Diagnosis of COVID-19 Infection in Pregnancy

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INTRODUCTION

Pregnancy is a well-known risk factor for development of respiratory infections. Pregnant women are at high risk of development of complications and severe forms of infections caused by coronaviruses including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Pregnant women were identified as a vulnerable group and were advised to take additional precautions during the COVID-19 pandemic. To reduce transmission risks for both pregnant women and healthcare workers, the International Federation of Gynecology and Obstetrics recommended the suspension of much routine antenatal care and replacement with video or telephone consultations whenever possible.

PHYSIOLOGICAL CHANGES WITH PREGNANCY

Many physiological changes that occur during pregnancy increase the susceptibility of the women to respiratory viral infections including COVID-19 infection. These changes include immunological, respiratory, coagulation, and endothelial cell responses.

Immunological Response

The immune system adapts during pregnancy to allow for the growth of a semiallogenic fetus, resulting in an altered immune response to infections during pregnancy (Cascella et al., 2020). The modulations of the maternal immune system in pregnancy may affect the response to infections, and specifically to viruses. The altered inflammatory response to viruses during pregnancy is thought to be mediated, at least in part, by the following:

1) A shift in CD4+ T cell population toward the Th2 phenotype over Th1 during pregnancy (a response that promotes humoral responses over cellular immune responses). For the immune response to viral infections, a decrease in Th1 reactivity can result in an altered clearance of infected cells (Zhou et al., 2020).
2) A decrease in circulating natural killer (NK) cells during pregnancy that may alter the ability to clear viruses (Bergsbaken et al., 2009).
3) A decrease in circulating plasmacytoid dendritic cells (pDCs). These cells are key for type 1 interferon production against viruses. Moreover, pDCs from pregnant women have also been shown to have an attenuated inflammatory response to the H1N1 virus. This is thought to be one of the reasons why

pregnant women were more severely affected by the H1N1 pandemic in 2009 (Vanders et al., 2013).
4) An increase in circulating progesterone levels that has immunomodulatory properties. Progesterone also can enhance lung repair of damage induced by influenza virus, making high levels during pregnancy potentially beneficial for the recovery after viral lung infections. However, in a mouse model of influenza A infection, treatment with progesterone or the progestin, levonorgestrel, also resulted in a decrease in virus-specific antibody levels, as well as a decrease in virus-specific CD8+ T cells in mice. When these mice were rechallenged with influenza A, this resulted in more severe disease (Hall et al., 2017).
5) Alterations in the innate immune system, including the pattern recognition receptors Toll-like receptors (TLRs) during pregnancy. COVID-19 infection causes pyroptosis (inflammation mediated) of host cells and release of damage-associated molecular patterns, which can be TLR ligands and further enhance inflammation (Young et al., 2014).

Respiratory Response

Physiological alterations to the chest shape and elevation of the diaphragm due to diaphragmatic splinting by the gravid uterus cause changes to the respiratory function. Although there is a 30%−40% increase in tidal volume, the reduction in chest volume leads to a decrease in functional residual capacity, end-expiratory volumes, and residual volumes from early in pregnancy. The reduction in total lung capacity and inability to clear secretions can make pregnant women more susceptible to severe respiratory infections (Goodnight and Soper, 2005).

Coagulation Response

In the general population, COVID-19 is associated with high rates of thromboembolic complications. One study in 184 critically ill COVID-19 patients (24% of them were female) reported thrombotic events in 31% of them (Ji et al., 2020). This is due to activation of coagulation pathways and potential progression to disseminated vascular coagulopathy (DIC) and fibrinolysis with resultant dynamic hypercoagulation occurring alongside thrombocytopenia. Pregnancy is a hypercoagulable state with increased thrombin production and an increase in intravascular inflammation. During pregnancy, there are higher levels of circulating coagulation and fibrinolytic factors, such as plasmin, and these may be implicated in the pathogenesis of SARS-CoV-2 infection (Creanga et al., 2017). Pregnant

women are at increased risk of thromboembolic events with associated mortality. Therefore, pregnant women with COVID-19 may have additive or synergistic risk factors for thrombosis. Current guidelines recommend that all pregnant women with confirmed COVID-19 should have thromboprophylaxis until 10 days postnatal and that their clinicians have a low threshold for investigation of possible thromboembolism (Royal College of Obstetricians and Gynecologists, 2020).

Endothelial Cell Function

Mortality in COVID-19 is predominantly due to acute respiratory distress syndrome (ARDS). Emerging evidence suggests that pulmonary endothelial cell dysfunction has an important role in the onset and progression of ARDS. In health, endothelial cells are surrounded by mural cells (pericytes) and limit inflammation by restricting immune cell entry and prevent coagulation via expression of anticoagulant factors. In ARDS, this endothelial barrier is damaged, leading to tissue edema, excessive inflammation, and hypercoagulability. Risk factors for COVID-19 (increasing age, obesity, diabetes mellitus, and cardiovascular disease) are all associated with endothelial cell dysfunction (Li et al., 2019). Maternal vascular adaptation to pregnancy is critical for optimal pregnancy outcomes. At implantation, the specialized uterine spiral arterioles are remodeled to form sinuses that become placental villi. Systemic vascular physiology also undergoes significant adaptations to pregnancy. Given the potential importance of endothelial cell function in the development and progression of COVID19, these women may be at particular risk, if infected, and an early systematic review found higher rates of preeclampsia in pregnant women hospitalized with COVID-19 (Wu and McGoogan, 2020).

CLINICAL FINDINGS

Diagnosis of SARS-COV-2 infection in pregnant women is more difficult than the general population as some of its symptoms are similar to pregnancy-induced manifestations (Dashraath et al., 2020). These manifestations include nasal congestion, which is present in 5% of COVID-19-infected patients and 20% of normal pregnant women in late pregnancy (estrogen induced nasopharyngeal hyperemia) and shortness of breath which is presented in 18% of COVID-19, while most pregnant women have this symptom as a result of increased oxygen needs from higher metabolism, hyperdynamic circulation, physiological anemia, and fetal consumption (Nelson-Piercy, 2015).

Incubation Period

The incubation period for COVID-19 infection is about 14 days after viral exposure. However, in most cases, it is approximately 4–5 days after exposure (Guan et al., 2020a). One study in 1099 reported a median incubation period of 4 days (Guan et al., 2020b). Another study that included 181 confirmed cases in China with clearly identified exposure reported a median incubation period of 5.1 days (2.5% and 97.5% of cases symptomatize within 2.2 and 11.5 days, respectively) (Lauer et al., 2020). A third one conducted on 1084 patients who had traveled or resided in Wuhan reported a median incubation period of 7.8 days (5–10% of cases developed symptoms after 14 or more days after exposure) (Qin et al., 2020b).

Signs and Symptoms

There are no specific clinical characteristics that can reliably distinguish between SARS-CoV-2 and other viral respiratory infections (Struyf et al., 2020). However, some features raise suspicion of COVID-19 infection as development of dyspnea several days after the onset of initial symptoms (Cohen et al., 2020).

Anosmia, myalgia, general malaise, headache, extreme tiredness, and fever were the most linked nonrespiratory manifestations to positive testing for SARS-CoV-2 in one study of healthcare workers (mainly 20- to 40-year-old women) (Tostmann et al., 2020). Other, unusual findings, as new-onset pernio-like lesions, also raise suspicion for COVID-19 infection. However, none of these findings definitively establish the diagnosis without microbiologic testing (Caliendo Angela, 2020).

All pregnant persons should be monitored for development of symptoms and signs of COVID-19 (which are similar to those in nonpregnant individuals), particularly if they have had close contact with a confirmed case or persons under investigation.

Asymptomatic infections are well documented in many studies (World Health Organization, 2020d). However, the proportion of asymptomatic infected patients vary among them. The difference was related to the absence of the longitudinal follow-up to assess for symptom development and the use of different definitions of "asymptomatic" depending on which specific symptoms were assessed in most studies. In one study based on data from three large cohorts that identified cases through population-based testing infections, the prevalence of asymptomatic cases was 30%–40% (Lavezzo et al., 2020; Oran and Topol, 2020). A study reported that 58% of the 712 confirmed COVID-19 cases were asymptomatic at the time of diagnosis

(Sakurai et al., 2020). In another study, among the 1271 cases confirmed to have COVID-19 infection, 88% were asymptomatic at the time of testing and 43% remained asymptomatic throughout the observation period (Kasper et al., 2020). High rates of asymptomatic infection have also been reported among pregnant women presenting for delivery (Campbell et al., 2020; Sutton et al., 2020).

Absence of symptoms does not mean absence of the disease or its objective clinical abnormalities (Hu et al., 2020; Wang et al., 2020c). In one study that included 24 asymptomatic COVID-19 patients, 50% of them had typical ground-glass opacities or patchy shadowing detected in chest computed tomography (CT) examination and another 20% had atypical imaging abnormalities (Hu et al., 2020). In another study of 55 asymptomatic COVID-19 patients, 37 of them (67%) had CT evidence of pneumonia on admission; 2 developed hypoxia; and all were recovered (Wang et al., 2020c).

