

Postmortem swabs in the Sars-CoV-2 Pandemic: Report on 12 complete clinical autopsy cases

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Abstract**Context**

Clinical autopsies have historically provided a fundamental contribution in the definition of the clinico-pathological 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 Sars-CoV-2 in the postmortem 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 rhino-pharyngeal and a tracheal swab in order to search for the virus in the upper airways and of two 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 that 9 out of 12 cases had at least one postmortem swab to be positive for Sars-CoV-2. Moreover we evaluated the time lapse between the antemortem and the postmortem swabs, the time between death and the postmortem swabs, and the time lapse between the postmortem swabs and the acceptance to the microbiology laboratory. Interestingly we did not find a relation neither between the results of the swabs and the time lapsed from their collection, or with the time lapsed 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 like Sars-Cov-2. Our study highlights the importance in performing multiple swabs in the postmortem, since Sars-CoV-2 swab positivity can be limited to only a single swab.

INTRODUCTION

Historically clinical autopsies gave a crucial contribution in the discovery and in the explanation of the clinico-pathological basis of infectious diseases, thus providing a radical contribution to their clinical management.

Nowadays we live in a historical 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 physiopathological 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 both to clinical and fetal/perinatal autopsies. Moreover, such unit has the appropriate means and structures meeting the structural safety criteria to perform autopsies on patients affected by Hazard group 3 infective pathogens³⁻⁶.

We remind that during the HIV/AIDS epidemic there was a great impulse in identifying the pathogen in postmortem tissues⁷⁻¹⁰, since it was clear the necessity both for clinicians and pathologists that its identification would have brought crucial information on the transmission and on clinical features of the virus. The demonstration of pathogens in tissues was also fundamental in the understanding the patterns of pathogenesis of viruses such as Hantavirus, Ebola, Marburg and Lassa ^{1,11-13}.

On the contrary, in the course of this pandemic, both scientific institutions and authorities showed a certain degree of diffidence in the execution of autopsies, strongly emphasizing the safety requirements for autopsy rooms and recommending tight criteria for the requests of clinical autopsy.

This happened probably because this activity could have the potential to represent a possible source of biological risk in all those cases where minimum requirements for their safe execution are not met^{14–16}.

In order to meet the urgent needs of the conspicuous number of patients with COVID-19 syndrome admitted in our hospital, in a perspective of resources optimization to the care and management of 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.

The moment we started performing complete clinical autopsies either on patients with a clinical suspicion of death from COVID-19, or with an outright diagnosis of Sars-CoV-2 related pneumonia, we adopted a strict protocol consisting of five postmortem swabs in order to look for the presence of the Sars-CoV-2 virus, according to the CDC guidelines⁴. Several studies in literature evaluated the technical feasibility of postmortem swabs in order to evaluate viral infections¹⁷.

Some groups already evaluated the presence of Sars-Cov-2 with postmortem swabs during autopsies^{18–22}. Our protocol, consisted of a rhino-pharyngeal and a tracheal swab in order to search for the virus in the upper airways, and of two swabs in the lower airways: one on the left lung parenchyma and one on the right lung parenchyma. A fifth swab on the parenchyma of both lungs was performed in order 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 whose examination was approached with a mini-invasive technique instead [using as a trans-ethmoidal probe a “T” biopsy jamshidi needle (Osteobell T, Biopsybell, Italy) in order to take samples - of about 0.5 x0.3 cm in size - of brain tissue].

In the course of the external examinations we performed the first swab (rhino-pharyngeal). While the Center for Disease Control and Prevention (CDC) recommendations prescribed to remove the heart lung block and to insert one swab for each lung as far as possible into the tracheobronchial tree⁴, our protocol prompted for a tracheobronchial swab (Figure 2) by obtaining an 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. After that, both 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.

Moreover, 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, MERS-CoV.

