COVID - 19

DIAGNOSIS OF COVID-19 IN CHILDREN

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Abstract: *The world is facing an unprecedented crises with the advent and spread of COVID-19. Fortunately children are less affected. Diagnosis begins with identifying the right suspect which in turn depends on local prevalence of infection and contact history. In high burden areas any acute illness with or without fever can be COVID-19. The gold standard for diagnosis is RT-PCR in respiratory specimen. Correct collection and transport of specimen is important. Since the sensitivity of RT-PCR is at best 70%, a negative test does not rule out the diagnosis. Sick children may have lymphopenia and elevated CRP, D-dimer, ferritin, CPK, LDH, IL-6. CT chest is more sensitive than CXR and may be abnormal even in those who are asymptomatic or have mild symptoms. The role of serologic tests in children at this time is limited to diagnosis of pediatric multi system inflammatory syndrome.*

Keywords: *SARS-CoV-2, COVID-19, Children, Diagnosis.*

COVID-19 has been ravaging the world since the past four months with devastating consequences.[1] Globally more than 6 million people have been affected and almost 4,00,000 lives lost.[2] Fortunately, disease in neonates, infants and children has been mild with less morbidity and mortality.[3] However, unusual manifestations such as the pediatric multi system inflammatory syndrome (PIMS) are now being reported.[4] It is possible that with the evolution of the pandemic, there may be a change in the spectrum and severity of disease in children and recognition of new manifestations. In this article we shall discuss the diagnosis of COVID-19 in general, with focus on children.

When to suspect?

The definition of 'Supect COVID-19' will vary with the local prevalence and contact history. With widespread closure of schools and day care centres and lockdowns since the onset of the pandemic, children are most likely to get infected from household contacts. Hence, history of sick family members is important. In published reports from China, a significant percentage of children with COVID-19 were infected from household contacts.[5] The common manifestations which merit evaluation for COVID-19 in the context of epidemiologic and contact history include:

- Any acute illness with no other explainable cause
- Fever with or without associated respiratory/ gastrointestinal manifestations
- Runny nose, sore throat, cough, loss of sense of taste or smell
- Myalgia, fatigue
- Abdominal pain, diarrhea and vomiting
- Irritability, drowsiness, seizures, stroke
- Breathlessness, tachypnea, hypoxia
- Manifestations of pediatric multi system inflammatory syndrome including fever, conjunctivitis, rash, hypotension

Asymptomatic household contacts of COVID-19 positive patients should be tested once between day 5 and 10 of exposure as per recent ICMR guidelines.[6] Similarly newborns born to mothers who were COVID-19 positive within 2 weeks of delivery or those who have been in contact with COVID-19 infected family members should be tested at birth and then before discharge.[7]

Diagnosis

The diagnosis of COVID-19 in the right clinical setting can be confirmed only by molecular tests as per current guidelines. Other laboratory tests and radiology offer supportive evidence. The role of antibody test is limited. Viral cultures are usually performed only for research purposes. Genome sequencing is also a research tool to determine viral virulence, aid in vaccine development and understanding epidemiologic characteristics of the virus such ascirculation of the virus/place of origin.[8]

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Molecular tests (Nucleic acid amplification tests/ NAAT) [9]

Basis

NAAT tests conducted on respiratory secretions are currently the gold standard for diagnosing COVID-19. The most common NAAT assays in commercial use are the RT-PCR tests. These tests have two targets. The first screening gene is the generic coronavirus gene coding for either the spike protein (S), nucleocapsid protein (N), envelope protein (E) or membrane protein (M). The second target is the gene specific to COVID-19 which could be the gene coding for RNA dependent RNA polymerase or spike protein (S) or the open reading frame, ORF 1 or 2. Hence, the sensitivity of kits may vary depending on the target genes used. The common kits in use in India are Altona Real star CoV-2 real-time PCR kit, Thermo TaqPATH COVID-19 and the indigenous Mylab PathoDirect COVID-19 kit. All these tests require first DNA extraction and then PCR. They need batching of samples, technical expertise and the testing time is 4-6 hours. Since most laboratories run only a few batches per day, the turn around time may actually be 24-36 hours depending on when the sample is submitted. Cepheid the manufacturers of Xpert MTB/ Rif have developed a RT-PCR assay for SARS-CoV-2 called Xpert Xpress SARS-CoV-2 which does not require separate DNA extraction and hence can deliver results within 2 hours after submission of the sample. It also does not need technical expertise and can be run as an individual test and samples need not be batched. It is particularly useful when rapid results are needed.