Many classifications were suggested for COVID-19 infection severity. The National Institutes of Health categorized nonpregnant COVID-19 patients into asymptomatic, mild illness, moderate, severe, and critical illness. Asymptomatic or presymptomatic disease include those having no symptoms but tested positive for SARS-CoV-2. Mild illness includes patients with any signs and symptoms (as fever, cough, sore throat, malaise, headache, muscle pain) without shortness of breath, dyspnea, or abnormal chest imaging. Moderate illness includes patients with evidence of lower respiratory disease by clinical assessment or imaging and $\geq$94% oxygen saturation of on-room air at sea level. Severe illness includes patients with respiratory rate >30 breaths per minute, oxygen saturation <94% on room air at sea level, the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO/FiO) < 300, or lung infiltrates >50%. Critical illness includes patients with respiratory failure, septic shock, and/or multiple organ dysfunction (National Institutes of Health, 2020).

Another classification was suggested by Wu categorized COVID-19 patients into mild, severe, and critical illness. Mild illness includes patients with no or mild symptoms (fever, fatigue, cough, and/or less common features of COVID-19). Severe illness includes patients with tachypnea (respiratory rate >30 breaths per minute), hypoxia (oxygen saturation $\leq$93% on room air or PaO/FiO <300 mmHg), or >50% lung involvement on imaging. Critical illness includes patients with respiratory failure, shock, or multiorgan dysfunction (Wu and McGoogan, 2020).

The Centers for Disease Control and Prevention (CDC) evaluated the prevalence of different symptoms in more than 386,000 nonpregnant and over 23,000 pregnant females. It reported the following.

Cough was present in 50.3% and 51.3% of pregnant and nonpregnant women, respectively (Zambrano et al., 2020). Cough was present in 28% of 55 pregnant women in another study. The latter compared the presence of cough in COVID-19-, SARS-, and MERS-infected pregnant women and found a prevalence of 28%, 76%, and 67%, respectively (Dashraath et al., 2020).

Fever was one of the presenting symptoms in 32% and 39.3% of pregnant and nonpregnant women, respectively, in the CDC report. Dashraath and colleagues reported fever in 84% of COVID-19 patients compared with 100% and 58% of patients infected with SARS and MERS, respectively (Dashraath et al., 2020). Although fever is a common finding in COVID-19 infection, it is not a universal finding at presentation even in hospitalized patients. In one study that included 1099 patients, fever was present in 44% of patients on admission and increased to 89% during hospitalization (Guan et al., 2020a). Another study in 5000 hospitalized COVID-19 patients reported an incidence of 31 for fever (Richardson et al., 2020). Even though the study reported fever in all patients, 20% of them had a low grade fever <100.4°F/38°C (Huang et al., 2020).

Shortness of breath was present in 25.9% and 24.8% of pregnant and nonpregnant women, respectively, in CDC report (Zambrano et al., 2020). Dashraath and colleagues reported dyspnea in 18% of COVID-19 patients compared with 35% and 58% of patients infected with SARS and MERS, respectively (Dashraath et al., 2020).

Smell and taste disorders as anosmia and dysgeusia are common manifestations of COVID-19 infection. New loss of taste or smell were reported by CDC in 21.5% and 24.8% of pregnant and nonpregnant women, respectively (Zambrano et al., 2020). In one metaanalysis, abnormalities in smell were reported in 52%, while taste abnormalities were reported in 44% of patients (Tong et al., 2020). In 202 patients with mild COVID-19 infection, changes in taste and/or smell were reported in 64%, severe alterations were documented in 24%, it was the only symptom in 3%, and it preceded other symptoms in 12% of patients (Spinato et al., 2020). However, the rate of objective smell or taste abnormalities may be lower than the self-reported rates. On objective testing, 38% of patients who reported complete anosmia had a normal smell

function (Lechien et al., 2020). These changes in taste and smell are temporary. In one study, 89% of patients with smell or taste alterations reported complete recovery or improvement within 4 weeks (Boscolo-Rizzo et al., 2020).

Other symptoms in CDC report included the following:

- Headache in 42.7% and 54.9% of pregnant and nonpregnant women, respectively
- Muscle aches in 36.7% and 45.2% of pregnant and nonpregnant women, respectively
- Sore throat in 28.4% and 34.6% of pregnant and nonpregnant women, respectively
- Other symptoms that occurred in >10% of each group included nausea or vomiting, fatigue, diarrhea, and rhinorrhea

Gastrointestinal (GI) symptoms including nausea, vomiting, diarrhea, and abdominal pains are not uncommon in COVID-19 patients. The CDC reported diarrhea in 19% and nausea and/or vomiting in 12% nonpregnant women (Zambrano et al., 2020). In a systematic review, the prevalence of overall GI symptoms was 18%, and the prevalence of diarrhea, nausea/vomiting, and abdominal pain were 13%, 10%, and 9%, respectively (Cheung et al., 2020).

Other manifestations include conjunctivitis (Ma et al., 2020), falls, general health decline, delirium, (especially in elderly and patients with neurocognitive impairments) (Annweiler et al., 2020), and cutaneous manifestations such as maculopapular, urticarial, and vesicular eruptions and transient livedo reticularis (Galván Casas et al., 2020). Sometimes, reddish-purple nodules may appear on the distal digits similar in appearance to pernio (chilblains) (mainly in children and young adults) named by some as COVID toes (de Masson et al., 2020; Galván Casas et al., 2020).

Patients with nonsevere illness may continue with the same grade of the disease or progress to more severe illness. Progression may occur over 7 days (Cohen et al., 2020). One study conducted on 138 hospitalized COVID-19 patients in Wuhan reported development of pneumonia and hospital admission after a median of 5 and 7 days since the onset of symptoms, respectively (Wang et al., 2020a). Another study reported a median time of 8 days between onset of symptoms and development of dyspnea (Huang et al., 2020).

Although pregnancy does not appear to increase the risk for acquiring COVID-19 infection, it appears to worsen the clinical course of the disease when compared with nonpregnant patients of the same sex and age (Allotey et al., 2020; Badr et al., 2020). Clinical deterioration may be rapid. Intensive care unit (ICU) admission was reported in 1%−3% pregnant patients with COVID-19 infection (Centers for Disease Control and Prevention, 2021). Although most (>90%) infected pregnant persons recover spontaneously without hospitalization or delivery, they are at increased risk of death when compared with symptomatic nonpregnant females of reproductive age (Vincenzo Berghella and Hughes, 2020).

COVID-19 Versus Flu

Influenza is a very common disease; it should be differentiated from COVID-19 infection. Both diseases have many similarities and differences. Both COVID-19 and influenza have airborne spread and share common manifestations such as fever, cough, shortness of breath, fatigue, sore throat, rhinorrhea, muscle pains, and headache. Many patients infected with influenza or COVID-19 have mild symptoms and recover spontaneously without hospitalization after rest and fluids or even no treatment at all. However, both infections may have serious sequelae as pneumonia, ARDS, multiple organ failure, stroke, and even death (both morbidity and mortality are higher in COVID-19 compared with influenza) (MayoClinic, 2020).

COVID-19 and influenza have several differences being caused by different viruses (SARS-CoV-2 and influenza A and B viruses). COVID-19 infection is more contagious with longer incubation period (symptoms appear 2−14 days and 1−4 days after exposure to SARS-CoV-2 and influenza virus, respectively). Some symptoms may differ as loss of taste and smell, which is common in COVID-19 not in influenza. COVID-19 may have different complications from flu as hypercoagulability and multisystem inflammatory syndrome in children (MayoClinic, 2020).

Laboratory testing and imaging of COVID-19

A real-time reverse-transcription polymerase chain reaction (rRT-PCR) assay is the current gold standard for diagnosis of SARS-CoV-2 infection. Other laboratory investigations can be used for diagnosis, determination of the possible treatment, evaluation of treatment efficacy, and prognosis of the infection. Chest imaging is of special importance for diagnosis and prognosis of the disease.

Whom to Test

Both symptomatic and asymptomatic individuals need testing for SARS-CoV-2 infection according to the following guidelines.

Asymptomatic individuals

Indications of testing included the following (Center for Disease Control and Prevention, 2020; Infectious Diseases Society of America, 2020b):

- Close contact with a known case (including neonates born to infected mothers). Testing 5–7 days after exposure is suggestive based on the average incubation period (Infectious Diseases Society of America, 2020a).
- Individuals at risk for severe disease living in congregate facilities that (e.g., long-term care facilities, correctional and detention facilities, homeless shelters). They should be screened in response to identified COVID-19 cases within the facility beside routine intermittent screening of employees and residents.
- Hospitalized patients at communities with high prevalence (+ve PCR in ≥10% of the community).
- Before aerosol-generating procedures and time-sensitive surgical procedures.
- Before immunosuppressive therapy.

The CDC recommended against retesting asymptomatic individuals who were previously diagnosed with SARS-CoV-2 within the prior 3 months because of the low likelihood that a repeat positive test during this interval represents an active reinfection.

Symptomatic individuals

Cases with clinical suspicion infections should be tested. Clinical suspicion is high in patients who developed new-onset fever and/or respiratory tract symptoms (e.g., cough, dyspnea), patients with severe lower respiratory tract (LRT) illness without any clear cause, those who developed dyspnea several days after the onset of initial symptoms (Cohen et al., 2020), and patients who present with extrapulmonary complications that may be linked to SARS-CoV-2 infection as cardiac injury, ischemic stroke, and other thromboembolic events (Caliendo Angela, 2020).

