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Review article

Diagnosis of COVID-19 in symptomatic patients: An updated review



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ABSTRACT

A group of pneumonia patients was detected in Hubei Province, in China in December 2019. The etiology of the disease was unknown. Later, the researchers diagnosed the novel Coronavirus as the causal agent of this respiratory disease. On February 12th 2020, the World Health Organization (WHO) officially named this disease Coronavirus disease 2019 (COVID-19). Consequently, the disease spread globally and became a pandemic. As there is no specific treatment for the symptomatic patients and several vaccines are approved by WHO, the efficacy and effectiveness of these vaccines are not fully understood yet and the availability of these vaccines are very limited. In addition, new variants and mutants of SARS-CoV-2 are thought to be able to evade the immune system of the host. So, diagnosis and isolation of infected individuals is advised. Currently, real-time reverse transcription-polymerase chain reaction (RT-PCR) is considered the gold standard method to detect novel Coronavirus, however, there are few limitations associated with RT-PCR such as false-negative results. This demanded another diagnostic tool to detect and isolate COVID-19 early and accurately. Chest computed tomography (CT) became another option to diagnose COVID-19 patients accurately (about 98% sensitivity). However, it did not apply to the asymptomatic carriers and sometimes the results were misinterpreted as from other groups of Coronavirus infection. The combination of RT-PCR and chest CT might be the best option in detecting novel Coronavirus infection early and accurately thereby allowing adaptation of measures for the prevention and control of the COVID-19.

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Diagnóstico de COVID-19 en pacientes sintomáticos: una revisión actualizada

R E S U M E N

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COVID-19
SARS-CoV-2
TC de tórax
RT-PCR
Test rápido de antígenos

En diciembre de 2019 se detectó un grupo de pacientes con neumonía en la provincia de Hubei, China, desconociéndose la etiología de la enfermedad. Posteriormente, los investigadores señalaron al nuevo coronavirus como agente causal de esta enfermedad respiratoria. El 12 de febrero de 2020, la Organización Mundial de la Salud (OMS) la designó oficialmente como enfermedad por coronavirus de 2019 (COVID-19). A continuación, dicha enfermedad se propagó a nivel global, y se convirtió en una pandemia. No existe tratamiento específico para los pacientes sintomáticos, y la OMS ha aprobado diversas vacunas. Sin embargo, la eficacia y la efectividad de las mismas no se comprende plenamente aún, siendo muy limitada su disponibilidad. Además, se piensa que las diferentes variantes y mutaciones del SARS-CoV-2 son capaces de evadir el sistema inmune del huésped. Por tanto, se recomienda el diagnóstico y aislamiento de las personas infectadas. Actualmente se considera la reacción en cadena de la polimerasa con transcriptasa inversa (RT-PCR) a tiempo real el método de referencia para detectar el nuevo coronavirus. Sin embargo, existen algunas limitaciones asociadas a RT-PCR tales como los resultados falso-negativos. En consecuencia, ello ha demandado otra herramienta diagnóstica para detectar y aislar la COVID-19 de manera temprana y precisa. La tomografía computarizada (TC) de tórax se ha convertido en otra opción para diagnosticar de manera precisa a los pacientes con COVID-19 (cerca del 98% de sensibilidad). Sin embargo no se aplica a los portadores asintomáticos, y a veces se han malinterpretado los resultados como en el caso de otros grupos de infección por coronavirus. La combinación de RT-PCR y TC de tórax podría ser la mejor opción para detectar la nueva infección por coronavirus de manera temprana y precisa, permitiendo, por tanto, la adaptación de las medidas para la prevención y el control de la COVID-19.

