Severe Acute Respiratory Syndrome Coronavirus 2 Pandemic

Review of the Literature and Proposal for Safe Autopsy Practice

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• ***Context.*—The novel coronavirus disease 2019 (COVID-19) pandemic is significantly changing methodologic approaches in all branches of the health system. From a forensic point of view, this event is partly changing the manner in which forensic pathologists and all those who work in autopsy services operate, but above all, it is changing the patterns established for years by which cadavers are analyzed postmortem.**

***Objective.*—To present a review of the literature and a proposal for COVID-19 autopsy protocols. To contain the infection risk, a revision of all the protocols that until now have been applied to the examination of bodies that require autopsy services is required.**

***Data Sources.*—Currently, the diagnosis and postmortem analysis of positive or suspected COVID-19 cases plays a crucial role in scientific research. A review of the main recommendations proposed by international scientific societies regarding the risk of infection during autopsy was carried out. Scientific papers currently available via the PubMed NCBI search engine on COVID-19 postmortem diagnosis were also examined.**

***Conclusions.*—Throughout the history of medicine, autopsy has been fundamental to the understanding of multiple pathogenic processes that are investigated postmortem. The purpose of the study is to propose an operating protocol that can be useful for all clinical and forensic autopsies, with particular reference to the correct methods to be applied to the examination of positive or suspected COVID-19 cases, regarding both the autopsy procedure and the collection and analysis of biological samples.**

(*Arch Pathol Lab Med.* 2020;144:1048–1056; doi: 10.5858/arpa.2020-0165-SA)

Coronaviruses (Coronaviridae) are a large family of microorganisms existing in nature in the form of positive-strand RNA viruses that infect vertebrates.[1,2] The family includes the subfamily Orthocoronavirinae, to which the human-transmitted *Betacoronavirus* genus belongs.[3] In particular, 7 viruses belonging to the *Betacoronavirus* genus are currently known to cause transmissible respiratory infections in humans, including serious diseases such as Middle Eastern respiratory syndrome and severe acute respiratory syndrome (SARS).[4] These diseases also include the new coronavirus 2019-nCoV (now called severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]), which is capable of causing a disease known as coronavirus disease 2019 (COVID-19).[5]

The new coronavirus was first identified in Wuhan, China, in December 2019. The existence of COVID-19 was discovered in a group of Chinese patients who presented with an atypical form of viral pneumonia and who frequented the same place, the Huanan Seafood Market. Subsequent microbiological investigations carried out through the analysis of bronchoalveolar fluids with a polymerase chain reaction technique confirmed the discovery of a new virus related to the SARS-related coronavirus species and therefore called SARS-CoV-2.[6]

The origin of the new coronavirus, according to the most credible hypothesis, is attributable to the transmission mechanism of spillover, consisting of a species jump or a zoonosis through which the virus would pass from an

Accepted for publication May 5, 2020.
Published online May 8, 2020.

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This research was supported by Fondi di Ateneo Linea D1 Università Cattolica del Sacro Cuore (Oliva). The authors have no relevant financial interest in the products or companies described in this article.

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animal, as a vector of the pathogen, to humans. In this regard, numerous hypotheses have been formulated in the scientific literature, Two snakes, *Bungarus multicinctus* and *Naja atra,* were detected as reservoirs of the zoonosis. This hypothesis was supported by the fact that numerous snakes were sold at the Huanan Seafood Market. Subsequently, other researchers identified the ideal reservoir candidate as a mammal, suggesting that 2019-nCoV may be a recombinant virus derived from the bat coronavirus and another coronavirus of unknown origin.[7] There is, however, a vast consensus in the scientific community on the lack of scientific evidence to support the existence of the pathogen in animals other than birds or mammals.[8] To date, the animal source of infection is not known with certainty, and numerous researchers still wish for the definitive closure of similar markets or the application of strict control measures to ensure hygiene and to implement public health protocols in these premises.[6]

The exact mechanism of action of SARS-CoV-2 is currently not completely clear. Recent studies show that the pathogen uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S-protein priming.[9] The mechanism of action would therefore be similar to that of the SARS-CoV virus, which involves the angiotensin 2–converting enzyme. Compared with the SARS and Middle Eastern respiratory syndrome viruses, SARS-CoV-2 seems to exhibit greater infectivity. Certainly, the cases described in the literature show that the virus produces a spectrum of symptoms and clinical signs that vary widely in terms of the incubation time, onset, and severity. The symptoms include dry cough, fever, asthenia, nasal congestion, and diarrhea, and the disease can progress to atypical interstitial pneumonia (with a ground-glass appearance), SARS, acute renal failure, and death.[10] Transmission occurs from person to person through saliva, close contact, or contaminated hands; thus, hand hygiene, maintaining a safe distance, and avoiding touching the eyes, nose, and mouth are recommended. The coronavirus pandemic represents a major international emergency that, to date, has resulted in many open questions about the pathogenesis, the clinical features, and especially the mortality rate in human subjects. In the health field, autopsy is always the gold standard for the precise diagnosis of the cause of death. In infected or suspected cases, the absence of data in the literature relating to the macroscopic and microscopic features of COVID-19 emphasizes the need to create environments that reduce (at least theoretically) the risks for the personnel involved in autopsy and body management.

