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Short Communication

COVID-19 autopsies: Procedure, technical aspects and cause of fatal course. Experiences from a single-center

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ABSTRACT

Autopsies on COVID-19 have provided deep insights into a novel disease with unpredictable and potentially fatal outcome. A standardized autopsy procedure preferably with an in-situ technique and systematic tissue processing is important. Strict safety measures include personal protective equipment with a standardized protocol for dressing and undressing, usage of FFP-3 masks and minimization of aerosol production. The use of an airborne infection isolation (AIIR) room is preferred. Viral RNA analysis using swabs from throat, both lungs and other organs provides information on cross-organ viral dynamics. To correctly determine the full extent of pathological organ changes an adequate processing procedure is of the utmost importance. Systematic dissection and processing of the lungs revealed pulmonary infarction caused by thrombosis and thromboembolism and bacterial bronchopneumonia as the most frequent cause of death. Fungal pneumonia (aspergillus) was found in one case. The quality of the tissue was sufficient for histopathological and immunohistochemistry analyses in all cases. Viral RNA from throat or lung swabs was detectable post mortem in 89 % of the cases and could also be detected from paraffin-embedded tissue by real-time PCR. Complete COVID-19 autopsies including extensive histopathological studies and viral RNA analysis require approximately three times more human and technical resources and time compared to standard non-COVID autopsies. Autopsies on COVID-19 are feasible, present a manageable risk, while following a strict protocol, and provide novel insights into disease pathogenesis and the clinician with important feedback.

1. Introduction

The global spread of the severe acute respiratory syndrome (SARS)-coronavirus-2 (CoV-2) causing the coronavirus disease 2019 (COVID-19) has plunged our modern world into an economic and healthcare crisis. Performing autopsies is a critical step to explore the underlying pathomechanisms of a disease and to determine the cause of fatality. Initially, there was a wide reluctance in performing autopsies in many countries, caused by a shortage of resources, staggering amounts of casualties but also an uncertain risk of infection for the autopsy team. During the last months, experience reports [7,10,15,25], examples of good practice [4] as well as autopsy procedure proposals have been published [6,27] underlining the importance of strict safety measures and a well-designed autopsy protocol [3,6,12,17]. In addition, the current literature offers little information on appropriate processing of COVID-19 samples. Hereby, we report our experience about practicability, usefulness and feasibility of COVID-19 autopsies and our processing method for COVID-19 tissue samples. We further analyze the most important pathological findings causing death and report on the quality of the tissue obtained by autopsy for conventional histology,

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immunohistochemistry and molecular analysis.

2. Methods

2.1. Case selection and pre-autopsy preparation

According to federal law in Austria an autopsy in a public hospital is mandatory without requirement of an informed consent by the relatives if it is ordered by health authorities or public prosecutor or if public or scientific interest is present, particularly if the cause of death is unclear. All performed procedures and investigations were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments and permitted on basis of the federal law. From March 1 to June 30 2020, we performed 28 autopsies on COVID-19 deceased corresponding to a 35 % autopsy rate of COVID-19 deceased in our hospital. All cases were clinically proven COVID-19 based on clinical presentation with symptoms and signs of respiratory infection, radiological findings of respiratory infection and positive PCR testing. The case selection for the autopsies was done randomly without medical exclusion criteria and determined by infrastructural, time and personnel constraints under the challenging pandemic situation. The average time between autopsy and death was 50.3 ± 28.6 h. All autopsies were performed in a high standard certified morgue with a special ventilation system (separate air circulation with supply air of 2120m^3/h, exhaust air of 2180 m^3/h and air exchange of 24x/h) considered as Airborne Infection Isolation Room (AIIR) by the same team consisting of two pathologists and one autopsy assistant. All safety measures were set up in advance together with the responsible specialist for hospital hygiene (K.V.). Before the start of the study all team members underwent in-house training, particularly, how to correctly dress and undress when dealing with infected patients according to clinical guidelines [22].

