Are two consecutive negative RT-PCR results enough to rule out COVID-19?

R. Waked[1], J. Makhoul[1], G. Saliba[1], N. Chehata[1], S. Mortada[2], A. Zoghbi[3], J. Choucair[1] and E. Haddad[1]

1) *Department of Infectious Diseases,* 2) *Department of Pulmonary and Critical Care and* 3) *Emergency Department, Faculty of Medicine, Saint Joseph University, Beirut, Lebanon*

Abstract

The coronavirus disease 2019 (COVID-19) pandemic is redefining the world we live in, and scientists are struggling to find the best severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostic tool. Routine testing is currently performed using real-time reverse transcription PCR (RT-PCR) of upper or lower respiratory tract secretions. We sought to demonstrate the importance of conducting RT-PCR using deep sampling when initial upper respiratory testing is negative in cases of high index of suspicion for COVID-19. We present the case of a 47-year-old man admitted for fever and bilateral pneumonia diagnosed via chest computed tomographic scan amidst the early peak of the COVID-19 pandemic, suggesting a SARS-CoV-2 infection. Two RT-PCR results from nasopharyngeal swab samples were negative. A bronchoscopy was then performed, and RT-PCR testing on bronchoalveolar lavage samples yielded positive results, confirming the diagnosis of COVID-19 pneumonia. RT-PCR samples of the lower respiratory tract likely contain a higher virus load and thus retain a higher sensitivity for SARS-CoV-2 detection.
© 2020 The Authors. Published by Elsevier Ltd.

Corresponding author: J. Makhoul, Department of Infectious Diseases, Faculty of Medicine, Saint Joseph University, Beirut, Lebanon.
E-mail: jennifermakhoul@outlook.com

Introduction

The number of confirmed coronavirus disease 2019 (COVID-19) cases around the globe is growing dramatically by the minute. Although the reason behind this uptick is chiefly due to the high contagiousness of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it also is a result of increased testing. The reference standard diagnosis is currently based on real-time reverse transcription PCR (RT-PCR) of respiratory specimens, most commonly using nasopharyngeal swabs. However, as of this writing, no diagnostic method has yet exhibited perfect sensitivity, with testing often leading to false-negative results and delayed diagnosis [1,2].

Case report

We report the case of a 47-year-old man with no relevant medical history who sought care at our tertiary-care hospital with spiking fever. Onset of symptoms was a week earlier, with the patient's fever temperature ranging 37.5°C to 39.5°C. He reported myalgia and asthenia but denied having any upper or lower respiratory symptoms. However, the patient had been in contact with a confirmed COVID-19 case 10 days before his hospitalization, although his household remained asymptomatic.

At admission the patient had a temperature of 40°C and an oxygen saturation of 97% on ambient air without any need for oxygen supplementation; there was no dyspnoea, cough or haemodynamic instability. His physical examination revealed bibasilar crackles.

Initial laboratory results showed no significant alterations except for an elevated C-reactive protein at 30.6 mg/L and procalcitonin at 0.14 μg/L. Leucocyte count and differential were within normal limits (Table 1).

A chest computed tomographic scan revealed several well-circumscribed central and peripheral ground-glass opacities associated with vascular ectasia, highly suggestive of a viral bilateral pneumonia, and particularly a COVID-19−related pneumonia, in view of the raging pandemic [3,4]. The index of severity was estimated at 5/25 [5]. There was no pleural or pericardiac effusion. A few lymph nodes less than 1 cm in size were noted in the mediastinum (Fig. 1).

Therapy with hydroxychloroquine and azithromycin was then empirically initiated while awaiting the SARS-CoV-2 RT-PCR results conducted using nasopharyngeal swabs.

The results of the first nasopharyngeal SARS-CoV-2 RT-PCR testing [6] conducted on day 7 of symptoms were negative. The test was repeated 2 days later with the same result.

Given the high index of suspicion of COVID-19 despite the two negative initial nasopharyngeal RT-PCR results, a bronchoscopy was performed the following day (day 10 after symptom onset); RT-PCR testing was done on the bronchoalveolar lavage sample. The latter was positive for SARS-CoV-2, confirming our suspected diagnosis of COVID-19 pneumonia.

