Allergy and Immunology

AIMS Allergy and Immunology, 4(3): 60–74.
DOI: 10.3934/Allergy.2020006
Received: 21 July 2020
Accepted: 09 September 2020
Published: 11 September 2020

http://www.aimspress.com/journal/Allergy

Review

An overview of the prominence of current diagnostic methods for diagnosis of COVID-19

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Abstract: Coronavirus disease 2019 (COVID-19) caused a fatal pandemic worldwide. This review aims to discuss laboratory, molecular, and serological methods and their advantages and disadvantages over each other in COVID-19 diagnosis. Moreover, computed tomography (CT) scan, that is used on suspicion of COVID-19 pneumonia and for determining the severity and progression of the disease, is also discussed. Different CT features categorize the patients into low to high-risk groups. Here, we described three kinds of CT classification based on CT patterns within different time courses of the disease. Chest CT imaging should be considered for screening, evaluating, and following up COVID-19 due to its high sensitivity. Approximately, shortly after the onset of symptoms, viral load can be diagnosed by real-time PCR technique through bronchoalveolar lavage, nasopharyngeal and/or oropharyngeal swab sampling. Proper sampling may delineate the result of this test. Although RT-PCR assay is currently considered the gold standard test, false-negative results should be considered. Furthermore, a positive test may indicate the infection with SARS-CoV-2, but not necessarily the disease, and the person may be a carrier or other organs may be involved other than the lungs. In contrast to CT imaging, RT-PCR assay has poor sensitivity, but it helps the decision-making on hospitalization and isolation. The emergence of reliable serological tests has promoted the diagnosis, treatment process, chronic or carrier status of an individual, and epidemiological studies. In addition, an earlier and more accurate diagnosis will be provided for asymptomatic or susceptible individuals.

Keywords: COVID-19; CT-scan; laboratory findings; real-time PCR; serological test

1. Introduction

The first cases of coronavirus infection disease 2019 (COVID-19) presented with pneumonia were reported in December 2019, in Wuhan, China [1]. This unprecedented disease is a highly contagious infectious disease that causes inflammation, especially in the respiratory system. The incubation period of severe acute corona virus-2 (SARS-CoV-2) until the emergence of symptoms is approximately 5.2 days [2,3]. Typical symptoms of COVID-19 include fever, cough, fatigue, and dyspnea. However, some patients may present with symptoms, such as sputum production, headache, hemoptysis, diarrhea, and vomiting [4–6]. It is of note, esophageal epithelial cells and absorptive enterocytes of ileum and colon are possible targets of COVID-19. Gastrointestinal symptoms such as diarrhea and vomiting have been observed in patients with COVID-19. However, few data are available for the gastrointestinal manifestations of COVID-19 [7]. Since two-thirds of patients with COVID-19 were positive for SARS-CoV-2 RNA in stool samples and viral shedding might take more than a month, the risk of fecal transmission should also be considered [8]. In addition, urogenital manifestations of COVID-19 may be observed. In particular, the elderly with chronic kidney disease are at high risk of severe infection and high mortality, thus monitoring the kidney function of patients with severe COVID-19 is recommended [4]. Age range of patients was very diverse but for most patients aged between 35 and 55 years and fewer cases among children and infants, and most of them were men [9].

The laboratory tests almost showed leukopenia (2.9×10^9 cells/L) of which 70% were neutrophils. Additionally, high levels of blood C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and D-dimer were evident [10]. Diagnosis of COVID-19 relies on both clinical and

para-clinical findings such as fever, decrease in lymphocytes and white blood cells, pulmonary infiltration on chest radiography, and no improvement in symptoms after 3 days of antibiotic consumption [11]. For suspected patients, real-time polymerase-chain-reaction (RT-PCR) should be performed to detect the positive nucleic acid of SARS-CoV-2 in sputum, throat swabs, and secretions of the lower respiratory tract samples [12]. Chest imaging can be used both for diagnosis of pneumonia and documentation of the lesion extension and follow-up changes. Ground-glass opacity (GGO) in the computed tomography (CT) images of patients with COVID-19 may not be visible on lain radiographs. Currently, according to the latest approved sources, RT-PCR test is the gold standard test for the diagnosis of COVID-19. However, owing to the limited sensitivity and specificity, the use of other laboratory parameters and serological tests, as well as CT imaging of lung are helpful where the results of molecular tests do not match with clinical manifestation [13,14]. Regarding the importance of these methods, this review aims to compare the features of the currently used diagnostic methods and their advantages and disadvantages over each other in the diagnosis of COVID-19.