Prior to the autopsy, clinical information was retrieved from the electronic hospital information system (MEDOCS) of the Styrian hospital corporation and carefully reviewed. The equipment for the autopsy consisted of a standard autopsy set and sampling material (Fig. 1) [13]. In all cases, regardless whether the last ante-mortem swab was positive for SARS-CoV-2 or not, swabs (Copan ESwab™ collection system) were taken from nasopharynx and both lungs to rule out possible false negative results or recurrence of viral positivity [16]. Swabs were also taken from other anatomic regions (brain, small intestine, colon, gallbladder/bile duct, lungs) and analyzed for SARS-CoV-2 positivity [9, 26]. Before each autopsy, a detailed checklist with all preparatory steps, paperwork and equipment and all necessary clothing steps were reviewed by the team (Table 1, Figs. 2, 3). Team members individually checked their health condition (in particular body temperature and signs and symptoms of an infection) and were responsible for reporting any changes.

Table 1
Personal protection and hygiene for COVID-19 autopsies in our department.

Universal personal protective equipment (PPE)	
- Surgical scrubs - Surgical cap - FFP3 mask - Face shield - Waterproof surgical gown - Waterproof surgical sleeve protectors - Plastic apron - OR shoes - Protective cut-resistant polyethylene gloves (proFood™ Knitted Spectra®) - Single use disposable latex gloves (2 pairs)	
Dressing	Undressing
1) Remove all personal items 2) Surgical scrubs and OR shoes 3) Surgical gown 4) Disposable apron 5) FFP3 mask 6) Surgical cap 7) Cut-resistant and latex gloves 8) Sleeve protectors 9) Second pair of latex gloves 10) Face shield	1) Remove apron 2) Remove outer pair of gloves 3) Remove sleeve protectors + hand disinfection 4) Remove face shield 5) Remove surgical cap 6) Remove gown 7) Remove gloves + hand disinfection 8) Remove OR shoes 9) Remove mask 10) Hand washing + disinfection

According to our internal hygiene standard for the treatment and care of COVID-19 patients and WHO (www.who.int).

2.2. Autopsy procedure

One pathologist remained clean and was responsible for taking swabs, documentation of findings and surveillance of the safety measures, particularly, to avoid contamination. A pharyngeal swab was taken before starting the autopsy. The autopsy technique consisted of a combination of en bloc and in-situ (Rokitansky) methods. A Y-cut was performed to open the thoracic and abdominal cavities. To avoid potential aerosol production the ribcage was opened manually along the chondral parts with a pair of rib shears and the sternal plate was removed. The thoracic cavity was then inspected for adhesions, pleural effusion, hemorrhage and other gross abnormalities. Subsequently, the neck region was prepared and removed en bloc together with the thoracic situs, which was separated from the abdominal situs at the diaphragm; the abdominal situs was left in place. During preparation, the neck was inspected for gross abnormalities such as enlargement of lymph nodes, salivary glands, and the thyroid. Pharynx, esophagus, and trachea were opened using scissors.

Subsequently, the bronchial system was opened and swabs from the bronchial system were taken. For each side fresh scissors were used to avoid cross-contamination. Heart and lungs were separated from the remaining parts of the thoracic situs and weighed. Before opening, the heart was grossly examined for pathological changes, particularly for

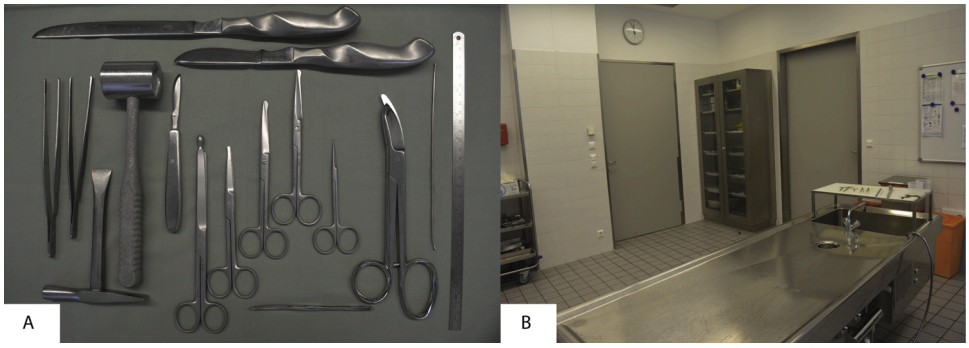

Fig. 1. Autopsy equipment (A) and room (B).

