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REVIEW ARTICLE

Laboratory diagnosis of COVID-19☆

Ekaterini S. Goudouris [a,b,c,d]

[a] Universidade Federal do Rio de Janeiro (UFRJ), Faculdade de Medicina, Departamento de Pediatria, Rio de Janeiro, RJ, Brazil
[b] Universidade Federal do Rio de Janeiro (UFRJ), Instituto de Puericultura e Pediatria Martagão Gesteira (IPPMG), Rio de Janeiro, RJ, Brazil
[c] Sociedade Brasileira de Pediatria, Departamento Científico de Imunologia Clínica, Rio de Janeiro, RJ, Brazil
[d] Associação Brasileira de Alergia e Imunologia, Brazil

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Abstract
Objectives: This was a non-systematic review of the literature on the laboratory diagnosis of COVID-19.
Data sources: Searches in PubMed and Google Scholar for articles made available in 2020, using the terms ''diagnosis'' OR ''diagnostic'' OR ''tests'' AND ''COVID-19'' OR ''SARS-CoV-2'' in the title.
Summary of findings: Tests for the etiological agent identify genetic material of SARS-CoV-2 or humoral responses to it. The gold standard for diagnosis is the identification of viral genome targets by real-time polymerase chain reaction (RT-PCR) in respiratory tract materials during the first week of symptoms. Serological tests should be indicated from the second week of symptoms onwards. A wide range of different tests is available, with variable sensitivity and specificity, most of which require validation. Laboratory tests such as complete blood count, C-reactive protein (CRP), D-dimer, clotting tests, lactic dehydrogenase (LDH), ferritin, and procalcitonin identify risk of disease with greater severity, thromboembolic complications, myocardial damage, and/or worse prognosis. Imaging tests may be useful for diagnosis, especially when there is a compatible clinical picture, and other tests presented negative results or were unavailable.
Conclusions: The identification of genetic material of the virus by RT-PCR is the gold standard test, but its sensitivity is not satisfactory. The diagnosis of COVID-19 should be based on clinical data, epidemiological history, tests for etiological diagnosis, and tests to support the diagnosis of the disease and/or its complications. New diagnostic methods with higher sensitivity and specificity, as well as faster results, are necessary.

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E-mail: egoudouris@gmail.com

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Introduction

Since December 2019, humanity is once again facing a pandemic, this time caused by a betacoronavirus, the SARS-CoV-2. The disease caused by this infection was named coronavirus disease 2019 (COVID-19).[1]

SARS-CoV-2 is a respiratory transmitting virus that causes a flu-like condition and, in some cases, severe acute respiratory syndrome (SARS).[1] However, the follow-up of COVID-19 patients has shown that the virus is capable of causing symptoms outside the respiratory tract, in addition to complications of an inflammatory nature in several organs, expanding the spectrum of associated clinical manifestations.[2] Early and accurate diagnosis of SARS-CoV-2 infection is essential for prevention and pandemic containment. The heterogeneity of the clinical presentation, from asymptomatic individuals to severe cases, and the relevant diversity of non-specific clinical manifestations of COVID-19, reinforce the need for complementary tests with good sensitivity and specificity.[3] The results of diagnostic tests have serious implications: return to work of a health professional, transfer to a COVID-19 area of an inpatient unit, or the reverse, possible contamination of family members, among other delicate situations.

As with any other infection, the gold standard for diagnosis is the identification of the infectious agent. In the case of viral infections, this identification can be made by visualizing viral particles at electron microscopy or identifying intracellular viral inclusions at light microscopy. Tissue cultures are necessary for the study of *in vitro* virus replication. These methods require technology that is usually available only in research centers. In commercial laboratories, immunoenzymatic assays or agglutination tests are available for detection of viral antigens and nucleic acid amplification tests for detection of virus genetic material.[4,5]

An indirect way to diagnose viral infections is the identification of a specific immune system response. The humoral response, or antibody production, is the simplest way to diagnose infectious conditions. There are different techniques for identifying antibodies that are directed against different parts of viruses.[4,5] However, it is important to note that the immune response to viral microorganisms occurs primarily by innate immunity, particularly by NK cells, and cellular immunity, especially cytotoxic T cells (TCD8+).[6]

To date, PubMed features over 35,000 articles on COVID-19. Many of them are presented as preprint, without peer review; some of these studies were conducted with poor methodology, providing unreliable results. Moreover, during the pandemic, knowledge has advanced greatly, and initially established concepts were modified, demonstrating that certain specificities of SARS-CoV-2 infection are not comparable with previously known viral infections.