Clinicians should have a low threshold for suspicion of COVID-19, and the likelihood is further increased if the patient lives in or is traveled to allocation with high prevalence of COVID-19 infection within the prior 14 days or is in close contact with a suspected or confirmed COVID-19 case in the prior 14 days (Caliendo Angela, 2020).

Many recommendations were described for COVID-19 testing priorities. The Infectious Diseases Society of America (IDSA) categorized priorities for RT-PCR testing (or antigen testing, if available) when testing capacity is limited into four levels (Infectious Diseases Society of America, 2020a).

First (high) priority was suggested for critically ill patients receiving ICU-level care with unexplained viral pneumonia or respiratory failure; individuals having fever or features of an LRT illness and close contact with patients with laboratory-confirmed COVID-19 within 14 days of symptom onset (including all residents of long-term care facilities with a confirmed case); individuals with fever or features of an LRT illness who are also immunosuppressed (including patients with HIV), are older, or have underlying chronic health conditions; and individuals with fever or features of an LRT illness who are critical to the pandemic response, including healthcare workers, public health officials, and other essential leaders.

Second priority was suggested for non-ICU hospitalized patients and long-term care residents with unexplained fever and features of an LRT illness with consideration of the number of confirmed cases in the community.

Third priority for outpatients who meet criteria for influenza testing (e.g., symptoms such as fever, cough, and other suggestive respiratory symptoms plus comorbid conditions, such as diabetes mellitus, chronic obstructive pulmonary disease, congestive heart failure, age >50 years, immunocompromising conditions) and testing of outpatient pregnant women and symptomatic children with similar risk factors is also included in this priority level with consideration of the number of confirmed cases in the community.

Fourth priority for community surveillance is directed by public health and/or infectious diseases authorities.

The WHO suggested that in the presence of limited resources in areas with community transmission, prioritization for testing should be given to people at risk of developing severe disease and vulnerable populations, who will require hospitalization and advanced care for COVID-19 health workers (including emergency services and nonclinical staff) regardless of whether they are a contact of a confirmed case (to protect health workers and reduce the risk of nosocomial transmission), the first symptomatic individuals in a closed setting (e.g., schools, long-term living facilities, prisons, hospitals) to quickly identify outbreaks and ensure containment measures. All other individuals with symptoms related to the close settings may be considered probable cases and isolated without additional testing if testing capacity is limited (World Health Organization, 2020f).

Diagnostic testing of SARS-CoV-2 is done through detection of the virus itself (viral RNA or antigen) or detection of the human immune response to the virus

(antibodies or other biomarkers). Standard infection confirmation is based on the detection of unique viral sequences by nucleic acid amplification tests (NAATs), such as rRT-PCR. The assays' targets include regions on the E, RdRP, N, and S genes (World Health Organization, 2020e).

The virus may be detectable in the upper respiratory tract (URT) 1−3 days before the onset of symptoms. The concentration of SARS-CoV-2 in the URT is highest around the time of symptom onset and then gradually decreases (Zou et al., 2020). There is a controversy about the association between virus loads and severity of infection (Lavezzo et al., 2020). The presence of viral RNA in the LRT, and for a subset of individuals in the feces, increases during the second week of illness (Weiss et al., 2020). The viral RNA detection is not consistent among individuals. The period of detection may be several days, weeks, or months (Li et al., 2020b). Prolonged presence of viral RNA does not necessarily signify prolonged infectiousness.

The optimum site for obtaining the specimen is dependent on the clinical presentation and interval since symptom onset. Specimens include respiratory, fecal, serum, semen, ocular fluid, and others.

Respiratory Specimen

These include upper and lower respiratory specimens. The variability of respiratory secretion composition and the adequacy of sampling efforts may result in false-negative PCR results (Tang et al., 2020).

Upper respiratory specimens are adequate for testing the early stage of infections, especially in asymptomatic or mild cases. Combined nasopharyngeal and oropharyngeal swabs increase the sensitivity for detection of respiratory viruses and improve the reliability of the result (Sutjipto et al., 2020). Two individual swabs can be combined in one collection tube, or a combined nasopharyngeal and oropharyngeal swab can be taken (Lieberman et al., 2010). A few studies have found that individual nasopharyngeal swabs yield a more reliable result than oropharyngeal swabs (Sutjipto et al., 2020).

Lower respiratory specimens are recommended during the delayed course of the disease and in patients with a negative upper respiratory sampling with strong clinical suspicion of COVID-19 (Liu et al., 2020). LRT specimens can consist of spontaneously produced sputum (induced sputum is not recommended due to high risk of aerosol transmission (World Health Organization, 2020b)) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. Caution should be exercised due to the high risk of aerosolization; therefore, strict adherence to IPC procedures during sample collection is required. The indication for an invasive procedure should be evaluated by a physician.

Fecal Specimens

Feces or rectal swabs have been shown to be positive for SARS-CoV-2 RNA from the second week after symptom onset and onward. It can be considered if both upper and lower respiratory specimens were negative along with high suspicion of infection (Ng et al., 2020). When testing faces, ensure that the intended extraction method and NAAT has been validated for this type of sample. Some suggested that fecal positivity for SARS-CoV-2 is prolonged compared with that of respiratory tract specimens (Wong et al., 2020).

Serum Specimens

If negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2−4 weeks later can be used to look for seroconversion or a rise in antibody titers. These two samples can be used retrospectively to determine whether the individual has had COVID-19, especially when the infection could not be detected using NAAT. Some studies linked the detection of virus in blood to the severity of the disease (Corman et al., 2020a,b).

Other specimens include oral fluid specimens (with variable detection rate compared with upper respiratory) (Williams et al., 2020), gargling/mouth washes (have limited data) (Guo et al., 2020b), ocular fluids (in patients with and without conjunctivitis) (Zhang et al., 2020b), urine (only in a limited number of patients) (Nomoto et al., 2020), semen (Li et al., 2020a), and cerebrospinal fluid (Moriguchi et al., 2020). Thus, SARS-CoV-2 can be detected in a wide range of other body fluids and compartments, but it is most frequently detected in respiratory material, and therefore, respiratory samples remain the sample type of choice for diagnosis of SARS-CoV-2. Postmortem specimens can be obtained through postmortem swab, needle biopsy, or tissue specimens from the autopsy, including lung tissue for further pathological and microbiological testing (Tian et al., 2020).

Specimens for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation and in the laboratory is essential.

SARS-CoV-2 infections should be tested with NAAT. A rRT-PCR assay is the current gold standard for

SARS-CoV-2 detection. The test uses specific primers and probes that target the RNA-dependent RNA polymerase (RdRp), envelope, and nucleocapsid genes of SARS-CoV-2, among which the RdRp assay has the highest analytical sensitivity (3.8 RNA copies/reaction at 95% detection probability) (Corman et al., 2020a,b).

Optimal diagnostics consist of a NAAT assay with at least two independent targets on the SARS-CoV-2 genome. When using a one-target assay, it is recommended to have a strategy in place to monitor for mutations that might affect performance (World Health Organization, 2020e).

RT-PCR is a quantitative method in which the amplification of DNA is detected in real time. It can detect the viral load. However, this usually requires laboratories to develop in-house test kits and to validate them with internal controls (Pan et al., 2020).

The practical limitations of RT-PCR testing include the need for a biosafety level-2 (BSL-2) facility, a requirement for kits with specific reagents and primers, the need to maintain a cold chain (as the specimens require storage at $2-8°C$), and the use of strict, validated protocols for testing; consequently, countries with resource limitations or acute spikes in the number of suspected cases may not be able to meet these demands. However, there are no good alternatives: antigen_antibody detection tests are not validated, and viral culture is impractical, as it takes at least 3 days for SARS-CoV-2 to cause cytopathic effects in selected cell lines (VeroE6 and Huh7 cells) (Zhou et al., 2020c). In addition, viral culture will require a BSL-3 facility, which is usually found only in tertiary medical or university research centers (Dashraath et al., 2020).

Pooling of Specimens for Nucleic Acid Amplification Test

Pooling of samples from multiple individuals can increase the diagnostic capacity for detecting SARS-CoV-2 when the rate of testing does not meet the demand in some settings (Abdalhamid et al., 2020). One strategy for pooling stated that if the pooled result is negative, all individual specimens in the pool are regarded as negative. If the pool test is positive, the follow-up steps depend on the strategy, but, in general, each specimen needs to undergo individual testing (pool deconvolution) to identify the positive specimen(s). Another strategy is matrix pooling in which the pools are made per row and per column, and tested by PCR; the position in the matrix identifies the positive specimen without additional testing if prevalence is sufficiently low. Depending on how robust the matrix testing method is in the specific context, it might still be advisable to retest the identified positive samples for confirmation. Pooling of specimens could be considered in population groups with a low/very low expected prevalence of SARS-CoV-2 infection, but not for cases or cohorts that are more likely to be infected with SARS-CoV-2. Routine use of the pooling of specimens from multiple individuals in clinical care and for contact tracing purposes is not recommended (World Health Organization, 2020e).

To perform reliable pooling, adequate automation is key (e.g., robotic systems, software supporting the algorithms to identify positive samples, laboratory information systems, and middleware that can work with sample pooling). Based on currently available data, intraindividual pooling (multiple specimens from one individual that are pooled and tested as a single sample) from URT samples can be used. Intraindividual pooling of sputum and feces with URT samples is not recommended because the former may contain compounds that inhibit rRT-PCR (World Health Organization, 2020e).

