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A Comprehensive Update on Diagnosis of SARS-CoV-2

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Abstract

An unmatched coronavirus; termed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been arisen in the end of 2019 in Wuhan, China resulted in a pandemic COVID-19 disease. In 2021, there were many mutations disseminated all over the world termed variants of concern. One of the critical main challenges is how to diagnose precisely the disease without confusion with another flu and respiratory distress causing viruses. The various clinical, radiological, laboratory, serological and molecular diagnostic approaches were discussed in this article.

Keywords: COVID-19 diagnosis, COVID19 clinical signs, COVID- 19 radiography, COVID- 19 serology, VOC, SARS-CoV-2.

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Introduction

There major present–day challenges face the health and research organizations are the hardness of containing the huge spread of COVID-19; a global human life threating disease caused by SARS-CoV-2 corona virus. The pattern of the disease that displays a varied range of clinical signs which may confused with other respiratory illnesses [1]. Later on several virus mutations occurred and spread globally [2], there is a great needing of acquiring efficient methods for precise identification of asymptomatic cases as well as vaccinated that result in spreading of the virus to close contacts. Hence, this identification facilitates the avoiding of unnecessary quarantines of negative persons and the spread of infection by positive ones [3, 4]. The diagnosis of COVID-19 may relies on clinical signs, so it is important to develop more complicated diagnostic techniques based on viral genomic sequencing as essential tool for determining the rate and degree of mutational variances associated with SARS-CoV-2 and for more efficient vaccine development [5].

This upright article pointed to the updated clinical and laboratory diagnosis that picked up from numerous published researches via searching through various databases.

1) Clinical signs, course and prognosis

The incubation period for COVID-19 was firstly calculated to be about five days, which was based on 10 patients only [6]. The wide majority of individuals of more progress clinical patterns had one or more coexisting medical situations, such as diabetes, hypertension, and cardiovascular disorders, with elevated case fatalities amongst elderly and feeble patients, [6, 7]. In the beginning of the disease (3-7 days of onset),it is difficult to differentiate COVID-19 from other respiratory diseases; as common signs are fever, cough (dry), fatigue, slight dyspnea, sore throat, headache and conjunctivitis. Occasionally, gastrointestinal involvement

was reported with diarrhea,nausea and vomiting, [8, 9]. Then, with the advanced stage of the disease dyspnea develop within 5 – 13 days, continuous severity on 8 – 14 day the case develops acute respiratory distress syndrome (ARDS) in 9 day, chest pain, muscle pain, acute respiratory failure, septic shock, refractory metabolic acidosis, and formation of multi thrombi, [10, 11].

Infected children with COVID-19 are either asymptomatic or mild to moderate symptoms that appear 3 to 7 days of the onset; include fever, dry cough and fatigue, diarrhea, headache, few upper respiratory symptoms including nasal congestion and runny nose, some children showed mild pneumonia. Most of infected children recovered within 1- 2 weeks of onset illness but few could progress badly towards lowering respiratory infections, rarely died, [12-14].

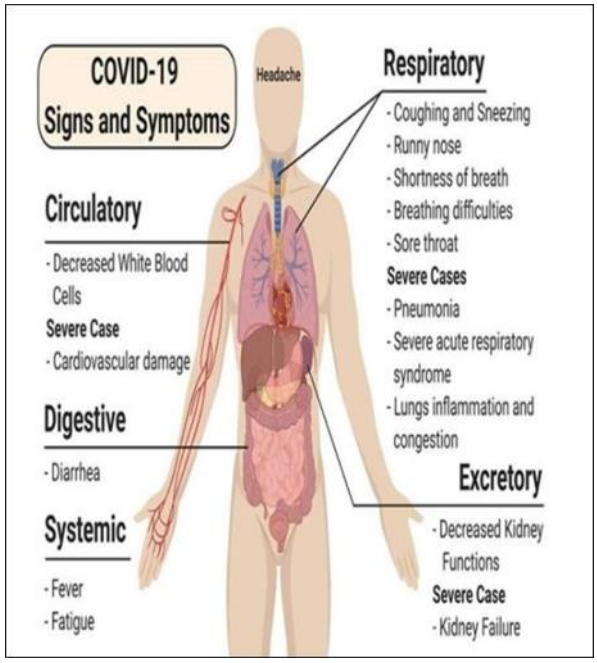

Figure (1) frequent symptoms of COVID-19

Moreover, there was an interesting observation; hyposmia, dysgeusia [15], as well as loss of taste and smell senses, lead to suggest that SARS-CoV-2 could have neuro-invasive potential, [16-18].