Objectives

This was a non-systematic review of the literature on the laboratory diagnosis of COVID-19, drawing attention to the knowledge already established, as well as the doubts that still need to be clarified.

Methods

A non-systematic review of the literature was carried out in PubMed, searching for articles submitted in 2020, with the terms ''diagnosisÖR ''diagnostic'' OR ''testsÖR ''diagnostic testsÄND ''COVID-19ÖR ''SARS-CoV-2ïn the title. Since many manuscripts have been made available in preprint version, without peer review, Google Scholar searches have also been performed, using the same terms.

This study included articles in English, Portuguese, French, or Spanish, using the checklists proposed by the User's Guide to Medical Literature (JAMA Evidence) as inclusion criteria.[7]

Results

The complementary tests used in the diagnosis of COVID-19 can be divided into tests for etiological diagnosis and support tests, which help in the diagnosis or indicate the risk or presence of complications.

Tests for etiological diagnosis

Tests for etiological diagnosis may be direct, identifying genetic material of SARS-CoV-2, or indirect, determining the humoral immune response to SARS-CoV-2.

The most commonly used method for identifying genetic material from SARS-CoV-2 is real-time polymerase chain reaction (RT-PCR). This method involves reverse transcription of the genetic material of the virus (RNA) to complementary DNA (cDNA), followed by amplification of some regions of the cDNA. Probes (DNA/RNA marked sequences to identify the genetic target in the material) and primers (DNA/RNA sequences that promote replication of the genetic material found in the sample) were created after the SARS-CoV-2 genome was sequenced. Several serial amplification cycles are performed to identify these targets: the more cycles are needed, the lower the viral load of the material under study.[8]

Four regions of the SARS-CoV-2 genome have been targeted: RdRp gene (RNA-dependent RNA polymerase), genes from structural proteins E (virus envelope) and N (virus nucleocapsid), and ORF1ab gene (open reading frame 1a and 1b).[3,8] Kits using different regions of the genome are commercially available. The sequential use of different probes and primers for the RdRp, E and N genes, known as the Charité-Berlin Institute protocol, presents good sensitivity and specificity.[9] There are other proposed protocols that follow the same logic of sequential use of probes and primes for different genetic targets.[10]

Regardless of the method used, the sensitivity and specificity of the different RT-PCR kits are not 100%. This is considered the gold standard for diagnosis of SARS-CoV-2 infection, but its sensitivity is estimated to be approximately 70% and specificity, 95%.[11,12] Many factors can interfere with the results, whether related to the virus, to the method itself (the collection procedure and handling of the material), or even to the viral load of the sample (type of material collected, duration of symptoms, and disease severity).[13]

Mutations in the virus genome can render the probes and primers obsolete, producing false negative results. To date, SARS-CoV-2 has undergone mutations, but without implications for the RT-PCR detection. Mismatch between primers and probes can also lead to false negative results, and ideally more than one region of the virus genome should be simultaneously or sequentially amplified.[13]

Factors related to the collection procedure and handling of the material are often responsible for false negative results. Dacron or polyester swabs should be used and immersed immediately after collection in appropriate and refrigerated storage medium. The material should be kept under refrigeration and quickly sent to the laboratory.[10,13]

