Overview of investigations in the diagnosis and prognosis of COVID-19

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Abstract

The Coronavirus disease 2019 continued to devastate across the lengths and breadths of the world since 30th January 2020 when World Health Organization declared CoVID-19 as pandemic, through the second wave as an unequal global public health problem. The clinical features expressed by COVID-19 patients are usually non-specific viz. cough, fever, fatigue, shortness of breath which could have been associated with any other seasonal flu; cannot be used for an accurate diagnosis even with the highest clinical acumen; many times, the infected contacts remain symptomless too. These issues led to an unprecedented scale of morbidity, mortality and disability including long-COVID and put laboratory medicine at the crossroads in search of the valid laboratory data. There is an urgent requirement of rapid, simple, and accurate battery of tests to diagnose SARS-CoV-2 infection to provide early interventions for optimum outcome at the primary care level. An increased understanding of the predictors of severity of outcomes is crucial especially in LMICs where intensive care setup might not match ever increasing demand. The gold standard for diagnosis is the identification of viral genome by real-time polymerase chain reaction in respiratory tract materials in the first week of symptoms. Laboratory tests such as complete blood count, C-reactive protein, D-dimer, interleukins, lactic dehydrogenase, troponin, and procalcitonin identify risk of disease with greater severity, myocardial damage, thromboembolic complications, and increased risk of abnormal blood clotting. Imaging tests may be useful for diagnosis, especially when the clinical pictures might not be very transparent and specific, and other tests results are unequivocal or negative.

Key Words: Covid-19, SARS-CoV-2, RTPCR, POCUS, D-Dimer, PCT

Introduction

Coronavirus disease 2019 (CoVID-19) is an infectious disease, initially reported in China and currently dispersion caused by a new coronavirus (SARS-CoV-2). The CoVID-19 pandemic is still spreading worldwide, affecting several million people. The World Health Organization (WHO) was informed of cases of pneumonia of unknown microbial etiology associated with Wuhan City, Hubei Province, China on 31 December 2019. Following the recommendations of the Emergency Committee, Director General of World Health Organization (WHO) declared on 30 January 2020, that the outbreak constitutes a Public Health Emergency of International Concern (PHEIC). Globally, 174,918,667 confirmed cases of CoVID-19, including 3,782,490 deaths, was reported to WHO till 12 June 2021; while a total of 2,156,550,767 vaccine doses have been administered till 10 June 2021. The laboratory diagnosis for SARS COV-2 remains the mainstay for early diagnosis and prompt intervention, since the WHO declared the pandemic, to interrupt the chain of transmission from the primary health care level, by incorporating issues of universal precaution, from the collection to reporting by dedicated capacity building and biomedical waste management system. This research group approached with the mindset to draft a holistic modelling incorporating all the battery of the diagnostic tests with higher sensitivity and specificity as urgent call of the day.


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Virological Diagnostic Parameters