2. Principle and implication of the current diagnostic methods for COVID-19

2.1. Real-time polymerase chain reaction (RT-PCR)

Coronaviruses are a family of RNA viruses containing positive-sense single-stranded RNA of almost up to 32kb (Figure 1) [15,16]. The viral genome of SARS-CoV-2 sequence was publicly released by NCBI with GenBank accession number NC-045512.2 [17] and the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947) [18], followed by four other new genomes deposited on 12 January in GISAID platform [19]. The genome is arranged in the order of 5'-replicase (ORF1a/b)-S-E-Membrane-N-poly(A)-3' [20]. Based on the available genome sequence, the RT-PCR assays can target RdRP, ORF1a/b, ORF1b-nsp14, E, N, and S genes of SARS-CoV-2 [15,21].

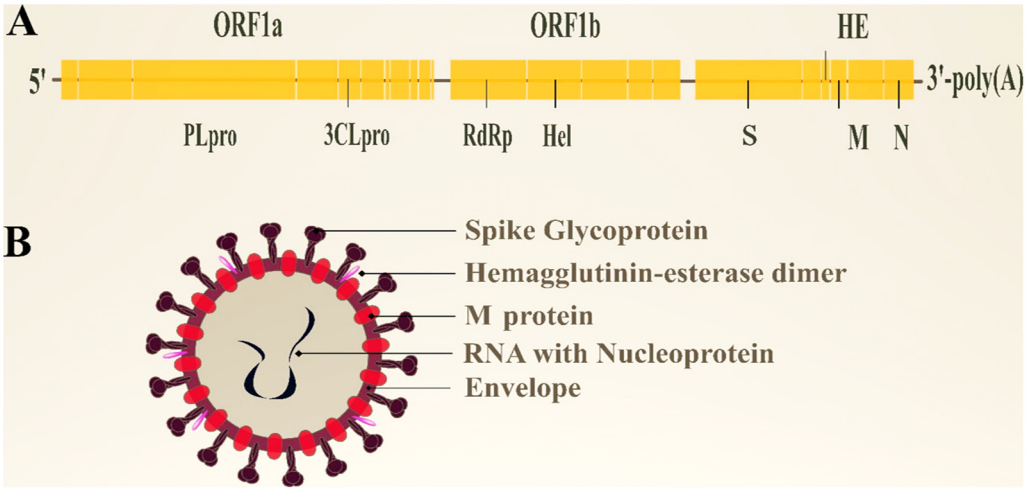

Figure 1. Schematic representation of the structure of SARS-CoV-2. (A) The genome size of SARS-CoV-2 is approximately 30Kb in length with a 5'-cap structure and

3’-poly-A tail. The single-stranded RNA genome of SARS-CoV-2 encodes two main genes, the ORF1a and ORF1b genes, which encode 16 non-structural proteins (nsp1–nsp16). (B) The genes encode the structural proteins comprised of spike (S), envelope (E), membrane (M), and nucleocapsid (N).

