small vessels is more prominent than microthrombus or large pulmonary vessel thromboembolism.

Like the lungs, it has been observed that the heart is one of the organs involved in COVID-19 to varying degrees. Common cardiac findings have been reported to be cardiomegaly and right ventricular enlargement [18, 34]. It has been argued that the presence of lymphocytes together with degenerated myocytes in rare areas may be a sign of the early stages of viral myocarditis [19, 34]. Although no findings suggestive of myocarditis were detected in any of the Istanbul cases, focal lymphocytic infiltration in the myocardium without myocardial necrosis was detected in the patient with bronchopneumonia. In six of the Istanbul COVID-19 autopsies, nonspecific chronic ischemia findings accompanied by varying degrees of coronary artery stenosis in the heart were detected. These cardiac findings indicate an underlying chronic heart disease which is one of the poor prognosis criteria rather than the direct effect of viral pneumonia.

As a conclusion, similar with the other published studies, pulmonary histopathological findings include edema, proteinous exudate, vascular congestion, inflammatory deposits accompanied by fibrinoid material, multinuclear giant cells, interstitial edema, reactive alveolar epithelial hyperplasia, fibroblastic proliferation, type II pneumocyte proliferation, and varying spectrum from acute respiratory distress syndrome (ARDS) to diffuse alveolar membrane damage [9, 13–15, 34, 36, 37]. It has been reported that there is diffuse alveolar damage with exudates especially in the case whose ground-glass appearance was described in radiological examination [32]. Viral particles in the pneumocyte

cytoplasm were demonstrated in electron microscopy studies [33, 37].

Among the reported cases, it has been argued that the detection of pathology suggesting cardiac Kawasaki disease with inflammatory involvement in the vessels and especially in the coronary arteries is not caused by viral alveolitis but due to the immune system response [29, 38].

In COVID-19-related deaths, it has been reported that the presence of some chronic diseases besides COVID-19 pneumonia is a poor prognosis criterion and increases mortality. These are stated as chronic lung diseases, coronary artery diseases, arterial hypertension, hepatitis C, renal failure, malignancies, and obesity [12, 13, 18, 19, 22]. In addition, pregnancy, one of the clinical groups that increase the susceptibility to thromboembolism, is stated to be in the risky group. In Istanbul cases, one patient has hypertension and diabetes, one has pulmonary cancer, one another has renal failure requiring hemodialysis, and one has a history of undiagnosed nonspecific lung disease. Medical histories of the cases whose cause of death was determined as viral infection (COVID-19) without autopsy revealed that one of these cases had hepatitis B, one had diabetes, two had hypertension and one had chronic bronchitis, one had diabetes with chronic heart and liver diseases, and two of them were bedridden patients. One patient had dementia and one had mental retardation. These cases were considered to be in the high-risk group for COVID-19 deaths. In other cases, no other chronic disease was identified that would put it in the risk group, except chronic cardiovascular disease and hypertension.

The age of 60 is cited as an important determinant for mortality. In the early stages of the pandemic, approximately 80% of COVID-19-related deaths were reported to be seen in cases over 60 years of age, and in later periods, this age limit was updated to 65 years and over [39]. It has also been reported that the period from the onset of symptoms to death is shorter over the age of 70 [15]. The risk of serious disease picture increases with age in addition to mortality [18, 39]. In the study in which Northern Italy COVID-19 autopsies were reported, the male to female ratio of cases was 6.6:1; the average age was reported to be 69 [33]. Considering all cases with positive PCR test results in the Istanbul Morgue Department, the most frequent case group was 41–70 age group and the average age was 58.2 ± 10.2 for males and 67.6 ± 23.1 for females (Table 1).

In conclusion, in autopsies of COVID-19 PCR-positive cases, pulmonary findings were prominent, and the main pathology was pneumonia; diffuse alveolar epithelial damage in the lung, type II pneumocyte hyperplasia, and hyaline membrane formation were found to be present in all cases regardless of the duration of the disease. Chronic ischemic cardiovascular disease findings in cases, lung cancer in one case, and chronic renal failure requiring hemodialysis in one

case indicate that the cases were in the risk group in terms of COVID-19 mortality.