1. RTPCR
The current diagnosis of COVID-19 infection mainly relies on reverse transcription polymerase chain reaction (RT-PCR), performed on naso and oropharynx specimens of suspected persons. This gold standard diagnostic test detects viral RNA by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR). Among the coronavirus causing human respiratory and intestinal infections, SARS-like bat coronavirus (SARS-CoV & SARS-CoV-2), comprises unique clade under subgenus Sarbeco virus. Coronaviruses have many molecular targets within their positive-sense, single-stranded RNA genome that can be used for PCR. These include genes encoding structural proteins, including envelope glycoproteins spike (S), envelope (E), Transmembrane (M), helicase (Hel), and nucleocapsid (N). In addition to the genes that encode structural proteins, there are species-specific accessory genes that are required for viral replication. These include RNA-dependent RNA polymerase (RdRp), hemagglutinin-esterase (HE). The CDC recommends two nucleocapsid protein targets (N1 and N2). WHO recommends first-line screening with an E gene assay, followed by a confirmatory assay using RdRp gene. The evidence in favour of the RT-PCR in the diagnosis of COVID-19 is still emerging with uncertainties about its efficacy and accuracy. A major advantage of real-time RT-PCR assays is that amplification and analysis are done simultaneously in a closed system to minimize false-positive results associated with amplification of product contamination. Estimates of diagnostic accuracy need to be interpreted with caution in the absence of a definitive reference standard to diagnose or rule out COVID-19. It is not clear whether a positive result always indicates the presence of infectious virus. As there is no clear-cut “gold standard” for COVID-19 testing, evaluating test results can be challenging. Clinical adjunction may be the best available ‘gold standard’ based on repeat swabs, history, clinical presentation, and chest imaging. The accuracy of the result depends on various factors including the site and quality of sampling, stage of disease, degree of viral multiplication or clearance, and disease prevalence. Sensitivity and specificity: the pooled sensitivity has been estimated to be 87.8%, with the specificity estimated to be in the range of 87.7% to 100%. False-positive results can be caused by a laboratory error or a cross-reaction with antibodies formed by current and past exposure to seasonal human coronavirus infections. False-positive results are more likely when the prevalence of SARS-COV-2 is moderate to low.

2. Rapid Antigen Test
Rapid antigen (Ag) test kits for covid-19 (oropharyngeal / nasopharyngeal swabs) have been introduced as an alternative to RT-PCR for use in the primary care set-ups. Minimum acceptance criteria regarding sensitivity and specificity by ICMR for Rapid Ag Test Kits and validated as a Point of Care Test (POCT) without transport to a laboratory setup as follows: Sensitivity ≥ 50% and Specificity ≥ 95%; in a laboratory setup with samples collected in Viral Transport Medium (VTM). Sensitivity ≥ 70% and Specificity ≥ 99%. Antigen based rapid tests which are US-FDA approved can be used directly after due marketing approval from DCGI [till 09.06.2021], 114 Antigen based Rapid Test Kits was validated (including 23 revalidated Kits) in our country. Positive results indicate presence of SARS CoV2 antigens, yet clinical correlation with history and other tests are necessary to diagnose infection status as the test detect both viable and non-viable antigenic material. False negative test result signifies below the detection limit of the test levels of antigen in the sample or collected or transported improperly. Thus, negative test results do not negate possibility of infection. Individuals with CT values greater than or equal to 34 are unlikely to have replication – competent virus. Negative results from patients with symptom onset outside of one to five days of symptom onset should be treated as presumptive.

3. Antibody or Serology Tests
These look for antibodies in blood to find past infections by finding proteins created by body’s immune system soon after infected/vaccinated Serology tests should not be used to diagnose a current infection with SARS COV-2 except when viral testing is delayed/ absent. An antibody test may not show if you have a current infection because it can take 1–3 weeks after the infection for your body to make antibodies. Sensitivity and specificity of the serological tests may depend on the testing technique, specificity of the antibody studied, duration of symptoms, and immune status of the individual.

Hematological Parameters

1. Neutrophils to Lymphocyte Ratio (NLR)
The reflections of the inflammatory changes can be vital in gauging the progression of the disease. Neutrophils are primarily responsible for activating the immune system, and systemic inflammation destroys CD4+ T lymphocytes and increases suppressor CD8+ T lymphocytes, thereby leading to an increased neutrophil-to-lymphocyte ratio (NLR). An increase in the apoptosis of lymphocytes leads to lymphopenia and elevated thrombopoietin (THPO) promotes megakaryocyte production. Neutrophils constitute the majority of the leukocytes and are primarily responsible for activating the immune system by migrating from the venous system and free oxygen radicals that can damage the nuclear material of the cell are released. Viral antigens are exposed, cell-specific and humoral immunities are stimulated by an antibody-dependent cell-mediated cell. There is a growing interaction with molecules like vascular endothelial growth factor (VEGF), interleukin-6, interleukin-8, tumour necrosis factor-alpha (TNF-α),...
interferon-gamma, and granulocyte colony-stimulating factor. VEGF-A and VEGF-C are particularly notorious in SARS COV-2. The release of neutrophil-chemo attractive elements and the resulting recruitment of neutrophils are a global host response to the viral infections done by fluorescence flow cytometry method and WBC subtypes evaluated for correlation from blood films stained by Leishman’s stain under light microscopy. The parameter derived from neutrophil to lymphocyte ratio (NLR) is an independent risk factor for severe CoVID-19, and neutrophilia forecasts poor outcomes.