THE INFECTION RISK IN THE AUTOPSY ROOM

Autopsy itself exposes health care professionals to high health risks. The main risks concern direct or indirect exposure to infectious agents. Those at direct risk include all those who work in autopsy services (forensic pathologists, paramedics, and autopsy technicians) and are in direct contact with the cadaver. Those at indirect risk include all the other individuals who could come into contact with the cadaver through secondary postmortem investigations, such as investigations of biological fluids, histologic analysis of organs, postmortem radiologic investigations, and testing of material from infected cadavers.

Autopsy is an irreplaceable source of information about the clinical conditions of an individual at the time of death because of the possibility of obtaining biological tissues and liquids for subsequent laboratory investigations.[11–14] In postmortem investigation, risk of infection represents a concrete and often underestimated danger. Given its invasiveness, autopsy is certainly a risky investigation, as it requires contact with and direct manipulation of organs and biological fluids. This risk is faced not only by forensic pathologists but also by all mortuary staff involved in the management of the body, including (1) reconnaissance of the body, (2) transportation, (3) autopsy, (4) sanitization of the rooms, (5) dressing, (6) exposure, (7) burial/cremation, (7) custody of the samples, and (8) analysis of the samples.

The data in the literature about the residence time of pathogens in the cadaver and in biological samples analyzed postmortem are not unequivocal. Burton[15] highlighted some potential transmission pathways of infections by cadavers, such as inhalation, inoculation, ingestion, contact with mucous membranes, and contact with skin lesions. In his review, the author emphasized the mortality of infections transmissible from cadavers to humans, such as infections with human immunodeficiency virus (HIV), tuberculosis, hepatitis B and C, transmissible spongiform encephalitis, gastrointestinal infections, meningitis from *Neisseria meningitidis*, and meningococcal septicemia.[15]

Of particular interest were the scientific studies performed on HIV and hepatic viruses. Human immunodeficiency virus is not very resistant in the external environment and can be easily removed from surfaces with suitable disinfectant solutions, such as alcohol, hydrogen peroxide, or formaldehyde. The infection risk of the cadaver, however, has been shown through various studies to be due to the ability of HIV to persist for a long time in organs and biological fluids.[16] Douceron et al[17] showed that the virus can be isolated from blood 16.5 days after death, from pericardial fluids 15.5 days after death, and from pleural fluids 13.8 days after death. Virus isolation was also carried out in bones, brain, spinal cord, spleen, and lymph nodes from a cadaver 6 days after death.[18] No study to date has shown a time interval at which the autopsy can be performed without the risk of contamination. This means that autopsy of HIV^+ cadavers always represents a high risk, so it is essential to adopt all necessary hygiene protocols. Hepatitis B is also a disease with a high risk of transmission. Several studies have shown the presence of the virus by detecting serologic markers in blood samples collected after death and in tissues stored in repositories.[19] Certainly, the availability of a vaccine administered at a large scale in the population has guaranteed a reduction in the rate of hepatitis B infections in workers. Unfortunately, such a scenario does not apply to hepatitis C. The data available to date suggest the lower infectivity of hepatitis C compared with that of hepatitis B, but occupational transmission of the infection has been demonstrated percutaneously.[20] The risk also increases in cases of skin lesions in the operator.

The risk of exposure to biological agents due to the possibility of contact with potentially infected material should, as a precaution, always be considered high with regard to hepatitis B and C viruses because of their long period of the survival in the external environment as well as their high infectivity.[21] This threat is increased greatly by the failure to use suitable personal protective equipment and is particularly high during the collection and transport of cadavers, because of possible contamination, and during activities in the autopsy room that may cause possible exposure through cuts, wounds, and splashes. As far as the risk of exposure to HIV is concerned, the possibility of

