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Review

Diagnosis of SARS-CoV-2: A review on the current scenario and future outlook

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Summary. – The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has undoubtedly created an emerging disease of topmost public health priority spilling throughout the globe. The diagnosis currently relies on a multiplex of criteria including the epidemiology, clinical manifestations and *in vitro* diagnostics. Presently, the real-time reverse transcriptase-PCR (rRT-PCR) is considered as the most reliable assay for the detection of SARS-CoV-2 and is being supplemented by other auxiliary tests, including serology and radiology. Many of these molecular and immunological tests have been validated by the Indian Council of Medical Research (ICMR) and the Food and Drug Administration (FDA) and commercial kits have been introduced in the field. But, considering the sensitivity and specificity based shortcomings and the lacunae in monitoring the spread of the virus, there is an immense need to develop integrated smart devices based on novel, safe, rapid and accurate diagnostic techniques and implement them on a large scale to curb this outbreak in the country and the world as a whole.

Keywords: clinical manifestations; COVID-19; diagnosis; PCR; SARS-COV-2; serology

Introduction

In the last two decades, the coronaviruses (CoV) have become the major pathogens of emerging respiratory disease epidemics such as the severe acute respiratory syndrome (SARS) in 2002 (China) and the Middle East respiratory syndrome (MERS) in 2012. The most recent outbreak of a novel coronavirus in the timeline began in 2019 with published literature tracing the first report of symptomatic individuals in Wuhan, China, in the form of pneumonia of unknown etiology. The International Committee on Taxonomy of Viruses (ICTV) termed it the SARS-CoV-2 virus as it is very similar to the one that caused the SARS outbreak (SARS-CoV) (Cascella *et al*., 2020).

The SARS-CoV-2 is a positive-sense, single-stranded RNA virus which belongs to the genus *Betacoronavirus*

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Abbreviations: ARDS = acute respiratory distress syndrome; CLIA = chemiluminescence immunoassay; CoV = coronavirus(es); COVID-19 = coronavirus disease 2019; CT = computed tomography; ELISA = enzyme-linked immunosorbent assay; EUA = Emergency Use Authorization; FDA = Food and Drug Administration; ICMR = Indian Council of Medical Research; Ig = immunoglobulin; LFIA = lateral flow immunoassay; MERS = Middle East respiratory syndrome; POCT = point-of-care testing; PPE = personal protective equipment; RdRp = RNA-dependent RNA polymerase; rRT-PCR = real-time reverse transcriptase-PCR; SARS = severe acute respiratory syndrome; SARS-CoV-2 = SARS coronavirus 2; VTM = viral transport medium; WHO = World Health Organization

of the *Coronaviridae* family (order *Nidovirales*). The WHO has designated the disease caused by this novel CoV as coronavirus disease 2019 (COVID-19) and it was declared as a public health emergency of international concern (PHEIC) on January 30, 2020 and a pandemic on March 11, 2020 (Vashist *et al.*, 2020). Till date, a total of 6 703 095 cases have been reported globally to be positive for SARS-CoV-2 with a case fatality rate of approximately 5.86% (COVID-19 Coronavirus Pandemic, 2020); while in India, there are 227 029 confirmed cases with a case fatality rate of 2.80% as on 4th June, 2020 (COVID-19 Coronavirus Pandemic, 2020). Among the various strategies which are being followed to contain the spread of this highly contagious virus in human population throughout the globe, there is an urgent need for prompt and intensive testing of suspected cases to diagnose SARS-CoV-2 so that quarantine measures can be taken and further spread of infection can be controlled.

Diagnosis of SARS-CoV-2

Clinical diagnosis of SARS-CoV-2 is mainly based on epidemiological history, clinical manifestations and some auxiliary examinations, such as CT scan, nucleic acid detection, blood culture and immunological techniques point-of-care testing (POCT) of IgM/IgG, ELISA (Li *et al.*, 2020c).

History

A detailed history should be taken from the persons suspected to be infected with SARS-CoV-2, including the residence, travel, smoking and underlying comorbidities. The details of the important risk factors which should be recorded for suspicion of SARS-CoV-2 infection include the following:

- Fever and at least one respiratory manifestation such as cough or dyspnoea (WHO, 2020a).
- History of travel or residence in a geographical region reported to have community transmission of the SARS-CoV-2 during the 14 days prior to the onset of symptoms (WHO, 2020a).
- Close contact with a suspected or confirmed person infected with SARS-CoV-2 during the 14 days prior to the onset of symptoms (WHO, 2020a).
- People aged 65 years and older are at higher risk of severe illness (Centers for Disease Control and Prevention, 2020).
- People with underlying health conditions i.e. comorbidities (e.g. chronic respiratory disease, cardiovascular disease, immunocompromised, obesity, diabetes, renal or liver disease, cancer) are at higher risk for severe illness (Centers for Disease Control and Prevention, 2020).
- Smokers are having 1.91 times the odds of progression in the severity compared to those who have never smoked (Patanavanich and Glantz, 2020).
- Severe disease and higher mortality has been seen in the male sex (Goyal *et al.*, 2020; Zheng *et al.*, 2020).