The presence of each of these genes using RT-PCR assay by nucleic acid amplification test of the nasopharyngeal (NP) and oropharyngeal (OP), Broncho alveolar lavage fluids (BALF), and other samples corroborates the presence of COVID-19 in patients. In general, 5–6 days after the onset of symptoms, viral load in respiratory tract is quite enough or sometimes at the highest level for detecting by molecular technique. Although BALF is more sensitive, NP and/or OP swabs are recommended for screening due to the invasiveness of the technique. In this regard, OP swabs are more used during outbreak but studies showed RNA was detected only in 32% of OP whereas in NP the detecting rate was 63% .If NP swab elicits tear, the swab hits the target site properly; likewise, proper OP swab sampling elicits gag reflex. The swab should also be made from non-toxic fiber such as polyester as well as nylon handles. The scarcity of personal protective equipment (PPE) may be also a limiting factor for getting proper samples. The importance of bronchoscopy in suspected COVID-19 patients with pneumonia and negative RT-PCR is under debate. The high clinical sensitivity (more than 90% positivity) in patients with COVID-19 has been reported in the recent investigations that presented BALF as one the best specimens for the final decision [22]. Based on guidelines, bronchoscopy is contradictory due to the high risk of spreading the virus during to sampling procedure. Bronchoscopy is recommended in conditions such as strong suspicion of mucus plugging, superinfection in immunocompromised patients, or in life-saving situations; however, it is not included in COVID-19 diagnostic guidelines [22–24].

Whether the sensitivity of RT-PCR test itself or the technique of taking a sample is not high enough remains debating. Although the clinical specificity of molecular tests for the SARS-CoV-2 sequence is high, current reports indicate a clinical sensitivity about 60–70% of patients in the best sampling conditions as well as standard pre-analytical processes such as RNA extraction and cDNA synthesis. A reason for this limitation is the time of sampling and copy number of the virus after the onset of clinical symptoms. The highest clinical sensitivity is associated with sampling up to one week after the onset of clinical symptoms [25,26].

However, RT-PCR has some restrictions in performance: (1) insufficient sample loading results in false-negative results due to low detection rate, (2) results are based on positive and negative results; therefore, the severity of infection, lung involvement, and progression cannot be determined, (3) lack of detection kits and reagents hinder the diagnosis process and prevent the rapid detection, (4) a prolonged lab performance at least 1 day of work may waste time, (5) errors made by lab professionals during test performance can lead to false-positive or negative results [27,28].

Despite the well-performance of CT imaging for COVID-19 diagnosis, the infection must be confirmed by RT-PCR or gene sequencing of isolated samples [15]. Here, we discuss the recent findings in RT-PCR for patients who had different CT manifestations at the initial COVID-19 development. Chun et al. reported that chest CT imaging of 3/21 (14%) symptomatic patients showed no abnormal CT manifestations at initial examination, which might be positive in terms of RT-PCR [29]. Similarly, Bernheim et al. evaluated 121 symptomatic patients with COVID-19 after the symptom onset (0–2 days). They found that 20/36 (56%) patients had normal CT features [30]. Fang et al. reported that 36/51 patients were positive RT-PCR for COVID-19 after initial onset,

and 3/51 had positive RT-PCR after 2–7 days from the symptom onset [28]. Further, Ai et al. reported that 580 (96%) of 601 patients with positive RT-PCR had abnormal CT manifestations, and 308 (75%) of 413 patients with negative RT-PCR had variation in their CT imaging. As such, findings revealed that 21 (3%) of 601 positive RT-PCR cases with clinical symptoms had normal CT features after the onset of the disease [15]. Consistent with these data, Xie et al. demonstrated that 5 (3%) of 167 patients with initial negative RT-PCR results had abnormal chest CT features [31]. Recently, a group of researchers assessed a novel RT-PCR assay targeting different regions of RNA-dependent RNA polymerase (RdRp)/helicase (Hel), S, and N genes, and compared their outcomes with targeting the region of RdRp-P2 in SARS-CoV-2. Amongst 273 samples isolated from 15 cases, 77 (28.2%) were positive for the RdRp-P2 assay, whereas all of these 77 samples were positive for the novel COVID-2019-RdRp/Hel assay. These findings highlight the high accuracy and sensitivity of RdRp/Hel assay for the detection of SARS-CoV-2 RNA in suspected patients [32]. In addition, based on suggested protocols, CT imaging of lungs is very helpful for managing inpatients with negative RT-PCR results and high clinical suspicion of COVID-19 [33].