Table 1. Reported Autopsy and Biopsy Findings in Coronavirus Disease 2019 (COVID-19)–Positive Cases				
Source, y	Cases	Materials and Methods	Organs	Autopsy Findings
Yao et al,[28] 2020	3	Minimally invasive autopsy Immunohistochemical staining	Lungs	Changes of the alveolar structure with development of hyaline membranes, macrophage and monocyte infiltrates, $CD4^+$ lymphocytes, and pulmonary fibrosis
			Other organs	Structural degenerations for chronic pathologies, in the absence of evidence of COVID-19 infections
Tian et al,[29] 2020	2	Lobectomy for adenocarcinoma Hematoxylin-eosin staining	Lungs	Inflammatory infiltrate with giant cells, proteinaceous exudates, and presence of hyaline membranes with massive pulmonary edema

contagion exposure via contact with cadaveric fluids cannot be theoretically excluded; it must in fact be considered to be higher in the first few hours following death, and it tends to decrease over time due both to the low potential for infection and the reduced survival of the virus in the environment.[22] The possibility of HIV contamination, except during the first few hours after death, is therefore low in terms of probability; however, obviously, the seriousness of HIV infection is still a factor. The risk is therefore more significant during body reconnaissance. Therefore, for health professionals involved in autopsies of infected patients, the risks include:

1. Contact with and manipulation of objects and surfaces soiled with feces and/or potentially infected biological sewage from cadavers (ie, with tetanus, hepatitis B and C, HIV infection) associated with the presence of possible cuts and wounds;
2. Contact with biological agents due to environmental pollution caused by autopsies performed on cadavers affected with *Mycobacterium tuberculosis*[23]; and
3. Contact with the surfaces, aerosols, organs, or biological fluids of cadavers affected by COVID-19.

MANAGEMENT OF THE RISK OF COVID-19 INFECTION DURING AUTOPSY

In light of the health emergency caused by SARS-CoV-2, the health personnel involved in autopsy in any capacity (clinical or forensic) are faced with the risk of infection during the performance of autopsy services. Therefore, the possibility of having to perform an autopsy on an infected body and the still-unexplored possibility of cadaver-operator transmission not only pose numerous questions about coronavirus microbiology in the cadaver but above all force the forensic pathologist to manage the risks of autopsy procedures to contain the infection.

In this regard, recommendations[24] from the Centers for Disease Control and Prevention have recently been published to guarantee the safety of operators involved in postmortem procedures. The document clarifies the methods that should be used to obtain the swab, emphasizes the use of safety devices during the autopsy, and suggests limiting the number of operators and the use of oscillating saws to a minimum. Considering the scientific relevance of collecting multiple specimens from deceased individuals with SARS-CoV-2, the Centers for Disease Control and Prevention also suggests taking trachea, central lung, bronchus, and lung parenchyma samples, which should be fixed in formaldehyde. Furthermore, because of the lack of official data on the resistance of the pathogen on surfaces,[25,26] it is recommended to perform complete disinfection of environments with active ventilation systems and to use specific products such as ethanol, hydrogen peroxide, or sodium hypochlorite. Finally, it is suggested to follow the routine procedures used for transporting the body, with body bags that are disinfected and used only once.

In addition, the Royal College of Pathologists[27] has listed some recommendations for the correct performance and management of necropsies in COVID-19 cases. In particular, collection of data about the circumstances of death, travel, and previous laboratory or microbiology results should be performed. Before the autopsy, it is recommended to don personal protective equipment such as a surgical suit, headgear, visor, FFP3 mask, and waterproof gloves that extend up to the forearm. During the autopsy, the use of rounded-tip scissors, the reduction of the number of blades in the work area to a minimum, the opening of a single cavity at a time, the use of a suction device for bone powders, the use of a sponge during the sectioning of organs that are still not fixed, and ensuring that needles are placed in a special container immediately after use are recommended. The collection of biological fluids should also be performed on the skin before cadaveric sectioning.

POSTMORTEM DIAGNOSIS OF COVID-19

To date, few scientific studies have described proven methodologies or autopsy protocols that can be used for postmortem diagnosis of the cadaver (Table 1). The only study on autopsy cases at present in the literature (Yao et al[28]) reported experimentation with the use of minimally invasive autopsy procedures to obtain small samples of lung, heart, kidneys, spleen, bone marrow, liver, pancreas, stomach, intestines, thyroid, and skin from 3 patients who died in China because of COVID-19. All the organs collected were stained with hematoxylin-eosin and analyzed with the immunohistochemical method to evaluate the expression of SARS-CoV-2 proteins. In addition, the authors carried out a real-time polymerase chain reaction procedure to search for RNA corresponding to SARS-CoV-2. The authors highlighted the alterations of the alveolar structure, including the development of hyaline membranes, macrophage and monocyte infiltrates, the accumulation of $CD4^+$ lymphocytes, and pulmonary fibrosis. In the other organs, the authors described structural degeneration consistent with chronic pathologies in the absence of evidence of COVID-19 infection.[28] In their study carried out on lung biopsy specimens, Tian et al[29] highlighted the presence of inflammatory infiltrates with giant cells and the presence of hyaline membranes with massive pulmonary edema.

