Postmortem Swabs in the Severe Acute Respiratory Syndrome Coronavirus 2 Pandemic

Report on 12 Complete Clinical Autopsy Cases

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• ***Context.***—**Clinical autopsies have historically provided a fundamental contribution in the definition of the clinicopathologic basis of infectious diseases. Even though we are witnessing the decline of the clinical autopsy, its importance remains unchanged as it is the most exhaustive way to investigate diseases. The identification of the virus in postmortem tissues is a fundamental step in the definition of its clinical features.**

Objective.—**To investigate the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in postmortem examination with swabs.**

Design.—**We performed postmortem swabs in 12 autopsy cases of patients with a clinical diagnosis of SARS-CoV-2–related pneumonia. Our protocol consisted of a rhinopharyngeal and a tracheal swab in order to search for the virus in the upper airways, and of 2 swabs on the parenchyma of each lung. We also performed a fifth swab on the parenchyma of both lungs in order to search for other viruses that could evolve in a clinical picture of interstitial pneumonia.**

Results.—**Overall, we found 9 of 12 cases had at least 1 postmortem swab positive for SARS-CoV-2. Moreover, we evaluated the time between the antemortem and postmortem swabs, the time between death and the postmortem swabs, and the time between the postmortem swabs and acceptance to the microbiology laboratory. Of note, we did not find a relationship between the results of the swabs and either the time elapsed from their collection or the time elapsed before their acceptance in the microbiology laboratory.**

Conclusions.—**A thorough knowledge of the eventual persistence of pathogens in deaths related to infectious diseases is fundamental for the safety of the operators during the autopsy practice, especially when referring to emergent pathogens, such as SARS-CoV-2. Our study highlights the importance in performing multiple swabs in the postmortem examination, because SARS-CoV-2 swab positivity can be limited to only a single swab.**

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Historically, clinical autopsies have made crucial contributions in the discovery and explanation of the clinicopathologic basis of infectious diseases, thus providing a radical contribution to their clinical management. Currently, we live in a historic period in which we are witnessing the decline of clinical autopsy, even though its importance has not changed and it remains the most thorough and precise way to study the complexity of diseases and to develop physiopathologic models to address clinical matters.[1,2]

Notwithstanding, in our institution (Policlinico Universitario Agostino Gemelli, Catholic University of Sacred Heart, Rome, Italy), in the pathology department, there is an operative unit that is exclusively dedicated to autopsy, with attention to both clinical and fetal/perinatal autopsies. Moreover, the unit has the appropriate means and structures meeting the structural safety criteria to perform autopsies on patients affected by hazard group 3 infective pathogens.[3–6]

We remind readers that during the HIV/AIDS epidemic there was a great impulse to identify the pathogen in postmortem tissues[7–10] because it was clear that its identification would bring crucial information on the transmission and clinical features of the virus for both