Taken together, although viral nucleic acid test by RT-PCR assay plays a central role in COVID-19 diagnosis, several cases with false-negative RT-PCR results were reported in the early stages of the infection. Definite cases are identified with RT-PCR assay, so a positive test may indicate the infection with SARS-CoV-2, but not necessarily the disease and the person may not be counted as a patient with lung manifestations, in turn, he/she may be a carrier or other organs may be involved except the lungs. In other words, compatible CT in the presence of negative RT-PCR result cannot rule out the disease. Accordingly, a positive nucleic acid test by RT-PCR assay has a prominent effect on whether the patient be hospitalized and isolated. However, the paucity of sensitivity, inadequate stability, and rather a long period of a procedure are the limitations of this method.

2.2. Laboratory tests

Clinical laboratories are central healthcare systems providing a diverse range of laboratory methods form the biological specimens of patients, which assist the healthcare professionals such as physicians in terms of diagnosis, treatment, and management of diseases. Sometimes, the interpretation of laboratory test results may be challenging and lead the healthcare technician to an unjustified decision [34]. These misinterpretations of test results may have several destructive outcomes because many lab results are found with minor deviations and insignificant abnormalities compared to control tests. Laboratory tests should prominently be considered in a manner consistent with early diagnosis, prevention, management, treatment follow-up, and disease progression [35]. Therefore, the evidence-based recommendations for the evaluation of new tests, especially in COVID-19, are required to use them in clinical purposes to improve diagnostic clinical pathways and disease management.

There are several laboratory tests for tracking the virus during the infection with COVID-19. However, the correlation between laboratory tests and COVID-19 remained elusive whether laboratory tests play an important role for patients with COVID-19. As previously mentioned, COVID-19 affects multiple organs and during a short period of time can alter the normal function of organs into failure leading to multi-organ dysfunction [36].

Chen et al. demonstrated that 73 of 99 patients had increased levels of CRP test, and 43/99

patients had abnormal levels of liver enzymes (AST and ALT), and most of the cases found elevated CK (13%) and LDH (76%). The absolute value of lymphocytes in the majority of cases was reduced. Six (6%) of 99 patients had an increased level of procalcitonin (PCT) and 7% of patients had increased BUN or Cr [37]. Consistent with these data, Wu et al. demonstrated that almost 45% of the patients were found with decreased WBC count and 32.50% with a decreased number of lymphocytes, which highlights the significant relationship between COVID-19 and T lymphocytes. Patients indicated elevated levels of CRP (77.50%), ESR (73.75), PCT (1.25%), AST and ALT (3.75%), CK (22.50%), LDH (21.25%), and D-dimer (3.75%). Whereas, 2.50% of the patients had a low level of albumin [38]. Similarly, Yang et al. reported that 44 patients with COVID-19 had elevated levels of CRP (75%), LDH (43.18%), Procalcitonin (29.55%), ALT (15.91%), GGT (15.91%), and AST (13.64%). While, many patients had decreased levels of troponin (40.91%), albumin (81.82%), prealbumin (50%), HDL (61.37%), and LDL (52.27%); and decreased lymphocytes count in 52.27% of the patients. In addition, they suggested that COVID-19 can affect T cells because many patients had decreased $CD3^+$, $CD4^+$, and $CD8^+$ T cell counts, thereby impairing the immune system homeostasis. As such, increased LDH and CRP levels, and decreased $CD4^+$ T cells could act as an indicator for the severity of pulmonary lesions on CT imaging [39]. Zhang et al. showed the relationship between eosinophil counts and 135 patients with COVID-19. Findings revealed that 52.9% of the patients had decreased eosinophils, which suggests eosinopenia as a diagnostic sign in suspected patients with COVID-19 infection. Other abnormal laboratory features include increased levels of serum CRP (91.9%), serum amyloid A (SAA) (90.2%) and D-dimer (43.2%), PCT (34.7%), and CK (6.7%) have been found in these patients [40]. In contrast, Shi et al. reported leukocytosis in 26 (32%) of 81 patients with COVID-19 and lymphocytosis in 54 (67%) of 81 patients [41]. Furthermore, Song et al. reported 37 (73%) of 51 patients had normal WBC, and normal (35.3%) or reduced (64%) lymphocyte count [42]. Taken together, data suggest that COVID-19 might mostly affect lymphocytes, especially TH1 cells. SARS-CoV-2 mainly invades and spreads through respiratory mucosa, induces consistent pro-inflammatory cytokines and chemokines, including IL-1β, IL-2, IL-6, IL-8, both IFN-α/β, TNF, CCL2-5, and IP-10 [43–45]. These inflammatory responses result in an imbalance in peripheral white blood cells and cytokine storm in the patient's body [46]. Therefore, cytokine assessment may be helpful in patients with severe COVID-19.