Therefore, according to 2 studies in the literature that analyzed different samples from deceased patients or biopsy samples of the lung, future autopsy procedures should be

Table 2. Diagnostic Measures for Coronavirus Disease 2019	
Investigations	**Materials and Methods**
Real-time polymerase chain reaction	Searching for RNA of SARS-CoV-2
Immunohistochemistry	Assessment of the expression of SARS-CoV-2 proteins
Electronic microscopy	Detection of coronavirus-related particle spread in the parenchyma

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

standardized based on unique scientific protocols that use clear, reproducible, and repeatable methodologies for sampling.

SAMPLING OF BIOLOGICAL LIQUIDS AND TISSUES IN COVID-19 CASES

In the studies mentioned above, the techniques used for the diagnosis of COVID-19 pathologies involved both laboratory and histopathologic investigations. Histopathologic sampling involved all organs, including the bone marrow. The procedures used included routine formalin fixation–paraffin embedding and staining with hematoxylin-eosin. In some cases, an immunohistochemical investigation was carried out that highlighted the positive staining of the alveolar epithelium and macrophages for the SARS-CoV-2 antigen, with electron microscopic detection of coronavirus-related particles throughout the parenchyma.[28] Histologic studies suggest that formalin and paraffin fixation would inactivate coronavirus. In particular, paraffin is applied at temperatures of at least 60°C for 2 hours, which is a high enough temperature and an appropriate amount of time to ensure the inactivation of the virus. The creation of frozen sections should therefore be avoided because of the risk of aerosol transmission of the pathogen unless a suitable cryostat is used.[30] The biological liquids and sampling methods used were not indicated in the works.

Regarding the laboratory investigation, the real-time polymerase chain reaction technique was used in the analyzed studies with biological samples extracted from nasal and oropharyngeal swabs, sputum, feces, and cell culture supernatants containing the virus. RNA was first extracted from the samples and subsequently amplified using real-time polymerase chain reaction with commercially available kits containing Taq polymerase (with deoxyribose triphosphates and a buffer solution of magnesium sulfate). The temperature was raised to 55°C for 10 minutes to allow the annealing of the primer, then to 95°C for 3 minutes for DNA denaturation, after which repeated cycles of 95°C and 58°C were performed (Table 2).[31]

PROPOSED PROTOCOL FOR SUSPECTED OR CONFIRMED CASES OF COVID-19

This protocol is focused on the management of suspicious or positive cases of COVID-19, the containment of infection, and the safety of the operators involved before, during, and after the autopsy. It also aims to provide a description of the most appropriate methods for the analysis and interpretation of the autopsy data for postmortem diagnosis of COVID-19 (Figure 1).

The current knowledge of the methodologies and results related to the postmortem diagnosis of COVID-19 is still limited because of the small number of analyzed cases. The proposed protocol could therefore prove useful to the scientific community not only for the optimal management of autopsy services but also for the postmortem diagnosis of the greatest number of coronavirus deaths by describing the methodologies used for the analysis of the samples taken during autopsy. Such research could be crucial to provide the scientific community with new information about the mechanisms of action of the virus and its pathogenic effects on organs and tissues in subjects who died and who had a COVID-19 diagnosis.

In light of the data in the literature, a 5-phase autopsy protocol is proposed for postmortem diagnosis and the conservation of samples for forensic and research purposes. First, it is essential that each organization create dedicated COVID-19 paths with insulated ventilation systems and biosafety level 3 autopsy rooms dedicated only to COVID-19 cadavers.

The operation protocol can be used both for cadavers for which there is a suspicion of COVID-19 because of the medical history and for cadavers for which there is already a clinical diagnosis.

Phase 1: Risk Assessment

If a cadaver arrives with suspected COVID-19, it is essential to analyze the medical and anamnestic documentation as well as to determine the location from which it came. In these cases, swabs are recommended.[32] The swabs must be obtained only after the operator dons all the necessary personal protective equipment (disposable gloves, visor, headgear, suitable gown, and FFP3 mask because of the risk of aerosolization of the virus). After opening the package, the swab must be inserted deep inside the nostril and rotated for a few seconds. The procedure must be repeated in the second nostril. The tube is then opened, the swab is inserted into it, and the distal end of the swab is broken at the edge of the tube. The test tube must be hermetically sealed. The second swab must be obtained at the level of the tonsils and oropharynx, while trying to avoid contact with other parts of the oral cavity, and maintained in position for a few seconds (Table 3). At the end of the procedure, the swab must be repositioned and broken in the manner already described. Once the closure of the tube has been verified, the test tubes must be placed in a special container and disinfected. Therefore, they must be labeled with the identification data, closed in a box, and transported within 2 hours for analysis, or stored in a refrigerator at 4°C pending analysis for a maximum of 48 hours.