Testing certain asymptomatic individuals may also be important for infection control purposes (for e.g., in congregate settings where COVID-19 cases have been identified, prior to time-sensitive surgical procedures, and prior to time-sensitive aerosol-generating procedures if PPE (personal protective equipment) supplies are limited, and in hospitalized patients at locations where prevalence is high (McIntosh, 2020).

Clinical manifestation

The clinical spectrum of SARS-CoV-2 infection ranges from asymptomatic or paucisymptomatic forms to clinical conditions characterized by respiratory failure, to systemic manifestations in terms of sepsis, septic shock, and multiple organ dysfunction syndromes (Cascella *et al.*, 2020). The prodromal symptoms on the initial presentation include fever, myalgia, cough and sore throat, which can become severe, and patients can flinch with shortness of breath and respiratory failure (Gupta *et al.*, 2019). The incubation period for COVID-19 is thought to be within 14 days following exposure (Chan *et al.*, 2020; Guan *et al.*, 2020; Li *et al.*, 2020a). Based on a modelling study from China, it has been estimated that symptoms develop in 97.5% of infected individuals within 11.5 days, with a median of 5.1 days (Lauer *et al.*, 2020).

As per a report from the Chinese Center for Disease Control and Prevention that included approximately 44 500 confirmed infections, approximately 81% of patients presented mild illness, 14% presented severe illness, and 5% presented a critical illness (Wu and McGoogan, 2020). The characteristics of the various forms are given below:

a. Asymptomatic form

Though the precise frequency of asymptomatic infections is still unpredictable, but these have been well-documented in the literature (Chan *et al.*, 2020; Gudbjartsson *et al.*, 2020; Kimball *et al.*, 2020; Liu *et al.*, 2020b; Mizumoto *et al.*, 2020; Sutton *et al.*, 2020; WHO, 2020a). Screening of a cruise ship for SARS-CoV-2 revealed 17% of positive cases up to February 2020, half of which were not showing any clinical symptoms at the time of diagnosis and 18% of these were estimated to be true asymptomatic cases i.e. did not develop the symptoms subsequently (Mizumoto *et al.*, 2020). Substantially higher proportions of asymptomatic forms have also been recorded. Screening of pregnant women at New York revealed 33 positive cases by the SARS-CoV-2 reverse transcription-PCR (RT-PCR)

test on a nasopharyngeal specimen, 29 (88%) of which were asymptomatic on presentation (Sutton *et al.,* 2020).

b. Mild form

The mild form of the SARS-CoV-2 infection may present with symptoms including dry cough, mild fever, nasal congestion, sore throat, headache, muscle pain, and malaise, most of which are related to the upper respiratory tract (Cascella *et al.,* 2020). Radiograph features are also absent in mild cases (Wang *et al.,* 2020). This form can progress to more severe forms as well.

c. Moderate form

The respiratory symptoms evident in the moderate form include cough, shortness of breath, and tachypnoea (Cascella *et al.,* 2020).

d. Severe form

This form is characterized by fever, severe dyspnoea, respiratory distress, tachypnoea (> 30 breaths/min), and hypoxia (SpO2 < 90% on room air). This is associated with severe pneumonia, acute respiratory distress syndrome (ARDS), sepsis, and septic shock (Cascella *et al.,* 2020). ARDS can be suggestive of the onset of respiratory failure. Based on the degree of hypoxia, ARDS can be mild (200 mmHg < PaO2/FiO2 ≤ 300 mmHg), moderate (100 mmHg < PaO2/FiO2 ≤ 200 mmHg) or severe (PaO2/FiO2 ≤ 100 mmHg) (Cascella *et al.,* 2020).

e. Critical form

If the disease progresses to critical form, it may result in respiratory failure, septic shock and/or multiple organ dysfunction (MOD) or failure (MOF). Data from the Chinese CDC regarding 72 314 case records suggest that approximately 49% of the critical patients and those affected by pre-existing comorbidities such as cardiovascular disease, diabetes, chronic respiratory disease, and oncological diseases, died (Wu and McGoogan, 2020).