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Introduction

In December 2019, a group of pneumonia patients was identified with an unknown etiology in Wuhan, Hubei Province, China.¹ Based on the sequence analysis, researchers identified the etiology of this unknown pneumonia as novel Coronavirus and named it 2019-nCoV.² It was named novel Coronavirus because of its unique genetic structure compared to other Coronavirus strains already existing in the world both in animals and humans which causes a range of diseases including respiratory, gastrointestinal, and neurological diseases.³ Among the Coronavirus strains that have been identified so far, six are known to cause human diseases, four strains 229E, OC43, NL63, and HKU1 typically produce the common cold symptoms and characteristically these are not fatal.^{4,5} The remaining two strains are severe acute respiratory syndrome Coronavirus (SARS-CoV) or SARS-CoV-1 and Middle East respiratory syndrome Coronavirus (MERS-CoV). These strains are zoonotic and fatal to severely infected human patients.⁵ Among the two zoonotic strains, the former was the causative agent of the SARS outbreaks in 2002–2003 in Guangdong Province, China⁶ and the latter was the causal agent of the MERS outbreak in the Middle East in 2012.⁷ To identify and distinctly differentiate among the diseases instigated by the Coronavirus strains currently prevailing, WHO declared a standard name for the 2019-nCoV as Coronavirus Disease-2019 (COVID-19) on 11 February 2020. On the other

hand, the International Committee on Taxonomy of Viruses (ICTV) termed this novel Coronavirus as SARS-CoV-2.⁸ The SARS-CoV-2 (COVID-19) is still spreading throughout the world since its first outbreak in China, affecting more than 200 countries and territories. A lot of infected patients are dying due to the lack of effective antiviral treatment facilities. So far, the epidemic of SARS-CoV-2 (COVID-19) has gained global attention and was declared a pandemic by WHO on 11 March 2020.⁹ Unfortunately, there is no fully approved treatment or vaccine for COVID 19 treatment/prevention. Although, some vaccines have been developed by companies such as Pfizer/BioNTech, Moderna, AstraZeneca/Oxford and have obtained emergency use authorizations, their availability is limited. Besides, frequent mutations of the virus and the recent outbreak of the new variant of SARS-CoV 2 in Britain, Italy, and few other countries, have been a cause of concern. Until mass immunizations are achieved, treatments are fully approved and the efficacy of vaccines on the new variants is understood, standard public health strategies such as diagnostics of suspected symptomatic patients followed by isolation of confirmed cases is crucial to control the spread and transmission of COVID-19.¹⁰

Vaccines developed by Pfizer, BioNTech, Moderna vaccine, and Oxford university COVID-19 vaccine are ahead of others and they have already passed Phase -III trial.^{11–16} But, frequent mutation of the virus^{17–20} and the recent outbreak of the new variant of SARS-CoV-2 in Britain,²¹ Italy, and a few other countries, is spreading quickly than previous variants.²¹

The situation became critical for scientists and physicians to control, prevent, and effectively treat the virus. Therefore, accurate diagnosis, isolation, and treatment of suspected patients are requisite to prevent or reduce the transmission of the virus to new hosts until the vaccines are proven to be efficient and preventative. Real-time reverse transcription polymerase-chain-reaction (RT-PCR) is currently being considered the gold standard method globally for accurate diagnosis of COVID-19 either in symptomatic patients or asymptomatic carriers. In addition to this, some scientists suggest chest computed tomography (Chest-CT) as an alternative tool for the diagnosis of COVID-19 effectively and accurately in symptomatic patients.²² This review focuses on various aspects of these two diagnostic methods including their suitability to detect COVID-19.

RT-PCR

RT-PCR is a widely utilized technique in diagnostic virology. During a public health emergency, a competent virology laboratory can rely on this molecular technique to ascertain new diagnostic tests before new reliable assays are offered. RT-PCR is the diagnostic technique used worldwide to detect SARS-CoV-2 and this test is approved by WHO and the Center for disease control and prevention (CDC) in the diagnosis of COVID-19.²³ Coronaviruses express several molecular targets that can be identified by RT-PCR. These are genes encoding for structural proteins e.g., glycoproteins spike (S), envelop (E), transmembrane (M), helicase (Hel), nucleocapsid (N), and accessory genes such as RNA-dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE), open reading frame 1a (ORF1a) and open reading frame 1b (ORF1b), etc.^{4,23} CDC recommended targeting two nucleocapsid protein genes (N1 and N2) while Corman and colleagues (2020) recommended the screening of the E gene first followed by a confirmatory RT-PCR assay targeting the RdRp gene.²³ Chan et al. (2020) reported that RT-PCR targeting the RdRp/Hel genes have higher sensitivity and specificity compared to S and N genes of SARS-CoV-2.²⁴ To avoid false-negative results and cross-reactivity with other endemic Coronaviruses at least two molecular targets should be screened during RT-PCR of SARS-CoV-2. This could potentially increase the probability of the RT-PCR technique to detect genetic variants of SARS-CoV-2 itself.²⁵ In this molecular test, samples are usually taken via the nasopharyngeal (the part of the throat behind the nose) together with the saliva. Based on the previously reviewed articles the sputum test is more reliable, but the sputum is not available if the patient does not have a productive cough. Therefore, the saliva-based SARS-CoV-2 shows promising results. It also allows self-sample collection²⁶ and is consistent with the method in which Vaz and colleagues demonstrated the accuracy, reliability, and non-invasiveness of the saliva test.²⁷