If the swab is negative but the clinical picture and radiologic findings are highly suggestive of COVID-19, the forensic pathologist should treat the case as positive. Indeed, for microbiological testing, a negative result certainly cannot exclude COVID-19 diagnosis because the virus could be present in lung tissue but be undetectable in the nasopharynx. Moreover, there is evidence of cases that rapidly progressed from a mild clinical presentation to severe worsening of respiratory symptoms despite a decreasing viral load in the nasopharyngeal samples.[32]

Phase 2: COVID-19–Positive Cadaver Storage

If a cadaver arrives with known positivity for COVID-19 and an autopsy cannot be carried out, the management of

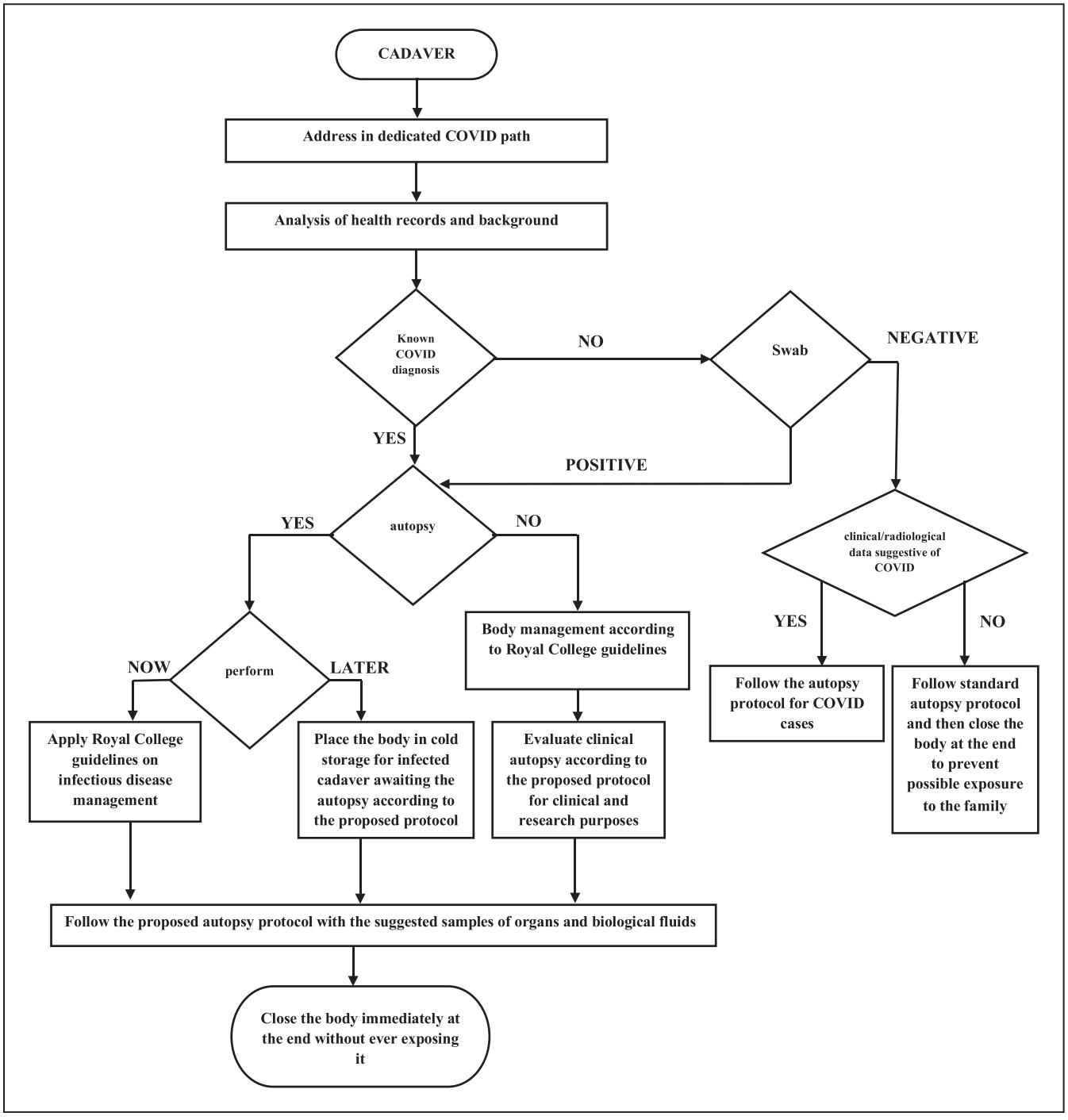

Figure 1. *Flowchart of the autopsy protocol for cases of suspected or confirmed COVID-19.*

the body according to the guidelines of the Royal College[27] is essential.