The clinical pictures of patients with COVID-19 induced sepsis have multiorgan involvement. These signs and symptoms include respiratory manifestations such as severe dyspnoea and hypoxemia, renal impairment with reduced urine output, tachycardia, altered mental status, and functional alterations of organs expressed as laboratory data of hyperbilirubinemia, acidosis, high lactate, coagulopathy, and thrombocytopenia. The reference for the evaluation of multiorgan damage and the related prognostic significance is the Sequential Organ Failure Assessment (SOFA) score, which predicts ICU mortality based on lab results and clinical data (Seymour *et al.,* 2019). A pediatric version of the score has also received validation (Matics and Sanchez-Pinto, 2017). The septic shock is associated with increased mortality, circulatory, and cellular/metabolic abnormalities such as serum lactate level greater than 2 mmol/l (18 mg/dl) and persisting hypotension despite volume resuscitation (Cascella *et al.,* 2020). Some laboratory findings associated with the critical illness include an exuberant inflammatory response, similar to cytokine release syndrome, with persistent fevers, elevated inflammatory markers (e.g. D-dimer, ferritin), and elevated proinflammatory cytokines (Huang *et al.,* 2020a; Mehta *et al.,* 2020).

Other complications include arrhythmias, acute cardiac injury, and shock (Arentz *et al.,* 2020; Cao *et al.,* 2020; Chen *et al.,* 2020a; Wang *et al.,* 2020). The onset of Guillain-Barré syndrome has also been reported 5 to 10 days after initial symptoms (Toscano *et al.,* 2020). Smell and taste disorders (anosmia and dysgeusia) have also been reported in patients infected with SARS-CoV-2 (Giacomelli *et al.,* 2020; Lechien *et al.,* 2020). Gastrointestinal symptoms (eg, nausea and diarrhea) have also been reported, sometimes even on initial presentation (Goyal *et al.,* 2020; Huang *et al.,* 2020a; Jin *et al.,* 2020; Wang *et al.,* 2020). The prevalence of diarrhea, nausea/vomiting, and abdominal pain have been reported to be 13, 10, and 9 percent, respectively (Cheung *et al.,* 2020b). Various other symptoms have also been reported, including headache, sore throat, and rhinorrhea (Chen *et al.,* 2020a; Guan *et al.,* 2020) and conjunctivitis (Colavita *et al.,* 2020). In addition to such symptoms, there have also been reports of dermatologic findings, including maculopapular, urticarial, and vesicular eruptions and transient livedo reticularis (Casas *et al.,* 2020; Galván *et al.,* 2020; Manalo *et al.,* 2020; Recalcati *et al.,* 2020).

The clinical features which are apparent in the symptomatic SARS-CoV-2 infection are shown in Fig. 1.

Laboratory features

The laboratory examinations which should be conducted in the patients suffering from a severe form of SARS-CoV-2 infection have been compiled in Table 1. Among these, the laboratory abnormalities which are most commonly found in patients with pneumonia include lymphopenia, leukocytosis, thrombocytopenia, elevated liver transaminases, elevated C-reactive protein, elevated lactate dehydrogenase, etc. apart from neutrophilia, decreased haemoglobin, decreased albumin and renal impairment (Chen *et al.,* 2020b; Goyal *et al.,* 2020; Huang *et al.,* 2020a; Li *et al.,* 2020b; Qin *et al.,* 2020; Wang *et al.,* 2020). In children, these are not common (Garazzino *et al.,* 2020).

Sample collection

The collection of samples in adequate quantity by proper methods is essential for the accurate diagnosis of SARS-CoV-2 infection. Table 2 shows the information on the collection and storage of samples from the presumably infected persons. If only one sample is to