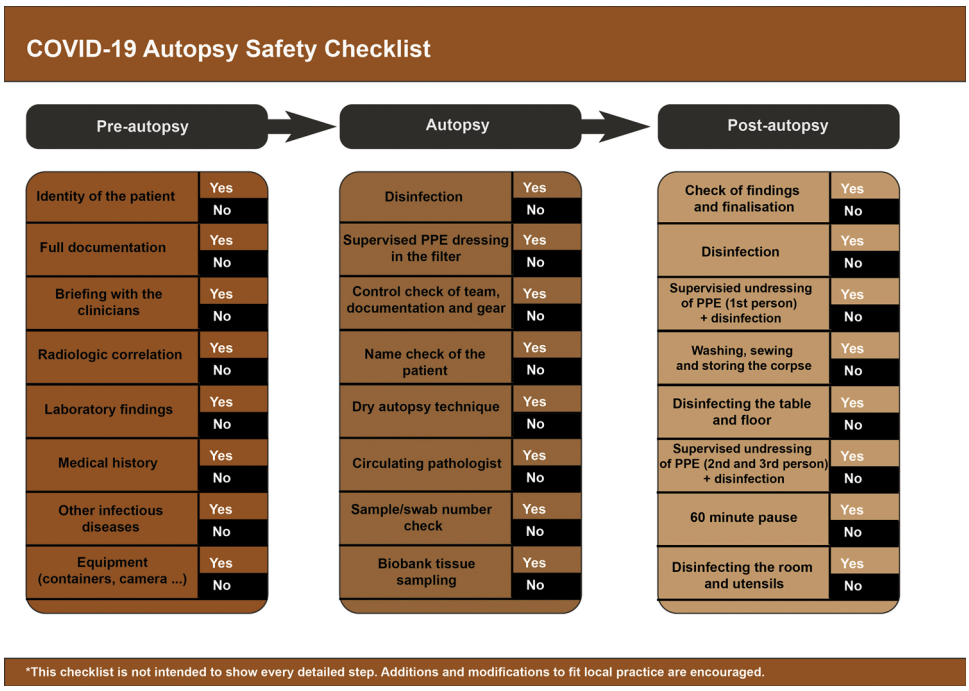

Fig. 2. Summarized COVID-19 autopsy checklist.

Fig. 3. Personal protective equipment for COVID-19 autopsies, consisting of a waterproof surgical gown to cover whole body and forearms, waterproof sleeve protectors, surgical scrubs, a scrub hat, an FFP-3 mask, a aface shield, a plastic apron, OR shoes and 2 pairs of single use disposable latex gloves (with cut-resistant gloves underneath. The neck was not completely covered but cleaned and disinfected after autopsy. With permission of the presented individuals.

changes of the pericardium and dilatation. The heart was opened along the blood flow from the right atrium to the pulmonary trunk and subsequently from the left atrium to the aorta enabling a good view of all 4 chambers. The coronary arteries were opened and examined for sclerosis and stenosis. The pulmonary trunk and the main branch of both pulmonary arteries were opened with scissors and examined for thromboembolic material. Finally, the myocardium was sliced from inside to outside to detect gross changes.

Inspection and preparation of the peritoneal cavity and the abdominal organs was performed in-situ before some organs were removed. First, the stomach and the duodenum were opened, the duodenal papilla (papilla of Vater) inspected, the common bile duct explored with a probe and opened. The gallbladder was opened by an incision with a fresh scalpel and a swab was taken. Subsequently the omental bursa was opened to access the pancreas. The spleen and subsequently the liver were removed, weighed and sectioned. Both adrenals were sectioned, both kidneys uncovered, sectioned and removed. Swabs were also taken from large and small intestines after opening by a fresh scalpel. Aorta, vena cava, pelvic vessels and pelvic organs were opened and inspected in-situ. The deep veins of the lower legs were opened from the calf, the veins of the upper leg from the anterior side.

The cranium was opened using a bone saw with integrated vacuum. The brain was removed, and the venous sinus opened. Brain stem and cerebellum were removed by cutting through the pons and sectioned, the cerebrum cut into two halves by a frontal section through the mamillary corpora and fixed totally for further neuropathological preparation. To avoid any aerosol formation during autopsy, a “dry technique” was used without use of water and the cut surfaces of the organs were cleaned by wiping them with a knife’s blade. For cleaning of the table, the instruments, the skin of the corpse and gloves of the autopsy team only disinfection solution was used.