Declarations

Ethics approval The study was approved by the Ministry of Health of Turkey and of Forensic Medicine Scientific Committee.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Yin Y, Wunderink RG (2018) MERS, SARS and other coronaviruses as causes of pneumonia. Respirology 23(2):130–137
2. Petrosillo N, Viceconte G, Ergonul O et al (2020) COVID-19, SARS and MERS: are they closely related? Clin Microbio Infect
3. De Groot RJ, Baker S, Baric RS et al (2012) Family Coronaviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (eds) Virus taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Academic Press, International Union of Microbiological Societies Virology Division, pp 806–817
4. Pillaiyar T, Meenakshisundaram S, Manickam M (2020) Recent discovery and development of inhibitors targeting coronaviruses. Drug Discov Today 25(4):668–688. https://doi.org/10.1016/j.drudis.2020.01.015
5. Mahase E (2020) Coronavirus: covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. BMJ 368:m641. https://doi.org/10.1136/bmj.m641
6. Gorbalenya AE, Baker SC, Baric RS et al (2020) The species severe acute respiratory syndrome related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 5:536-544
7. CDC - COVID-19 (2020) Guidance postmortem specimens. Collection and submission of postmortem specimens from deceased persons with known or suspected COVID-19. Cent Dis Control Prev
8. Chan P, Chan D, To K et al (2001) Evaluation of extraction methods from paraffin wax embedded tissues for PCR amplification of human and viral DNA. J Clin Pathol 54:401–403
9. Buja LM, Wolf DA, Zhao B et al (2020) The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): Report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. Cardiovasc Pathol 48:107233. https://doi.org/10.1016/j.carpath.2020.107233
10. Sapino A, Facchetti F, Bonoldi E et al (2020) The autopsy debate during the COVID-19 emergency: the Italian experience. Virchows Archiv:1
11. Pomara C, Li Volti G, Cappello F (2020) COVID-19 deaths: are we sure it is pneumonia? Please, autopsy, autopsy, autopsy! J Clin Med 9(5):1259
12. Edler C, Schröder AS, Aepfelbacher M et al (2020) Dying with SARS-CoV-2 infection—an autopsy study of the first consecutive 80 cases in Hamburg, Germany. Int J Legal Med 1
13. Magro C, Mulvey JJ, Berlin D et al (2020) Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res 220:1–13. https://doi.org/10.1016/j.trsl.2020.04.007
14. Carbone M, Green JB, Bucci EM, Lednicky JA (2020) Coronaviruses: facts, myths, and hypotheses. J Thorac Oncol 15(5):675–678
15. Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun 102433
16. Shi H, Han X, Jiang N et al (2020) Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis
17. Sessa F, Bertozzi G, Cipolloni L et al (2020) Clinical-forensic autopsy findings to defeat COVID-19 disease: a literature review. J Clin Med 9(7):2026
18. Menter T, Haslbauer JD, Nienhold R et al (2020) Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. Histopathology 77(2):198–209
19. Wichmann D, Sperhake JP, Lütgehetmann M et al (2020) Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Ann Intern Med
20. Lai CC, Shih TP, Ko W-C et al (2020) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents 105924
21. Tian S, Hu N, Lou J et al (2020) Characteristics of COVID-19 infection in Beijing. J Infect
22. Schaller T, Hirschbühl K, Burkhardt K et al (2020) Postmortem examination of patients with COVID-19. JAMA 323(24):2518–2520. https://doi.org/10.1001/jama.2020.8907
23. Avula A, Nalleballe K, Narula N et al (2020) COVID-19 presenting as stroke. Brain Behav Immun 87:115–119. https://doi.org/10.1016/j.bbi.2020.04.077
24. Mao L, Wang M, Chen S et al (2020) Neurological manifestations of hospitalized patients with COVID-19 in Wuhan, China: a retrospective case series study. https://www.medrxiv.org/content/. https://doi.org/10.1101/2020.02.22.20026500v1. Accessed 29 June 2020
25. Asadi-Pooya AA, Simani L (2020) Central nervous system manifestations of COVID-19: a systematic review. J Neurol Sci 413:116832. https://doi.org/10.1016/j.jns.2020.116832
26. Benhamou D, Keita H, Ducloy-Bouthors AS (2020) Coagulation changes and thromboembolic risk in Covid-19 obstetric patients. Anaesth Crit Care Pain Med. https://doi.org/10.1016/j.accpm.2020.05.003
27. Lodigiani C, Iapichino G, Carenzo L et al (2020) Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan Italy Thrombosis research
28. Maiese A, Manetti AC, La Russa R et al (2020) Autopsy findings in COVID-19-related deaths: a literature review. Foren Sci Med Pathol 1-18
29. McGonagle D, Plein S, O'Donnell JS et al (2020) Increased cardiovascular mortality in African Americans with COVID-19. Lancet Respir Med
30. Falasca L, Nardacci R, Colombo D, Lalle E, Di Caro A, Nicastri E, Antinori A, Petrosillo N, Marchioni L, Biava G (2020) Postmortem findings in Italian patients with COVID-19: a descriptive full autopsy study of cases with and without comorbidities. J Infect Dis 222(11):1807–1815
31. Maiese A, Passaro G, Matteis AD et al (2020) Thromboinflammatory response in SARS-CoV-2 sepsis. 88(2):78-80
32. Hanley B, Lucas SB, Youd E et al (2020) Autopsy in suspected COVID-19 cases. J Clin Pathol 73(5):239–242
33. Carsana L, Sonzogni A, Nasr A et al (2020) Pulmonary postmortem findings in a large series of COVID-19 cases from Northern Italy. medRxiv:2020.2004.2019.20054262. https://doi.org/10.1101/2020.04.19.20054262
34. Fox SE, Akmatbekov A, Harbert JL et al (2020) Pulmonary and cardiac pathology in African American patients with COVID-19:

an autopsy series from New Orleans. Lancet Respir Med. https://doi.org/10.1016/S2213-2600(20)30243-5

35. Grimes Z, Bryce C, Sordillo EM et al (2020) Fatal pulmonary thromboembolism in SARS-CoV-2-infection. Cardiovascular pathology: the official Journal of the Society for Cardiovascular Pathology 48:107227–107227

36. Tian S, Hu W, Niu L et al (2020) Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. J Thorac Oncol 15(5):700–704. https://doi.org/10.1016/j.jtho.2020.02.010

37. Yao X, Li T, He Z et al (2020) A pathological report of three COVID-19 cases by minimally invasive autopsies. Zhonghua bing li xue za zhi. Chinese J Pathol 49:E009-E009

38. Riphagen S, Gomez X, Gonzalez-Martinez C et al (2020) Hyper-inflammatory shock in children during COVID-19 pandemic. The Lancet 395(10237):1607–1608

39. CDC COVID-19 Response Team (2019) Severe outcomes among patients with coronavirus disease (COVID-19)—United States, February 12–March 16, 2020 (2020. MMWR Morb Mortal Wkly Rep 69(12):343–346

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.