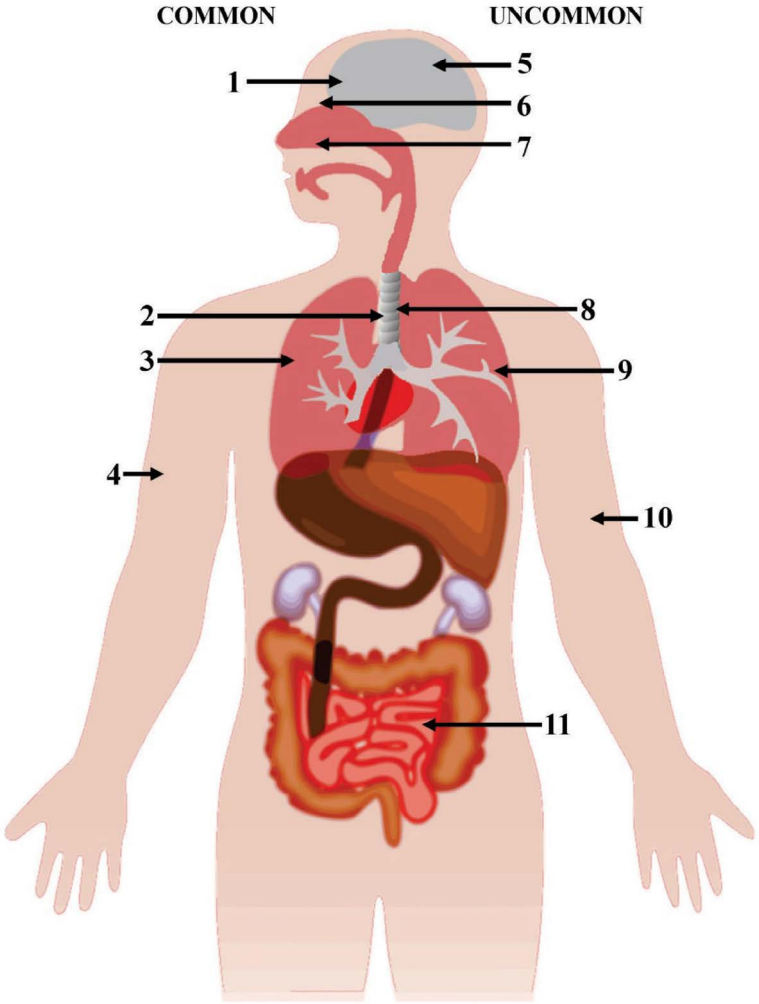

Fig. 1

Clinical manifestations of symptomatic SARS-CoV-2 infection in humans

Commonly evident symptoms include fever, altered sense of smell and/or taste (1), cough, sputum expectoration, sore throat (2), dyspnoea (3), fatigue, myalgia (4); whereas the uncommon symptoms include confusion, dizziness, headache (5), conjunctivitis (6), rhinorrhoea, nasal congestion (7), haemoptysis (8), chest pain, bronchial breath sounds, tachypnoea, crackles/rales on auscultation (9), cutaneous manifestations, cyanosis (10), and gastrointestinal symptoms (11).

be collected, the nasopharyngeal swab is recommended (Hong *et al.*, 2020).

For the patients which are asymptomatic or have mild manifestations, the nasopharyngeal and oropharyngeal swabs should be collected and placed in the viral transport medium (VTM) to increase sensitivity (Centers for Disease Control and Prevention 2020; WHO, 2020a). If the sample from the upper respiratory tract of patients is negative, especially in patients with severe disease, samples from the lower respiratory tract should be tested for confirmation. As the serological tests become available, the paired serum samples (acute and convalescent) can be collected. Additional samples like blood, stool and urine may be collected, but the frequency of shedding of virus and hence the diagnostic value of such samples is still unknown. The samples should be transported to the laboratory at 2–8°C or; at -70°C on dry ice in case of delay. In the laboratory, samples can be stored at 2–8°C, but repeated freezing and thawing should be avoided (WHO, 2020a).

Note: As adapted from the guidelines on response to coronavirus disease 2019 published by the Korea Centers of Disease Control and Prevention (Korea Centers of Disease Control and Prevention, 2020), the cases can be defined as "confirmed case" as per the diagnostic criteria (positive for COVID-19 rRT-PCR, virus isolation) irrespective of clinical manifestations; and "suspected case" which is under scanner as per the clinical and epidemiological findings, but not yet confirmed.

Table 1. Laboratory findings associated with SARS-CoV-2 infection (adapted from Beeching *et al.*, 2020)