2.3. Serological tests

Several serological immunoassays have been developed by research use only (RUO) and in-vitro diagnostic (IVD) companies for the detection of SARS-CoV-2 viral antigens as well as antibodies in the biologic fluids such as plasma and serum. The most widely used diagnostic method of COVID-19 in commercial immunoassays are enzyme Immunoassay (EIA) and automated Electrochemiluminescence (ECL) Chemiluminescence immunoassay (CLIA), and lateral flow immunoassay (LFIA) tests [47]. To the best of our knowledge, detectable antibodies are developed in infected patients almost two weeks after the virus exposure. The IgM antibody levels rise faster than IgG antibodies, but they are reduced and may be totally undetectable afterward. Although several studies have shown that IgG levels can persist for 6 weeks or more, Moreover, few studies have reported a significant reduction of IgG in recovered patients. IgM antibodies can be detected in the serum or plasma samples from 10 to 30 days, while IgG/IgA antibodies can be detected from 20 days

after SARS-CoV-2 exposure or onset of symptoms. In general, the use of total antibodies is more sensitive in identifying people exposed to the SARS-CoV-2 [48–50].

Based on the majority of reports, a greater extent of antibodies is produced against antigens of the SARS-CoV-2, including nucleocapsid (NC) and spike (S) proteins (Figure 1). Although the design of diagnostic commercial kits is mostly based on NC protein, published reports up to now were indicated the antibodies against S antigen have a protective effect in patients with COVID-19. In the meantime, the role of IgA in protecting against SARS-CoV-2 should not be overlooked because the first site of immune-exposing is the mucosal tissue; thus the production of local or systemic IgA may have a high immune protection potential [47,48,50]. Furthermore, the initiation of antibody response after the infection is highly host-dependent and time-consuming. In this regard, preliminary studies in patients with COVID-19 suggest the majority of patients seroconvert between 2 and 3 weeks after exposure to the SARS-CoV-2; however, few patients may develop antibodies later or sooner. As a result of this natural delay in immune response, antibody detection is not useful in the diagnosis of suspected cases of acute infection. These serological tests may be helpful for confirmation of suspicious results of nucleic acid test, determination of virus exposure in asymptomatic individuals, and identification of people with close contact with confirmed patients who suffered from COVID-19 in the past and those who had symptoms more than two weeks ago who had a lower chance for obtaining a positive molecular test and seroconversion studies in a population [47,51,52].

Given clinical sensitivity and specificity of antibody-based diagnosis, the most important factor is the shelf life of the specific antibodies. There are reports that the shelf life of antibodies and memory B cells varies depending on the induced epitopes. However, most studies have focused on the presence of a longer time for IgG versus IgM. These studies have also shown at least 6 to 8 weeks of persistence for SARS-CoV-2 IgG antibodies. However, several studies also indicated that specific antibodies are not produced in some patients with positive RT-PCR. In this regard, further studies need to respond to the controversial questions, especially clinical trials in the development of vaccines [53–56].

2.4. Computed tomography (CT) scan

2.4.1. CT findings

Chest CT imaging is the mainstay of diagnostic imaging for COVID-19 pneumonia because of its high positive rate, rapid, and time efficiency, accuracy, and non-invasiveness capacity [15,57]. However, the specificity of CT is low and is useful for differential diagnosis and the atypical patterns (Table 1). CT scans are used only to evaluate pneumonia, and not all COVID-19 patients have pneumonia. Pulmonary involvement may last for weeks or months, so using a CT scan may not show the time of lung involvement in an asymptomatic person. In addition, pulmonary CT scans may be helpful along with epidemiology, exposure, and other symptoms. Otherwise, this view is not completely specific to COVID-19, and influenza and other respiratory infections and even non-infectious diseases can mimic some of these features. COVID-19 has diverse imaging features at different stages, which are significantly related to the time course of the infection [27]. Therefore, a comprehensive evaluation of CT features in the disease process could help classify patients from low to high-risk groups.