If a cadaver with known positivity for COVID-19 arrives and an autopsy must be performed immediately, it is fundamental to first apply the Royal College guidelines for infectious disease management.[27] Subsequently, it is advisable to follow the autopsy protocol and to sample liquids as described below.

Table 3. Categories of Samples Collected for Testing

Swab in nostrils
Swab in tonsils and oropharynx
Swab with bronchoalveolar fluid
Biological fluids
Sections of lungs

If a positive COVID-19 cadaver arrives and an autopsy must be performed after a period of time, it is necessary to put the body in a cold room by following the recommendations, with particular attention paid to placing the body in a closed body bag with an identification tag ascertaining the chain of custody and placing it in a cold room for infected cadavers. Therefore, it is recommended that the morgue be equipped with insulated rooms with specific cold rooms for

Table 4. Samples for Histopathology	
Organ	**Samples**
Heart-lung block including the glottis	In toto
Liver	2 fragments
Spleen	2 fragments
Stomach	2 fragments
Pancreas	2 fragments
Kidneys	4 fragments
Brain	8 fragments (if possible, only after manual opening of the skull)
Thyroid	2 fragments
Prostate	2 fragments
Bone marrow from the iliac wing	At least 2 × 3 cm
Small and large bowel	2 fragments
Bladder	2 fragments
Aorta and iliac vessels	2 fragments
Ileus psoas	1 fragment
Quadriceps in the medial section	1 fragment of 3 × 3 cm

infected cadavers. The body awaiting an autopsy must be placed in a cold room for infected cadavers at a temperature between 2°C and 4°C. Once the body has been removed from the cold room for autopsy, the body bag cannot be reused.

If the cadaver has bone fractures, it is essential to avoid unusual movements of bone structures that have sharp edges, which can create risks for the forensic who pathologist during evisceration procedures.

If the cadaver has a BMI higher than 30, evaluate the risk of the presence of abundant visceral fat as a limiting and potentially dangerous factor that could cause the hand of the forensic pathologist that is holding the scalpel to slip during the examination.

If the body has abundant bodily fluids, evaluate the risk of splashes and the danger that the hand of the forensic pathologist who is holding the scalpel might slip during the examination.

In cases where an autopsy is requested for a suspected COVID-19–positive cadaver and swabs are obtained to evaluate positivity, it is recommended to place the body at the end of the procedure in a clean body bag in a cold storage room for infected cadavers at a temperature between 2°C and 4°C until the microbiological result is obtained. It is recommended to wait for the microbiological outcome of the swabs before exposing the body; to date, the exposure of cadavers is prohibited in Italy until April 3, 2020, and there is a ban on funerals.[33]

Phase 3: Postmortem Radiologic Testing

Supplementary investigations, such as postmortem radiologic testing before autopsy, should be carried out only for forensic purposes or, if necessary, when there is no medical history. The recommended procedure is to isolate the cadaver in 2 closed body bags, and after the computed tomography or magnetic resonance imaging examination, sanitization of the environments must be performed.

Phase 4: Autopsy and Sample Collection

For autopsy of all positive COVID-19 cases, it is recommended to perform the autopsy according to the standard protocol.[13] Only one study[34] so far has outlined how to perform an autopsy in these cases by providing general recommendations. Additionally, in these cases, it is recommended to obtain the following samples (Table 4):

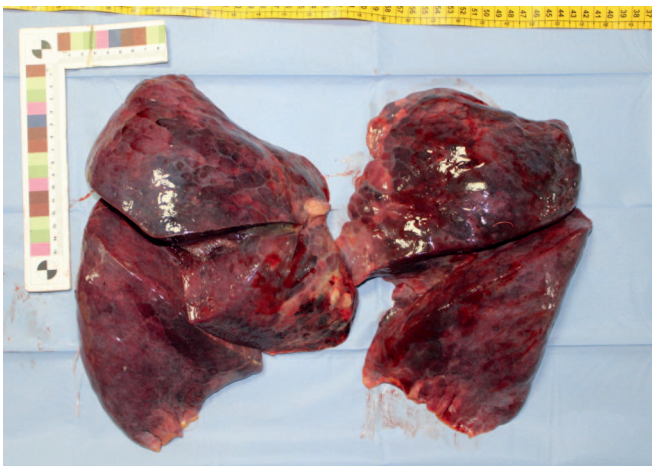

Figure 2. *Macroscopic image at autopsy of lungs with SARS-CoV-2 pneumonia. Photograph from Dr Isabella Aquila.*

1. The heart-lung block in toto including the glottis, as recommended for asphyxial deaths[35]; this would allow us to fully examine the macroscopic and microscopic alterations caused by the coronavirus in the respiratory tract (Figures 2 and 3) and heart and compare them with the first histopathologic findings that were described.[28]

Figure 3. *Section at autopsy of lung with SARS-CoV-2 pneumonia. Photograph from Dr Isabella Aquila.*