Test	Results	Relevance
Pulse oximetry	Low oxygen saturation (SpO_2 <90%) may be seen.	Recommended in case of respiratory distress and cyanosis. Although 'silent hypoxia' and respiratory failure can also occur in patients without any symptoms of respiratory distress (Xie *et al.*, 2020).
Arterial blood gas (ABG)	Low partial oxygen pressure may be seen.	Recommended in case of respiratory distress and cyanosis in patients having low oxygen saturation (SpO_2 <90%) to detect hypercarbia or acidosis.
Complete blood count (CBC)	Lymphopenia, leukocytosis, and thrombocytopenia are most commonly seen. Neutrophilia and decreased haemoglobin may be seen (Chen *et al.*, 2020; Huang *et al.*, 2020; Qin *et al.*, 2020; Wang *et al.*, 2020).	Lymphopenia, leukocytosis, and thrombocytopenia may serve as markers for early identification of severe disease associated with poor prognosis (Huang *et al.*, 2020; Tan *et al.*, 2020). High neutrophil-to-lymphocyte ratio also indicates the risk for severe illness and poor prognosis (Lagunas-Rangel *et al.*, 2020; Liu *et al.*, 2020).
Coagulation test	Elevated D-dimer and fibrinogen, prolonged prothrombin time (Chen *et al.*, 2020; Huang *et al.*, 2020; Micco *et al.*, 2020; Wang *et al.*, 2020).	Non-survivors have been found to have significantly higher D-dimer levels, longer activated partial thromboplastin time and prothrombin time as compared to survivors (Tang *et al.*, 2020).
Metabolic tests	Elevated liver transaminases; decreased albumin; renal impairment (Chen *et al.*, 2020; Huang *et al.*, 2020).	Abnormalities in liver function may be more common in patients with SARS-CoV-2 infection as compared to other types of pneumonia (Zhao *et al.*, 2020).
Serum procalcitonin	May be elevated.	May be elevated in case of secondary bacterial infections (Huang *et al.*, 2020; Chen *et al.*, 2020), more commonly in children (Xia *et al.*, 2020). May be useful in limiting the overuse of antibiotics in SARS-CoV-2 related pneumonia (Metlay and Waterer, 2020).
Serum C-reactive protein	May be elevated.	May be elevated in case of secondary bacterial infections, or in hyper inflammation (Huang *et al.*, 2020; Chen *et al.*, 2020). May serve as markers for early identification of severe disease (Tan *et al.*, 2020).
Serum ferritin	May be elevated.	Indicates the development of cytokine release syndrome (Mehta *et al.*, 2020).
Serum lactate dehydrogenase	May be elevated.	Reported in 73% to 76% cases (Chen *et al.*, 2020; Huang *et al.*, 2020). May be more common in patients with SARS-CoV-2 infection as compared to other types of pneumonia (Zhao *et al.*, 2020).
Serum creatine kinase	May be elevated.	Reported in 13% to 33% cases (Chen *et al.*, 2020; Huang *et al.*, 2020). Indicates muscle or myocardium injury.
Serum troponin	May be elevated.	Elevated in case of cardiac injury (Huang *et al.*, 2020; Gupta *et al.*, 2020).

Table 2. Collection and storage of samples (adapted from Druce *et al.*, 2011)

Sample type	Collection devices	Storage and shipment	Collection time
Upper respiratory tract Nasopharyngeal swabs Oropharyngeal swabs Nasopharyngeal wash	Dacron or polyester flocked swabs*. Sterile container*.	2–8°C within 5 days; -70°C for more than 5 days. 2–8°C within 2 days; -70°C for more than 2 days.	Collect on presentation. Sampling repeated for monitoring.
Lower respiratory tract Sputum Endotracheal aspirate Bronchoalveolar lavage	Sterile container.	2–8°C within 2 days; -70°C for more than 2 days.	Collect on presentation. Sampling repeated for monitoring.
Lung tissue from biopsy/ autopsy	Sterile container with saline or VTM*.	2–8°C within 24 hours; -70°C for more than 24 hours.	
Serum	Serum separator tubes (collect 3–5 ml whole blood).	2–8°C within 5 days; -70°C for more than 5 days.	Paired samples, initially in the first week of illness or contact and then 2–4 weeks later.
Whole blood	Collection tube.	2–8°C within 5 days; -70°C for more than 5 days.	Collect on presentation. Sampling repeated for monitoring.
Stool	Stool container.	2–8°C within 5 days; -70°C for more than 5 days.	Collect on presentation. Sampling repeated for monitoring.
Urine	Urine container.	2–8°C within 5 days; -70°C for more than 5 days.	Collect on presentation. Sampling repeated for monitoring.

*VTM is the viral transport medium containing antifungal and antibiotic supplements. If VTM is unavailable, sterile saline may be used.

Table 3. Testing priorities for the diagnosis of SARS-CoV-2 infection (ESC Guidance, 2020)

Priority	Cases	Remarks
I	Hospitalized patients. Symptomatic healthcare workers.	Hospitalized patients to be provided with optimal care. Risk of nosocomial infections to be reduced. Integrity of healthcare system to be maintained.
II	Symptomatic patients in long-term care facilities. Symptomatic patients of 65 years age and older. Symptomatic patients with underlying health conditions. Symptomatic first responders.	Patients at higher risk of complications to be promptly identified.
III	Symptomatic infrastructure workers. Symptomatic individuals who are not in categories of I and II priority. Health care workers and first responders. Individuals with mild symptoms.	Testing the community with rapidly increasing hospital cases (as per the available resources). Ensuring the health of essential workers.
IV	Individuals without symptoms.	Non-priority.