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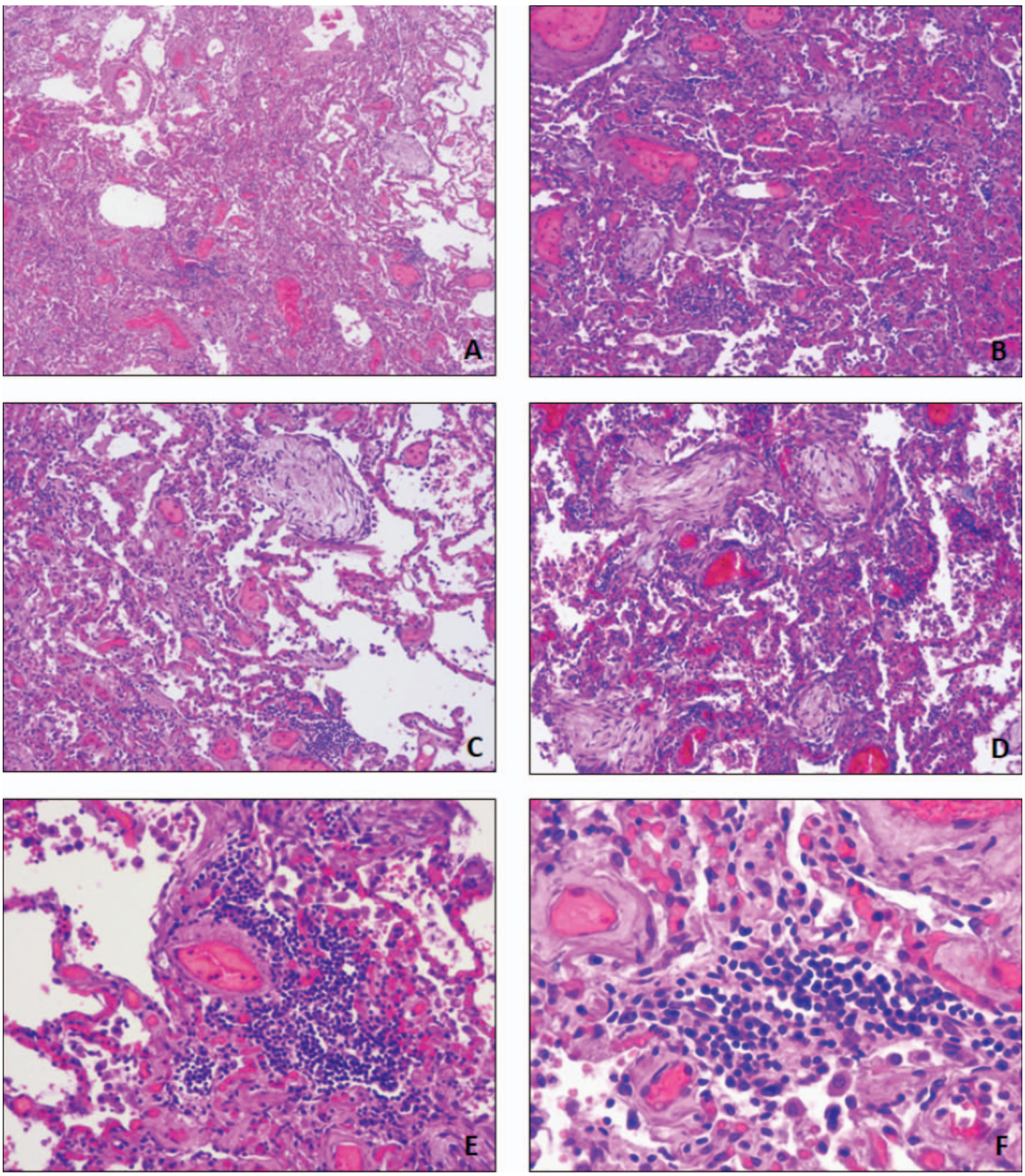

Figure 1. *A through F, Representative panel of the histopathologic aspects of severe acute respiratory syndrome coronavirus 2 in our cases. Organizing phase of diffuse alveolar damage (B through D), associated with chronic interstitial and perivascular inflammatory infiltrate (E and F). Microthrombotic (F) aspects were also present (hematoxylin-eosin, original magnifications [objective lens] ×4 [A], ×10 [B through D], ×20 [E], and ×40 [F]).*

clinicians and pathologists. The demonstration of pathogens in tissues has also been fundamental in understanding the patterns of pathogenesis of viruses, such as hantavirus, Ebola virus, Marburg virus, and Lassa virus.[1,11–13]

To the contrary, in the course of this pandemic, both scientific institutions and authorities have shown a certain degree of diffidence in the execution of autopsies, strongly emphasizing the safety requirements for autopsy rooms and recommending tight criteria for requests for clinical autopsy. This is probably because this activity could represent a possible source of biological risk in cases where minimum requirements for its safe execution are not met.[14–16]

To meet the urgent needs of the conspicuous number of patients with COVID-19 syndrome admitted to our hospital, in a perspective of resource optimization for the care and management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–affected patients, autopsies on patients with certain or suspected SARS-CoV-2 infections started to be requested in our hospital only by the end of April 2020.