Taken together, Radiological Society of North America recommends four categories for reporting CT imaging findings potentially related to COVID-19. Clinicians should consider the prevalence, exposure, risk factors, and clinical presentation for diagnosis of COVID-19 pneumonia. "Typical", "indeterminate", "atypical appearance" and "negative for pneumonia" are four classifications in reporting the CT findings. Typical features have been frequently reported in cases with confirmed COVID-19 pneumonia. Peripheral bilateral or multifocal rounded GGO with or without consolidation or obvious interlobular thickening (crazy-paving) as well as reverse halo or compatible findings with organizing pneumonia are regarded as the "typical appearance". If CT findings are not typical, and multifocal, diffuse, perihilar, or unilateral GGO with or without consolidation have not typical distribution and are not rounded and peripheral, those are considered "indeterminate appearance", which may be associated with COVID-19 pneumonia as well as several other infectious and non-infectious conditions. Lobar or segmental consolidation, separate small nodules, cavity, smooth interlobular septal thickening associated with pleural effusion without typical and indeterminate are named as "atypical appearance" [58].

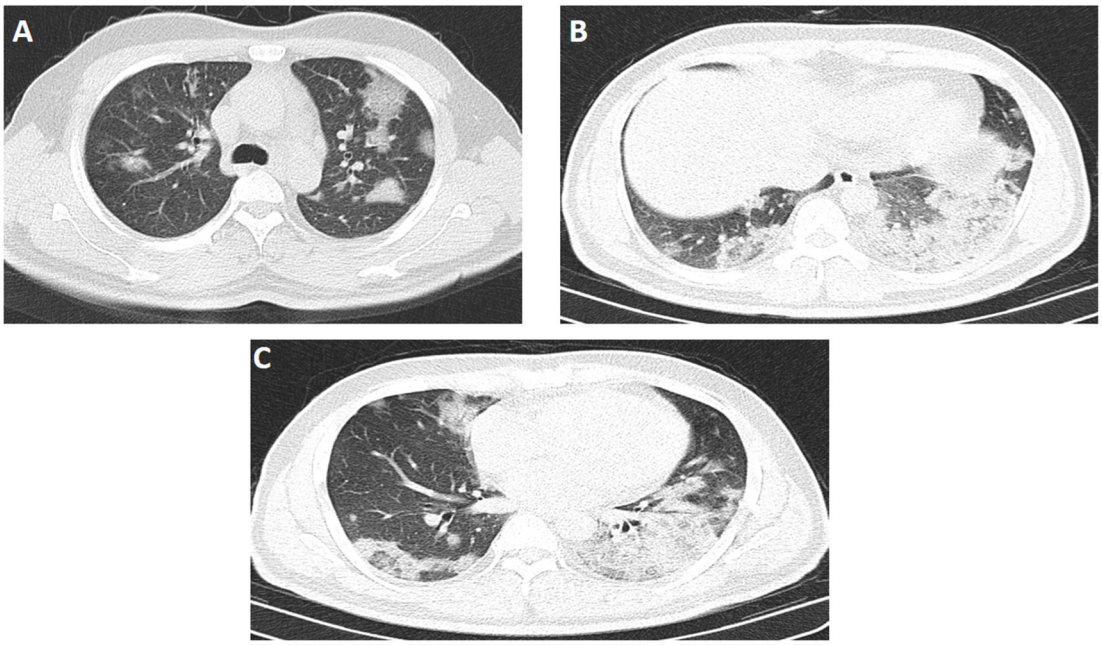

Figure 2. Different CT imaging features of patients with COVID-19. (A) "Typical Appearance": Peripheral multifocal GGO and interlobular thickening (crazy-paving); (B) "Typical Appearance": Peripheral rounded GGO with consolidation in the left lower lobe, evolving reverse halo in the right lower lobe compatible findings with organizing pneumonia, and (C) "Typical Appearance": Multifocal GGO in the right middle lobe, typical reverse halo in the right lower lobe and air space consolidation in the left lower lobe.