Sensitivity of the test depends on the type and quality of the specimen obtained, the time of testing in relation to the course of the disease, and the specific assay:

Test performance by specimen type

LRT specimens are more likely to yield positive tests than URT specimens as they may have higher viral loads. In one study of 205 COVID-19 patients, the highest rates of positive viral RNA tests were reported from bronchoalveolar lavage (95%, 14 of 15 specimens) and sputum (72%, 72 of 104 specimens), compared with oropharyngeal swab (32%, 126 of 398 specimens) (Wang et al., 2020b). Some studies have suggested that viral RNA levels are higher and more frequently detected in nasal compared with oropharyngeal specimens (Kujawski et al., 2020; Wang et al., 2020b).

Self-collected specimens can reduce the need for personal protective equipment with relatively good accuracy with certain self-collected specimens (i.e., nasal swabs and saliva specimens). The sensitivity of NAAT with self-collected nasal or nasal midturbinate specimens may be similar to that with nasopharyngeal specimens collected by a healthcare provider (Tu et al., 2020).

Studies suggest that the relative sensitivity of self-collected saliva specimens compared with nasopharyngeal specimens is 85% or higher (Wyllie et al., 2020). In one study of 70 patients hospitalized with COVID-19 who underwent repeat testing every few days, RT-PCR testing of first-morning saliva (enough to fill one-third of a sterile cup) yielded on average one-half log higher

viral RNA levels than that of nasopharyngeal specimens and was more frequently positive in the first 10 days (Wyllie et al., 2020).

Test performance by illness duration: The detection of SARS-CoV-2 RNA is variable among the course of the disease (Zhou et al., 2020c; Guo et al., 2020a,b). Analysis of seven studies reported that estimated rates of false-negative results were 100%, 38% , 20%, and 66% on the day of exposure, day 5 (the first day of symptoms), day 8, and day 21, respectively (Kucirka et al., 2020). Heterogeneity across studies and assumptions made in the analysis (e.g., about incubation period and time of exposure) reduce confidence in these results. Other studies have also suggested that viral RNA levels are high prior to the development of symptoms (Furukawa et al., 2020).

Test performance by assay type: There are also differences in the limit of detection among the major commercial NAAT assays, and retesting samples on different platforms may yield conflicting results (Nalla et al., 2020). Additionally, point-of-care NAAT assays may not be as sensitive as laboratory-based tests (Dinnes et al., 2020). In an August 2020 systematic review of 11 studies evaluating four rapid, point-of-care molecular tests using confirmed SARS-CoV-2 samples, sensitivity ranged from 68% to 100%, with an average of 95.2% (Dinnes et al., 2020). However, most studies were judged to be at high risk of bias.

Cycle threshold: The cycle threshold refers to the number of cycles in an RT-PCR assay needed to amplify viral RNA to reach a detectable level. The cycle threshold value indicates the relative viral RNA level in a specimen (with lower Ct values reflective of higher viral levels). Resulting laboratories generally do not provide the cycle threshold value with the qualitative NAAT result, although it can be obtained upon request for some testing platforms. However, the clinical application of the cycle threshold is uncertain. Ct values are not standardized across RT-PCR platforms, so results cannot be compared across different tests (Caliendo Angela, 2020).

Test interpretation and additional testing (Caliendo Angela, 2020).

Positive NAAT result confirms the diagnosis of SARS-CoV-2 infections without any need for additional diagnostic testing (additional testing may be required for management in hospitalized patients). Some patients may have positive NAATs following documented viral RNA clearance. It is unknown whether this finding indicated relapse or recurrent infection.

Negative NAAT result: In most cases, a single negative NAAT excludes the diagnosis of SARS-CoV-2 infection.

In case of high suspicion of SARS-CoV-2 infection and confirming the presence of infection is important for management or infection control and the initial testing was negative, it is suggested to repeat the test 24−48 h after the initial test. Repeat testing within 24 h is not recommended.

The IDSA and WHO recommend to reserve LRT specimen NAAT for hospitalized patients who have an initial negative test on an URT specimen but for whom suspicion for LRT SARS-CoV-2 infection remains.

For patients who present 3−4 weeks into the course of illness and have negative NAAT, checking a serologic test may be informative.

In many cases, because of the limited availability of testing and concern for false-negative results, the diagnosis of COVID-19 is made presumptively based on a compatible clinical presentation in the setting of an exposure risk (residence in or travel to an area with widespread community transmission or known contact) in the absence of other identifiable causes.

False-negative tests can occur in the following conditions:
- Poor quality of the specimen with insufficient material
- Late collection of the specimen.
- The specimen was obtained from a body compartment that did not contain the virus at that given time
- Inappropriate handling and/or shipping of the specimen
- Technical reasons inherent in the test, e.g., PCR inhibition or virus mutation

Indeterminate NAAT result: The interpretation of an inconclusive or indeterminate result depends on the specific NAAT performed; the clinician should confer with the performing laboratory about additional testing.

In some cases, an inconclusive or indeterminate result indicates that only one of the two or more genes that the NAAT test targets was identified. These results can be considered presumptive positive results, given the high specificity of NAAT assays. If the patient is early in the disease course, repeat testing can be helpful to confirm.

Rapid Diagnostic Tests Based on Antigen Detection

Rapid diagnostic tests (RDTs) that detect the presence of SARS-CoV-2 viral proteins (antigens) in respiratory tract specimens are being developed and commercialized. Most of these are lateral flow immunoassays (LFIs), which are typically completed within 30 min. In

contrast to NAATs, there is no amplification of the target that is detected, making antigen tests less sensitive. Additionally, false-positive results may occur if the antibodies on the test strip also recognize antigens of viruses other than SARS-CoV-2, such as other human coronaviruses. The sensitivity of different RDTs compared with rRT-PCR in specimens from URT (nasopharyngeal swabs) appears to be highly variable (Dinnes et al., 2020; Porte et al., 2020), but specificity is consistently reported to be high. Currently, data on antigen performance in the clinical setting are still limited: paired NAAT and antigen validations in clinical studies are encouraged to identify which of the antigen detection tests that are either under development or have already been commercialized demonstrate acceptable performance in representative field studies. When performance is acceptable, antigen RDTs could be implemented in a diagnostic algorithm to reduce the number of molecular tests that need to be performed and to support rapid identification and management of COVID-19 cases. How antigen detection would be incorporated into the testing algorithm depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS-CoV-2 infection in the intended testing population. Higher viral loads are associated with improved antigen test performance; therefore, test performance is expected to be best around symptom onset and in the initial phase of a SARS-CoV-2 infection (World Health Organization, 2020e).

WHO recommended against the use of SARS-CoV-2 Ag-RDTs in (1) asymptomatic individuals unless he had contact with a confirmed case as pretest probability is low; (2) communities with sporadic cases as positive test results would likely be false positives; molecular testing is preferred; (3) if patient management is not dependent on test results; (4) airport or border screening at points of entry as disease prevalence is highly variable among travelers and both positive and negative tests would require confirmatory testing to increase positive predictive value and negative predictive value for decision-making; and (5) screening before blood donation as positive result would not necessarily correlate with presence of viremia. Asymptomatic blood donors do not meet the definition of a suspect case (World Health Organization, 2020a).

Antibody Testing

Serological assays that detect antibodies produced by the human body in response to infection with the SARS-CoV-2 can be useful in various settings. For example, serosurveillance studies can be used to

support the investigation of an ongoing outbreak and to support the retrospective assessment of the attack rate or the size of an outbreak (World Health Organization, 2020c). Commercial and noncommercial tests measuring binding antibodies (total immunoglobulins [Ig], IgG, IgM, and/or IgA in different combinations) utilizing various techniques including LFI, enzyme-linked immunosorbent assay (ELISA), and chemiluminescence immunoassay have become available. As SARS-CoV-2 is a novel pathogen, our understanding of the antibody responses it engenders is still emerging, and therefore, antibody detection tests should be used with caution and not used to determine acute infections. Nonquantitative assays (e.g., lateral flow assays) cannot detect an increase in antibody titers, in contrast to (semi)quantitative or quantitative assays. Lateral flow antibody detection assays (or other nonquantitative assays) are currently not recommended for acute diagnosis and clinical management, and their role in epidemiologic surveys is being studied. Serology should not be used as a stand-alone diagnostic to identify acute cases in clinical care or for contact tracing purposes. Interpretations should be made by an expert and are dependent on several factors including the timing of the disease, clinical morbidity, the epidemiology and prevalence within the setting, the type of test used, the validation method, and the reliability of the results. Seroconversion (development of measurable antibody response after infection) has been observed to be more robust and faster in patients with severe disease compared with those with milder disease or asymptomatic infections. Antibodies have been detected as early as in the end of the first week of illness in a fraction of patients but can also take weeks to develop in patients with subclinical/mild infection (Deeks et al., 2020; Okba et al., 2020; Zhao et al., 2020). A reliable diagnosis of COVID-19 infection based on patients' antibody response will often only be possible in the recovery phase, when opportunities for clinical intervention or interruption of disease transmission have passed. Therefore, serology is not a suitable replacement for virological assays to inform contact tracing or clinical management. The duration of the persistence of antibodies generated in response to SARS-CoV-2 is still under study (Seow et al., 2020). Furthermore, the presence of antibodies that bind to SARS-CoV-2 does not guarantee that they are neutralizing antibodies or that they offer protective immunity.