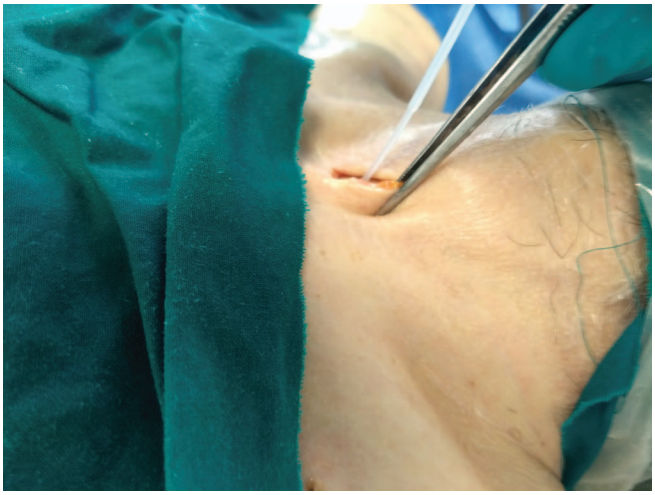

Figure 2. *Picture depicting the performing of a tracheobronchial swab through a small vertical incision in the neck to the trachea.*

The moment we started performing complete clinical autopsies on patients with either a clinical suspicion of death from COVID-19 or an outright diagnosis of SARS-CoV-2–related pneumonia, we adopted a strict protocol consisting of 5 postmortem swabs to look for the presence of SARS-CoV-2, according to the Centers for Disease Control and Prevention[4] guidelines.

Several studies in the literature have evaluated the technical feasibility of postmortem swabs to evaluate viral infections.[17] Some groups[18–22] have already evaluated the presence of SARS-CoV-2 with postmortem swabs during autopsies. Our protocol consisted of a rhinopharyngeal and a tracheal swab to search for the virus in the upper airways and 2 swabs in the lower airways: 1 on the left lung parenchyma and 1 on the right lung parenchyma. A fifth swab on the parenchyma of both lungs was performed to look for other viruses that could evolve in a clinical picture of interstitial pneumonia (Figure 1).

MATERIALS AND METHODS

We performed complete autopsies on 12 patients with a clinical diagnosis of SARS-CoV-2–related pneumonia, except for the brain, examination of which was approached with a mini-invasive technique instead (using as a transethmoidal probe a T biopsy Jamshidi needle [Osteobell T, Biopsybell] to take samples, approximately 0.5 × 0.3 cm in size, of brain tissue).

In the course of the external examinations, we performed the first swab (rhinopharyngeal). Whereas the Centers for Disease Control and Prevention[4] recommendations prescribe removing the heart-lung block and inserting 1 swab for each lung as far as possible into the tracheobronchial tree, our protocol prompted for a tracheobronchial swab (Figure 2) by obtaining access to the trachea through a small vertical incision performed in the midline of the neck from the thyroid cartilage up to the space above the suprasternal notch. The choice of the vertical incision is chiefly dictated by the greater simplicity of recomposition. Afterward, the skin, the subcutaneous tissue, and the neck muscles are retracted. The thyroid isthmus is therefore removed. After the exposition and identification of the cricoid cartilage, the trachea is cut open with a vertical incision.

We did proceed to the swabbing of both the right and the left lung parenchyma after their slice opening in the course of the gross examination. A further swab was performed on both lungs in order to evaluate the eventual presence of adenovirus, coronavirus (229E, NL63, OC43, HKU1), metapneumovirus, rhinovirus/enterovirus, influenza A virus, respiratory syncytial virus, bocavirus, and Middle East respiratory syndrome coronavirus.