Auxiliary diagnostic tests

The clinical signs in patients infected with SARS-CoV-2 are highly atypical. Therefore, auxiliary examinations are necessary for the diagnosis of COVID-19, keeping in mind the epidemiological history as well. The testing capacity may not be sufficient for widespread testing, such as drive-through testing in South Korea. Thus, suspected cases can be categorized priority-wise (Table 3).

Molecular or nucleic acid-based techniques

The two types of nucleic acid-based techniques that are being used for the detection of SARS-CoV-2 are rRT-PCR and high-throughput sequencing. The isolation and culture of viruses from blood followed by high-throughput sequencing is the highly specific method for identification of SARS-CoV-2 (Zhou *et al*., 2020), apart from electron microscopy. But this method has limited clinical application because of equipment requirements and cost involved. So, rRT-PCR is considered effective as well as a reliable method to detect such pathogenic viruses in blood and respiratory secretions (Corman *et al*., 2020). Although it is advisable to perform viral sequencing of a percentage of samples from the clinical cases to monitor the genetic mutations in virus (WHO, 2020b).

The rRT-PCR assays are the most widely used *in vitro* diagnostic tests, which are being used for the confirmatory diagnosis of SARS-CoV-2 throughout the globe. In certain RT-PCR protocols, two genes are being examined using a two-step interpretation algorithm (Chu *et al*., 2020; National Institute for Viral Disease Control and Prevention, 2020) with the identification of one gene as a screening test and of the second gene for a confirmatory test. One such novel and the highly specific assay was developed by the Tib-Molbiol, Germany, targeting the envelope (E) gene for screening and RNA-dependent RNA polymerase (RdRp) gene for the confirmation (Corman *et al*., 2020).

Similarly, a first-line screening assay that uses the TaqMan fluorogenic probe and is based on the E gene has been designed by the Indian Council of Medical Research (ICMR, 2020b). Out of a total of 106 Virus, Research and Diagnostic Laboratories (VRDLs) established under the Department of Health Research/ICMR, 13 operational and 18 new VRDLs are carrying out this E gene-based screening and it is eventually proposed to involve the rest of the VRDLs in SARS-CoV-2 testing (Gupta *et al*., 2020). The positive samples are immediately sent in triple packaging to the reference laboratory i.e. ICMR- National Institute of Virology (ICMR-NIV), Pune for confirmatory assays based on the ORF 1b, RdRp gene, E gene and N gene (ICMR, 2020b).

In certain protocols, three or more genes are examined and the test is considered positive only when all genes are detected for e.g., an emergency test targeting the N1, N2, N3 and RP genes has been approved by the FDA for the diagnosis of SARS-CoV-2. The presence of only one or two genes is interpreted as indeterminate. Another example is the Xpert® Xpress SARS-CoV-2 test developed by the Cepheid. This emergency test approved by the FDA and ICMR targets the N2 and E genes. The presence of both the genes as well as the N2 gene alone is considered positive, whereas if only E gene is present, it is interpreted as presumptive positive (Gupta *et al*., 2019). With a turnaround time of just 45 min, it can be referred to as a frontline point-of-care test for the diagnosis of SARS-CoV-2 in the nasopharyngeal swabs, nasal wash and aspirate from the suspected individual (Cepheid, 2020; Vashisht, 2020).

Various other assays including Roche COBAS-6800/8800 targeting E and RdRp gene; TaqPath ABI targeting S, N and orf-1 ab genes; and Truenat (make-in-India product for screening) are also available. Many commercial diagnostic kits have also been validated by the ICMR-NIV such as Altona diagnostics based on the E and S genes; KILPEST based on the E, RdRp and N genes; MY LAB Patho detect kit (make-in-India product); SD Biosensor kits based on the

E and RdRp ORF 1 ab genes; and Seegene based on the E, RdRp and N genes (Gupta *et al*., 2019). The IIT, New Delhi has also designed a probe-free kit aimed at easier and cheaper diagnosis with lesser turnaround time (Gupta *et al*., 2020b; Vashisht, 2020).

Due to the worldwide efforts of the research community, prominent advances are being made for the prompt diagnosis of SARS-CoV-2. One such development is the Vivalytic COVID-19 test by Bosch, Germany, which is a rapid point-of-care molecular test that can simultaneously detect SARS-CoV-2 along with nine other respiratory viruses, including influenza A and B (Vashisht, 2020). Another benchmark is the development of the Abbott ID Now™ COVID-19 test, which detects SARS-CoV-2 from the nasal, nasopharyngeal, throat and oropharyngeal swabs in as little as five minutes (ID NOW, 2020; Vashisht, 2020). This test based on the isothermal nucleic acid amplification targeting the RdRp gene, has received FDA Emergency Use Authorization (EUA). It utilizes a portable touchscreen-operated instrument, i.e., ID Now and can be used in locations such as hospitals, offices and hotspots of the outbreak (Vashisht, 2020).