The predictive ability of serologic assays varies widely according to the severity of the disease, timing of the test, the target viral protein, and characteristics of the studied individual (e.g., young or old). Antibody

detection tests for coronavirus may also cross-react with other pathogens, including other human coronaviruses (Okba et al., 2020; Gorse et al., 2020), or with preexisting conditions (e.g., pregnancy, autoimmune diseases), and thus yield false-positive results. Virus neutralization assays are the gold standard test for detecting the presence of functional antibodies. These tests require highly skilled staff and BSL-3 culture facilities and, therefore, are unsuitable for use in routine diagnostic testing. Diagnostic testing for SARS-CoV-2: Implementation and interpretation of antibody testing in the clinical laboratory. When implementing serological assays in the clinical laboratory, an in-house validation or verification of the specific assays is advisable. Even if commercial tests have been authorized for use in emergencies, an in-house verification (or if required by local authorities a validation) is still required. Protocols and examples with suggestions as to how to do this are now available (Theel et al., 2020). Each serological test is different. With regard to commercial tests, follow the manufacturer's instructions for use. Studies show that several commercial assays measuring total Ig or IgG have performed well. Most of these studies show no advantage of IgM over IgG, as IgM does not appear much earlier than IgG (Deeks et al., 2020). The additional role of IgA testing in routine diagnostics has not been established. For confirmation of a recent infection, acute and convalescent sera must be tested using a validated (semi)quantitative or quantitative assay. The first sample should be collected during the acute phase of illness, and the second sample should be collected at least 14 days after the initial sera were collected. Maximum antibody levels are expected to occur in the third/fourth week after symptom onset. Seroconversion or a rise in antibody titers in paired sera will help to confirm whether the infection is recent and/or acute. If the initial sample test is positive, this result could be due to a past infection that is not related to the current illness. The first known case of reinfection with SARS-CoV-2 has been documented [182]. Only limited information is available on the interpretation of SARS-CoV-2 antibody tests after a previous infection with SARS-CoV-2 and on the dynamics of SARS-CoV-2 serology if a subsequent infection with another coronavirus occurs. In these two sets of circumstances, interpretation of serology may be extremely challenging (World Health Organization, 2020e).

Viral Isolation

Virus isolation is not recommended as a routine diagnostic procedure. All procedures involving viral isolation in cell culture require trained staff and BSL-3 facilities.

A thorough risk assessment should be carried out when culturing specimens from potential SARSCoV-2 patients for other respiratory viruses because SARS-CoV-2 has been shown to grow on a variety of cell lines (Chu et al., 2020).

Genomic Sequencing for SARS-CoV-2

Genomic sequencing for SARS-CoV-2 can be used to investigate the dynamics of the outbreak, including changes in the size of an epidemic over time, its spatiotemporal spread, and testing hypotheses about transmission routes. In addition, genomic sequences can be used to decide which diagnostic assays, drugs, and vaccines may be suitable candidates for further exploration. Analysis of SARS-CoV-2 virus genomes can, therefore, complement, augment, and support strategies to reduce the disease burden of COVID19. However, the potentially high cost and volume of the work required for genomic sequencing means that laboratories should have clarity about the expected returns from such investment and what is required to maximize the utility of such genomic sequence data. WHO guidance on SARS-CoV-2 genomic sequencing is currently being developed (World Health Organization, 2020e).

Pathogen Detection in Secondary Infection

Patients with COVID-19 infection are at high risk for catching secondary bacterial and/or fungal infections especially in those having the severe form of the disease. Microbiological examination should be considered in these cases. The site of the obtained specimen for microbiological examination differs according to clinical suspicion. Sites include respiratory secretions, blood or serum, GIT secretions, cerebrospinal fluid, and others.

The elevation of C-reactive protein (CRP) has poor specificity for the diagnosis of secondary infection. Elevated procalcitonin levels are of great significance for clinical diagnosis of sepsis. In a cohort study that included 731 hospitalized COVID-19 patients, secondary bacterial infection was diagnosed in 9.3%, 7.9% had one or more secondary bloodstream infections (BSIs), and 3% had at least one possible lower respiratory tract infections (pLRTIs). The overall 28-day cumulative incidence was 16.4% (95% CI: 12.4%−21.0%). Most of the BSIs were due to gram-positive pathogens (76/106 isolates, 71.7%), specifically coagulase-negative staphylococci (53/76%, 69.7%), while among gram-negatives (23/106%, 21.7%) *Acinetobacter baumanii* (7/23%, 30.4%) and *Escherichia coli* (5/23%, 21.7%) predominated. pLRTIs were caused mainly by gram-negative pathogens (14/26%, 53.8%). Eleven patients were diagnosed with putative invasive aspergillosis. At multivariable

analysis, factors associated with secondary infections were low baseline lymphocyte count ($\leq$0.7 vs. >0.7 per 10^9/L, subdistribution hazard ratios (sdHRs) 1.93, 95% CI: 1.11−3.35), baseline PaO_2/FiO_2 (per 100 points lower: sdHRs 1.56, 95% CI: 1.21−2.04), and ICU admission in the first 48 h (sdHR 2.51, 95% CI: 1.04−6.05). They concluded that patients hospitalized with COVID-19 had a high incidence of secondary infections and identified the early need for ICU, respiratory failure, and severe lymphopenia as risk factors for secondary infections (Ripa et al., 2021).

In case of the suspected fungal infection, in addition to fungal culture, G test, and GM test, Cryptococcus antigen detection can also be performed.

Laboratory Investigations
Total leukocytic count
At the onset of the disease, the total peripheral blood leukocyte is normal or decreased, and the lymphocyte count is decreased. Patients with a lower absolute lymphocyte value generally have a poor prognosis, and peripheral blood lymphocytes in critical patients show a progressive decrease. Elevated neutrophil to lymphocyte ratio (NLR) is an independent risk factor affecting the occurrence of severe illness (Liu et al., 2020b).

A retrospective study found several differences in WCC between severe and nonsevere COVID-19 patients (Qin et al., 2020a). Both groups experienced an increase in leukocytes with the severe group having a significantly greater rise (5.6 vs. 4.9 × 109/L; P < .001). Neutrophils were predominantly driving this increase as the severe set (4.3 vs. 3.2 × 109/L; P < .001). Interestingly, the levels of lymphocytes, monocytes, basophils, and eosinophils were less, resulting in a greater neutrophil-to-lymphocyte ratio (NLR; 5.5 vs. 3.2; P < .001). Another study conducted in China concludes similar findings of high neutrophil and low LC count in severely affected patients, suggesting NLR could be a potential biomarker for early detection of severe COVID-19 (Wang et al., 2020a). However, other factors may disrupt the accuracy of the WCC results observed. These include glucocorticoid therapy and other underlying viral/bacterial infections (Yip et al., 2005). A descriptive study in China reported depleted lymphocytes levels in the majority of COVID-19 patients (Chen et al., 2020). Another study has found low blood lymphocyte percentage (LYM%) in critically ill patients, suggesting low lymphocytes count indicates poor prognosis. However, since the virus can target lymphoid tissue and mechanisms of IL-6, other causes of low lymphocytes count must be investigated (Tan et al., 2020b).

Similar to NLR, the clinical benefits of LC count as a biomarker for COVID-19 remain uncertain (Kermali et al., 2020).

Platelet Count
A metaanalysis of 1799 patients reveals those with severe COVID-19 infection had significantly lower platelet counts (WMD −31 × 109/L; 95% CI: −35 to −29 × 109/L) (Lippi et al., 2020). When using mortality as an endpoint, nonsurvivors evidently had a significantly lower platelet count (WMD, −48 × 109/L; 95% CI: −57 to −39 × 109/L). Using thrombocytopenia as an endpoint also revealed a fivefold greater risk of COVID-19 (OR, 5.13; 95% CI: 1.81−14.58). A retrospective study that used Cox proportional hazard regression analysis found that platelet count is an independent risk factor for mortality among COVID-19 patients, where a 50 × 109/L increase is associated with 40% deceased mortality (HR 0.60, 95% CI: 0.43, 0.84) (Liu et al., 2020d). Here, thrombocytopenia at admission was more likely to occur in nonsurvivors than in survivors. Although many risk factors were accounted for in this study, the possibility for unmeasured confounder cannot be excluded. Another study corroborates the previously documented work. The nadir platelet count was significantly associated with mortality—and the lower the nadir, the stronger the association (Yang et al., 2020). Again, thrombocytopenia was more likely to occur in nonsurvivors than survivors. This study is from adequate sample sizes providing statistical power; however, similar to the previous studies, they are all retrospective making the correlation seen difficult to extrapolate from. Testing the platelet count is a routine part of laboratory tests, and the literature suggests it has inherent value in providing more detail on the patient's condition (Kermali et al., 2020).