The tests are semi quantitative in nature wherein, the viral load can be estimated from the cycle threshold (Ct) i.e. the number of cycles that need to be run to amplify the RNA. The usual cut off for most test kits is 40 (for the Xpert Xpress kit it is 45). If the cycle threshold is above the cut off, test is negative. The lower the Ct, the higher is the viral load. The Ct values also tend to correlate with infectivity. High Ct values are associated with non viable virus and low risk of transmission. However these Ct values are assay dependent and have been variably reported as >24/ >34 between different assays [10]

Collection of samples

The molecular test is performed on respiratory secretions which can be collected from the upper respiratory (nasal swab, oropharyngeal swab, nasopharyngeal swab) or lower respiratory (sputum, endotracheal aspirate, bronchoalveolar lavage) tracts. The person collecting the swab should wear proper personal protective equipment (PPE) including eye protection, N 95 mask, gloves and gown while collecting the specimen. The samples have to be collected by synthetic swabs and immersed in viral transport medium and transported on ice. Saliva is also being evaluated and found comparable in efficacy to other respiratory specimens and obviates the need for swab sticks and the transmission risks associated during collection.[11] If the samples are not processed immediately, they should be frozen at -20^{o}C.

Sensitivity

Sensitivity of the molecular methods is difficult to assess since they themselves are the gold standard for diagnosis. The sensitivities reported in literature are based on test positivity in clinically suspect cases with epidemiologic, contact and radiologic corroboration.

The sensitivity of the molecular tests depends on many factors including the site of collection, method of collection/ transport and duration of illness prior to collection. A study from China which evaluated 1000 swabs from multiple sites reported the best sensitivity from lower respiratory tract specimens (bronchoalveolar lavage 90%, sputum 70%) followed by nasopharyngeal swabs (50%) and lowest from nasal and oropharyngeal swabs (20-30%). [12] The sensitivity is higher in the early phase of the illness and decrease as the illness progresses, particularly after the first week. The tests are often negative in children and adolescents with the Kawasaki disease like multisystem inflammatory syndrome.[4]

While there is scanty data about the sensitivity in children, one study from China that evaluated more than 2000 children with suspect COVID-19 disease reported virologic positivity only in 33% of the cases. [13] Children with high clinical suspicion of the disease but initial negative molecular tests should be treated as COVID-19. Tests can be repeated on a daily basis in suspect cases. If lower respiratory samples are available, they should be tested. However, doing bronchoscopies for getting access to lower respiratory specimens for diagnostic purposes is not recommended as these are invasive requiring technical expertise, aerosol generating procedures and associated with heightened risk of transmission to health care workers.

Specificity

The RT-PCR tests are highly specific. However, since they detect only RNA sequences of the virus, they can remain positive for weeks and months. Therefore in children who are asymptomatic and test positive on throat swab (such as before elective surgeries) it cannot be inferred when they were infected and whether they are

currently infectious or not. Therefore positive results should be interpreted with caution in patients who are asymptomatic and have no history of close contact with COVID-19 patients. This is also true for children who have tested positive in the past and then are readmitted with some acute illness and test positive again. These were earlier attributed to reinfections but now it is well established that COVID-19 produces at least short term immunity against the virus. [14] Hence, these children who retest positive after recovery should not be managed/ isolated as COVID-19.

