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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Postmortem detectability and viability of SARS-CoV-2 virus in various biological specimens

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SUMMARY

Introduction Without a comprehensive postmortem investigation it is impossible to determine the cause of death among the SARS-CoV-2-suspected and -positive patients. We present two cases to discuss the postmortem detectability of SARS-CoV-2 virus and RNA stability in biological samples.

Outline of cases Case No. 1: a 40-year-old man on whom the autopsy was performed four days after death. The body was stored at 4°C. Bilateral pneumonia was confirmed grossly and histopathologicaly. Molecular testing was positive for IgM antibodies, but negative for SARS-CoV-2 RNA. Case No. 2: a 28-year-old professional basketball player who suffered from SARS-CoV-2 about a month earlier. The autopsy was performed two days after death. The body was stored at 15°C. Gross autopsy findings revealed advanced putrefactive changes and an enlarged heart, with visible fibrotic focuses. The histopathological finding corresponded to the sudden cardiovascular death due to the cardiac dysrhythmia most probably formed in one of the fibrotic focuses. Tests for SARS-CoV-2 RNA and antibodies (IgM, IgG) were positive in the analyzed samples.

Conclusion This report suggests that SARS-CoV-2 virus can be isolated in the biological samples even after a long post-mortem prolongation of molecular analyses. We emphasize the necessity of wider studies that will define the infectiveness and biological stability of the virus in postmortem tissues.

Keywords: forensic medicine; forensic pathology; COVID-19; virus detection; biological samples

INTRODUCTION

To date, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused more than 304 million cases worldwide, with more than 5.4 million deaths [1].

It is well known that, in living people, respiratory viruses like SARS-CoV-2 are highly contagious, and are mainly transmitted by respiratory droplets exchanged during immediate interpersonal physical contacts. Also, it has been shown that it persists on inanimate surfaces up to nine days, which suggests its possible postmortem transmission and detectability in different biological samples. Therefore, it is clear that the biological samples should be handled with care [2–5]. The United States Centers for Disease Control and Prevention (CDC) published official guidelines for collection of postmortem specimens of confirmed or suspected COVID-19 cases [6].

Without comprehensive postmortem investigation, it is impossible to determine the exact cause of death among the SARS-CoV-2-suspected and -positive patients, which, again, highlights the role of postmortem human COVID-19-associated deaths investigations.

In order to discuss the postmortem detectability of SARS-CoV-2 virus and its RNA stability in different biological samples, we present two case reports. One case shows that SARS-CoV-2 virus can be retrospectively detected in the biological samples of the lower respiratory tract during a relatively long postmortem period, and the other that the virus RNA is lost over time, with the prolongation of the postmortem period.

CASE REPORTS

Case 1 presentation

On April 9, 2020, a 40-year-old man was found dead in front of his house. The external exam showed no evidence of mechanical and other injuries that would suggest a violent manner of death.

The deceased had no chronic conditions, but had a history of heroin abuse. Heteroanamnestic data indicated that he was not feeling well during the previous several days. He complained of weakness and shortness of breath, which is why he went to the emergency medical center and was prescribed symptomatic therapy. According to his step-sister, he was constantly in contact with his neighbors, who were SARS-CoV-2-positive.

The autopsy was performed four days after death, according to the standard procedure. Gross autopsy findings revealed heavy, grossly firm, and rubbery, shiny "ground glass"-like lungs, with severe bilateral edema. On the cut section, the lungs were dark red without

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purulent discharge, with a large number of blood clots in small-caliber blood vessels around the described pulmonary changes. The hilar lymph nodes were slightly enlarged. The liver was also slightly enlarged, while the findings in other organs were unremarkable.

Toxicological analyses indicated the presence of opioid analgesic codeine, along with its metabolites and analgesic metamizole metabolite, 4-acetilaminoantipirine in therapeutic concentrations, which were interpreted as metabolic products of analgoantipyretic drugs.

Initially, the death was attributed to pneumonia of unknown origin, but after additional diagnostic procedures conducted two months later, it was proven that death was caused by COVID- 19.

Histopathological analysis

Histopathological analysis (HP) of the lungs revealed prominent intraalveolar protein- rich edema, capillary congestion, and formation of hyaline membranes. Alveolar lumen was filled with a moderate number of multinuclear giant cells presenting with a viral cytopathogenic effect. A number of embolized thrombi were present in middle-caliber pulmonary artery branches. The HP finding corresponded to the viral etiology interstitial pneumonia. HP findings of other organs were unremarkable.