Elevated C-Reactive Protein
Elevation of CRP (plasma protein produced by the liver) occurs in various inflammatory conditions. A rise in CRP level is associated with an increase in disease severity. In a retrospective study, most patients with severe COVID-19 infection showed significantly higher levels compared with the nonsevere cohort (57.9 mg/L vs. 33.2 mg/L, P < .001) (Qin et al., 2020a). A second retrospective cohort study found the likelihood of progressing to severe COVID-19 disease increased in patients with CRP levels >41.8 mg/L (Liu et al., 2020a). CRP is suggested as one of the first biomarkers within blood plasma that changes to reflect physiological complications. Compared with erythrocyte sedimentation rate (ESR), CRP levels are significantly greater during

early periods of severe cases and proved to be a more sensitive biomarker in reflecting disease development. Compared with CT scans alone, CRP values are more reliable for earlier identification of case severity (Tan et al., 2020a).

Interleukin-6

Cytokine release syndrome (CRS) is an overexaggerated immune response involving an overwhelming release of proinflammatory mediators. This mechanism underlies several pathological processes including ARDS (Mahajan et al., 2019). CRS was linked to disease severity in SARS and MERS infections (Mahallawi et al., 2018). Understanding their role in COVID-19 disease may help facilitate the design of novel immunotherapies. Studies have revealed that levels of IL-6, the most common type of cytokine released by activated macrophages, rise sharply in severe manifestations of COVID-19 (Chen et al., 2020). One metaanalysis reviewing six studies shows mean IL-6 concentrations were 2.9-fold higher in patients with complicated COVID-19 compared with those with noncomplicated disease (n = 1302; 95% CI: 1.17−7.19) (Coomes and Haghbayan, 2020). In its analysis, the outcomes of the studies include ICU admission, onset of ARDS, and mortality. Since the proportionate rise of IL-6 is correlated with disease severity, this study can prove groundbreaking. Although clinicians can use this to identify severity earlier and commence oxygen therapy sooner, the varying outcomes make it somewhat difficult to ascertain what level of IL-6 corresponds to what negative outcome. Furthermore, many studies recruited participants from the same center, giving rise to the potential of selection bias (Kermali et al., 2020).

Indicators reflecting body's inflammation and immune status, such as CRP, procalcitonin, ferritin, erythrocyte sedimentation, total lymphocytes and subpopulations, IL-6, and blood lactic acid, can facilitate the clinical stages judgment, which can be used as a clinical warning indicator for severe and critical cases, and provide a basis for the formulation of treatment strategies (National Health Commission & State Administration of Traditional Chinese Medicine, 2020). Normal procalcitonin and significantly elevated ESR and CRP are seen in most patients. There are significant elevation of IL-6 and IL-10 expressions in patients with severe disease and significant reduction of the numbers of CD8+ T lymphocytes. Serial measures of IL-6, IL-10, and CD8+ T lymphocyte levels can be used in the assessment of prognosis of the severity of the disease (Liu et al., 2020b).

Lactate Dehydrogenase

Lactate dehydrogenase (LDH) secretion is triggered by necrosis of the cell membrane, signaling viral infection or lung damage, such as the pneumonia induced by SARS-CoV-2 (Han et al., 2020). There is convincing evidence linking LDH levels to the development of COVID-19 disease (Ferrari et al., 2020). A multicenter study involving 1099 patients reported supporting evidence correlating extent of tissue damage and inflammation with increasing levels of LDH (Guan et al., 2020b). Furthermore, when LDH levels were correlated with CT scans, significantly higher levels reflected the severity of pneumonia (Xiong et al., 2020). There is increasing confidence in using LDH as a biomarker to measure severity of COVID-19 infection. Another study found that there was a significant rise in LDH levels among refractory COVID-19 patients (Kermali et al., 2020).

D-Dimer

D-dimer is released in the blood as a result of lysis of cross-linked fibrin, and its increasing levels indicate activation of coagulation and fibrinolysis (Zhang et al., 2018). A retrospective study was conducted on 191 COVID-19 patients and found that D-dimer levels >1.0 μg/mL ($P = .0033$) were associated with higher mortality, and levels ≥2.0 μg/mL on admission were the optimum cutoff to predict in-hospital mortality for COVID-19 (Zhang et al., 2020a). Studies have reported that nearly 90% of inpatients with pneumonia had increased coagulation activity marked rising D-dimer levels (Milbrandt et al., 2009). Furthermore, Huang et al. found that levels of D-dimer on admission could be used to triage patients into critical care (Huang et al., 2020). The researchers found that median D-dimer levels were higher in ICU patients compared with non-ICU patients (2.4 mg/L vs. 0.5 mg/L; $P = .0042$). D-dimer levels can be used as a prognostic marker and help clinicians monitor those who are likely to deteriorate earlier. However, this study confirmed the diagnosis of COVID-19 using LRT specimens and did not use paired nasopharyngeal swabs to investigate the viral RNA detection rate between the URT and LRT specimens. Secondly, with a cohort size of 41 patients, it is difficult to assess predictors of disease severity and mortality with multivariable-adjusted methods (Kermali et al., 2020).

Cardiac Troponin

There is growing evidence of higher mortality rates among those with underlying cardiovascular disease due to COVID-19 infection [22,52,53]. Some have

investigated the use of high-sensitivity cardiac troponin I (hs-TnI) as a marker of disease progression and mortality. A retrospective study performed in China of patients with confirmed COVID-19 based on SARS-CoV-2 RNA detection revealed a univariable odds ratio for death at 80.1 (95% CI: 10.3−620.4, $P < .0001$) for hs-TnI (Zhou et al., 2020a). This risk was higher compared with other biomarkers such as D-dimer and lymphocyte count. Another study of 416 hospitalized patients with COVID-19 reported that hs-TnI was elevated in one in five patients on presentation (Shi et al., 2020). These patients were more likely to require invasive (22% vs. 4%, $P < .001$) or noninvasive (46% vs. 4%, $P < .001$) ventilation and develop ARDS (59% vs. 15%, $P < .001$) or acute kidney injury (9% vs. 0%, $P < .001$). Early recognition of myocardial injury indicated by elevated hs-TnI aids in appropriate triage to a critical care area and informs the use of inotropes and vasopressors. However, elevated levels are common in hospitalized patients and are likely to be due to nonischemic causes of myocardial injury. This may lead to inappropriate use of cardiology consultation and downstream testing and increased risk to cardiac physiology staff (Kermali et al., 2020).

Renal Markers

There is also evidence that chronic kidney disease is associated with severe forms of COVID-19 infection (Henry and Lippi, 2020). Studies have demonstrated significantly higher levels of renal biomarkers such as serum urea, creatinine, and markers of glomerular filtration rate in severe cases (Xiang et al., 2020). A study of 701 patients revealed that elevated serum creatinine levels on admission correlated with severity due to significant abnormalities in the coagulation pathway (Xiang et al., 2020). They also found that these patients were more likely to require mechanical ventilation or be placed in ICU. Univariate Cox regression analysis found elevated creatinine levels were also associated with in-hospital mortality (HR 2.99, 95% CI: 2.00, 4.47). Proteinuria, hematuria, and elevated urea levels had similar, if not larger, hazard ratios. Interestingly, another study showed a potential role for urinalysis over serum markers of kidney function (Zhou et al., 2020b). Here, abnormalities in the routine urine test on admission correlated strongly with disease severity. They go on to suggest that urinalysis may reveal kidney impairment more readily than evaluation of serum renal biomarkers. However, these tests were only carried out on admission and so patients in earlier stages of the infection had changes in serum levels obscured by compensatory kidney function. Hence, renal abnormalities on admission may indicate higher risks of deterioration, ensuring appropriate triaging (Kermali et al., 2020).

Elevated alanine aminotransferase, aspartate aminotransferase, LDH, phosphocreatine kinase (CK), and myoglobin (Mb) in COVID-19 patients are suggestive of multiple organ dysfunctions.

The WHO reported that the laboratory features associated with severe COVID-19 infection include the increased levels of D-dimer >1000 ng/mL, CRP >100 mg/L, LDH >245 units/L, troponin >2× the upper limit of normal, ferritin >500 mcg/L, CPK >2× the upper limit of normal, and decreased absolute lymphocyte count <800/mL (Guan et al., 2020a; World Health Organization, 2020f).

A systematic review of pregnant women with suspected or confirmed COVID-19 revealed the occurrence of lymphopenia in 35%, leukocytosis in 27%, elevated procalcitonin level in 21%, and abnormal liver chemistries in 11% (Allotey et al., 2020).

Screening for Disseminated Intravascular Coagulation

DIC is a clinical syndrome in which pathogenic factors damage the microvascular system, leading to activation of coagulation cascade, systemic formation of microvascular thrombosis, massive consumption coagulopathy, and secondary hyperfibrinolysis.

The diagnosis of DIC depends on clinical characteristics and laboratory findings. Clinically, the patient has bleeding tendency from multiple sites. However, it rarely happens to the critical ill COVID-19 patient in whom microcirculatory disturbances and multiple organ failure are commonly observed. The specific manifestations included progressive and rapid deterioration of pulmonary functions, deterioration of the liver and kidney functions, disturbed conscious level, myocardial damage, and shock, which cannot be explained by other causes. No single indicator can diagnose DIC. The value of laboratory parameters for the early warning on COVID-19 with DIC varies. The sensitivities of various tests arranged in decreasing pattern include increased D-dimer, thrombocytopenia, prolonged prothrombin time, decreased fibrinogen, and prolonged activated partial thromboplastin time. Their values regarding specificities are arranged as follows: progressive decreases in fibrinogen > progressive decreases in platelets > prolonged APTT > increased D-dimer (Mei and Hu, 2020).

