Letter to the Editor

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# Ultrasound-Guided Minimally Invasive Autopsy of Respiratory Muscles as a Safe and Cost-Effective Technique in COVID-19 Pandemic Era

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Dear Editor,

An outbreak of coronavirus disease in 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a pandemic and public health emergency [1, 2]. COVID-19 has been largely studied as it negatively affects the lungs leading to pneumonia, acute respiratory distress syndrome, and pulmonary thrombotic phenomena [3–7]. However, the impact of this virus on respiratory muscles' cytopathology had not yet been fully addressed.

Due to the large number of COVID-19 cases in Brazil, the Clinical Hospital of the Faculty of Medicine of the University of São Paulo has allocated all of its beds to receive patients with COVID-19, and unfortunately, it is expected that a large number of deaths will still occur. Although severe manifestations of COVID-19 that may lead to death are more common in older patients [3–5], several unexpected deaths have been reported in previously healthy young adults and teenagers [8, 9]. Thus, recently, we read an interesting issue published in your journal (Acta Cytologica from January to February 2020)

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concerning techniques in cytopathological specimens [10] that encouraged us to highlight the ultrasound-guided minimally invasive autopsies as a link between clinicians and cytopathologists.

Safety and cost-effective techniques in autopsy are essential in COVID-19 pandemic era. Thus, ultrasoundguided minimally invasive autopsies are both simple and significantly less risky in comparison to conventional autopsy, which allows to characterize the pathology of the SARS-CoV-2 [6, 11]. In traditional autopsy, the professional is exposed to more risks as a thoracotomy is needed for respiratory muscles sampling. However, ultrasound-guided minimally invasive autopsies are safer as it is considered a "closed-body autopsy" [11]. This method targets small tissue fragments by needle puncture for diagnosis [12]. For the procedure, a portable SonoSite M-Turbo R (Fujifilm, Bothell, WA, USA) ultrasound with C60x (5-2 MHz Convex) multifrequency broadband transducer was employed as lower frequency ultrasound waves permit a more in-depth visualization of all the organs. After locating the target tissue, we used a Tru-CutR

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**Fig. 1.** Histopathological findings in both diaphragm and intercostal muscles. Black arrows show a cluster of fibers with degeneration and atrophy. White arrows show edema due to muscle injury that leads to collagen deposit. Scale bar,  $50 \mu m$ .

semi-automatic 14 G coaxial needle with 20 cm length for tissue puncture and collection, still using SonoSite for needle guidance. Samples from both diaphragm and intercostal muscles were fixed in formalin and embedded in paraffin [13]. Further, sections were stained with hematoxylin and eosin for general structure evaluation using parameters from The National Toxicology Program from the US Department of Health and Human Services [14].

From ethical approval of the Clinical Hospital of the Faculty of Medicine of the University of São Paulo (protocol #3951.904), we showed in Figure 1 our histopathological findings. We observed fiber degeneration that is characterized as cell swelling, hypereosinophilia, vacuolation, loss of striation, fragmentation, and rupture of cell cytoplasm. This process leads to atrophy, increasing the extracellular space (edema) that will be occupied by collagen fibers in response to muscle injury, which decreases muscle function. Recently, an autopsy study showed that diaphragm muscle had increased collagen deposit in critically ill patients with COVID-19 infection [15], which is in accordance with muscle injury, as seen in our study using ultrasound-guided minimally invasive autopsy. Despite the decline in postmortem examination rate due to COVID-19, autopsy remains the gold standard technique able to provide valuable knowledge about different pathologies. Thus, we hope that both our methodology and histological findings may shed a light in CO-VID-19 pathogenesis on respiratory muscles.

## **Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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## **Author Contributions**

R.A.B.N., M.D., P.H.N.S., and W.J.F. collected the samples, analyzed the results, and drafted the letter.

#### References

- 1 Anderson RM, Heesterbeek H, Klinkenberg D, Hollingsworth TD. How will countrybased mitigation measures influence the course of the COVID-19 epidemic? Lancet. 2020 Mar 21;395(10228):931-4.
- 2 Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Health. 2020 Mar;25(3): 278–80.
- 3 Ludwig S, Zarbock A. Coronaviruses and SARS-CoV-2: a brief overview. Anesth Analg. 2020 Jul;131(1):93–6.
- 4 Wan S, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, et al. Clinical features and treatment of CO-VID-19 patients in northeast Chongqing. J Med Virol. 2020 Jul;92(7):797–806.
- 5 Zheng Y, Xu H, Yang M, Zeng Y, Chen H, Liu R, et al. Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu. J Clin Virol. 2020 Jun;127:104366.
- 6 Duarte-Neto AN, Monteiro RAA, da Silva LFF, Malheiros DMAC, de Oliveira EP, The-

odoro Filho J, et al. Pulmonary and systemic involvement of COVID-19 assessed by ultrasound-guided minimally invasive autopsy. Histopathology. 2020 Aug;77(2):186–97.

- 7 Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of CO-VID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020 Apr; 8(4):420–2.
- 8 Du W, Yu J, Wang H, Zhang X, Zhang S, Li Q, et al. Clinical characteristics of COVID-19 in children compared with adults in Shandong Province, China. Infection. 2020 Jun; 48(3):445–52.
- 9 Jeng MJ. COVID-19 in children: current status. J Chin Med Assoc. 2020 Jun;83(6):527–33.
- 10 Baloch ZW, Gupta PK. Cytopathology comes of age. Acta Cytol. 2020;64(1–2):5–6.
- 11 Monteiro RAA, Duarte-Neto AN, Silva LFFD, Oliveira EP, Filho JT, Santos GABD, et al. Ultrasound-guided minimally invasive autopsies: a protocol for the study of pulmonary

and systemic involvement of COVID-19. Clinics. 2020;75:e1972.

- 12 Veras FP, Pontelli M, Silva C, Toller-Kawahisa J, de Lima M, Nascimento D, et al. SARS-CoV-2 triggered neutrophil extracellular traps (NETs) mediate COVID-19 pathology. J Exp Med. 2020 Dec;7(21712):e20201129.
- 13 Nucci R, Jacob-Filho W, Busse A, Maifrino L, de Souza R. Anatomopathological assessment of the diaphragm in formalin-fixed, paraffinembedded sections. J Morphol Sci. 2018; 35(3):173–6.
- 14 National Toxicology Program [Internet]. U.S. Department of Health and Human Services: skeletal muscle. 2020 [cited 2020 Jul 30]. Available from: https://ntp.niehs.nih.gov/ nnl/musculoskeletal/skel\_musc/index.htm.
- 15 Shi Z, de Vries HJ, Vlaar AP, van der Hoeven J, Boon RA, Heunks LM, et al. Diaphragm pathology in critically ill patients with COVID-19 and postmortem findings from 3 medical centers. JAMA Intern Med. 2021;181(1):122–4.