Multiplex RT-PCR testing using the BioFire FilmArray technique [7] on the bronchoalveolar lavage sample was negative for other common respiratory viruses (wild coronavirus subtypes HKU1, NL63, 229E and OC43), adenovirus, rhinovirus, enterovirus, influenza A (H1, H3, H1-2009), influenza B, parainfluenza 1/2/3/4, human metapneumovirus and respiratory syncytial virus and bacteria (*Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae*). Culturing the bronchoalveolar lavage sample yielded no microbial growth.

Lopinavir and ritonavir were then added to the patient's treatment regimen following local institutional COVID-19 treatment guidelines.

The patient's disease course progressed clinically well without the need for mechanical ventilation. However, his C-reactive protein increased to 53.8 mg/L during his hospitalization, and ceftriaxone was added to treat a likely bacterial secondary infection. Only one fever spike (temperature 39.5°C) occurred the following day; he remained apyretic for the rest of his hospital stay.

The patient's hospitalization in our COVID-19 unit was uncomplicated, and he was discharged on day 7 of hospitalization with instructions to remain in strict home confinement. The SARS-CoV-2 RT-PCR using nasopharyngeal swab was repeated on days 24 and 29 after symptom onset, following the US Centers for Disease Control and Prevention's guidelines on discontinuation of isolation [8]. Both test results were negative.

TABLE 1. Initial laboratory results at admission

| Test	Unit	Value at admission	Reference value
Haemoglobin	g/dL	15.7	13−17
White blood cells	μL of blood	6100	4000−9000
White blood cell differential	%	62.3% neutrophils, 32.1% lymphocytes	50−78% neutrophils, 20−45% lymphocytes
Platelets	μL of blood	135 000[a]	150 000−400 000
Creatinine	μmol/L	104	58−110
Blood urea nitrogen	mmol/L	10.3[a]	2.5−7.5
C-reactive protein	mg/L	30.6[a]	<3.5
Procalcitonin	μg/L	0.14[a]	<0.1
Ferritin	μg/L	753	30−400
Lactase dehydrogenase	U/L	309[a]	120−246
Lipase	U/L	230	23−300

[a]Abnormal value.

Discussion

Daily medical practice commonly focuses on expertise and clinical judgement to guide diagnosis and treatment. With the coronavirus pandemic hitting hospitals around the globe, physicians must remain alert and maintain a high index of suspicion when it comes to COVID-19 [9].

Studies have found RT-PCR to have an imperfect sensitivity, estimated to be around 63% for nasopharyngeal sampling [10]. The disparity in results could be due to low virus shedding in the upper respiratory tract early on, damaged specimen (as with heat exposure) [11] or inappropriate sample collection. A combination of clinical presentation and radiologic evidence can help dodge underdiagnosis [12]. Chest computed tomographic scan has a higher sensitivity in detecting COVID-19 compared to RT-PCR [13], even in paucisymptomatic patients, but it has low specificity [14], as the imaging pattern could be similar in non−coronavirus-associated viral pneumonia.

Because virologic evidence of SARS-CoV-2 is required to confirm diagnosis of COVID-19, repeat testing using RT-PCR is indicated, preferably via deeper specimen collection, such as sputum or bronchoalveolar lavage [10,15], if initial results are negative in cases of high clinical, epidemiologic or radiologic suspicion.