Blood Gas Analysis

Blood gas analysis includes the measurement of the pH, partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), and electrolyte concentration in the blood. It provides references for a quick judgment on the presence of respiratory dysfunction and acid—base imbalance in COVID-19 patients. It is used for assessing the severity of the disease and helps to guide the prognosis and treatment of patients with COVID-19 (Dukić et al., 2016). The radial artery is the preferred site for sample collection, followed by the brachial artery, dorsalis pedis artery, and femoral artery. The blood should be sampled when the patient is rested and quiet to prevent over breathing or breathe holding; if the body temperature is not easy to control, it is necessary to input the body temperature value for correction during the test. If the patient's oxygen administration mode changes, a stable oxygen state shall be ensured for at least 20—30 min before blood collection, and the oxygen inhalation parameters shall be input during the test to ensure the accuracy of the test results (Dukić et al., 2016).

Chest Imaging

Chest imaging has been considered as part of the diagnostic workup of patients with suspected COVID-19 (Manna et al., 2020). Imaging has been also considered to complement clinical evaluation and laboratory parameters in the management of patients already diagnosed with COVID-19 (World Health Organization, 2020b). or the use of chest imaging in acute care of adult patients with suspected, probable, or confirmed COVID-19, including chest radiography, CT, and lung ultrasound.

The WHO provided the following recommendations for the use of chest imaging in COVID-19 patients (World Health Organization, 2020g):

1. Chest imaging is not recommended for the diagnosis of COVID-19 in asymptomatic contacts of patients with COVID-19. RT-PCR should be done to confirm diagnosis.
2. Chest imaging is not recommended for the diagnosis of COVID-19 in symptomatic patients with suspected COVID-19 infection when RT-PCR testing is available with timely results. RT-PCR should be done to confirm diagnosis.
3. Chest imaging for diagnosis of COVID-19 in symptomatic patients with suspected COVID-19 infection is used when RT-PCR testing is not available, but results are delayed or initial RT-PCR testing is negative, but with high clinical of suspicion of COVID-19.
4. For nonhospitalized patients with suspected or confirmed COVID-19 with mild symptoms, the WHO suggests using chest imaging in addition to clinical and laboratory assessment to decide on hospital admission versus home discharge.
5. For nonhospitalized patients with suspected or confirmed COVID-19 with moderate-to-severe symptoms, the WHO suggests using chest imaging in addition to clinical and laboratory assessment to decide on regular ward admission versus ICU admission.
6. For hospitalized patients with suspected or confirmed COVID-19 with moderate-to-severe symptoms, the WHO suggests using chest imaging in addition to clinical and laboratory assessment to inform the therapeutic management.
7. For hospitalized patients with COVID-19 whose symptoms are resolved, the WHO suggests not using chest imaging in addition to clinical and/or laboratory assessment to inform the decision regarding discharge.

In the general population, CT of chest has a sensitivity of 97%, specificity of 25%, positive predictive value of 65%, and negative predictive value of 83% (Ai et al., 2020). CT can be done during pregnancy as it exposes the fetus to 0.03 mGy dose and doses up to 50 mGy are not associated with fetal teratogenicity (American College of Obstetricians and Gynecologists, 2017).

Chest Computed Tomography Scan
Preparation prior to admission

1. Reserve a CT scanner for suspected or confirmed cases, if available. Preference is given to movable CT scanner (if available) or the CT scanner that can lift the examination bed through the console, a separate control room (operating room) is required; if not, when disinfecting after examination, air disinfection of other computer rooms connected to the control room (operating room) is also required.
2. If a central air-conditioning fresh air system is used in the examination room, adjust the air supply and exhaust to the maximum; if an ordinary central air-conditioning is used, turn off the central air-conditioning in the examination room and operation room, and turn on the standby separate air-conditioning; if no spare separate air conditioner is available, turn on the central air conditioner after examination and disinfecting.
3. To reduce the viral transmission, a disposable medical middle sheet is needed during the examination to isolate the equipment from patients.

4. Two technicians are required, with one operating the CT scanner, and the other one enter to be in the examination room for positioning. (According to the requirements of the National Center for Disease Control and Prevention, both technicians for the operation and positioning require secondary or higher protection.)

Preparation for patient

The patient must wear a mask and lie down in a supine position. The technician trains the patient to hold his or her breath at the end of inspiration during the examination.

Scope and direction of scanning

Scan from apex pulmonis to costophrenic angle. For severe and critical patients (who are difficult to hold their breath), the scanning can be from costophrenic angle to apex pulmonis to reduce respiratory motion artifacts caused by difficulty in holding breath in the lower lung field, so as to ensure image quality.

Scanning parameters

The technician uses a low-dose chest CT protocol to scan the patient. The automatic tube voltage selected $100-120$ kV of tube voltage, smart mAs of $20-50$ mAs, collimator with $0.5-1.5$ mm width, layer thickness, and layer spacing of $1-5$ mm. For severe and critical patients, a larger pitch ($1.0-1.5$ pitches) can be used to reduce scanning time and respiratory motion artifacts.

Keys for CT Diagnosis (Xing et al., 2020).:

Early-stage manifestations. The presence of Multiple lesions affecting both lungs is common and unilateral affection is rare. Peripheral affection of the lungs or under the pleura is more common in the lower fields. Lesions are fan-shaped and irregular, but flaky or nearly round lesions can be noticed. Lesions generally do not affect the whole lung segments (patchy distribution). The density is uneven, often limited to small patches or large ground glass opacities, in which thickened blood vessels and thick-walled bronchi are seen, with or without localized grid-like interlobular septal thickening. The consolidation range is small and limited, with air bronchial signs visible.

Advanced stage manifestations. There is increase in the distributions of the lesions, and fused lesions can be seen, involving larger parts of lung lobes. There is increased lesion density due consolidation patches that are irregular, wedge-shaped, or fan-shaped, and the boundary is unclear. Bronchial vascular bundle thickening or multifocal lung consolidation can be seen under the pleura. The lesion progresses and changes rapidly, and the morphological changes are evident in the short-term review, which can be combined with the necrosis of lung tissue to form a small cavity. There are air bronchograms, usually with no pleural effusion and mediastinal and hilar lymph node enlargement.

Severe-stage manifestations. Sign of "white lungs" with elevation of diaphragm can be seen when most of the lungs are affected. Other features such as air bronchial signs and bronchiectasis are seen with uneven distribution of the lesions. The areas without consolidation can be patchy with ground glass opacities, bilateral pleural thickening, and thickened interlobular pleura, with pleural effusion.

Absorption-stage manifestations. The clinical improvement usually precedes the characteristic imaging changes. The lesions become narrower, decrease in size, and become less in density; the consolidations gradually vanish, with complete absorption of the ground glass opacities; and the pleural effusion is absorbed or organized by the body (Fig. 3.1).

3-2 Bedside X-ray

Bedside chest plain X-ray has become the main imaging method for patients with severe and critical COVID-19.

The findings are like those in CT in patients with severe pneumonia, including the shadows of consolidation, which may be patchy, reticular, stripe, hilar and mediastinal changes, pneumothorax, pleural effusions, thickening of the pleura, etc.

COVID-19 follow-up using imaging techniques

Examination status. Primary examination or reexamination is included.

Areas of the lesions. The method of 18-segment segmentation is used in lungs to record the number of involved lung segment regardless the lesion of the size. However, the term of significantly involved lung segment is defined when the lesion is at least 1/2 of the lung segment in size. Lesion area of 7/15 means there are 15 involved lung segments, of which there are seven significantly involved lung segments.