COVID-19 is a systemic multi-organ system infection with significant impacts on the hematopoietic system and haemostasis. Lymphopenia can be considered to be a cardinal laboratory sign, and is potentially prognostic and NLR predict the severity of cases.

Markers Related to Coagulopathy in COVID-19

Research groups are working out in different part of the world to identify interdependence of various coagulation related parameters with high index of suspicion of coagulopathy to predict the early diagnosis and prognosis of moderate and severe CoVID-19 patients.

1. Activated Partial Thromboplastin Time (APTT)

Test involve recalcification of plasma in presence of standardized amount of Cephalins (Platelet substitute) and particulate activator (Silica); aPTT explores coagulation factors XII, XI, IX, VIII, X, V , II, I except platelets. Normal range 30-40 seconds [Critical values that should prompt a clinical alert: aPTT more than 70 seconds signifies spontaneous bleeding crisis in patients.]

2. Prothrombin Time (PT)

Coagulopathy is an alarming signal in in stage-2 with mildly prolonged PT/INR and aPTT with critical progression towards classic disseminated intravascular coagulation if unattended. Reference range: 11.0-12.5 seconds; 85%-100% (although the normal range depends on reagents used for PT). Following full anticoagulant therapy: >1.5-2 times control value; 20%-30%. INR = (Patient’s PT/Mean normal PT) 1.1. The reference range (not on anticoagulation) of 0.8-1.1 regardless of the ISI or particular performing laboratory; above 4.9-5.5 considered possible critical values to predict increase risk of bleeding at 20 seconds.

3. Fibrinogen

The liver produces important proteins involved in the coagulation process including fibrinogen. Quantitative estimation is done by Clauss method using freeze dried Human Thrombin with Heparin inhibitor and Calcium. The most common pattern of coagulopathy observed in patients hospitalized with COVID-19 is characterized by elevations in fibrinogen levels.

Radiological Parameters

1. Point of Care Lung Ultrasound (POCUS)

Point-of-care ultrasound (POCUS) has been demonstrated to identify, various pathologies of the lung, integration of it was done during patient care in these pandemics and was found to be a great adjunct to clinical decisions. Lung ultrasound is used as a diagnostic tool in some centres as an alternative to chest x-ray and chest CT. There is only very low-certainty evidence supporting its diagnostic accuracy, yet it might be helpful as a supplemental or alternate imaging modality. Ultrasonography (USG) is sensitive but not specific for the diagnosis of CoVID-19. Pooled results found that lung ultrasound correctly diagnosed CoVID-19 in 86.4% of people with the disease. USG may have more utility for excluding CoVID-19 than for differentiating it from other causes of respiratory illness. B-lines are the prominent pattern in patients with CoVID-19 occurring with a pooled frequency of 97%. Pleural line abnormalities are also common with a pooled frequency of 70%. The characteristic CoVID-19 LUNGS findings are

A. B-Lines and interstitial syndrome — early disease: focal B-lines, Severe Disease: diffuse confluent B-lines (light beam), multilobar distribution;
B. Pleural line abnormalities — Irregularities, unsmooth, thickening, and fragmentation;
C. Subpleural consolidations — Associated with B-lines, Presence of air Broncho grams;
D. Small sub pleural effusions.