In the early diagnosis of COVID-19, CT examination might play a key role for suspected patients but characterizing the most typical patterns of COVID-19 on CT images seem necessary [15,57]. Investigators examined the relationship between symptom onset and the initial chest imaging in 121 sympathetic patients during different times, including early (0–2 days),

intermediate (3–5 days), and late (6–12 days) phases. Data revealed that 20/36 (56%) of early patients were found with normal CT. Interestingly, CT features of patients with a longer time after the onset of symptoms were more common, including consolidation, bilateral and peripheral disease, greater total lung involvement, linear opacities, crazy-paving pattern, and the reverse halo sign. Bilateral lung involvement was observed in 10/36 early patients (28%), 25/33 intermediate patients (76%), and 22/25 late patients (88%) [30].

2.4.2 Stages of the disease and associated CT Findings and current guidelines

Ground-glass opacification (GGO) on the initial CT imaging could act as a hallmark of early-phase infection [59]. Further, a study corroborated that 25/80 cases (31.25%) had no abnormal density shadow in the parenchyma of both lungs. The rest 36 cases (45%) and 19 cases (23.75%) presented with bilateral pneumonia and unilateral pneumonia, respectively [38]. Similarly, COVID-19 on CT imaging of 81 patients manifested as bilateral, subpleural, ground-glass opacities with air bronchograms, ill-defined margins, and a slight predominance in the right lower lobe. Importantly, the study highlighted that abnormal CT features were observed even in asymptomatic individuals, and lesions could turn into a diffuse GGO predominance or consolidation within 1–21 days after the initial symptoms [41]. These data were demonstrated by Chung et al. who showed bilateral pulmonary parenchymal ground-glass and consolidative pulmonary opacities, sometimes with a rounded morphology and a peripheral lung distribution were the most typical CT manifestations of the disease [29]. In addition, Xie et al. highlighted that GGO and/or mixed GGO and mixed consolidation patterns were the typical CT manifestations in patients with COVID-19 pneumonia who have been misdiagnosed with initial negative RT-PCR results [31]. Yang et al. reported 17 (11.4%) of 149 patients had normal CT features on admission. Moreover, 12/17 patients had negative CT findings until the latest follow-up in 10.3 days, whereas the rest 5 patients had positive CT images after almost 7 days [60].

The investigators have found that the frequency of CT manifestations is relatively associated with the disease time courses. Herein, Pan et al. conducted a study to assess chest CT features from the initial identification (day 0) to the patient's recovery (day 26). The findings confirmed the correlation between the time course of COVID-19 pneumonia and CT manifestations. The results have important implications for developing four stages in lung CT within different time courses. The stages are classified into four stages, including stage 1 (0–4 days): GGO in 75% patients; stage 2 (5–8 days): elevated crazy paving pattern in 53% of patients; stage 3 (9–13days): consolidation in 91% of patients; and stage 4 (≥14 days): with no crazy paving pattern but gradual resolution of consolidation in 75% of patients [61]. Nevertheless, Jin et al. defined the lung CT stages as follows: early-stage (1–3 days): single or multiple scattered patchy or agglomerated ground-glass opacities, separated by honeycomb-like or grid-like thickened of interlobular septa in 54.2% of patients; rapid progression stage (3–7 days): a fused and large-scale light consolidation with air-bronchogram inside in 20.5% of patients; consolidation stage (7–14): multiple patchy consolidations in slighter density and smaller range in 31.2% of patients; and dissipation stage (2–3 weeks): grid-like thickening of interlobular septum, thickening and strip-like twist of bronchial wall and a few scattered patchy consolidations in 20.5% of patients [62]. These findings suggest that CT imaging can be classified into four stages in different time courses of infection, and each step provides key CT manifestations to consider the severity and progression of the disease. A study on 1014 patients showed that the