Sensitivity of RT-PCR

Although RT-PCR is considered the standard test to diagnose COVID-19 worldwide, two different groups of scientists from China reported 3% and 30% false-negative results after the RT-

PCR test.^{28,29} One research group from China found a RT-PCR positive result

the third time after a patient's results showed false-negative results in two consecutive tests.³⁰ Another research group from Thailand reported false-negative RT-PCR results in a COVID-19 case up to day 7 of infection. Three consecutive tests were done on days 1, 5, and 7, however, the patient finally tested positive for COVID-19 on the 8th day of infection (4th RT-PCR) assay.³¹ Kucirka et al., (2020) revealed the variations of the false-negative results in SARS-CoV-2 based RT-PCR tests considering the timing of the tests from the day of exposure. Their findings showed that 67% of the false-negative results were recorded four days after exposure, 38% on the day of illness onset, 20% three days after illness onset, and 21% four days after illness onset.³² Two clinical transmissions of SARS-CoV-2 from patients with negative RT-PCR swab test to others were recorded in China and the authors suggested another alternative test such as serological analysis to detect the COVID-19 accurately.³³

However, the main reasons for false-negative results in RT-PCR might include the use of low-sensitivity primers, testing kits, and RT-PCR technology. Sample source, sample collection by unskilled personnel, and sampling mistiming (low virus load in patients during the sampling time) also contribute to false-negative results.²⁸

Chest CT (Computed Tomography)

Chest CT is the imaging technology based on X-ray and computer technology to visualize the organs and structures in the chest. This is more reliable than a regular X-ray to identify the abnormalities in the chest organs. During the CT scan, an X-ray beam transfers through the patient's chest, and takes many high-resolution medical images of the lungs and other organs by radiation detectors, and displays them on a monitor.³⁴ Chest CT has been using in clinical practices to diagnose diseases and disorders since 1972.³⁵ This method is quite rapid than molecular tests and it is a vital diagnostic tool to detect viral pneumonia in COVID-19 suspected patients.³⁶ Chest CT enables the detection of viral pneumonia in the lungs of COVID-19 patients by producing sharp images of the lungs. This information is used by radiologists to diagnose and determine the stage of infection. The National Health Commission of the People's Republic of China encouraged COVID-19 diagnosis based on clinical and chest CT findings³⁷ because of the limited number of RT-PCR kits in some diagnostic centers as well as the possibility of false-negative RT-PCR.

A radiology research group from China³⁸ stated that the gradual changes observed in the lungs by chest CT among COVID-19 patients include, unilateral or bilateral subpleural ground-glass opacity in the lower lobes of the lungs in the early stage (0–4 days) of COVID-19 infection. During 5 to 8 days of the course of the infection, bilateral multilobe distribution with diffuse ground-glass opacity, crazy-paving pattern, and consolidation can be observed. During the peak infection time of SARS-CoV-2 (9–13 days of onset of symptom) diffuse ground-glass opacity, crazy-paving pattern, consolidation, and residual parenchymal bands appear. After 14 days of the initial onset of symptoms, the consolidation

can be gradually absorbed, and ground-glass opacity can be observed. As a result of recovery, the crazy paving disappears in this stage. The absorption stage may last up to 26 days of infection.³⁸ A research group in Italy reported that the typical chest CT pattern of COVID-19 infection had ground-glass opacities with multilobe and posterior involvement, bilateral distribution, and subsegmental vessel enlargement.³⁹ Chest CT can be an effective tool to diagnose and manage COVID-19 patients besides RT-PCR. It is useful in recognizing potential abnormalities in the respiratory system of patients with negative RT-PCR results. This makes it advantageous over RT-PCR in measuring disease severity and monitors treatment outcomes. This diagnostic tool can help minimize fatality among severely infected patients through early detection of major complications.^{40–42}