Table 5. Procurement of Bodily Fluids and Storage		
Fluid	**Tubes**	**Storage**
Peripheral blood	1 tube with EDTA K2 or EDTA K3	−20°C
Peripheral blood	1 tube with EDTA K2 or EDTA K3 centrifuged for 5 minutes at 2500 rpm	−80°C
Peripheral blood	1 tube with anticoagulant for genetic investigation	−80°C
Urine	1 test tube	−20°C
Vitreous humor	2 test tubes	−20°C
Cerebrospinal fluid	1 test tube	−80°C
Visceral liquid (pleural, peritoneal, pericardia) if available	1 test tube for each	−20°C

2. Samples (at least 2) of liver, spleen, stomach, pancreas, kidneys, thyroid, prostate, bone marrow, small and large intestine, bladder, aorta, and psoas. The sampling of all organs would allow us to obtain more information on the pathologic alterations caused by the coronavirus in other body areas.[28] Brain examination (until the presence/absence of viruses in bone bioaerosol is clarified) should be limited to selected cases. In any case, the opening of the skull will have to be done manually.
3. A sample of the quadriceps from the medial section approximately 3 × 3 cm; this sample would allow us to verify the role of IL-6 in the inflammatory response to the virus in the muscle and therefore to evaluate the correlation, so far only hypothetical, between the virus and athletic activity.[36] All tissue samples should be fixed in 10% buffered formaldehyde.

In all positive COVID-19 autopsy cases, it is recommended to perform the autopsy according to the standard protocol[13] for the collection of biological liquids (Table 5). In particular, it is recommended to obtain 2 tubes (EDTA K2 or EDTA K3) of peripheral blood, 1 of which should be immediately centrifuged for 5 minutes at 2500 rpm and frozen at –80°C for enzyme-linked immunosorbent assay investigations[37]; we recommend freezing the second tube at –20°C. We also suggest obtaining an additional tube of blood with anticoagulant for genetic investigations. It is recommended to obtain 1 test tube of urine when urine is present, 1 test tube of cerebrospinal fluid, and 2 test tubes of vitreous humor and visceral liquid (pleural, peritoneal, or pericardial) if present. Freezing of these samples at –20°C is recommended, except for the cerebrospinal fluid, which should be frozen at –80°C.

The autopsy reports for the COVID-19–positive cases must contain all the autopsy findings and the results of the laboratory investigations, including both positive and negative results. In particular, the role of histopathology, immunohistochemistry, and enzyme-linked immunosorbent assay in the analysis of samples for scientific research purposes is emphasized.

Phase 5: Sample Preservation

So that clinicians can understand how to fix the obtained biological samples without compromising the subsequent analyses or putting the operator at risk, we have provided a table (Table 6) summarizing the virucidal action of the major fixatives used routinely in laboratories. With the exception of a single fixative, phosphate-buffered saline/100% ethanol (1:1 volume ratio), all fixatives were able to eliminate the viral agent. In addition, the World Health Organization SARS report[38] indicates that the virus is stable in both stool and urine at room temperature for at least 1 to 2 days and is also stable for at least 4 days in the fecal material of patients with diarrhea (characterized by a pH higher than that of normal feces).

Based on this information, it is appropriate for all biopsy samples sent to outside laboratories to be fixed in one of the fixatives indicated. Depending on the molecular analysis to be carried out later, such as enzyme-linked immunosorbent assay of biological liquids, it would be convenient to pretreat the obtained biological sample with 0.5% glutaraldehyde. This fixative does not interfere with the protein structure of the biological sample and favors the immunologic reaction underlying the mechanism of the enzyme-linked immunosorbent assay.[39] For the analysis of nucleic acids, it would be advisable to pretreat samples with 78% ethanol[40] to avoid degradation and guarantee a certain degree of preservation. In both cases, once the sample has been prefixed, to safeguard the results it is advisable to proceed quickly with the execution of the analysis. In the case of the preservation of biological tissues or liquids for research purposes, it is recommended that fixation be performed at the time of collection in one of the fixatives given in Table 6. In particular, for tissues, conventionally, the use of 1% formaldehyde is recommended; for liquids, 0.5% glutaraldehyde is recommended. For the subsequent recovery of

Table 6. Anti–Severe Acute Respiratory Syndrome Coronavirus Activity of Different Fixatives				
Sample Type	**Fixative**	**Temperature**	**Timing**	**Source, y**
Liquid	0.5% glutaraldehyde	37°C	Day 1	Darnell et al,[43] 2004
	10% formalin	37°C	Day 1	Darnell et al,[43] 2004
Tissue	Formalin and paraffin embedded	65°C	2 h	Henwood,[30] 2020
Cells	Methanol/acetone	Ice/cold	10–20 min	Thevarajan et al,[44] 2020
	1% paraformaldehyde	Room temperature	10–20 min	Thevarajan et al,[44] 2020