A low viral load, usually found in asymptomatic individuals or in those with mild clinical conditions, may also be responsible for a false negative result.[14,15] Individuals with more severe clinical conditions have greater elimination of viruses.[16] Although it has been described that there may be elimination of viruses from two to three days before to up to six weeks after the onset of symptoms,[8] very early (before three days of symptoms) or late material collection (after the seventh day) may produce false negative results, due to lower viral load.[1,13,15] The type of material and the collection technique also interfere with the result. In several studies, bronchoalveolar lavage was the material with the highest positivity, followed by sputum, nasopharyngeal swabs, and nasal swabs. Oropharyngeal swabs did not present good positivity.[15,17] The identification of genetic material of the virus in feces is less common and has uncertain significance, since infecting virus was not detected in this material.[18] Viral particles were not isolated in urine or blood.[18] Saliva tests have also been implemented, but have lower sensitivity than the nasopharyngeal swab and require validation.[19]

False positive results are most commonly related to errors in sample handling during or after swab collection, leading to inadvertent contamination.[13]

Tests to identify genetic material of the virus using simpler techniques, which do not require personal and sophisticated devices and that produce faster results, have been developed.[3] One example is the qualitative detection of the E and N proteins genes through the GeneXpert (Cepheid Company) platform, in which the amplification process takes place within a cartridge and provides results in 45 min.[20]

Point-of-care tests for SARS-CoV-2 proteins, most commonly using lateral flow assays, are useful for diagnosis in regions where there are no specialized laboratories.[3,21]

The presence of genetic material in respiratory tract secretions has no direct relationship with virus viability or infectivity, since inactive or dead virus particles can be identified.[8] Therefore, a patient with positive RT-PCR test is not always able to infect other people. The viability of SARS-CoV-2 and consequent infectivity can be assessed directly, *in vitro*, by its ability to contaminate cells and, indirectly, through the threshold cycles (the lower the Ct, the higher the viral load) or identification of sub-genomic RNA (which are transcribed only by viable viruses).[18]

Serological tests identify the presence of humoral response to SARS-CoV-2. Antibodies of IgA, IgM, and IgG isotypes specific to different virus proteins are detected by enzyme-linked immunosorbent assay (ELISA) or chemiluminescence immunoassays (CLIA), and the latter has been shown to be more sensitive.[21] It is known that the priority immune response to the virus is related to the cytotoxic activity of NK cells and CD8 + T lymphocytes. There is evidence of robust cellular response to SARS-CoV-2, regardless of the results of serological tests;[22] however, tests to evaluate the specific cellular immune response for SARS-CoV-2 are not yet commercially available.

Antibodies against S protein, where the receptor-binding domain (RBD) is located, are very specific for SARS-CoV-2;[10] their levels presented a good correlation with the virus's neutralization capacity.[23] However, the role of antibodies directed to other proteins in the pathogenesis of COVID-19, even promoting a greater penetration of the virus into cells, still need to be elucidated.[24]

Sensitivity and specificity of serological tests vary according to the testing technique, specificity of the antibody studied, duration of symptoms at the time of collection, and immunocompetence of the individual.[4] However, actual sensitivity and specificity values for these tests are difficult to define considering that a gold standard for diagnosis with high sensitivity is not yet available.[11] Most of the tests in use were not evaluated in scientific publications.[21]

The assessment of specific antibodies to N protein is more sensitive and less specific, since this protein is more abundant in coronaviruses. Antibodies directed to S protein are more specific to SARS-CoV-2, because in this protein is RDB.[8]

In addition, other factors that interfere with the results are duration of symptoms when the blood is collected and severity of the clinical picture. IgM is identified from the fifth day of symptomatology, and more significantly, from the eighth day onwards. The specific IgA dosage appears to be more sensitive and the values seem to increase earlier than those of IgM.[21] Specific IgG values begin to be detectable from the tenth day of symptom onset, and more significantly, from the 14th day onwards.[21] These tests are therefore not appropriate for the early diagnosis of COVID-19. They are, however, relevant when RT-PCR is not available or is negative in the face of a suggestive clinical picture, when the patient has been symptomatic for over 14 days,[8,21] or to assist in the diagnosis of COVID-19-related multisystemic inflammatory syndrome.[25]

