Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Persistence of SARS-CoV-2 viral RNA in nasopharyngeal swabs after death

To the Editor,

We read with great interest the letter of Landi et al.[1] in which the authors reported solid data about the persistence of virus particles in SARS-CoV-2 patients after the acute phase of epidemic infection. In particular, authors found that 20,6% of patients were tested positive again after at least one month from post-acute care admission (on a total of 29 examined patients).

Results showed by Landi and colleagues[1] strongly suggest that a significant number of recovered SARS-CoV-2 patients still could be potentially asymptomatic carriers of the virus. According to these data authors also suggest the modification of the current Italy guidelines for the management of SARS-CoV-2 patients[2] proposing two consecutive negative RT-PCR test results together with the clinical evidence of symptoms improvement before discharge from the hospital and discontinuation of quarantine.

In our opinion this study highlighted a great issue for the public health in terms of both virus spread control and safety of healthcare professionals. Indeed, asymptomatic carriers of the virus could transmit the infection during the frequently post-admission visits to which they are often subjected.

Our recent investigation can extend the considerations reported by Landi et al.[1] shedding new light on the capability of SARS-CoV-2 particles to also persist in deceased persons. In fact, we studied the persistence of SARS-CoV-2 by real time-PCR in 24 h post-mortem swabs of subjects who died from SARS-CoV-2 infection. RT-PCR analysis targeted the common envelope (E) gene, the specific nucleocapsid (N) and RNA-dependent-RNA-polymerase (RdRp) genes complying with the international validated protocols[3] both at T0 and after 24 h. The results of rRT-PCR test are showed as cycle-threshold (Ct) values. Ct-values less than 35 were considered as positive tests. Experimental procedures were performed according to the authorization of the independent ethics committee of the "Policlinico Tor Vergata" for the study of the presence of SARS-CoV-2 in the organic tissues and fluids obtained from autoptic examinations (#77.20).

15 patients with SARS-CoV-2 interstitial pneumonia were included in this study, 8 males and 6 females (age range 46–92, mean 73,1 ± 11,7). The duration of hospitalization ranged from 1 to 108 days (mean 22,1 ± 13,4). All patients had more than one associated disease (8 had heart failure, arrhythmia or ischemic heart disease, 6 hypertension, 6 dementia, 3 chronic renal failure, 2 neoplasms, 2 diabetes, 2 obesity, 1 recent kidney transplantation, 1 stroke).

RT-PCR investigations showed that SARS-CoV-2 viral RNA was still detectable in 60% of cases 24 h after death. Noteworthy, our data showed an increase of the viral load in more of 50% of positive individuals 24 h post-mortem (decrease of Ct value). It is important to note that more of 20% of deceased were positive for all evaluated genes (E, N and RdRp) also after 24 h. This fact allows to consider the autoptic exam of a SARS-CoV-2 positive deceased an highly dangerous procedure also after 24 h.

From biological point of view, this unexpected finding can be explained by the increase in chest pressure due to the formation of intestinal gas with consequent compression on the diaphragm (Fig. 1A) and / or by post-mortem cell lysis (Fig. 1B). In this case, there would be an indirect and greater release of the alveolar contents and virus-containing secretions in the upper airways. In addition, it is possible to hypothesize the existence of a post-mortem time window in which spike positive alive human cells can be infected by SARS-COV-2 and release great amount of viral particles, especially in absence of circulating immune cells (Fig. 1C). Indeed, several studies on animal models demonstrated that many cells remain alive and thriving after an organism's death. Amazingly, some of these increase their activity in the first hours after death.[4] This interesting discovery has been dubbed the "Twilight of Death," which refers to the time frame between death and body's decomposition where not all of the body's cells are yet dead. The authors suggest that the death phenomenon is more like a slow shutdown process and not the simple off-switch many imagine it to be. Thus, pathological process such as viral infections, could continue, or even increase, in the first hours after death. However, we cannot exclude a false negative result on the first test. According to literature, false negatives may be due mainly to inappropriate sample taking or transport, or a low viral load, below the sensitivity limit of the technique.[5] To minimize the risk of sampling errors, in this study all nasopharyngeal swabs were taken by a single well-trained operator using a previously described standard operating procedure.[6] Therefore, we believe that further possible explanations cannot be excluded, especially when considering the potential role of post-mortal transformative phenomena.

The peculiar results emerged from our study can be considered of great scientific interest and justify the need to adopt appropriate protective measures by both the medico-legal team in the surveys on the investigative scenes and the staff of the morgue.[7,8] Nonetheless, even in a hospital setting when a diagnostic autopsy is required, our results highlight the importance of obtaining not only one post mortem swab, but at least two, if not three, consecutive swabs before a patient can be considered SARS-CoV-2-negative with a reasonable degree of certainty. This is particularly true for patients who died during the transfer to the hospital or without medical intervention, for whom autopsy is required with no exhaustive clinical data.