Table 7. Anti–Severe Acute Respiratory Syndrome Coronavirus Activity of Different Biocidal Agents			
Biocidal Agents	**Activity**	**Time of Action**	**Source, y**
0.1% sodium hypochlorite	Good	30 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
62%–71% ethanol	Good	30 s	Kampf,[47] 2020
0.5% hydrogen peroxide	Good	30 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
Quaternary ammonium compounds	Good	30 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
Phenolic compounds	Good	30 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
70% propanol	Good	30 s	Rabenau et al,[46] 2005; Coluccio et al,[48] 2015
0.5% glutaraldehyde	Good	120 s	Rabenau et al,[46] 2005; Coluccio et al,[48] 2015
Formalin	Good	120 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
1% formaldehyde, paraformaldehyde	Good	120 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
Alcohol solutions with >70% alcohol	Good	120 s	World Health Organization,[45] 2020; Rabenau et al,[46] 2005
0.05%-0.2% benzalkonium chloride	Less	...	Henwood,[30] 2020
0.02% chlorhexidine digluconate	Less	...	Henwood,[30] 2020
PreservCyt, CytoLyt, SurePath	Less	...	Pambuccian,[49] 2020
UVC (200–280 nm)	Good	Distance 3 cm >15 min	Darnell et al,[43] 2004
UVB (280–320 nm)	Less	...	Darnell et al,[43] 2004
UVA (320–400 nm)	Less	...	Darnell et al,[43] 2004
Gamma radiation (3000, 5000, 10 000 rad)	Less	...	Darnell et al,[43] 2004

samples frozen in liquid nitrogen or at –80°C, the labeling procedure must ensure that the source (COVID-19–positive or –negative), the type of sample, the fixation procedure performed, and the collection date, which always coincides with the date of fixation and conservation, are recorded. It is appropriate to specifically dedicate rooms and equipment to the preservation and analysis of COVID-19–positive biological samples even if they have been previously fixed.

Because viruses belonging to the SARS-CoV family are transmitted by air (via droplets <5 μm in diameter with displacement >1 m), they can be present in aerosols generated by certain procedures that are carried out during autopsy and survive on dry surfaces for a prolonged period; therefore, it is necessary to sanitize all surfaces. The use of disinfectants with proven activity against the envelope of the virus that disrupts its viability is recommended. Table 7 describes the chemical and physical tools required for good microbiological practices and the procedures for laboratory biosafety reported in the literature.

The protocol is summarized in Table 8.

CONCLUSIONS

The COVID-19 pandemic has affected the operational protocols used in all sectors of public health. In forensic pathology, autopsy remains indispensable for assessment for judicial purposes but is also a hazardous procedure because of the infection risk it entails.[41,42] Besides the published recommendations for hygiene during autopsy, there are no guidelines for the postmortem diagnosis of COVID-19, nor have the procedures for collecting biological samples for judicial or scientific research purposes been defined. Our protocol aims to help operators in this field to perform postmortem analyses of cadavers associated with suspected or positive cases of COVID-19. To date, no studies are available on the occupational outcomes of the forensic operators involved. In fact, it is not known how long the virus can survive in a cadaver, nor is it possible to estimate the percentage of transmission in cadaver operators. It is hypothesized, in light of the literature relating to viral infections, that in such cases, the body could potentially be infectious even after many days despite storage in the cold room. In the absence of evidence regarding the

Table 8. Five-Phase Autopsy Protocol for Coronavirus Disease 2019 (COVID-19) Cases		
Phase		
No.	**Description**	**Recommendations**
1	Risk assessment	Carefully analyze the entire medical documentation If COVID-19 can be suspected, perform postmortem swab Always interpret microbiological results according to clinical and radiologic data
2	COVID-19–positive cadaver storage	Always follow Royal College guidelines on infectious disease management Always carefully maintain a chain of custody If autopsy must not be immediately performed, store the body in a cold room (2°–4°C) for infected cadavers, in a closed body bag with an identification tag Follow this procedure also when you are waiting for microbiological results
3	Postmortem radiologic testing	Perform supplementary investigations (eg, postmortem radiologic testing) only if necessary
4	Autopsy and sample collection	Follow autopsy standard protocol Collect samples according to procedures in Table 4
5	Sample preservation	Almost all fixatives can eliminate SARS-CoV-2 Pretreat the sample if particular analysis must be carried out (see Table 6)

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

occupational risk of infection, the autopsy must always be considered a hazard for the operator any time it is carried out. This protocol aims to have clinical implications, as it is applicable to the diagnosis (clinical autopsy) of suspected or confirmed COVID-19 cases that are not of judicial interest.

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