Some studies report patients with mild (or even asymptomatic) COVID-19 present lower levels of SARS-CoV-2-specific antibodies or may even do not develop detectable levels, while patients with more severe conditions have higher levels of these.[26–28] These data raise questions about the protective capacity of antibodies and may suggest the participation of specific antibodies in the pathogenesis of COVID-19.[14,24]

One study demonstrated that the positivity of serological tests was not accompanied by a rapid drop in virus elimination, which may indicate that the positivity of these tests does not necessarily imply prompt resolution of the disease or absence of infectivity.[18]

It has recently been shown that specific IgG levels suffer significant decline after two/three months.[27] Considering that the immune response to the virus is primarily cellular, it is not yet known what are the implications of this reduction in the protection against the virus.

Regardless of the test used for diagnosis, either identification of genetic material of the virus or serologic test,

the interpretation of the results is based on the accuracy of the test itself, and also on the estimated risk of the disease before the results. This risk is modified by the prevalence of COVID-19 in a given region.[11] This means that tests developed in regions where the prevalence of SARS-CoV-2 infection is high tend to have lower sensitivity when used in regions where the prevalence is lower.

A single negative test in an individual with a characteristic clinical picture should not discard the possibility of COVID-19.[11] In turn, a positive RT-PCR has greater strength to confirm the diagnosis than a negative test has to discard it, since it presents high specificity, with only moderate sensitivity.[11]

Point-of-care tests for antibodies against SARS-CoV-2 using lateral flow assays (usually immunochromatography) are quite numerous and many of them have not been adequately validated.[20] Moreover, they were tested in the laboratory using plasma or serum, but have been applied with whole blood, which can greatly modify their sensitivity.[21] They are not recommended to be used for the individual diagnosis of COVID-19, but may be useful in implementing public policies.[29]

Support tests

These are laboratory or imaging tests that demonstrate characteristic manifestations of COVID-19, its complications, and/or risk factors for complications.

Laboratory tests

Complete blood count – lymphopenia, eosinopenia, and neutrophil/lymphocyte ratio $\geq$ 3.13 are related to greater severity and worse prognosis. Thrombocytopenia is related to a higher risk of myocardial damage and a worse prognosis.[2] Lymphopenia results from a multifactorial mechanism that includes the cytopathic effect of the virus, induction of apoptosis, IL1-mediated pyroptosis, and bone marrow suppression by inflammatory cytokines.[30]

High values of C-reactive protein (CRP), ferritin, D-dimer, procalcitonin, lactic dehydrogenesis (DHL), prothrombin time, activated partial thromboplastin time, amyloid serum protein A, creatine kinase (CK), glutamic-pyruvic transaminase (SGPT), urea, and creatinine are risk factors for more severe disease, thromboembolic complications, myocardial damage, and/or worse prognosis.[2,30–32]

Immunological markers that may also represent risk factors for greater severity and/or worse prognosis are: decreased values of CD4 + T and CD8+ lymphocytes, and NK cells and increased values of IL6, IL-8, IL-10, IFN-γ, TNF-IL-2R, TNF-α, GM-CSF, and IL-1 β.[2,32]

Imaging tests

Imaging tests for the diagnosis of COVID-19 have gained relevance, given the unavailability of tests for etiological diagnosis.[3] The alterations described in these tests can also be found in influenza or mycoplasma infections, in inflammatory processes of different origins, or in eosinophilic lung diseases.[33] Although the findings in these tests are not specific to COVID-19, given a compatible clinical picture and/or the presence of confirmed or possible history of contact, they may help in the diagnosis.