sensitivity, specificity, accuracy of chest CT were 97% (580/601), 25% (105/413), and 68% (685/1014), respectively. Moreover, the negative predictive and positive predictive values were 83% (105/126) and 65% (580/888), respectively. Nevertheless, owing to the overlap of CT imaging properties between COVID-19 and other viral pneumonia types, false-positive patients with COVID-19 can be diagnosed on chest CT. Nevertheless, wing to the increasing number of CT scans and the fact that there has always been concern about the amount of radiation, based on the studies done on some other diseases, it is proposed to reduce the radiation dose for CT scans and perform the so-called low-dose CT. It was suggested at the beginning of the epidemic and various studies established its diagnostic value is not less than the standard dose [63].

3. Conclusions

Confrontation with the COVID-19 virus was one of the most unfortunate events of the last century. The rapid development of diagnostic and preventive protocols was one of the valuable experiences that human society gained during this period. The test status performed by above-mentioned methods varies with the disease stage at the time of examination, immune response, and the symptom onset. Until now, many undetermined questions have remained, especially the persistence duration of immunity for those asymptomatic and symptomatic individuals. Serological immunoassay methods cannot directly confirm virus presence and the stage of COVID-19, only provide data concerning the recent and late infection, but along with other diagnostic tests can be beneficial for physicians to track the disease burden. Besides, Serological-based examination further needs to be assessed thoroughly for cross-reactivity with other viruses since different potential pathogens may manipulate the test results. Rapid SARS-CoV-2 IgG/IgM test is considered a primary and fast screen tool of SARS-CoV-2 infection. Despite the short time testing manner and easy performance, it has several disadvantages. Thus, serological immunoassay performance is much more recommended for screening and follow up purposes for high-risk population and infected patients. Evidence demonstrated that both RT-PCR and CT imaging had almost similar diagnostic value with some tangible variation in their sensitivity and specificity performance. In some cases, CT featuring illustrates the prime stage of SARS-CoV-2 infection before the positivity of RT-PCR and serological tests, which highlights the pivotal role of CT imaging at the initial stage of infection. To further corroborate the CT findings, RT-PCR should be considered a complementary test component over all the diagnostic methods; however, a negative RT-PCR result does not show the free-SARS-CoV-2 status of patients because RT-PCR is time course-dependent. Therefore, we believe that the combination of CT imaging and RT-PCR test can shed light on the stage and burden of the infection, and serological tests are more suitable for screening on a large scale of population. Meanwhile, laboratory tests are strongly recommended as complementary factors for tracking the infection status and the success of treatments (Table 1).

Table 1. Comparison of different diagnostic methods of COVID-19 according to their specificity and sensitivity.

Diagnostic methods	After clinical onset	Test time	Specificity	Sensitivity	References
ELISA	IgM (2–4 weeks)	1–2 h	60–98%	60–90%	[7,48,64]
Antibody	IgG (2–7 weeks)				[48,65,66]
RT-PCR	Likely positive (1–2 weeks)	1–8 h	90–98%	30–65%	[25,26,67,68]
	Likely negative (more than 2 weeks)				
Chest CT with RT-PCR positive result	Time-interval of 4 days or more	1–2 h	25–35%	50–95%**	[15,25,28]

**: In the case of involvement of lungs in COVID-19 patients.

Conflict of interests

All authors declare no conflicts of interest in this paper.

Author contributions

Muhammad Sadeqi Nezhad and Farhad Seif designed the study and drafted the manuscript. Hossein Aazami was involved in search and data collection. Monireh Kamali and Ilad Alavi Darazam provided and interpreted CT scan data. Azam Samei, Pegah Babaheidarian, and Monireh Mohsenzadegan provided and interpreted laboratory data. Majid Khoshmirsafa and Yaghoub Mollaei Kandelousi provided and interpreted serological data. Majid Khoshmirsafa and Mohsen Fateh supervised the study. Farhad Seif revised the manuscript for important intellectual contents. All authors read and approved the final manuscript.

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