Repetition of molecular tests

As discussed earlier, molecular tests can remain positive for a long time after infection, even weeks and months. Earlier, it was assumed that the patient is infectious as long as SARS-CoV-2 RNA is detectable in the upper respiratory tract and deisolation required demonstration of two negative swabs 24 hours apart.[15] However, this approach resulted in multiple tests and prolonged hospital stay and was very resource intensive. [16] Meanwhile, it was reported that viable virus was seldom cultured after 7-10 days of the illness if the patient had recovered.[17] Hence, now as per new national guidelines, patients with COVID-19 can be deisolated once 10 days have elapsed from the time of symptom onset and three days since clinical recovery, whichever is longer. [18] However, there is little information about infectivity in patients with severe and protracted illness and current guidelines recommend demonstrating one negative swab prior to discharge in severe cases. [18]

Hematologic and biochemical parameters

The changes in hematologic and biochemical parameters in COVID-19 have been extensively studied. These studies have been mainly in hospitalized adults and abnormalities depend on the severity of disease.[19 20] The white blood cell count is usually normal or low with lymphopenia and elevated absolute neutrophil: lymphocyte ratio. The platelet counts are normal or mildly decreased. There is elevation of C reactive protein but procalcitonin is normal. In patients with severe disease there is elevation of creatine phosphokinase (CPK), lactate dehydrogenase (LDH), D-dimer, ferritin and interleukin-6 (IL-6). There may be mild derangement of liver enzymes, elevation of creatinine and prolongation of prothrombin time/ activated partial thromboplastin time. There may be elevation of troponin in some patients indicating myocardial involvement. In children, there is less derangement of the hematologic and biochemical parameters discussed above.[21] This may also be related to the fact that the disease is less severe in children.

Hence, it is reasonable to say that these tests are primarily of value to assess the severity of COVID-19 and do not really aid the diagnosis of the disease. Poor prognostic markers in adults have been absolute lymphocyte count of < 1000, absolute neutrophil count: absolute lymphocyte count of > 3.5, elevation of CRP beyond 100 mg/ L, increase in D-dimer to more than 6 times normal and levels of IL-6 beyond 7-10 times normal. [19]

The pediatric multi system inflammatory syndrome is associated with marked rise in inflammatory parameters. These children have usually an elevated white cell count with neutrophilia and lymphopenia, but normal/ low platelet counts.[4] The latter is unlike Kawasaki disease where platelet counts increase progressively. These children have high erythrocyte sedimentation rate and high CRP levels. There is mild derangement of the liver enzymes. These children also show other laboratory abnormalities like raised triglycerides, fibrinogen, ferritin and D-dimer and elevated troponin I. Hyponatremia was also a common feature. Some of these children also had elevated levels of IL-6. The ECHO in some showed evidence of coronary artery aneurysms and reduced ejection fraction.

Radiology [22]

Chest radiology plays an important diagnostic role in COVID-19 disease. However, radiology in COVID-19 presents logistic challenges due to transmission risks to health care workers and radiation risks to the patients.

The CxR's are usually normal in mild/ early disease. In those with severe disease, it is abnormal with bilateral infiltrates and sometimes complete white out of the lungs.

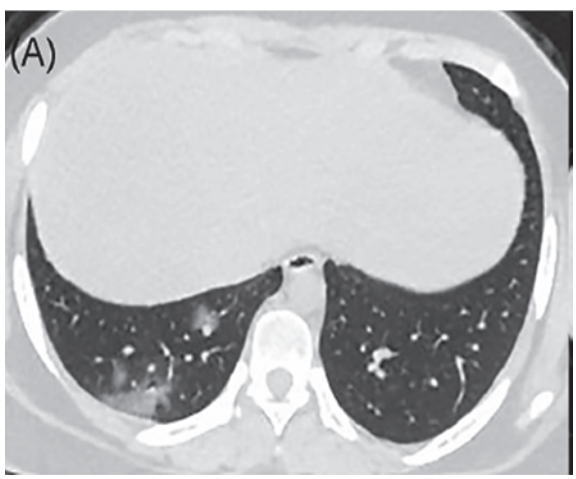

Fig.1. CT scan of a mildly symptomatic child showing a peripheral ground glass opacity