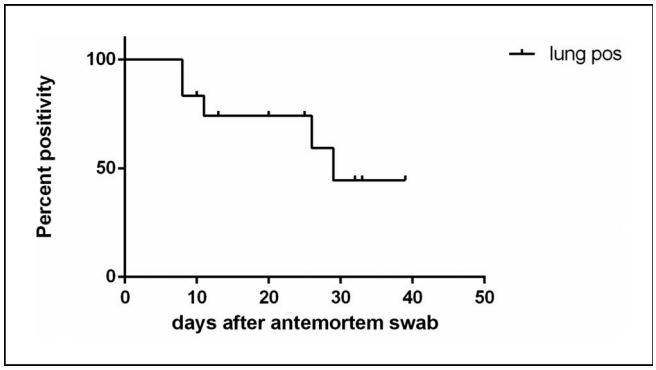

Figure 3. *Kaplan-Meier plot for lung positive swabs (lung pos). The plot illustrates the reduction in the percentage of positive lung swabs as days pass after the antemortem swabs.*

Right after their collection, all the swabs were deposited in a refrigerator set at –20°C. Afterward, swabs were brought and accepted by the microbiology laboratory in an average time of 113.67 hours (range, 3–310 hours), abiding to the needs and to the dutiful priority given to the emergency department and all wards in our hospital dedicated to the diagnosis and care of patients suspected for a SARS-CoV-2 infection.

Samples were collected in viral transport medium (UTM, Copan, Italy) and analyzed with real-time reverse transcription polymerase chain reaction (PCR) for SARS-CoV-2 RNA. Processing was performed on CE-IVD–marked NIMBUS automated liquid handling workstations from nucleic acids extraction to PCR setup (Seegene, Arrow Diagnostics), according to the manufacturer's directions.

RNA of SARS-CoV-2 was detected by multiplex real-time reverse PCR assay using Allplex 2019-nCoV Assay (Seegene, Arrow) on the CFX96 real-time detection system (Biorad) with automatic data system analysis software (Seegene viewer) for identifying positive samples (cycle threshold value <40 is interpreted as positive for SARS-CoV-2 RNA). The Allplex 2019-nCoV assay is a multiplex real-time PCR assay for simultaneous detection of 3 target genes of SARS-CoV-2 in a single tube. The assay is designed to detect RdRP and N genes specific for SARS-CoV-2 and E gene for all of *Sarbecovirus* including SARS-CoV-2, as recommended by the US and Chinese Centers for Disease Control and Prevention, and approved for emergency use authorization by the Korea Centers for Disease Control and Prevention.

Reported positive percentage agreement was 100.00% (95% CI, 92.75%–100.00%) and negative percentage agreement was 93.07% (95% CI, 85.76%–96.93%) in upper respiratory specimens, including nasopharyngeal and oropharyngeal. In lower respiratory specimens, the positive percentage agreement reported was 100.00% (95% CI, 92.75%–100.00%), and the negative percentage agreement reported was 96.84% (95% CI, 90.39%–99.18%). Procedures to prevent specimen contamination and PCR carryover were rigorously observed at all stages.

Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software) and MedCalc version 10.2.0.0 (MedCalc Software). The study of the relationship between time and the results of the swab was performed with the Kaplan-Meier estimator (Figure 3) and the Pearson correlation coefficient, with 95% CI. P values <.05 were considered statistically significant.

RESULTS

Patients were on average 82.3 years old (range, 54–93 years) and all had a clinical diagnosis of COVID-19 that was confirmed either with radiologic findings or with antemor-

Results of the Swabs									
Case No.	Age, y/Sex	Time Between Antemortem and Postmortem Swabs, d	Time Between Death and Postmortem Swabs, h	Time Between Postmortem Swabs and Microbiology Acceptance, h	Antemortem Swab Results	Postmortem Swab Results			
						Nasal	Tracheal	Right Lung	Left Lung
1	93/F	8	93	146	Pos	Neg	Pos	Pos	Pos
2	80/F	11	52	310	Pos	Pos	Pos	Pos	Pos
3	84/F	13	52	240	Pos	Pos	Neg	Neg	Neg
4	78/F	26	48	216	Pos	Pos	Neg	Neg	Pos
5	90/F	8	13	168	Inconclusive	Pos	Neg	Neg	Pos
6	80/M	33	14	168	Pos	Pos	Neg	Neg	Neg
7	92/F	25	32	48	Pos	Pos	Neg	Neg	Neg
8	81/M	39	24	48	Pos	Neg	Pos	Neg	Neg
9	54/F	20	21	5	Pos	Neg	Neg	Neg	Neg
10	82/M	10	22	5	Pos	Neg	Neg	Neg	Neg
11	86/M	32	36	7	Pos	Neg	Neg	Neg	Neg
12	87/F	29	120	3	Pos	Pos	. . .	Pos	Pos