Conclusion

When a clinician strongly suspects a COVID-19−related infection but faces negative RT-PCR results, it is critical to perform repeat testing for SARS-CoV-2, preferably using deep respiratory specimens, such as bronchoalveolar lavage. While RT-PCR remains the reference standard for diagnosis, a negative result should be interpreted with a grain of salt. Its negative predictive value is based on pretest probability. Combining several diagnostic tests would increase the diagnostic accuracy.
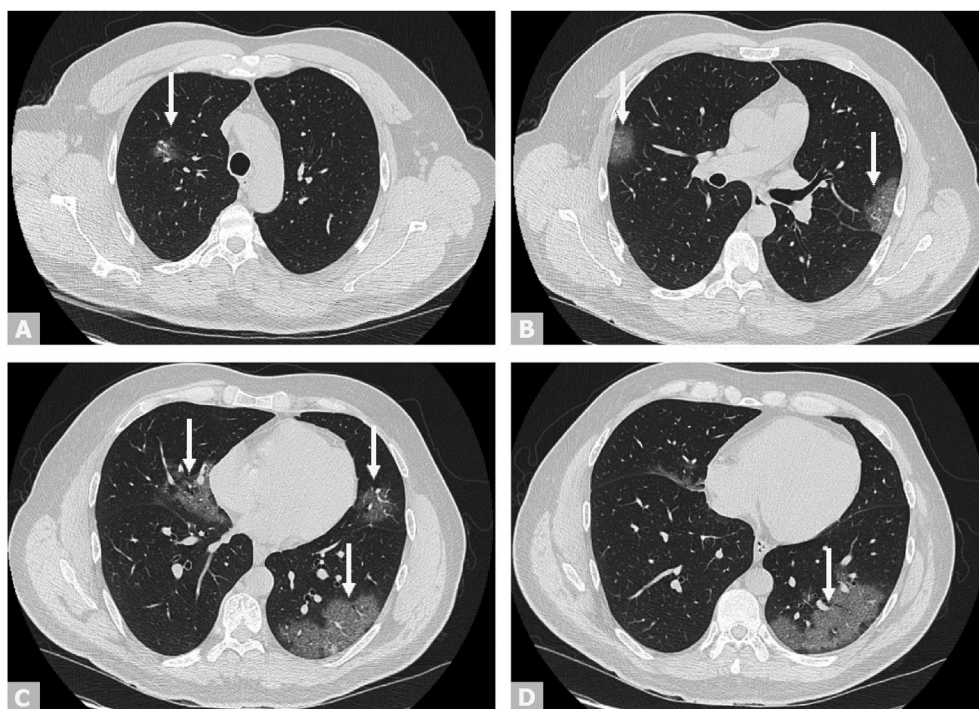
FIG. 1. Axial view of chest CT scan revealing bilateral ground-glass opacities (arrows) indicating COVID-19 pneumonia. COVID-19, coronavirus disease 2019; CT, computed tomography.

When in doubt, testing should be repeated, especially when a high index of suspicion for SARS-CoV-2 persists.

Conflict of interest

None declared.

References

[1] Wang X, Tan L, Wang X, Liu W, Lu Y, Cheng L, et al. Comparison of nasopharyngeal and oropharyngeal swabs for SARS-CoV-2 detection in 353 patients received tests with both specimens simultaneously. Int J Infect Dis 2020;94:107−9.

[2] Zitek T. The appropriate use of testing for COVID-19. West J Emerg Med 2020;21:470−2.

[3] Zhou S, Wang Y, Zhu T, Xia L. CT features of coronavirus disease 2019 (COVID-19) pneumonia in 62 patients in Wuhan, China. AJR Am J Roentgenol 2020;214:1287−94.

[4] Simpson S, Kay FU, Abbara S, Bhalla S, Chung JH, Chung M, et al. Radiological Society of North America expert consensus statement on reporting chest CT findings related to COVID-19. Endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA. J Thorac Imaging 2020;35:219−27.

[5] Zhao W, Zhong Z, Xie X, Yu Q, Liu J. Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) pneumonia: a multicenter study. AJR Am J Roentgenol 2020;214:1072−7.

[6] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020;25(3):2000045.

[7] bioMérieux. BioFire FilmArray product information. Available at: https://www.biomerieux-usa.com/clinical/biofire-film-array.

[8] US Centers for Disease Control and Prevention (CDC). Coronavirus disease 2019 (COVID-19). Available at: https://www.cdc.gov/coronavirus/2019-ncov/hcp/disposition-in-home-patients.html.

[9] Kokkinakis I, Selby K, Favrat B, Genton B, Cornuz J. COVID-19 diagnosis: clinical recommendations and performance of nasopharyngeal swab-PCR. Rev Med Suisse 2020;16:699−701.

[10] Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323:1843−4.

[11] Pan Y, Long L, Zhang D, Yuan T, Cui S, Yang P, et al. Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads. Clin Chem 2020 01;66:794−801.

[12] Wang Y, Hou H, Wang W, Wang W. Combination of CT and RTPCR in the screening or diagnosis of COVID-19. J Glob Health 2020;10:010347.

[13] Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology 2020;296:E32−40.

[14] Waller JV, Kaur P, Tucker A, Lin KK, Diaz MJ, Henry TS, et al. Diagnostic tools for coronavirus disease (COVID-19): comparing CT and RT-PCR viral nucleic acid testing. AJR Am J Roentgenol 2020.

[15] Hornuss D, Laubner K, Monasterio C, Thimme R, Wagner D. COVID-19 associated pneumonia despite repeatedly negative PCR-analysis from oropharyngeal swabs. Dtsch Med Wochenschr 2020;145:844−9.