2.3. *Tissue sampling and processing*

Samples for histopathological examination were taken from all major organs and fixed in 4% buffered formalin. The lungs were fixed in toto and for improved fixation the bronchial system was filled with 4% buffered formalin using a syringe. After fixation for at least 48 h the

lungs were systematically processed by serial slicing from the apex to the basis (Fig. 4). This allowed a careful gross examination of the various anatomical regions and an extensive tissue sampling allowing the detection of different stages of diffuse alveolar damage as well as thrombosis of pulmonary vessels and smaller infarcted areas [19]. For the lung samples giant sections were performed which provided valuable information on the distribution of pathological changes (Fig. 5). In addition, samples from organs of interest were snap frozen and stored in liquid nitrogen for research projects. A biomedical technologist assisted on request in freezing and fixation of tissue.

2.4. Post-autopsy procedure

After autopsy, the second pathologist supported to the other team members to undress. This was performed in the same room but with a minimum distance of 3 m from the autopsy table. All disposable material was discarded in the autopsy room. The autopsy assistant sewed the body, washed it with disinfectant, put it into a body bag and stored it in the cooling chamber. The equipment was soaked for 30 min in disinfectant (1% PERFEKTAN® ENDO solution for 30 min) and later washed in a sanitizing dishwashing machine at 95 °C. Immediately after the last autopsy, the table and floor were soaked in disinfectant for 30 min before any further cleaning was done. Subsequently, the autopsy table, floor and surroundings were systematically cleaned using disinfectant (0.5 % OPTISAL® PLUS solution for 60 min). No pure water was used in the cleaning procedure.

All members from the autopsy team as well as all other co-workers of the pathology department stayed healthy during the whole period of performing COVID-19 autopsies. The two team members performing the autopsies were tested twice negative for SARS-CoV-2 antibodies (in mid-April, one month after starting the autopsies and 3 months later in mid-July) using a commercially available assay (Liaison® SARS-CoV-2, DiaSorin, Saluggia, Italy).

2.5. Ancillary analysis performed on autopsy tissue

Immunohistochemistry was performed on a Ventana Benchmark™ platform using various antibodies including CD3 (clone 2GV6 ready to use), CD68 (clone KP1 Dako, 1:3000), TTF1 (SPT24, Novocastra, 1:100) and CK7 (OVTL 12/30 Dako, 1:100). The usual procedure standardized for surgical material was used.

Viral and cellular RNA from formalin-fixed paraffin-embedded (FFPE) lung tissue specimens were extracted using the FavorPrep™ FFPE Tissue DNA Extraction Micro Kit (Favorgen Biotech, Vienna, Austria) according to the manufacturer's instruction. RNA was eluted in 50 µL nuclease-free H20. SARS-CoV-2 RNA (E-gene) and cellular β-actin RNA were quantified using published qPCRs [8,28].

3. Results

3.1. Clinicopathological characteristics of the study cohort

The clinicopathological characteristics of the deceased patients who underwent autopsy and the most important pathological findings are detailed in Table 2. Diffuse alveolar damage was found bilaterally at various stages including organizing pneumonia and fibrosis and frequently associated with thrombosis of segmental and subsegmental pulmonary arteries and hemorrhagic infarction of the lung tissue (Fig. 6). In addition, pulmonary thromboembolism was found in about 20 % of the patients, but only half of these cases was associated with deep venous thrombosis of the legs. Focal to extensive bronchopneumonia (Fig. 6) was found in 57 % and was associated with fungi resembling aspergillus in one case. Severe pulmonary emphysema and chronic bronchitis (COPD) were pathologically diagnosed in a third of the cases. Myocardial hypertrophy associated with coronary artery sclerosis and myocardial fibrosis was another frequent finding, cardiac amyloidosis was present in 4 cases (proven by Kongo red stain and immunohistochemistry for amyloid A and amyloid P). Ischemic changes of the bowel associated with mucosal ulcerations were found in 24 % of the cases. Acute tubular injury was found in all cases and frequently associated with benign nephrosclerosis. Lung infarction, bronchopneumonia and ARDS with microthrombi were considered the most frequent cause of death. One patient died from a brain stem infarction in addition to bilateral ARDS and bronchopneumonia.