Plain chest X-rays are less sensitive than computed tomography, but may evidence sparse bilateral consolidations accompanied by ground glass opacities, peripheral/subpleural images, predominantly in the lower lobes.[33]

Computed tomography of the chest presents greater sensitivity and reveals multifocal, bilateral, peripheral/subpleural ground glass opacities, generally affecting the posterior portions of the lower lobes, with or without associated consolidations.[33,34] Children have a similar presentation to that found in adults, albeit with a milder involvement.[33] The halo sign, described as a consolidation area involved by ground glass opacities, was identified in 50% of the children.[33] An inverted halo sign, in which areas of ground glass opacities are surrounded by condensation halo, has also been described.[35]

Pulmonary ultrasonography has good sensitivity; the typical findings are B-lines, consolidations and pleural thickening.[36] The advantages of this method are its lower cost, absence of radiation exposure, and the fact that it does not require sedation or transportation of unstable patients.[37]

Most studies on diagnostic methods presented here refer to adults; however, studies specific to the pediatric age group show very similar data.[38]

The data presented suggest that the diagnosis of COVID-19 should be based on clinical manifestations, contact history, imaging tests, laboratory tests, and not only on serological tests and the search for the genetic material of the virus. In addition, strategies to increase sensitivity, specificity, and speed of diagnosis are fundamental.[11]

Conclusions

The gold standard for the diagnosis of SARS-CoV-2 infection is the identification of viral genetic material by RT-PCR, in different samples, with greater sensitivity in bronchoalveolar lavage and nasopharyngeal swab. Many factors related to the individual, the collection procedure, and the test technique interfere with the sensitivity of these tests. Therefore, a negative test in a patient with a characteristic clinical picture should not discard the possibility of COVID-19.

The available serological tests are different from each other and many factors influence their sensitivity and specificity. Not all patients who have SARS-CoV-2 infection will have detectable levels of antibodies, particularly if they have milder symptoms. The absence of antibodies does not imply the absence of contact or protection against the virus, since there may be an efficient specific cellular immune response. In turn, the presence of antibodies does not rule out the possibility that the individual is still infectious, as no immediate reduction in the elimination of the virus has been identified.

The support laboratory and imaging tests show alterations that are characteristic of COVID-19, but they lack specificity.

The diagnosis of COVID-19 should be based on clinical and epidemiological history, tests for etiological diagnosis, and tests to support the diagnosis of infection and/or its complications.

New diagnostic methods with higher sensitivity and specificity, as well as faster results, are necessary and are being developed.

Conflicts of interest

The author declares no conflicts of interest.