Pulmonary USG has the advantages of portability, bedside evaluation, decrease health care worker exposure, easier sterilization process, and absence of ionizing radiation exposure, lower cost and repeatability during follow-up. Further, USG is more readily available in resource-limited settings; used in pregnant women and children; does not require sedation or transportation of unstable patients. Limitations of lung USG in CoVID-19 are non-specific findings and cannot detect lesions deeply seated lesions. The abnormality must extend to the pleural surface to be visible with on USG examination; USG findings of CoVID-19 are predominant in posterior and inferior lung fields. Further, USG is an operator-dependent technique and needs close contact with the patient, which may contribute to SARS COV-2 transmission to the sonographer.

Interpretation of POCUS

POCUS help in initial screening and segregation of non-severe cases from severe ones. The utility of POCUS is more marked in temporary healthcare facilities like isolation wards, where availability of a routine X-ray and CT machines is not possible. POCUS be used in CoVID-19 patient management, starting from the triage area to ICU care.
2. CT Scan of Chest

Computerized tomography (CT) of chest is a conventional, non-invasive imaging method to investigate and detect lung involvement with high accuracy and speed.\textsuperscript{10,21} Chest CT may play a role in diagnosis in a limited number of hospitalized patients, particularly when initial molecular testing has been inconclusive. However, it is not diagnostic for CoVID-19. British Society of Thoracic Imaging (BSTI) recommended CT imaging in patients with clinically suspected CoVID-19 who are seriously ill if chest x-ray is uncertain or normal. Chest CT is sensitive and moderately specific for the diagnosis of CoVID-19. Therefore, chest CT may have more utility for excluding CoVID-19 than for differentiating it from other causes of respiratory illness. CT imaging abnormalities may be present in asymptomatic patients. The most common findings are ground-glass opacity, either in isolation or coexisting with other findings such as consolidation, interlobular septal thickening, or crazy-paving pattern. The most common distribution pattern is bilateral, peripheral/subpleural, posterior distribution of the opacities, with lower lobe predominance. Extensive/multilobar involvement with consolidations is more common in older patients and those with severe disease. Ground-glass opacity has the highest diagnostic performance for CoVID-19 pneumonia, followed by ground-glass opacity plus consolidation. The simultaneous presence of ground-glass opacity and other features of viral pneumonia had optimum performance to detect CoVID-19. CT scan generally shows an increase in the size, number, and density of ground-glass opacities in the early follow-up period, with a progression to mixed areas of ground-glass opacities, consolidations, and crazy-paving peaking at day 10 to 11, before gradually resolving or persisting as patchy fibrosis. WHO recommends chest imaging in the following scenario:

[A] Symptomatic patients with suspected CoVID-19 when RT-PCR is not available, RT-PCR test results are delayed, or initial RT-PCR testing is negative but there is a high clinical suspicion for CoVID-19 (for diagnosis);

[B]. Patients with suspected or confirmed CoVID-19 who are not currently hospitalized and have mild symptoms;

[C]. Patients with suspected or confirmed CoVID-19 who are not currently hospitalized and have moderate to severe symptoms;

[D]. Patients with suspected or confirmed CoVID-19 who are currently hospitalized and have moderate to severe symptoms.

3. Chest X Ray

Chest x-ray is moderately sensitive and moderately specific for the diagnosis of CoVID-19.\textsuperscript{10} Pooled results found that chest x-ray correctly diagnosed CoVID-19 in 80.6% of people who had the disease. However, it incorrectly identified CoVID-19 in 28.5% of people who did not have the disease. Although chest x-ray appears to have a lower sensitivity compared with chest CT, it has the advantages of being less resource-intensive, associated with lower radiation doses, easier to repeat sequentially, and portable.\textsuperscript{2,3} Chest X-rays are less sensitive than chest CT and may show evidence of bilateral consolidations, peripheral/subpleural ground glass opacities which are predominantly seen in the lower lobes.