Viral RNA was detectable in 25 cases in swabs from throat and/or lungs, whereas 3 cases with positive ante mortem swabs were negative post mortem. Viral organotropism showed positivity in swabs from large and/or small intestine in 37 % (7 out of 19 cases) and from the brain in 10 % (1 out of 10 cases). Parts of these viral data had been previously published [19,26].

3.2. Qualitative analysis of the tissue samples

The quality of the tissues varied depending on the time lapse between autopsy and death and the various organs but allowed proper histopathological analysis in all cases (Fig. 6). Immunohistochemistry could be performed without need for a special protocol and the immunostainings could be evaluated without difficulties. The results for TTF1, CD68 and CD3 are shown on Fig. 7. In the investigated lung tissues SARS-CoV-2 RNA was detected with a cycler threshold (Ct) value of 32.5 and 33.9 respectively. This correlated roughly with the results from the swabs from the bronchial mucosa revealing Ct values of 30.99 and 37.21, respectively. The quality of the RNA extraction from formalin-fixed, paraffin-embedded tissue was confirmed by the detection of ß-actin RNA with a Ct-value of 24.3 and 24.9 respectively.

Fig. 4. The lungs were sliced after fixation in total (A) and systematically examined grossly and by multiple histological samples. On the cut surface pulmonary arteries occluded by thrombotic material are visible (B).

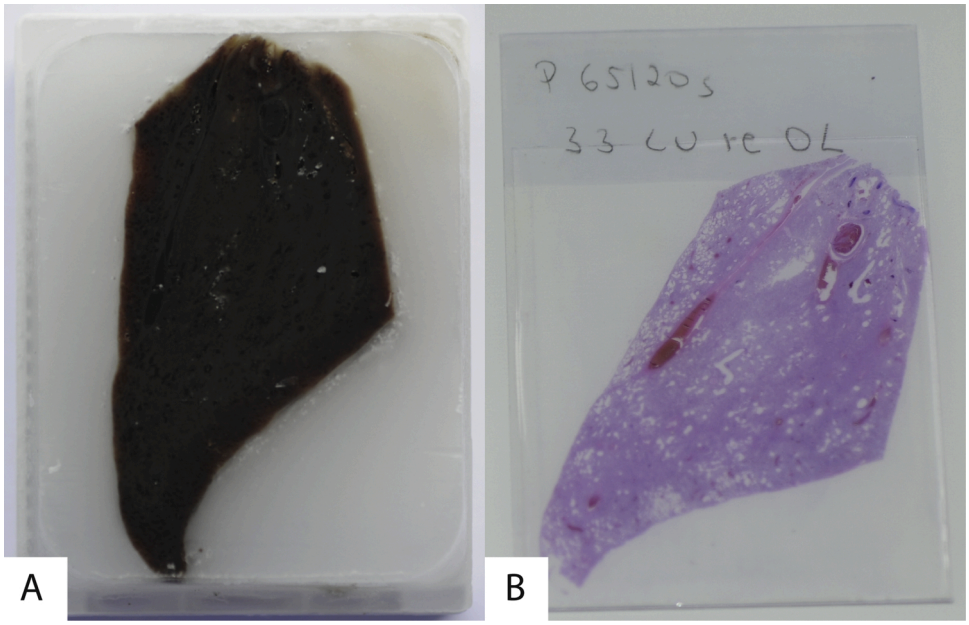

Fig. 5. Large tissue samples (A) from the lungs were blocked for giant sections (B) allowing better demonstration of the pathological changes.

Table 2
Clinicopathological and viral RNA analysis of the 28 autopsy cases.