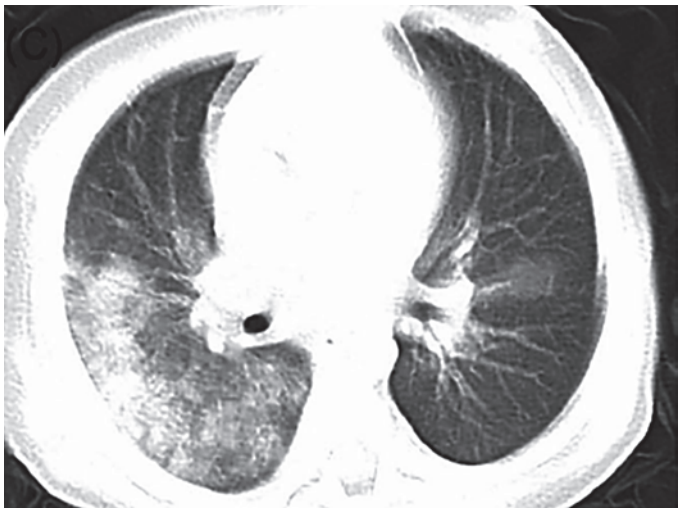

Fig.2. CT image in a child with severe COVID-19 disease

CT scans of the lungs are infinitely more sensitive than CxR. Some series reported abnormal CT scans in 20% of children who were clinically asymptomatic. In those who had symptoms, chest CTs were abnormal 2/3rd of the time.[22] Disease could be bilateral/ unilateral with predominant involvement of lower lobes than the upper lobes and lesions more peripheral than central. The most common radiologic finding is that of ground glass opacity (Fig.1). Other findings include consolidations, crazy paving pattern and the halo and reverse halo signs. Findings can be severe in patients with clinical evidence of pneumonia and hypoxia (Fig.2). Pleural effusions were rare. As compared to adults, CT findings are less common and less severe in children which basically correlates with the fact that disease is less severe in children. Symptomatic adults had abnormal CT, 90% of the times unlike children, where the CT was abnormal 60% of the time. Also, adults were more likely to have bilateral involvement unlike children where almost half the times there was unilateral involvement. Some studies in adults reported superior sensitivity of CT scan as compared to RT-PCR in diagnosis of COVID-19.[23] This was however not observed in children.

Point of care lung ultrasound is also emerging as a useful diagnostic investigation in COVID-19.[24] Since the lesions are peripheral in COVID-19 they are readily picked up by lung USG and termed as straight beam sign.

Serologic diagnosis [9,25,26]

Kinetics of immune response

COVID-19 is associated with a gradual development of an immune response. IgM antibodies appear 1-2 weeks after infection almost followed immediately by IgA antibodies and then IgG antibodies between 2-3 weeks. However, the immune response is not uniform in all individuals and severe infections are associated with stronger immune responses. People with mild disease or those who are asymptomatic may not develop an immune response at all. This is possibly attributed to the innate immune response wiping out the virus before the adaptive immune response can kick in.

Detection of immune response

It is expected that IgM detection should be more useful since these antibodies appear early; however IgM antibodies cross react with other circulating coronaviruses. On the other hand, IgG response though a little delayed persists for a long time and is more specific. Detection of virus neutralizing antibodies by special assays is the best approach since these antibodies correlate with future protection. However, viral neutralizing assays are only possible in research laboratories. IgA antibodies are also reliable but are not included in most commercial kits.

Several antibody detection kits that are based on principle of enzyme-linked immunosorbent/ Chemiluminescence immunoassay (ELISA/ CLIA) have been developed that detect both IgM and IgG antibodies or combined antibodies or only IgG antibodies. They differ in sensitivity and specificity and in-house validation of kits is strongly recommended. Rapid serologic tests based on lateral flow assays have been developed. These can give the results within 10-15 minutes but have not been validated sufficiently and are possibly less sensitive than ELISA.