Although these nucleic acid-based assays have good sensitivity but the negative results cannot always rule out the possibility of SARS-CoV-2 infection. Such false-negative results can arise due to various factors such as inadequate poor quality of the sample, late or very early sampling, improper transportation of sample and technical errors in the test.

Immunoassays

Several serological tests have been developed for the diagnosis of infection caused by SARS-CoV-2; most of which are based on the IgM and IgG. IgM antibodies can be detected in the serum at 10 to 30 days after SARS-CoV-2 infection and then diminishes, while IgG can be detected after 20 days and persists for a long time after the infection (Tan *et al.,* 2020c). The ICMR has also licensed antibody-based blood tests for the rapid diagnosis of SARS-CoV-2 infection with the advisory to retest after 10 days or confirm by rRT-PCR in case of negative results (ICMR, 2020a).

Various formats of diagnostic immunoassays are being developed, such as lateral flow immunoassay (LFIA) based COVID-19 test developed by BioMedomics, USA which detects the IgM and IgG antibodies in the suspected individuals in around ten minutes (Vashisht, 2020). This test is rapid and easy to perform in field conditions using just 20 µl of blood (finger-pricked) or 10 µl of serum. Similarly, the test named SARS-CoV-2 rapid developed by Pharmacyt AG, Germany gives the results in 20 minutes from just two drops of the blood pricked from the finger (Vashisht, 2020). Another LFIA based assay by Chembio Diagnostics, USA i.e. DPP COVID-19 IgM/IgG test has received FDA EUA. The results are obtained in 15 minutes using the blood pricked from finger and the optical readings are provided in MicroReader 1 and 2 analyzers instead of visual detection (Vashisht, 2020). A large number of rapid IgM/IgG tests have been developed by several IVD companies, such as Beijing Lepu Medical Technology, Biomerica, Guangzhou Wondfo Biotech, Jiangsu Medomics Medical Technologies, Innovita Biological Technology, Sona Nanotech, Sugentech, Sure Bio-Tech Xiamen AmonMed Biotechnology and Zhenjiang Orient Gene Biotech (Vashisht, 2020). However, these rapid tests need to be assessed strictly for their clinical accuracy before providing authorization. There have been reports from various European countries suggesting that the analytical performance of most of the rapid test kits procured from China was not good and they did not work in over 70% of SARS-CoV-2 cases (Vashisht, 2020).

The main drawback of IgM and IgG based tests is that they are detectable after two weeks of the onset of infection. Thus more assays involving other biomarkers need to be developed for the rapid diagnosis of SARS-CoV-2 infection in early stages. The automated chemiluminescence immunoassay (CLIA) are advantageous over LFIA tests because of the analysis of very high throughput of samples and the ability to incorporate many clinical tests based on other biomarkers, such as C-reactive protein (CRP). Examples of CLIA based tests include the DZ-Lite SARS-CoV-2 CLIA IgM and IgG tests by Diazyme, USA that have received FDA EUA and have a throughput of 50 tests per hour (Vashisht, 2020).

Commercial enzyme-linked immunosorbent assay (ELISA) kits based on the IgM and IgG antibodies have also been developed by the manufacturers, such as DRG Diagnostics GmbH, Epitope Diagnostics, Euroimmun and IBL International (Vashisht, 2020).

Although the serological tests have the limitation of cross-reactivity to other coronaviruses (Meyer *et al*., 2020), the validated tests conducted on paired serum samples (acute and convalescent-phase) can aid in retrospective assessment of the ongoing outbreak of SARS-CoV-2.

Antigen based detection

These tests detect the protein fragments of the virus by testing from the nasal swabs. Many antigen-based commercial kits are being developed for the purpose of surveillance (Administrator, 2020). The test is rapid as compared to rRT-PCR but despite being highly specific for the SARS-CoV-2, the sensitivity is lower; therefore, the negative results should be preceded by rRT-PCR for confirmation (US Food and Drug Administration, 2020).

Radiological techniques

Considering certain false negative results of rRT-PCR, it needs to be complemented with other detection methods

such as radiology as well. The chest x-ray is recommended in all the patients with suspected pneumonia and reveals unilateral lung infiltrates in 25%, and bilateral lung infiltrates in 75% cases (Song *et al.,* 2020; Huang *et al.,* 2020a; Chen *et al.,* 2020b).