Patients' Characteristics	N = 28
Age	80.7 (57–96)
Sex	M:F = 16:12
Chronic medical illnesses (clinical history)	
Diabetes mellitus	8 (29 %)
Obesity	4 (14 %)
Arterial hypertension	23 (82 %)
COPD	5 (18 %)
Cerebrovascular diseases	8 (29 %)
Dementia	11 (39 %)
Previous or current malignancy	7 (25 %)
Autopsy findings	
Diffuse alveolar damage	28 (100 %)
Severe COPD	9 (32 %)
Pulmonary artery thrombosis	28 (100 %)
Pulmonary thromboembolism	6 (21 %)
Pulmonary Infarction	21 (75 %)
Bronchopneumonia	16* (57 %)
Pulmonary fibrosis	20 (71 %)
Arteriosclerosis (moderate or severe)	28 (96 %)
Coronary arteriosclerosis (moderate or severe)	24 (86 %)
Left ventricular hypertrophy	26 (93 %)
Myocardial fibrosis	14 (50 %)
Cardiac amyloidosis	4 (14 %)
Heart weight (g)	430 (210–700)
Ischemic bowel changes	6** (24 %)
Acute tubular injury of the kidney	28 (100 %)
Cause of death	
Bronchopneumonia (with infarction or hypostasis)	14 (50 %)
Pulmonary infarction (by thrombosis and/or thromboembolism)	9 (32 %)
ARDS (including pulmonary artery thrombosis)	4 (14 %)
Brain stem infarction	1 (4%)
Positivity for SARS-CoV-2 RNA	
Pharynx	21 (75 %)
Right lung	22† (81 %)
Left lung	21 (75 %)
Intestine	7† (37 %)
Brain (including liquor)	1† (10 %)

Legend: * fungal pneumonia in 1 case; **not assessed in 3 cases; †not all cases were analyzed.

3.3. Comparison of the resources required for usual non-COVID and COVID-19 autopsies

COVID-19 autopsies require more time compared to standard non-COVID autopsies due to several reasons. This is less caused by the dissection technique itself but, particularly, by the systematic dissection and histological analysis of the lungs and the samples from the various organs after fixation in formalin. In addition, the stricter safety standards for COVID-19 autopsies require a more extensive preparation of the morgue including the care for the various instruments and a more time-consuming cleaning procedure. The autopsy team is larger by an additional pathologist and might be even supported by a biomedical technologist assisting with fixation and freezing of tissue. Further time-consuming factors are dressing and undressing of the personal, documentation and snap freezing of tissue for biobanking. The extensive processing of tissue for histopathology and ancillary studies such as immunohistochemical investigations and viral analysis by RT-PCR also require more time and resources in the laboratory. In summary, COVID-19 autopsies take at least three times longer than a standard non-COVID autopsy until all analyses are completed. Except for the increased time for the personal additional costs arise from a more extensive examination of tissue, ancillary studies and safety measurements. Notably, part of our equipment is recycled such as the surgical gowns, face shields, cut-resistant gloves, FFP3 masks and OR ORshoes, which saves costs and makes the equipment more easily available.

4. Discussion

As evident from the published research reports COVID-19 autopsies provide deeper mechanistic and clinical insights into this challenging disease, particularly, with severe and fatal course [5,14,20,23]. Pathological changes of the various organs and the cause of death have become more clear as well as the impression of COVID-19 being a complex multisystemic disease [11]. Thus, the importance of a standardized autopsy procedure and a systematic examination of tissue samples became apparent [27]. Only complete autopsies provide full insights into the pathological changes in the various organs and organ systems and help to recognize and to understand their context. Notably, a systematic gross and extensive histological examination of the main target organ, the lungs, provided us major insight into the most likely