Molecular testing

Nasopharyngeal swab and lower respiratory tract specimens (trachea and both lungs), as well as the femoral vein blood, were collected during the autopsy. Before the postmortem examination, the body was kept in the refrigerator at 4°C. After the RNA isolation from the swabs, using QIAamp Viral RNA mini Kit (QIAGEN N.V., Venlo, The Netherlands) on manufacturer's instructions, the presence of RNA sequence specific for OFR1ab gene of SARS- CoV-2 was tested using Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2 (BGI Genomics, Shenzhen, Guangdong, China). This kit also employs human housekeeping gene β-actin as the internal control. Reverse transcription, amplification and detection was performed in ViiA7 (Applied Biosystems, Waltham, MA, USA), using the cycling profile recommended by the manufacturer. Signals specific for the internal control were the only targets detected in all the samples. Lateral flow immunochromatographic test for IgM and IgG SARS-CoV-2-specific antibodies (Wuhan UNscience Biotechnology Co. Ltd., Wuhan, Hubei, China) showed positive results for the IgM, but not for the IgG antibodies against novel coronavirus, suggestive for the acute phase of coronavirus infection. Negative results for the viral RNA could be explained by the prolonged period from death to sampling.

Case 2 presentation

A 28-year-old professional basketball player suffered a cardiorespiratory arrest during training, which was followed by an unsuccessful cardiopulmonary resuscitation. Heteroanamnestic data indicated that he suffered from SARS-CoV-2 about one month previously. He had mild symptoms (low fever, periodic dysrhythmias, and fatigue), but was not involved in any training activities during the SARS-CoV-2 infection symptoms, nor during one month afterwards. At the time of his return to training he had no complaints about any health issues.

The autopsy was performed two days after death, according to the standard procedure. While waiting for the autopsy, the body was stored in a "cold room" at 15°C, because it could not be refrigerated due to the excessive body length (over 2 m). External examination showed advanced putrefaction and several recent injection wounds on the left forearm suggesting attempted resuscitation. Gross autopsy findings revealed an enlarged heart (dimensions 17 × 14 cm, weight 570 g), with macroscopically visible fibrotic focuses, while the findings in other organs were unremarkable.

Specimens for toxicological and histopathological analyses were taken. Samples for toxicological analysis included heart blood (peripheral blood was not available because of the advanced putrefaction), as well as kidney and liver samples, which were negative for the presence of therapeutic or any other drugs of abuse.

Histopathological analysis

Although HP analysis showed advanced putrefactive changes, basic anatomical structure was still recognizable. There was a prominent intraalveolar edema and heavy capillary congestion of lungs. The alveolar lumen in better conserved tissues was filled with a moderate number of macrophages. In the heart, large fields of perivascular and interstitial fibrosis were visible and could be attributed to old myocarditis changes and advanced atherosclerotic changes in the intramyocardial blood vessels. The HP finding corresponded to the sudden cardiovascular death due to the cardiac dysrhythmia most probably formed in one of the fibrotic focuses.

Molecular testing

Nasopharingeal swab and heart blood samples were collected immediately after the admission of the body to the Institute of Forensic Medicine. Collected biological samples were analyzed using SARS-CoV-2 One-Step RT-PCR Kit (NZYTech, Lisbon, Portugal), targeting viral RNA-dependent RNA polymerase of the virus and human RNAse P gene (internal control) in WiiA7 (Applied Biosystems), as well as with serological testing for COVID-19-specific IgM/IgG antibodies. Both tests performed were positive for SARS-CoV-2 RNA and antibodies (both IgM and IgG) in the analyzed biological samples. The high Ct value from the RT-PCR test suggested low viral load in the samples. Three days later, during which time the body was kept in a room at 15°C, the same biological samples were taken during the autopsy and analyzed immediately, but were negative for SARS-CoV-2 RNA possibly due to the postmortem degradation of the samples. The lateral flow tests for antibodies were unreadable, due to the extensive hemolysis of the blood samples.

DISCUSSION

One of the crucial roles of forensic medicine and pathology during any epidemic is to perform autopsies along with all additional analyses in order to provide new insights of the pathogens' transmission and their clinical features. In contrast, in the course of this pandemic, medical public and scientists are under the impression that there is a certain degree of modesty in the performing of autopsies. This fact can potentially be explained by the very demanding safety requirements for autopsy rooms and by tight criteria recommendations for clinical autopsy requests. Despite numerous scientific publications, there is no reliable data concerning virus pathogenicity, postmortem transmission, and its viability in cadavers.

Given the fact that virus detection is more probable in cases where the viral load is higher, nowadays nasopharyngeal swab represents the golden standard sample for SARS-CoV-2 virus detection not only in live persons, but also as a part of postmortem isolation. Following the above-mentioned principles, a positive SARS-CoV-2 nasopharyngeal swab taken during early postmortem period, according to the CDC recommendations, would mean that the person was infected and, if other clinical data suggest so, died from COVID-19 disease or its complications. On the other hand, there is not enough scientific evidence that would prove that negative nasopharyngeal swab for SARS-CoV-2 virus taken during early postmortem period will definitely exclude COVID-19 as a cause of death [7].