Biomarkers

1. D – Dimer

D-Dimer is a fibrin degradation product that is often used to measure and assess clot formation. D-Dimer fragments are produced when plasmin cleaves fibrin to break down clots in the human body by the degradation of cross-linked (by factor XIII) fibrin showing ongoing activation of the coagulation system. A single fibrinogen molecule is a symmetrical dimer that is made up of three pairs of different polypeptide chains, which include α, β and γ. Each of the intertwined polypeptide chains that comprise a single fibrinogen molecule is held together by disulfide bonds. The formation of fibrin begins with the cleaving of α and β polypeptide chains of the fibrinogen molecule, which is achieved by thrombin.\textsuperscript{8} This cleaving event causes the fibrin monomers to spontaneously polymerize, which results in the formation of double-stranded fibrin protofibrils. If an injury occurs, the fibrinolytic system will activate to limit the size of the clot. This system begins with the release of the plasminogen activator from the vascular endothelial cells to allow this molecule to bind to the fibrin surface of plasmin. Fibrin-bound plasmin will then degrade the fibrin network into several soluble fragments, of which will include the D-dimer of the (DDE) complex.\textsuperscript{21} D-dimer is estimated by immune turbid metric assay and abnormal coagulation function with elevated D-dimer suggest progression of CoVID-19.\textsuperscript{22,23} The raised D-dimer can help to easily assess the risks of pulmonary complications, correlates with disease severity and a reliable prognostic marker for in-hospital mortality in patients admitted for CoVID-19.\textsuperscript{21}

2. C-Reactive Protein (CRP)

CRP protein is produced by the liver and it is an early marker of infection and inflammation which has been shown an important marker in patients with severe CoVID-19.\textsuperscript{2} The normal concentration of CRP in blood is < 5 mg/L, that rises rapidly and significantly within 6 to 8 hours, peak in 48 hours and a decrease suggest reduction in inflammatory response, making it a useful marker to monitor inflammatory response in patients with CoVID-19.\textsuperscript{10,24}

3. Procalcitonin (PCT)

Procalcitonin is a glycoprotein calcitonin pro-hormone released by the thyroid parafollicular cells. In case of a microbial infection, PCT levels are significantly raised as...
it is released by all parenchymal tissue under the influence of endotoxins and pro-inflammatory cytokines. In normal physiological state serum PCT is recorded significantly below 0.05ng/mL. PCT follows an inclining levels detected 2–6 hours after the stimulus. PCT has emerged as a promising prognostic biomarker in CoVID-19. (21) Many studies during pandemic have shown higher levels of PCT in severe CoVID-19 cases. PCT is an inflammatory biomarker that rises in bacterial infection and falls in response to antibiotic treatment, and has greater sensitivity and specificity for bacterial infection than CRP. PCT is a prohormone whose secretion by extra-thyroidal tissues is stimulated by inflammatory cytokines and endotoxins but inhibited by IFN-γ, leading to a specificity for bacterial infection PCT has been identified as marker of poor prognosis in CoVID-19 infection, and it is unclear if a raised PCT is part of the inflammatory syndrome associated with CoVID-19 or primarily reflects bacterial co-infection requiring antibiotic treatment. (10) PCT measurement is done by Electro chemiluminescence method. Reference value of PCT in adults and children older than 72 hours is 0.15 ng/mL or less (may be below the level of detection).25 Procalcitonin appears to be a useful biomarker in identifying CoVID-19 patients with super-added bacterial infection, and supports antibiotic treatment in CoVID-19 patients with a significantly raised. PCT is used as a biomarker of lower respiratory tract bacterial infection therefore guiding antibiotic therapy in the intensive care unit.