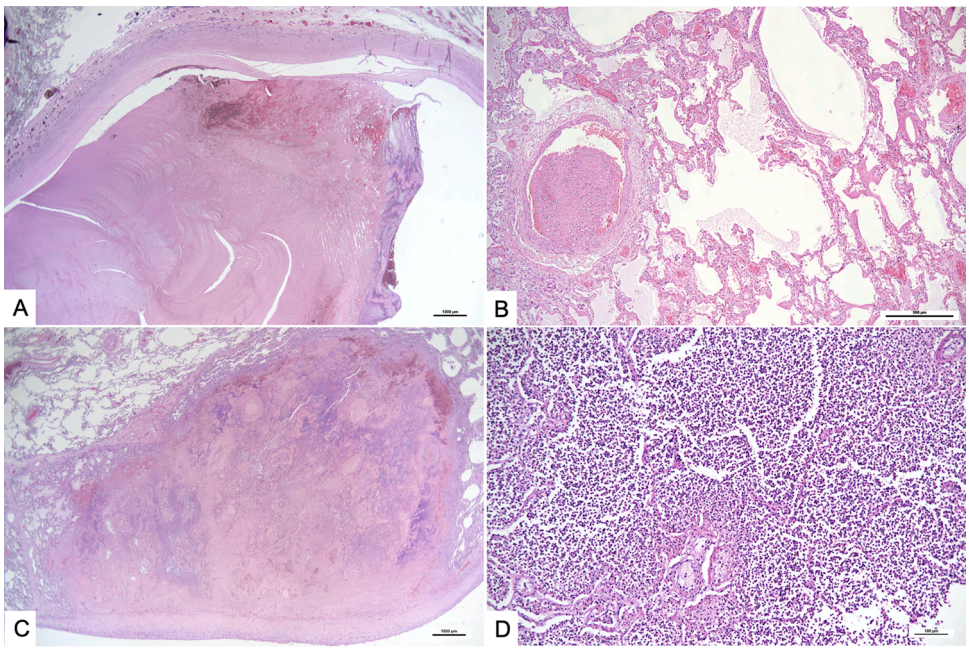

Fig. 6. Major causes of death in COVID-19: Thromboembolism in large pulmonary arteries (A), thrombosis of asmall pulmonary artery ("microthrombosis") associated with diffuse alveolar damage (B), lung infarction (C) and bacterial bronchopneumonia (D). HE, original magnification 10x (A, C), 40x (B), 100x (D).

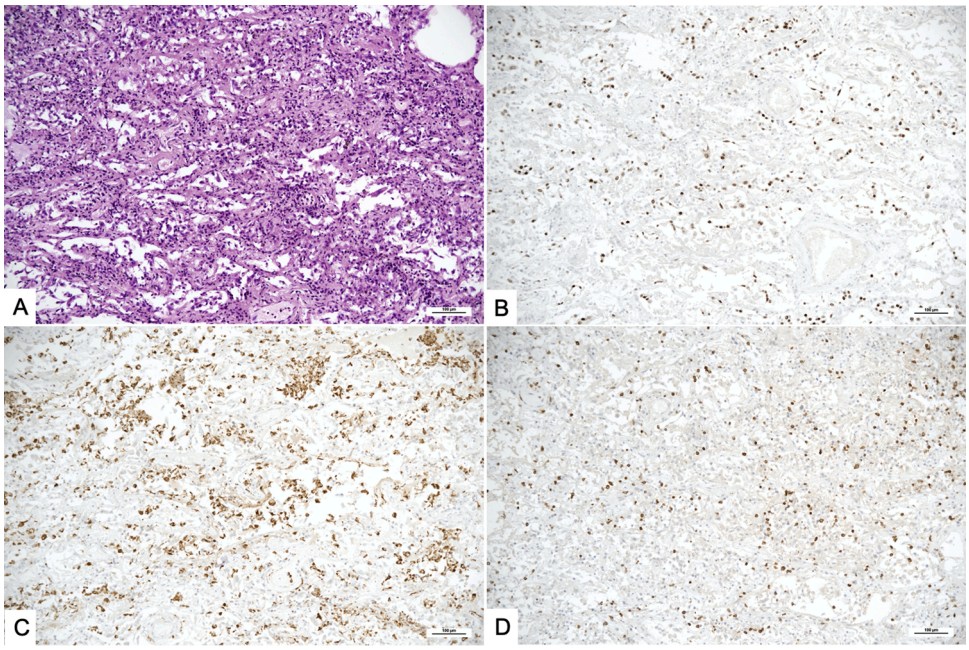

Fig. 7. Preserved quality of tissue for histopathological and immunohistochemical analysis despite autolysis: Lung tissue with proliferative phase of diffuse alveolar damage (A) with TTF1 immunoreactivity of alveocytes (B) showing extensive macrophage reaction by CD68 (C) and mild lymphocytic infiltrate by CD3 (D) immunoreactivity. SARS-CoV-2 RNA could be detected from the same tissue block by RT-PCR. HE (A), DAB (B-D). Original magnification 100×.

cause of fatal course [19].