Computed tomography (CT) scans are considered as the primary imaging module in some of the countries, such as China. Although the CT scans are not confirmatory for the diagnosis of SARS-CoV-2 infection, they may be helpful in the management of individual cases and detecting the complications (Beeching *et al.,* 2020). Especially the high-resolution CT (HRCT) for the chest is essential for early diagnosis and evaluation of disease severity of patients with SARS-CoV-2 (Pan *et al.,* 2020). In a cohort study involving more than 1000 patients in a hyperendemic area in China, the sensitivity of chest CT was found to be higher (88%) as compared to initial rRT-PCR (59%) (Ai *et al.,* 2020).

But, it should be emphasized that an individual with SARS-CoV-2 infection can have a normal chest CT. Conversely, the abnormal chest CT is also non-specific as the findings are indistinguishable from viral pneumonia of different aetiologies, and this is a major shortcoming of this technique. The typical features which have been found in the lungs of patients infected with SARS-CoV-2 include the ground-glass opacity or consolidation at multiple sites in the bilateral lobular and subsegmental areas (at the periphery or posterior aspect especially in the lower lobes (Huang *et al.,* 2020a; Salehi *et al.,* 2020; Xu *et al.,* 2020); apart from air bronchograms, crazy-paving pattern and reverse halo/perilobular pattern (British Society of Thoracic Imaging, 2020). The atypical features of the disease which are generally found in the later stages include smooth or irregular thickening of the interlobular area or septa, adjacent pleura and involvement of the subpleura as well; and rarely bronchiectasis, cavitation, pneumothorax, pleural and pericardial effusion and lymphadenopathy (Huang *et al.,* 2020a; Salehi *et al.,* 2020; Xu *et al.,* 2020).

Lung ultrasound

Lung ultrasound technique can aid in the diagnosis of SARS-CoV-2 infection since it is highly sensitive for the detection of abnormalities such as ground-glass opacity, pleural thickening and subpleural consolidation; besides having the advantage of portability and reproducibility (Beeching *et al.,* 2020). It can be used in pregnant women and children as well (Denina *et al.,* 2020; Inchingolo *et al.,* 2020). The typical features observed in lung ultrasound of patients infected with SARS-CoV-2 include air bronchograms, B-lines, white lung, pleural line thickening and consolidations (Soldati *et al.,* 2020; Moro *et al.,* 2020; Cheung *et al.,* 2020a; Moore and Gardiner, 2020).

Other diagnostic assays

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay is a prospective test for the detection of the RNA of SARS-CoV-2. The technique is simple, quick and the viral DNA produced is more in quantity as compared to rRT-PCR (Beeching *et al.,* 2020). The RT-LAMP based assays are currently being developed and evaluated (Baek *et al.,* 2020; Lu *et al.,* 2020; Park *et al.,* 2020). Other such emerging formats which are being researched include the CRISPR technology (Sheridan, 2020), lab-on-a-chip and microfluidics (Vashisht, 2020).

Future perspective of smart diagnostics

In the present-day era of information and technology, smartphones equipped with the global positioning system (GPS) and internet are omnipresent, which can be efficiently exploited as the analysis and surveillance platform. Examples of such smart fitness devices that have already been anticipated in other diseases include the fitbit wearable device for influenza-like illness (Cecile and Mauricio, 2020; Radin *et al.,* 2020) and iHealth Align device for blood glucose monitoring (iHealth Align, 2020). In a remarkable move, the Aarogya Setu mobile App has been launched in India on 2nd April 2020, which enables bluetooth based contact tracing and mapping of likely hotspots apart from disseminating the relevant information about SARS-CoV-2 (Aarogya Setu, 2020). Furthermore, there is a need for the development of an automated smartphone-based POC device equipped with the molecular and immunological tests based on the SARS-CoV-2 biomarkers so that the diagnosis as well as reporting of this highly communicable disease can transform to software-based analytics.

Conclusion

The SARS-CoV-2 has become a global pandemic defying the geographical borders and putting the lives of billions at risk, especially those at the extreme of ages and immunocompromised. Diagnosis of the SARS-CoV-2 infection currently relies on a combination of epidemiological criteria, evident clinical manifestations and *in vitro* diagnostic assays. Although there have been commendable advances in the diagnostic assays but, considering the sensitivity and specificity based disadvantages in the presently available diagnostics, it becomes imperative to highlight that the nations need to invest more in the research and smart integrated diagnostics so that safe, rapid and reliable technologies for the detection of SARS-CoV-2 can be developed and implemented in large scale for the accurate diagnosis and containment of this outbreak.

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