References

1. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA. 2020. Epub ahead of print 2020/07/11.
2. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: current state of the science. Immunity. 2020;52:910–41.
3. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. Diagnosing COVID-19: the disease and tools for detection. Nano ACS. 2020;14:3822–35.
4. Murray PR. The clinician and the microbiology laboratory. In: Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas and Bennett's principles and practice of infectious diseases. Philadelphia, USA: Elsevier Inc.; 2015. p. 191–223.
5. Pretorius M, Venter M. Diagnosis of viral infections. In: Viral infections in children, Volume I; 2017. p. 151–82.
6. Rouse BT, Muller SN. Host defense to viruses. In: Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM, editors. Clinical immunology: principles and practice. 5th ed. Elsevier Limited; 2019. p. 365-74e.
7. Guyatt G, Rennie D, Meade MO, Cook DJ. User's guide to the medical literature – essentials of evidence-based clinical practice. 3rd ed McGrawHill Education; 2014.
8. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA. 2020;323:2249–51.
9. 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;25:1–8.
10. Oliveira BA, Oliveira LC, Sabino EC, Okay TS. SARS-CoV-2 and the COVID-19 disease: a mini review on diagnostic methods. Rev Inst Med Trop São Paulo. 2020;62:e44.
11. Watson J, Whiting PF, Brush JE. Interpreting a COVID-19 test result. BMJ. 2020;369:m1808.
12. Padhye NS. Reconstructed diagnostic sensitivity and specificity of the RT-PCR test for COVID-19. medRxiv. 2020, preprint. doi: 10.1101/2020.04.24.20078949.
13. Tahamtan A, Ardebili A. Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert Rev Mol Diagn. 2020;20:453–4.
14. Matricardi PM, Dal Negro RW, Nisini R. The first, holistic immunological model of COVID-19: Implications for prevention, diagnosis, and public health measures. Pediatr Allergy Immunol. 2020;31:454–70.
15. Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. medRxiv. 2020, preprint. doi: 0.1101/2020.02.11.20021493.
16. Liu Yang, Yan Li-Meng, Wan Lagen, Xiang Tian-Xin, Le Aiping, Liu Jia-Ming, et al. Viral dynamics in mild and severe cases of COVID-19. The Lancet Infectious Diseases. 2020;20:656–7.
17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323:1843–4.
18. Wolfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Muller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581:465–9.
19. Williams E, Bond K, Zhang B, Putland M, Williamson DA. Saliva as a noninvasive specimen for detection of SARS-CoV-2. J Clin Microbiol. 2020;58:e0076–20.
20. Vieira LM, Emery E, Andriolo A. COVID-19: laboratory diagnosis for clinicians. An updating article. Sao Paulo Med J. 2020;138:259–66.
21. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev. 2020;6:CD013652.
22. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin J-B, Olsson A, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. bioRxiv. 2020, preprint. doi: 10.1101/2020.06.29.174888.
23. Alvim RG, Lima TM, Rodrigues DA, Marsili FF, Bozza VB, Higa LM, et al. An affordable anti-SARS-CoV-2 spike elisa test for early detection of IgG seroconversion suited for large-scale surveillance studies in low-income countries. medRxiv. 2020. Preprint. doi: 10.1101/2020.07.13.20152884.
24. Zohar T, Alter G. Dissecting antibody-mediated protection against SARS-CoV-2. Nature reviews Immunology. 2020;20:392–4.
25. Riollano-Cruz M, Akkoyun E, Briceno-Brito E, Kowalsky S, Posada R, Sordillo EM, et al. Multisystem inflammatory syndrome in children (MIS-C) related to COVID-19: a New York city experience. J Med Virol. 2020 [Epub ahead of print]. doi: 10.1002/jmv.26224.
26. Kellam P, Barclay W. The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. J Gen Virol. 2020 [Epub ahead of print]. doi: 10.1099/jgv.0.001439.
27. Seow J, Graham C, Merrick B, Acors S, Steel KJA, Hemmings O, et al. Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection. medRxiv. 2020, preprint. doi: 10.1101/2020.07.09.20148429.
28. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020;26:1200–4.
29. Ricco M, Ferraro P, Gualerzi G, Ranzieri S, Henry BM, Said YB, et al. Point-of-care diagnostic tests for detecting SARS-CoV-2 antibodies: a systematic review and meta-analysis of real-world data. J Clin Med. 2020;9:1515–30.
30. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Bruggen MC, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy. 2020;75:1564–81.
31. Kermali M., Khalsa R.K., Pillai K., Ismail Z., Harky A. The role of biomarkers in diagnosis of COVID-19 - a systematic review. 2020;254:117788.
32. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Invest Clin. 2020;130:2620–9.
33. Foust Am, Phillips Gs, Chu Wc, Daltro P, Das Km, Garcia-Peña P, et al. International expert consensus statement on chest imaging in pediatric COVID-19 patient management: imaging findings, imaging study reporting and imaging study recommendations. Radiology: Cardiothoracic Imaging. 2020;2:e200214.
34. Caruso D, Zerunian M, Polici M, Pucciarelli F, Polidori T, Rucci C, et al. Chest CT features of COVID-19 in Rome. Italy. Radiology. 2020;296:E79–85.
35. Farias LPG, Strabelli DG, Sawamura MVY. COVID-19 pneumonia and the reversed halo sign. J Bras Pneumol. 2020;46:e20200131.
36. Sofia S, Boccatonda A, Montanari M, Spampinato M, D'Ardes D, Cocco G, et al. Thoracic ultrasound and SARS-COVID-19: a pictorial essay. Ultrasound J. 2020;23:217–21.

37. Zhang Y, Xue H, Wang M, He N, Lv Z, Cui L. Lung ultrasound findings in patients with coronavirus disease (COVID-19). AJR Am J Roentgenol. 2020:1–5.

38. Hoang A, Chorath K, Moreira A, Evans M, Burmeister-Morton F, Burmeister F, et al. COVID-19 in 7780 pediatric patients: a systematic review. EClinicalMedicine. 2020;24:100433.