Abbreviations: Neg, negative; Pos, positive.

tem swabs. Eleven of 12 cases were found to be SARS-CoV-2 positive by antemortem swabs; 1 case yielded an inconclusive result, yet the postmortem swabs were found to be positive in this case.

The average time elapsed between the antemortem and the postmortem swab was 21.16 days (range, 8–39 days), whereas the time between death and the execution of swabs (in the course of the autopsy) was on average 43.92 hours (range, 12–120 hours).

According to our observations on the 12 cases subjected to complete autopsy, in relation to the time interval between the postmortem swab collection and the microbiological analysis, we clearly found evidence that the virus could be found in samples up to 310 hours (range, 3–310 hours) from the postmortem sampling. Nine cases of 12 were found to have at least 1 postmortem swab positive for SARS-CoV-2. The median cycle threshold value of all positive specimens was 28.5 (interquartile range, 26.0–31.0). Of note, this result was found in either nasal or tracheal swabs, whereas lung swabs were found to be positive in only 5 of 12 cases. The results are summarized in the Table.

Overall, we found a correlation between negativity of the lung swabs and the number of days elapsed from the antemortem swabs, calculated with the Pearson correlation coefficient ($R^2 = 0.9633$, $r = -0.9815$, $P < .001$).

We also found a negative correlation between positivity of the other swabs in aggregate and the number of days passed from the antemortem swabs. This correlation was also calculated with the Pearson correlation coefficient ($R^2 = 0.9502$, $r = -0.9748$, $P < .001$).

DISCUSSION

A thorough knowledge of the eventual persistence of pathogens in deaths related to infectious diseases is fundamental in order to secure an approach to complete autopsy performance in which the operators can be fully aware of the eventual biological risks before exposition. This postulate is particularly evident when referring to emergent pathogens, such as SARS-CoV-2. An effective way in which postmortem staff can effectively reduce the risks associated with necropsies is through awareness of the infective status of the bodies.[10]

A noteworthy result of this study is that we did not find a relation between the results of the swabs and either the time elapsed from their collection or the time elapsed before their acceptance in the microbiology laboratory; however, the exiguity of our cases limits the conclusiveness of this finding, and wider studies would be necessary in order to define the infectiveness of the virus in postmortem tissues.

Afterward, the whole staff involved in the performing of these complete autopsies was also subject to nasopharyngeal swabs for SARS-CoV-2 that resulted in negative yields (the autopsies we reported implied an exposition time for the medical and technical staff spanning from 45 minutes to 2 hours).

Our data highlight the high degree of importance of performing multiple swabs, as it was clear from our experience that in the postmortem, SARS-CoV-2–positive yields can also be limited to only 1 of 4 swabs. Moreover, we cannot stress enough the importance of a solid experience of the staff involved in the autopsies of SARS-CoV-2–related deaths and eventually in other emergent or re-emergent infectious diseases, in order to effectively reduce the infection risk for the operators, as was also highlighted by the Italian Instituto Superiore di Sanità (Italian National Institute of Health) guidelines for the execution of clinical autopsies.[16]

The SARS-CoV-2 pandemic will probably have an impact in the near future on clinical autopsy conduct. We believe that such a context should not imply a reduction in the number of complete clinical autopsies, but it should represent an opportunity for a profound revaluation of this essential diagnostic tool. As it was pointed out, in this historic moment more autopsies are needed in order to establish the actual extent of organ involvement induced by SARS-CoV-2, thus resulting in better and more tailored clinical management schemes.[23]

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