Interestingly, the course of the disease is asymptomatic or mild or in about 80–90% of cases, but becomes serious only in around 10% of cases, with dyspnea, hypoxemia and extensive (>50%) radiological involvement of the lung parenchyma. The bad consequence develops in around 5% of cases; a critical condition, pneumonia with respiratory failure, if shock with multi -organ failure occur, often lead to death in 2- 5%, [19, 20].

The mortality rate is variable, probably due to various patient features and risk health conditions. It is also probable that quick saturation of intensive care facilities may have affected mortality rates, especially in high epidemic regions, [20]. Until now, the mortality due to COVID-19 is appeared to be around 3%, lower than homologue other human respiratory coronaviruses; SARS-CoV (10%)and MERS-CoV (35%) [21].

COVID-19 infection may be related to complications on pregnant women as, premature

rupture of membranes (PROM) and preterm labor but in general the signs were mild to moderate and prognosis of the disease has almost gone well [22-24].

Regarding vertical transmission, recent quantitative study revealed that out of 936 neonates from mothers with COVID-19; 27 neonates had a positive result for SARS-CoV2 viral RNA test using nasopharyngeal swab, indicating a pooled proportion of 3.2% (95% confidence interval, 2.2–4.3) for vertical transmission [25]. On the other hand, 42 SARS-CoV-2-positive asymptomatic pregnant women fulfilled the inclusion criteria. Twenty-five (59%) women developed mild disease after discharge. Neonatal death occurred in three (7%) cases, of which one had a positive SARS-CoV-2 test at birth and none had coronavirus disease 2019-related symptoms. There were five (12%) cases with strong evidence of intrauterine transmission of SARS-CoV-2, according to the WHO criteria, as amniotic fluid samples and neonatal samples at birth and at 24 h after birth were positive for SARS-CoV-2 [26]. Fifty one studies reported 336 newborns screened for COVID-19; only 15 (4.4%) were positive for throat swab RT-PCR. Among neonates with throat swab SARS-CoV-2 positive only five (33.3%) had concomitant placenta, amniotic fluid, and cord blood samples tested, of which only one amniotic fluid sample is positive for RT PCR. Five neonates had elevated IgG and IgM but without intrauterine tissue tested. Four neonates had chest imaging suggestive of COVID-19 pneumonia [27].

2) Radiological findings

Indelibly, Computed Tomography (CT) of the chest is rapid, painless, noninvasive and precise, and helps in the diagnosis of obscure chest symptoms etiology. Regarding first week of COVID-19 onset patient's; the typical CT feedbacks were unilateral or multifocal ground glass opacities, especially on the peripheral and lower lung lobes, in addition to bilateral multiple lobular and sub-segmental areas of consolidation, particularly in ICU patients. More severity of the disease more number of lung segments involved and as the disease progress, the opacities tended to flow together and thicken [28-30]. The Non-typical CT findings comprise pleural effusion (only about 5%), masses, cavitation and lymphadenopathies; therefore, these would suggest alternative diagnoses. The CT sensitivity is variable; ranged from (86–97%) in patients with positive RT-PCR, and to (about 50%) in patients that show only constitutional and non-respiratory symptoms, [31].

3) Laboratory findings

The most prevalent clinic- laboratory disorders recorded amongst 1099 hospitalized COVID-19 patients with pneumonia comprised lymphocytopenia (83%), thrombocytopenia (36%) and 34% had leucopenia [32], hypertransaminasemia and elevation in lactate dehydrogenase level have also been stated [10]. The other significant observations were increased inflammation indicators; including ESR and C-reactive protein (CRP) level which may reaches 6 times in clinical sever cases and linked to increased mortality risk (29, 33].