The first case presented in this report highlights the fact that the persistence of SARS-CoV-2 RNA in the lower respiratory tract swabs can be detected as late as two months after death regardless of the fact that the swabs were not stored according to the CDC recommendations in cases of a delayed testing (at -70°C or below). Contrary to the first, in the second presented case report, after initial SARS-CoV-2 virus isolation, only two days later, when the autopsy was performed, the virus was not detectable in the same biological samples any more, even though all samples were taken and stored according to the CDC recommendations. Thus, previously mentioned facts raise a number of questions concerning postmortem SARS-CoV-2 virus viability, especially in the light of post-mortem period prolongation, and its detectability not only in different biological samples. According to CDC information concerning novel SARS-CoV-2, there is a lack of data on the frequency of detection of SARS-CoV-2 by RT-PCR on postmortem swabs collected in different intervals after death. Generally, it is said that, based on the knowledge from previous MERS-CoV and SARS-CoV epidemics, if SARS-CoV-2 testing on postmortem swab samples is considered a suspected COVID-19 case, SARS-CoV-2 RNA in the majority of cases may still be detected up to three days postmortem. Also, some scientists have shown that the sensitivity of postmortem tests may be reduced with a longer postmortem interval or embalming [8]. On the other hand, a group of German scientists showed that SARS-CoV-2 RNA may be detectable even in decomposed corpses [9].

An Italian autopsy study did not find a correlation 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 for virus isolation [8]. Therefore, it can only be concluded that the available scientific results are limited and, at the very least, unconvincing, suggesting the necessity of more thorough studies concerning this issue.

We want to highlight the fact that there are many factors that can affect the postmortem virus survival time. Besides the previously mentioned, according to some studies, refrigeration of the corpse may also prolong survival time of the coronavirus [10]. A lack of adequate antiviral therapy during the immediate pre-mortem period may play some role in the lasting persistence of SARS-CoV-2 RNA. In the first case report, the deceased did not receive specific antiviral therapy since there were no certain clinical data confirming SARS-CoV-2 infection. Also, collection of swabs from the lower respiratory tract provides a higher probability of viral RNA detection than swabs from the nasopharynx [11]. However, special attention should be paid to the interpretation of PCR testing in postmortem specimens. Positive nasopharyngeal swabs or lower airway specimens do not always mean that the deceased had the infection, because the viral RNA may persist in clinical samples without the virus being viable [12].

Our case reports suggest that post-mortem SARS-CoV-2 virus can be isolated in the biological samples even after a considerable post-mortem delay of the molecular analyses. Regardless of the facts stated in these case reports, the authors want to highlight the necessity of wider studies in order to define the infectiveness and biological stability of the virus in postmortem tissues. It is necessary to form firm arguments, supported by strong pathohistological and molecular evidence that would be the foundation for future clinical and postmortem clinical studies. This information will also ensure reducing the risks of infection for medical staff involved in autopsy procedures by increasing their knowledge and awareness of the postmortem infective status of the body.

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Постмортална детектабилност и вијабилност вируса *SARS-CoV-2* у различитим биолошким узорцима

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САЖЕТАК

Увод Без свеобухватних постморталних истраживања није могуће утврдити узрок смрти код преминулих особа за које се сумњало да су позитивни и код оних који су били позитивни на *SARS-CoV*-2. У циљу разматрања о могућностима постморталне детекције вируса *SARS-CoV*-2 и стабилности његове РНК, приказана су два случаја.

Приказ случајева Први случај представља четрдесетогодишњи мушкарац чије је тело обдуковано четири дана после смрти. Тело је чувано на температури од 4° *C*. Макроскопским и микроскопским прегледом уочено је обострано запаљење плућа. Молекуларне анализе показале су присуство *IgM* антитела, али је *PCR* тест на РНК *SARS-CoV*-2 био негативан. Други случај представља двадесетосмогодишњи професионални кошаркаш који је боловао од инфекције вирусом корона око месец дана пре смрти. Обдукција је извршена два дана касније. Тело је чувано на температури од 15°*C*. Макроскопски налаз је показао узнапредовале трулежне промене и увећање срца са видљивим фокусима фиброзе. Хистопатолошки налаз је одговарао напрасној срчаној смрти због поремећаја срчаног ритма генерисаног највероватније на месту неког од фокуса фиброзе. Тестирањем на РНК *SARS-CoV-2* и антитела (*IgM*, *IgG*) добијени су позитивни резултати.

Закључак Овај рад указује на то да вирус може бити изолован молекуларним методама у биолошким узорцима чак и после веома продуженог постморталног интервала. Истиче се неопходност спровођења обимнијих студија које би дефинисале период инфективности и биолошке стабилности вируса у постморталним ткивима.

Кључне речи: судска медицина; патологија; ковид 19; детекција вируса; биолошки узорци