In this and previous studies we were able to demonstrate that besides diffuse alveolar damage resulting in organizing pneumonia the lungs are altered by vascular occlusion due to thrombosis of pulmonary arteries at the segmental and subsegmental level. In addition, we found in about 20 % of the cases thrombotic material also in large pulmonary arteries which we considered embolic due to the location and since it did not fill out the whole vessel lumen. In addition, half of the cases with pulmonary thromboembolism were associated with deep leg venous thrombosis. In this expanded collective we were able to find pulmonary thromboembolism but less frequently compared to thrombosis of small pulmonary arteries and less prominent compared to another study [29]. Notably, pulmonary infarction and infarction-like changes could be found in almost all deceased due to a meticulous and systematic dissection of the lungs after fixation. It is likely that pulmonary infarction is favored by hypertensive and ischemic cardiopathy since left ventricular hypertrophy, coronary artery sclerosis and myocardial fibrosis were frequent findings. Infarction and hypostasis seem to propagate the frequent development of bacterial bronchopneumonia as another major cause of death. A fungal component was found only in

one case although COVID-19–associated pulmonary aspergillosis (CAPA) has recently received increasing attention [2]. Apart from advancing our understanding of the pathogenesis of COVID-19, the autopsies have provided important feedback for the clinicians, in particular the presence of pulmonary thrombosis, which has resulted in adaptation of clinical anticoagulation guidelines [21]. Moreover, the frequent presence of bronchopneumonia led to the application of more effective antibiotic regimens.

Guidelines for clinicians how to manage COVID-19 patients presented an important source of knowledge for developing the safety procedure. We consider the use of a fitted respirator mask (FFP-3) a prerequisite; these masks are strongly recommended for performing aerosol-generating procedures, particularly, outside a special room with negative pressure [1]. Recommendations whether an AIIR is required for COVID-19 autopsies varies among societies, institutions and experts [4,13,15,20,25] and should be the preferred procedure in our opinion. However, if the aerosol formation is kept minimal, personal protective equipment (PPE) is correctly used and the morgue is properly ventilated and equipped, an autopsy may also be performed outside an AIIR. This has also been addressed by the guidelines from the Royal College of Pathologists [13]. The safety measures require a standardized approach, careful preparation and adequate training of the involved personnel [13,15,24]. We consider particularly the protective gear, the mask and the strict procedure of undressing as crucial elements for safety. Our surgical gowns provide advantages to the full body protective suits since undressing is easier and safer and the gowns can be recycled. It is very important to use a second pair of gloves since the outer pair often may be torn and to use cut-resistant gloves at least on the hand not holding the knife. Furthermore, avoiding the production of aerosols by a dry autopsy technique, by opening the thorax with rib shears and by using an automated saw with integrated vacuum contributed to the safety as well as the disinfectant solutions for cleaning. There is no evidence that postponing the autopsy would reduce the infectivity of the corpse since viral RNA can be detected up to several days after death without significant reduction of the viral load as determined by cycle threshold (Ct) values but in a subset of cases with Ct values greater than 30 [26]. There is some evidence that infectivity is unlikely if the Ct values are greater 30 [18]. In a single case, cultivation of the virus post-mortem could be demonstrated but without information on the Ct value [5]. To have sufficient personal protective equipment (PPE) available local production (e.g. face shields) and recycling (e.g. FFP3 masks, gowns) are very useful and were established by the management of our hospital corporation.

We consider the following points as important and advisable for COVID-19 autopsies: 1) a dedicated team with at least one experienced pathologist and autopsy assistant each; 2) training of the entire team in all safety measures; 3) all steps of the procedure being documented and easy to follow; 4) a quick briefing and rundown with the help of a check-protocol before starting the autopsy; 5) avoiding the use of water during autopsy to minimize the possibility of aerosol formation; 6) performing the autopsy confidently, adequately and with purpose whichever technique is used; 7) documenting and regulating every step of dressing, autopsy, tissue sampling and undressing e.g. by a check protocol and with the help of a third person (e.g. a pathologist).

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CRediT authorship contribution statement

Kristijan Skok: Methodology, Investigation, Writing - original draft, Writing - review & editing. **Klaus Vander:** Methodology, Writing - review & editing. **Lisa Setaffy:** Investigation, Writing - review & editing. **Harald H. Kessler:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **Stephan Aberle:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **Ute Bargfrieder:** Investigation, Writing - review & editing. **Michael Trauner:** Formal analysis, Writing - original draft, Writing - review & editing. **Sigurd F. Lax:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Supervision.

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