Moreover, decreased calcitonin, and remarkable elevation in D- dimer, ferritin levels, prothrombin time, and lactate dehydrogenase was noticed in hospitalized patients [34]. In severe cases, laboratory finding show what is termed "cytokine storm"; high elevation cytokines that include IL2, IL6, IL10 and TNFα [10, 35]. Interestingly, increased troponin was also recorded in 7% of patients who thereafter died because of fulminant myocarditis so the high troponin level is considered a very bad prognosis [36].

4) Molecular diagnosis

4.1. Real-Time Reverse-TranscriptionPCR assays (RT-PCR)

It is established that RT-PCR is the gold diagnostic test that uses various clinical samples

which proved to contain the virus nucleic acid (RNA) particles; nasal swab (72%), oropharyngeal swab about (32 %) tracheal aspirate, fibro-bronchoscope brushbiopsy (46%) or bronchoalveolar lavage(BAL) specimens (93%). Primarily, the collection of upper respiratory samples via nasopharyngeal and oropharyngeal swabs forbronchoscopy were preferred, but its drawback may constitute the hazard of aerosol infection for both patients and healthcare staff [37, 38].

Furthermore, numerous studies have demonstrated that SARS- CoV-2 RNA can also be detected in stool specimens (29 %) and blood (1 %) while less extent; ocular secretions, urine, anal swabs, semen and breast milk samples [39-44]. The test is believed to be reliable either in the first days after symptom onset during the initial stages of the illness or even during pre-symptomatic and post-symptomatic phases [45].

There are many RT-PCR techniques targeting different genes in 2019n-COV; (N, E, *orf*1ab and RdRp genes) [13]. Both RT- PCR N gene and nsp2 assays were observed to be highly specific and more sensitive, take one hour and 15 min for each PCR run, [37, 46]. In the initial time of the COVID-19 pandemic, dual- or multi-gene detection approaches were adopted for RT-qPCR assays to ensure assay specificity [47]. As the pandemic progressed and the disease prevalence increased, many laboratories globally implemented a workflow using single-target detection of SARS-COV-2 [30]. To reduce false negatives coupled with technical errors, targeting human "housekeeping" transcripts like RNase P mRNA should be involved during the testing of patient's samples by RT-qPCR [48].

RT-PCR test validity in asymptomatic persons who have been in close contact with symptomatic individuals is even less obvious; the rate of positivity could reach 50% without any indication of symptoms or assured infection [49].

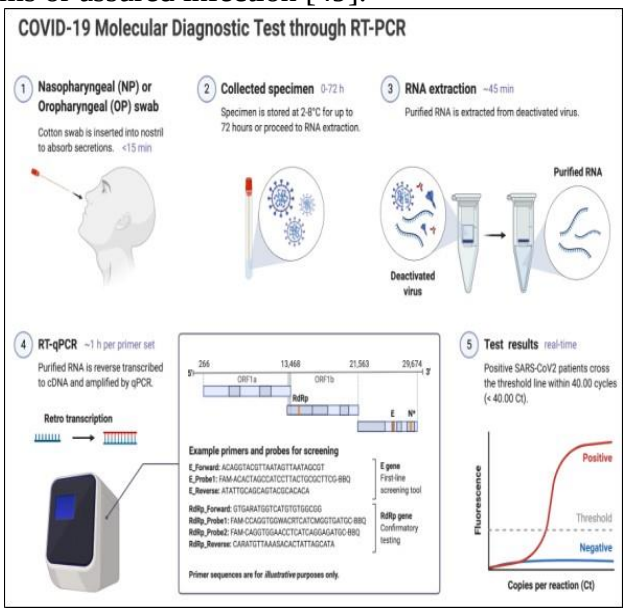

Figure (2)RT-PCRsteps:
https://covidtestingproject.org/faq.html

4.2. Real – Time Loop-Mediated Isothermal Amplification assays (RT-LAMP)

RT-LAMP method is a rapid and sensitivity method which used 4 – 6 different target sequence for recognizing 6 – 8 sequence of target gene within 1 hour. Moreover, the primers in this method consist of an outer forward primer (F3), an outer backward primer (B3), a forward inner primer (FIP) and a backward inner primer (BIP). A loop forward primer (LF) and/or a loop backward primer (LB) that were designed to accelerate the reaction that and

finally result is seen by naked eye [50].