The sensitivity of Chest CT

CT images of SARS-CoV-2 infected patients had similarities with SARS-CoV-1. Although a few differences were noticed, these two pathogens of the Coronavirus group revealed mainly ground-glass opacities, with consolidation that was seen sporadically.⁴³ Moreover, the CT findings of COVID-19 and adenovirus infection have similarities with the SARS-CoV-1 infection.⁴⁴

One research group from China reported that chest CT may show false-negative results for COVID-19 patients at the initial stages of infection,⁴⁵ while another group reported that about 3% of COVID-19 patients had negative RT-PCR findings during the early stages of infection despite chest CT findings being typical of viral pneumonia.²⁹ Furthermore, Zhou and colleagues reported that COVID-19 could be detected in the early stages of infection by chest CT even when RT-PCR results are negative and chest CT is more applicable in early recognition and rapid detection of COVID-19 in patients than other diagnostic tests.⁴⁶ Based on a molecular study among 51 COVID-19 patients, Fang et al.,²⁸ reported that the sensitivity of the chest CT was superior to RT-PCR (98% vs 71%, respectively) and 15 out of 51 patients showed positive chest CT and negative RT-PCR at the initial stage while all of them showed positive RT-PCR results between 2–7 days. However, the small sample size was the limitation of this study. Another research group reported that the sensitivity of chest CT is low in asymptomatic patients (54%) compared to patients (79%) with viral pneumonia.⁴⁷ Consequently, Xie and colleagues tested 167 COVID-19 patients by both CT imaging and RT-PCR and reported that 155 patients (93%) showed positive results in both diagnostic methods, while 5 patients showed positive CT imaging and negative RT-PCR results at the early stage of infection and became RT-PCR positive between 2 to 8 days later. The remaining seven patients showed positive RT-PCR and negative CT imaging at the initial stages of infection.

These researches showed that chest CT had a high accuracy rate in the diagnosis of COVID-19 (96.1%) and it might be a useful method for the quick and early diagnosis of COVID-19 besides RT-PCR.³⁶ On the contrary, chest CT is still limited in detecting specific viral infections and distinguishing among different viruses of the same group.⁴⁴ Furthermore, Caruso and colleagues (2020) reported that chest CT had a

low specificity rate of (56%) and high sensitivity (97%) as a diagnostic tool among Italian COVID-19 patients.³⁹ Also, another research group from China stated that chest CT had high specificity (96%) when done by well-trained radiologists in clinical settings.⁴⁸ Moreover, Bernheim and colleagues reported that chest CT could not detect COVID-19 in suspected patients after the onset of symptoms (0–2 days). It is not a reliable method when used independently to test/detect SARS-CoV-2 infection in clinical settings.³⁷

SARS CoV-2 cannot be detected appropriately by chest CT alone in asymptomatic patients (Table 1). Besides, the results of chest CT might be misinterpreted as SARS CoV-1 or other respiratory viral infection and its specificity rate is not satisfactory compared to RT-PCR (Table 1). Furthermore, the false-negative results in chest CT can be minimized by well-trained radiologists.³⁶ Skilled radiologists can minimize the harmful health effects of X-ray radiation by reducing the dose of the radiation without affecting the image quality.⁵² Ultimately, chest CT is useful as a diagnostic tool that may compensate for initial false-negative RT-PCR results in COVID-19 suspected patients.