Evolution of pulmonary lesions. Using a serial chest CT scan presented different patterns with six manifestations: progression, stability, stalemate, improvement, sequelae, and complete radiological resolution.
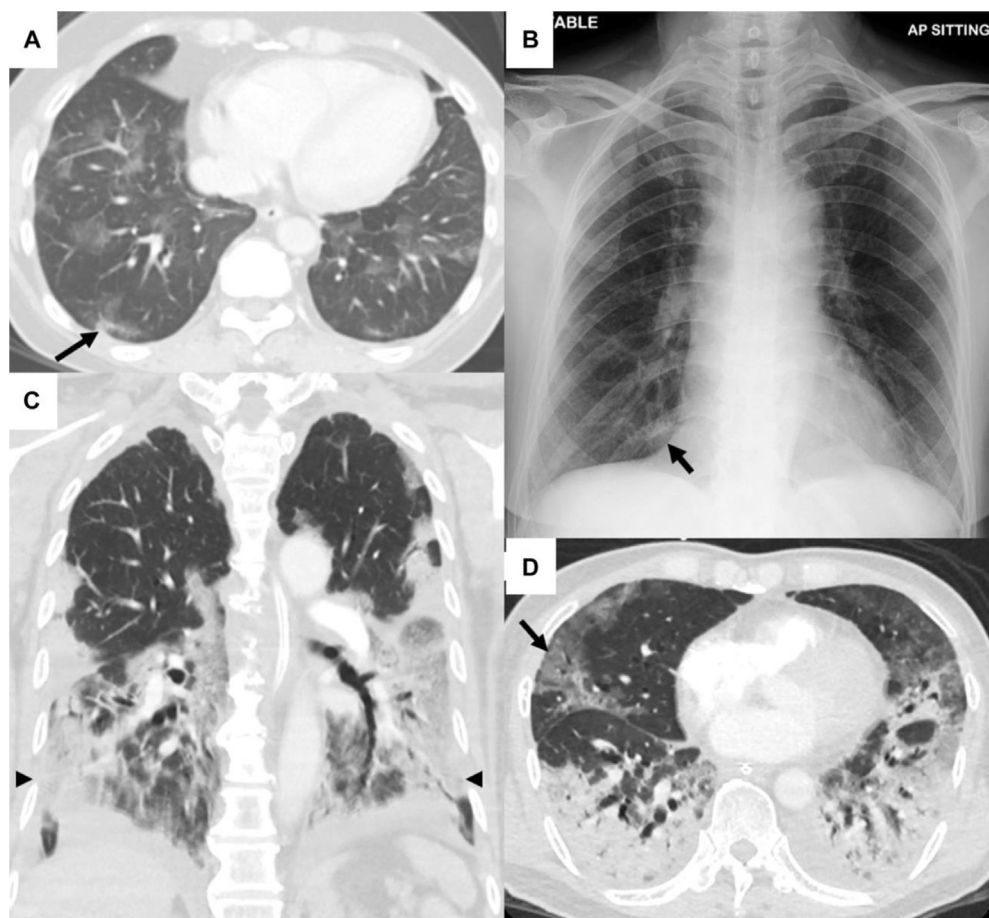
FIG. 3.1 Imaging of two patients with COVID-19. **(A)**, Contrast-enhanced CT of one patient in the axial plane across the lower lobes of the lungs shows patchy GGO in a lobular distribution. Early changes of consolidation are present in the posterior segment of the right lower lobe (arrow). **(B)** Corresponding chest radiograph does not reveal significant abnormality other than a small focus of consolidation in the medial right lower zone (arrow), which would have been easily missed owing to projection adjacent to the right cardiophrenic angle and overlapping rib shadow. **(C** and **D)** CT pulmonary angiogram of a different patient with severe pneumonia in the axial and coronal planes showing extensive multilobar GGO (arrows) with areas of confluent consolidation (arrowheads) mostly distributed in the posterior and basal regions of the lower lobes. No pulmonary embolism was detected. These findings are not specific to COVID-19 and may be seen in other viral and atypical pneumonias. COVID-19, coronavirus disease 2019; CT, computed tomography; GGO, ground-glass opacity (Ashokka et al., 2020).

Progression: Increased lung lesions with increased consolidation.

Stability: No evident changes from previous chest CT scans.

Stalemate: New lesions could be seen with partial absorption of old lesions.

Improvement: Decreased lung involvement with decreased density of the old lesions.

Sequelae: The patient improved clinically but with the typical CT changes, such as bronchiectasis and pleural effusion.

Complete radiological resolution: All lesions disappeared completely on chest CT scan (Fig. 3.2).
Criteria for discharge

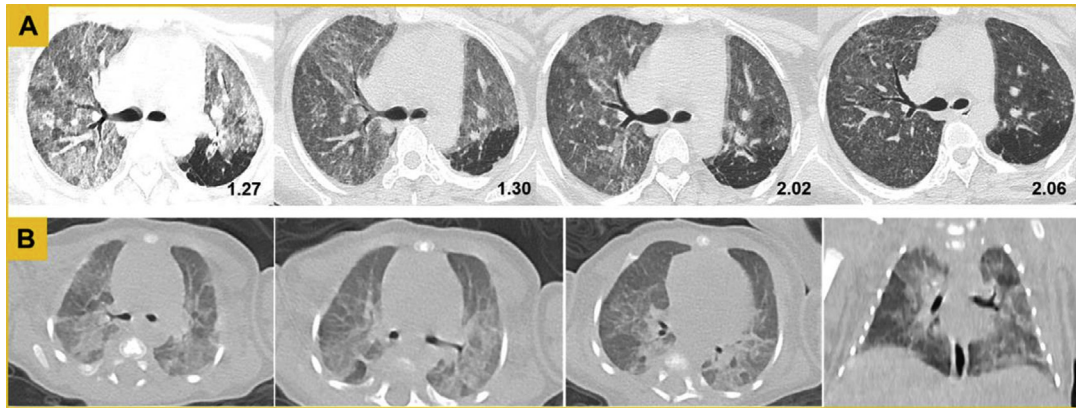1. Pulmonary lesions are significantly decreased in size or completely absorbed.

FIG. 3.2 Chest computed tomography (CT) screening from the mother (patient 6) and her twin neonate. **(A)** CT findings from the mother. The first axial image showed extensive ground-glass opacities (GGOs) and nodules. On the following days, the intensity decreased, indicating the lesions were gradually absorbed after effective treatment. **(B)** CT findings from the twin neonate. The axial and coronal images at the 19th day of birth presented with extensive GGO along the bronchovascular bundle or in the peripheral area, and the localized consolidation in dorsal segment of right lower lobe (Huang et al., 2020).

2. Only a few shadows of fibrotic stripes seen in the follow-up images.
3. No de novo lesion is seen.

The follow-up is recommended after discharge after 14 days or according to the clinical needs.

Ultrasonography

COVID-19 disease shows rapid transmission, progression, and high rate of critical cases (Xu et al., 2020). Ultrasound plays an important role in the diagnosis, efficacy assessment, and follow-up of COVID-19 patients.

Ultrasonography. Ultrasound for COVID-19 patients is highly recommended to be performed as bedside service of isolation ward area and fever clinics to decrease transmitted infections caused by machine transport. The ultrasound assessment for COVID-19 patients is focused on lungs and heart and for the fetal assessment in pregnant ladies with COVID-19. Moreover, critically ill patients may suffer from multiple organ dysfunction that needs imaging of these organs such as liver and kidneys, and this is of help for assessment of the condition of the patients.

To reduce the exposure time of the technician, relevant image data could be collected fast and fully in an isolation ward area and analyzed after the technician leaves the infectious ward area. To reduce the time of exposure of the sonograoher, ultrasound evaluation should be targeted through rapid assessment not comprehensive routine evaluation.

Thoracic examination

Diagnosis and localization of pleural effusion. It also helps in identification and localization for the patients who are candidates for catheter drainage, which can be ultrasound-guided.

Additional diagnosis of pneumonia. The consolidations caused by COVID-19 will enhance the ultrasound penetration of the lung tissue.

Abnormal signs of pneumonia by ultrasound include disappearance of line A which is a horizontal artifact indicating normal lung surface and lung sliding sign, disappearance of line B which is a kind of comet-tail artifact indicating subpleural interstitial edema, lung pneumonic patches, air bronchogram, and pleural effusion (Rouby et al., 2018).

Abnormal signs of pneumothorax by ultrasound. Abnormal signs of pneumothorax by ultrasound include disappearance of lung sliding sign of the pleura and appearance of "lung point," which is highly specific ultrasound sign of pneumothorax. It involves visualizing the point where the visceral pleura begins to separate from the parietal pleura at the margin of a pneumothorax (Lichtenstein and Mezière, 2008).

Echo heart. Cardiac insult occurs in approximately 31% of critically ill patients. Rapid assessment of the functions of the right and left sides of the heart includes the following:

(1) Visual measurement of left heart function is recommended in patients with normal ventricular wall motion.

(2) M-mode assessment of left heart function is recommended for diffuse attenuation of ventricular wall motion.

(3) Abnormal regional wall motion can be assessed using the uniplanar or biplanar Simpson method.

(4) The maximum systolic excursion of the tricuspid annular plane is measured by visual inspection of right ventricular wall motion or M-mode method, if necessary, and the right ventricular fractional area change rate is estimated by the two-dimensional method.

(5) Rapid identification of pericardial effusion and localization: Observation of subxiphoid and parasternal sections is recommended.

(6) Exclusion of other cardiac structural abnormalities: valvular heart disease, cardiomyopathy, myocardial infarction, infective endocarditis, aortic dissection, and other diseases. Comprehensive routine measurement is not necessary.

Assessment of pulmonary artery pressure. Pulmonary artery pressure changes can be dynamically observed via ultrasound so as to adjust diagnostic and therapeutic strategies in a timely manner. Pulmonary artery systolic pressure is estimated using tricuspid regurgitation method or pulmonary venous reflux in the absence of right ventricular outflow tract stenosis.

Ultrasound monitoring supported by intensive care unit and extracorporeal membrane oxygenation. In ICU patients, left atrial pressure and vein width are dynamically monitored to determine whether fluid therapy shall be terminated. During the extracorporeal membrane oxygenation support, echocardiography can detect the size of the cardiac chamber, monitor whether the blood flow is emptied, and evaluate cardiac function and lung changes; ultrasound is used to determine the presence or absence of lung recruitment before weaning.

Examination for peripheral vascular thrombosis. Ultrasound shall diagnose deep venous thrombosis and arterial embolism in the early phase and determine the distribution range of thrombosis, etc., so as to decrease the possibility of systemic and pulmonary emboli, keeping in mind the increased risk of deep venous thrombosis in critically ill patients with COVID-19. Severe patients with COVID-19, especially the elderly and those with underlying diseases, may have multiple other risk factors that cause more increased risk of embolism.

Fetal assessment. The ultrasound assessment of fetal well-being and fetal growth remains the corner stone of assessment of fetus, being the second susceptible patient in the pregnant lady with COVID-19 disease.

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