The RT-LAMPis more preferred than RT-PCR; it takes a shorter time, does not require highly skill personal or high instrumentation plus it can be easily conducted in any site (not need certified laboratories). On other hand, its drawbacks are the implication of an internal PCR inhibition control and requiring of a complex primer model [51, 52].

4.3. Amplicon-Based Metagenomic Sequencing

Touching its name, this SARS-CoV-2diagnostic technique based on a dual approach; the use of amplicon-based sequencing in accompanied to meta- genomics sequencing. Metagenomics sequencing is used mainly to address the background microbiome of infected persons, permitting the ability to rapidly identify not only SARS-CoV-2 virus but also other pathogens implemented in secondary infections that exaggerate the severity of COVID-19 symptoms. An Amplicon and metagenomics MinION based sequencing were applied to rapidly (within 8 h) sequence the genome of both SARS-CoV-2 and the other microbiome present in nasopharyngeal swabs obtained from COVID-19 patients by the ISARIC 4C consortium [53, 54].

4.4. CRISPR-Based Assays

A group of bacterial enzymes can recognize certain nucleic acid sequences found in prokaryotic organisms called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) so called CRISPR- associated enzymes at which can beprogrammed to target and cut viral RNA sequences [55]. Commercially, two companies, Mammoth Biosciences and Sherlock Biosciences, established independently gene-editing CRISPR methodology for detection of SARS-CoV-2 [56, 57].

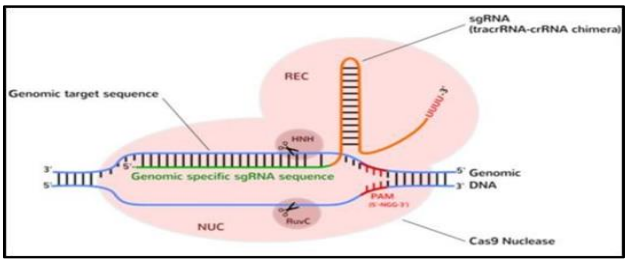

Figure (3): The CRISPR-Cas9 nuclease programmedwith sgRNA
https://www.bio-connect.nl/crispr-cas9-genome-editing-transfection-reagents/cnt/page/5171

More recently, De Puig et al. (2021) evolved a low-cost test relied on the SHERLOCK system to construct diagnosis of SARS-CoV-2 and emerging variants. The authors realized high sensitivity within 1 h and exposed multiplex detection of SARS-CoV-2 and mutations associated with VOC, including B.1.1.7, B.1.351 and P.1 represented alpha, beta, and gamma respectively [58].

Fortunately, the CRISPR-based methods do not require complex instrumentation andboth low-cost and rapid (1 h), have greatpotential for point-of-care diagnosis [1].

5. Serological and ImmunologicalAssays

Despite, RT-PCR-based viral RNA detection has been broadly used in diagnosis of

COVID-19, it cannot be used to check the progress of the disease phases and cannot be applied to vast identification of past infection and immunity [5]. Serological testing is known as an assessment of body fluids for presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, wherefore shares an essential role in epidemiology and vaccine development. The body fluids specimens are primarily, the blood serum or plasma, also may comprise other biological fluids as saliva, sputum, etc...That analysis may be applied on either short- term (days to weeks) or long-term (years or permanence) tracks of antibody response, as well as antibody redundancy and diversity. The low specificity issue is a very significant concern that the false positive results (cross reaction) may lead to deceptive antibody prevalence [59].

Different serology-based assay platforms have been developed to date, including lateral flow assay (LFA), enzyme-linked immunosorbent assay (ELISA) [5], chemiluminescence enzyme assay (CLIA) [60], immunofluorescence assay (IFA) [61, 62] and biosensor [63].

Conclusion

A new respiratory human to human infection has been rushed all over the world. The disease appeared firstly in China, caused by acoronavirus greatly related to the previousSARS virus, so termed SARS-CoV-2. Because of the COVID-19 patient is either asymptomatic or shows flu-like signs, so become as potential source of infection. Eventhe radiological picture may not give the actual situation, so the early and precise diagnosis is needed to prevent the spread of infection. Combination of PCR and ELISA method make the detection of SARS-COV2 more sensitive and may overcome the cross- reactivity of the novel corona virus with SARS-CoV but these methods need more development in specificity and sensitivity.

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