Abbreviations

Ct: cycle-threshold
E: envelope

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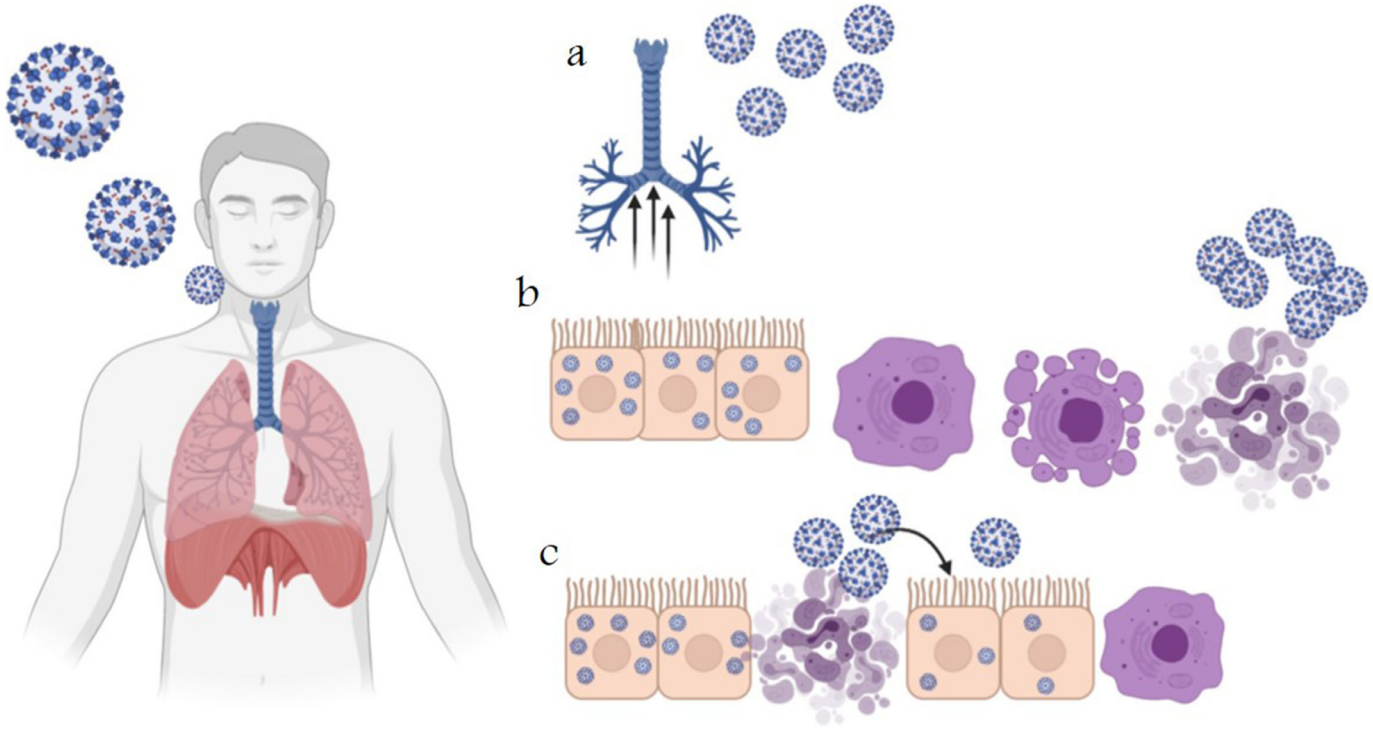

Fig. 1. *Hypothesis about the SARS-CoV-2 persistence in human body after death.* A) The increase in chest pressure due to the formation of intestinal gas with consequent compression on the diaphragm can push out the SARS-CoV-2 particles from lower to upper airways. B) The lysis of SARS-CoV-2 infected cells that occur after death can release a large amount of viral particles in the upper airways. C) In the first hours after death the SARS-CoV-2 can infect alive cells thus increasing the viral load. Created in Biorender.com.

N: nucleocapsid
RdRp: RNA-dependent-RNA-polymerase
RT-PCR: real-time- Polymerase Chain Reaction

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Policlinico Tor vergata (Rome, Italy) (#77.20).

Consent for publication

Not applicable.

Consent for publication

The author gives consent for publication of this paper.

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Authors' contributions

MS, SM and AM coordinated the overall study.

MS, SM, FS, OS, and AM were involved in the concept and the design of the study.

MS, SM, and AM drafted the manuscript.

FS, BC, MC, LA, MM, EG, MT, SB, and LTM contributed to the acquisition and the interpretation of the data.

All the authors revised the manuscript and approved its final version.

Declaration of Competing Interest

All authors have no conflict of interest.

Data for reference

All the data and material are available by emailing the corresponding aut

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