Clinical application of serologic tests

While serologic tests hold great promise, they have not been commonly used in the clinical setting. Indications of these tests include:

- Diagnosis of COVID-19 infection in those patients who have COVID-19 like illness and who test negative by RT-PCR methods. These tests could thus prove useful in those who present with prolonged symptoms when the viral shedding is less. This is especially the case in the multi system inflammatory syndrome where RT-PCR is negative but antibody tests are positive.

- To estimate the prevalence of infection in population and health care workers. This would indirectly help in assessment of herd immunity and effectiveness of infection control measures in hospitals. Recent studies also indicate that infection with COVID-19 leads to short term protection against reinfections. [14] If more evidence accumulates that presence of antibodies equates with protection against future infection,

deployment of such people in front line work would also be possible.[27] The main drawback of this approach is that mildly symptomatic people or those who have been asymptomatically infected may not mount a detectable immune response and thus test falsely negative. Hence, absence of antibodies does not indicate absence of previous infection.

- To estimate the level of protection given by convalescent plasma before transfusion to patients with COVID-19. Ideally the titre of neutralizing antibodies should be determined but these assays are not always universally available.

Conclusion

COVID-19 is challenging our lives and resources like never before. Currently, the most common cause of fever with or without any other focus is COVID-19. While we can draw comfort from the fact that children tend to have milder disease as compared to adults, diagnosis of COVID-19 in them is equally important. The RT-PCR in appropriately collected nasopharyngeal swab is the diagnostic method of choice. False negative results can be seen in 30-50% of the cases. Hematologic and biochemical markers and radiology play a supporting role and help in assessment of disease severity. The role of antibody tests is yet to be elucidated.

Points to Remember

- ***The gold standard test for diagnosis of COVID-19 at this time is RT-PCR in respiratory tract specimens.***
- ***The sample has to be collected and transported properly.***
- ***A negative RT-PCR does not rule out the diagnosis of COVID-19.***
- ***Presence of lymphopenia, high CRP/ ferritin/ D-dimer/ CPK/ LDH may indicate severe disease.***
- ***CT may be useful in the right clinical setting for quick triaging of suspect cases and evaluation of RT-PCR negative cases.***

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CLIPPINGS

A new therapeutic strategy in severe COVID-19.

Patients with severe COVID-19 have a hyperinflammatory immune response suggestive of macrophage activation. Bruton tyrosinase kinase (BTK) regulates macrophage signaling and activation.

Aclabrutinib, a selective BTK inhibitor, was administered off- label to 19 patients hospitalized with severe COVID-19 (11 on supplemental oxygen; 8 on mechanical ventilation), 18 of whom had increasing oxygen requirements at baseline. Over a 10-14 day treatment course, acalabrutinib improved oxygenation in a majority of patients, often within 1-3 days, and had no discernible toxicity. Measures of inflammation-C-reactive protein and IL-6-normalized quickly in most patients, as did lymphopenia, in correlation with improved oxygenation. At the end of acalabrutinib treatment, 8/11(72.7%) patients in the supplemental oxygen cohort had been discharged on room air and 4/8 (50%) patients in the mechanical ventilation cohort had been successfully extubated, with 2/8(25%) discharged on room air. Ex vivo analysis revealed significantly elevated BTK activity, as evidenced by autophosphorylation and increased IL-6 production in blood monocytes from patients with severe COVID-19 compared with blood monocytes from healthy volunteers.

These results suggest that targeting excessive host inflammation with a BTK inhibitor is a therapeutic strategy in severe COVID-19 and has led to a confirmatory international prospective randomized controlled clinical trial.

Roschewski M, Lionakis MS, Sharman JP, Roswarski J, Goy A, Monticelli MA, Inhibition of Bruton tyrosine kinase in patients with severe COVID-19. Science Immunology 05 Jun 2020:Vol. 5, Issue 48, eabd0110 DOI: 10.1126/sciimmunol.abd0110.