Right after their collection, all the swabs were deposited in a refrigerator set at -20 C. Afterwards swabs were brought and accepted by the microbiology lab in an average time of 113.67 h (range 3-310 h), abiding to the needs and to the dutiful priority given to the

emergency department and of all those 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 RT-PCR for SARS-CoV-2 RNA. Processing was performed on CE-IVD marked NIMBUS Automated Liquid Handling Workstations, from NA Extraction to PCR Setup (Seegene, Arrow Diagnostics, South Korea), according to the manufacturer's directions. SARS-CoV-2 RNA was detected by multiplex Real-time RT-PCR assay using Allplex 2019-nCoV Assay (Seegene, Arrow Diagnostics, South Korea) on CFX96 Real-time detection system (Biorad, Italy) with automatic data system analysis software (Seegene viewer) for identifying positive samples (Cycle threshold value less than 40 is interpreted as positive for SARS-CoV-2 RNA). 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 the Chinese Centers for Disease Control and Prevention, and approved for emergency use authorization from Korea Centers for Disease Control and Prevention.

Reported Positive Percent Agreement (PPA) was 100.00% (95% CI: 92.75% ~ 100.00%), while the Negative Percent Agreement (NPA) was 93.07% (95% CI: 85.76% ~ 96.93%), in upper respiratory specimens including nasopharyngeal and oropharyngeal.

In lower respiratory specimens the Positive Percent Agreement (PPA) reported was 100.00% (95% CI: 92.75% ~ 100.00%), while the Negative Percent Agreement (NPA) 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 relation between time

and the results of the swab was performed with the Kaplan-Meyer estimator (Figure 3) and the Pearson's correlation coefficient, with 95% Confidence Intervals. P values $<.05$ were considered statistically significant.

RESULTS

Patients were on average 82.3 years old (range 54-93 years), they all had a clinical diagnosis of COVID-19 that was confirmed either with radiological findings or with antemortem swabs. Eleven out of 12 cases were found to be Sars-CoV-2 positive to antemortem swabs, while one case yielded an inconclusive result, nevertheless the postmortem swabs were found to be positive in this case.

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

According to our observations on the 12 cases subjected to complete autopsy, in relation to the time interval elapsed between the post-mortem swabs collection and the microbiological analysis, we clearly found evidences that the virus could be found in samples up to 310 hours (range 3-310) from the post mortem sampling. Nine cases out of 12 were found to have at least one postmortem swab to be positive for Sars-CoV-2.

The median Cycle threshold values (Ct) of all positive specimen was 28.5 (IQR 26.0-31.0). Interestingly this result was found either in nasal or tracheal swabs, while lung swabs were only found to be positive in 5 out of 12 cases. The results are summarized in Table 1.

Overall we found a correlation between negativity to the lung swabs and the number of days passed from the antemortem swabs, calculated with Pearson's correlation coefficient (R square: 0.9633, r : -0.9815; $P < .001$).

We also found a negative correlation between positivity to the other swabs in aggregate and the number of days passed from the antemortem swabs. This correlation was also calculated with Pearson's correlation coefficient (R square: 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 the complete autopsy performance in which the operators could be fully aware of the eventual biological risks before the exposition. This postulate is particularly evident when referring to emergent pathogens like Sars-Cov-2. An effective way in which postmortem staff can effectively reduce the risks associated with necropsies is through the awareness of the infective status of the bodies¹⁰.

A noteworthy result of this study is that we did not find a relation neither between the results of the swabs and the time lapsed from their collection, or with the time lapsed 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 post-mortem tissues.

Afterwards, 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 highlights the high degree of importance in 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 one out of four 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 it was also highlighted by the Italian I.S.S. (Istituto Superiore di Sanità, Italian National Institute of Health) guidelines for the execution of clinical autopsies¹⁶.

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 reevaluation of this essential diagnostic tool. As it was pointed out, in this historical 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 schemes²³.

Figure legends:

Figure 1 A-F. Representative panel of the histopathological aspects of Sars-Cov-2 in our cases (A-E). Organizing phase of diffuse alveolar damage (B,C,D), associated with chronic interstitial and perivascular inflammatory infiltrate (E-F). Microthrombotic (F) aspects were also present. (A-F Hematoxylin and Eosin stain; objective lens: A 4x; B 10x; C 10x; D 10x; E 20x; F 40x)

Figure 2. Picture depicting the performing of a tracheobronchial swab through an access from the neck to the trachea.

Figure 3. Kaplan Meier plot. Survival proportions: survival of Data 1.

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