Rapid antigen test

RT-PCR-based molecular diagnosis of Covid-19 has been done globally since the genetic sequence of SARS-CoV-2 was made available. The molecular test requires robust laboratory facilities and skilled laboratory personnel. The results of RT-PCR should be available within 2–4 h of sample collection but some countries experience delays of up to seven days.⁵³ This delay may facilitate the transmission of the virus to a wide range of new hosts because the suspected patients will resume their daily activities until they receive the test results. This leads to an increase in the number of new patients and the pandemic becomes uncontrollable. An alternative tool/assay that can alleviate the limitations of RT-PCR is the Rapid antigen test. It can detect the SARS-CoV-2 virus within 15–20 min. It is cost-effective, easily applicable outside the laboratory facilities, no need for skilled personnel, and applies to many patients. Although the rapid antigen test kits are highly specific, their sensitivity is not as high as other molecular tests.⁵⁴ WHO explained that although the sensitivity of the antigen test is lower than the molecular test, it is suitable due to its rapid performance, low cost, and applies to symptomatic patients with high viral load and having increased chances of transmitting the pathogen to others.⁵⁵ As of November 2020, six antigen test kit have received emergency use authorization from the US Food and Drug Administration (FDA) and two antigen test kits have received emergency use authorization from WHO respectively.^{56,57} Additionally, WHO recommended that the Rapid antigen test kit should have the minimum 80% sensitivity and 97% specificity.⁵⁵ Another advantage of using antigen test is its ability to detect large numbers of asymptomatic carriers who frequently moves from one region to another, for example in the airport, school, industry, workplace, and mass gatherings. The antigen test is readily applicable in places where molecular tests are not available to diagnose and isolate symptomatic patients or suspected asymptomatic carriers. This test can also be a preliminary screening test before

Table 1 – Comparison of RT-PCR and Chest CT.

Parameters	RT-PCR	Chest CT	References
Sensitivity	79–83%	77–97%	39,49
Specificity	100%	56–96%	39,49
Time	Comparatively time-consuming (5–6h)	A quick test compared to RT-PCR	46,50
Patient availability	Not always, samples could be collected from home	The patient must be present in the Radiology Center	
Cost-utility	Comparatively low (72 USD)	Slightly high (97 USD)	51
Effectiveness	Less effective alone as a single test due to false negative result	Comparatively more effective than RT-PCR to detect infection rate and isolate patients at clinical settings	28,29
Early detection	Detectable immediately after the onset of infection	Normal CT findings immediately after the onset of infection (0–2 days)	37,45
The severity of the disease and treatment outcome	Unable to detect	Detectable	40–42

RT-PCR and Chest CT within large groups of patients. Unlike molecular tests, saliva samples are not preferred in antigen tests due to low viral load, instead, nasopharyngeal swabs are suggested by researchers because some saliva samples whose viral load was not detected by the antigen test showed positive results in subsequent molecular tests.⁵⁸ Similarly, rapid antigen test should not be used single-handedly to detect COVID-19 and it is not a replacement for molecular tests since it is appropriate only when the patient has a high viral load.

Rapid antibody test

A rapid antigen test is mostly applicable within 7 days of the onset of the infection when the viral load is high enough to be detected by the test. However, if the patient comes to the laboratory or hospital after 7 days of the onset of the infection when viral load is reduced to a level that is undetectable by the Rapid antigen test, the best option is to perform a combination of rapid antibody tests and molecular test.^{59,60} The antibody concentration of the infected patients becomes detectable after the first week of the onset of the symptoms, and a combination of antibody tests with molecular tests is suggested in this period.^{61,62} Serological testing alone is less effective in diagnostic facilities to control a pandemic because 44% of SARS-CoV-2 virus transmission happens during the pre-symptomatic stage⁶³ before the antibodies become detectable. If the molecular test or CT scan is not available or the demands for confirming the COVID-19 patients exceed the capacity of RT-PCR, rapid antigen tests and the serological assay can be convenient. This combination of techniques should have high sensitivity and will be useful in the rapid detection and isolation of COVID-19 patients.

Conclusion

Rapid accurate diagnosis is crucial for providing effective treatment, prevention, and control of any disease. So far, RT-PCR is the gold standard method being used worldwide to diagnose COVID-19 among symptomatic patients and/or asymptomatic suspected carriers. On the other hand, chest CT, rapid antigen test, and serological assays could be other options besides RT-PCR to increase the accuracy of diagnosis

in symptomatic patients. Simultaneously, chest CT can be a second option and readily applicable after RT-PCR for clinically suspected SARS-CoV-2 infections, especially when the initial RT-PCR result is negative. A combination of rapid antigen tests with serological assays is helpful to screen many patients in clinical settings when the availability of RT-PCR is limited. We recommend a combination of RT-PCR and chest CT as the best method to detect COVID-19 accurately among hospitalized symptomatic patients.

Author contribution

MSP designed the study; MNZ, HH, and MSP collected the literature; MNZ prepared the initial manuscript; MSP, HH, JAG, SAA, AP, NSJ, and MTR critically revised the manuscript; and MSP completed the final draft.

Conflict of interest

None to declare.

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