4. High Sensitivity Cardiac Troponin Test (cTn)2,8,21,25

Myocardial injury is frequent and prognostic in CoVID-19. Most hs-cTnT increases are modest and due to myocardial injury, they have important prognostic implications. A single hs-cTnT <6ng/L at presentation may facilitate the identification of patients with a favorable prognosis. Cardiac troponin (cTn) is the preferred biomarker for the detection of myocardial injury and hs-cTn assays as a continuous marker of risk with the potential to facilitate triage and risk-stratification.8 Myocardial injury is frequent in COVID-19 patients undergoing hs-cTnT measurement and associated with adverse outcomes. To facilitate triage of COVID-19 patients, a single hs-cTnT <6ng/L at presentation may help identify patients with a favorable prognosis. Early diagnosis of cardiac complications in these patients is possible through the measurement of cardiac troponin which is considered the gold standard marker for myocardial injury.3 The most recent international guidelines recommend the use of high sensitivity cardiac troponin I (hs-cTnl) and T (hs-cTnT) in the diagnosis of myocardial damage and acute myocardial infarction in COVID 19.

5. Metabolic/ Inflammatory Markers1,3,6,8,10,21

Lymphopenia, leucocytosis, thrombocytopenia, decreased eosinophils, decreased haemoglobin, with high NLR and ESR are significantly associated with severe disease, and predict disease progression. Severe cases present with lymphopenia and thrombocytopenia, but not leukopenia. Elevated erythrocyte distribution width is associated with a significantly higher risk of mortality in hospitalized patients. Absolute counts of major lymphocyte, particularly CD4+ and CD8+ T-cell counts, are significantly decreased in patients with severe disease.1,3 Serum lactate dehydrogenase is increased and also useful in progression of COVID-19 patients. Elevated interleukin-6 level is significantly associated with severe disease and cytokine storm. Blood specimens should be collected for culture in patients with Severe or critical disease to rule out other causes of lowers respiratory tract infection and sepsis. Fasting hyper glycaemia independently predicts poor prognosis and is associated with an increased risk of mortality, regardless of whether or not the patient has diabetes. Severely ill patients may have high levels of cytokines IL2, IL4, IL6, IL7, IL10 and tumour necrosis factor (TNF). In patients with the severe acute respiratory syndrome, the so-called cytokines storms was observed, with the release of the previously mentioned cytokines in leading to multiple organs failure and eventually to death.9,10,21 Measured by CLIA (Chemiluminescence) method, the normal ferritin levels: 12-300 ng/mL of blood (males) and 12-150 ng/mL for females; higher levels are indicative of iron accumulation in the organs; prognostically leads to destruction and loss of normal function. Higher values reported in COVID and other chronic inflammatory medical conditions Elevated ferritin is significantly associated with severe disease, useful for predicting disease progression and indicate development of cytokine release syndrome.27-29 We have to find suitability of inflammatory biomarkers as user-friendly and cost-effective for prognosis of moderate and severe cases of COVID-19 in-patients even at the primary care levels.30 Laboratory need to frame innovative approaches of inflammatory biomarkers of SARS-CoV-2 in the Clinical Practice Guidelines from diagnosis, interventions including tackling of opportunistic infections and handle an array of ever-increasing list of long-COVID with futuristic vision.

Future Directions and limitations

Presently many diagnostic tools are available and also inerasably being available for the diagnosis of COVID-19. This narrative review provides of common investigations used for the diagnosis and predicting disease severity and outcome.

Conclusions

The identification of genetic material of the virus by RT-PCR is the gold standard test. POCUS and HR-CT Chest is non-specific yet lifesaving, diagnostic tools. Several laboratory parameters are associated with worse outcomes in CoVID-19: increased NLR levels, elevated liver enzymes, ferritin, IL-6, LDH, CRP, D-dimer, PT, troponin, and creatine phosphokinase, along with acute kidney
injury. Increased NLR, CRP and LDH in SARS-CoV2-infected patients, upon hospital admission, predict severe acute lung injury and mortality. Accurate, efficient, rapid and cost-effective diagnostic tools are needed for timely identification, isolation and management of CoVID-19 patients. In summary, the diagnosis of CoVID-19 is based on clinical presentation, diagnostic tests to identify the causative agents and also on related test to suspect complication.

References