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REVİEW

Laboratory Diagnosis of COVID-19: Review of Literature

Abdurrahman ŞARMIŞ[1]

[1] Department of Microbiology, İstanbul Medeniyet University, Faculty of Medicine.

Abstract

Ongoing COVID-19 pandemic outbreak is an emerging health issue affecting every country in the World. Problems at rapid diagnosis of the disease is also another important concern of health facilities. NAATs (Nucleic acid amplification tests) from nasopharyngeal and lower respiratory airway samples have more specifity at diagnosis; but the feasibility of sampling these tests for every suspected patients during ongoing pandemic outbreak is not easy due to intensity of hospitals. Routine simple tests on admission may give an idea about hospitalization of severe cases or cases who can be severe later. This review is focused on nonspecific and Microbiological tests of COVID-19 to help clinicians make decisions about management of diagnosis and treatment process of patients fastly and accurately in the middle of pandemic intensity.

Keywords: RT-PCR, COVID-19, Diagnosis, Antibody Tests

Özet

Devam etmekte olan COVID-19 pandemisi Dünya'daki tüm ülkeleri yaygın bir şekilde etkileyen bir sağlık problemidir. Hastalığın hızlı tanısındaki sorunlar da sağlık kuruluşlarının önemli endişelerinden biridir. Nazofarengeal ve alt solunum yolları örneklerinden yapılan NAAT (Nükleik asit amplifikasyon testleri) tanıda daha fazla özgüllüğe sahiptir; ancak, hâli hazırda devam etmekte olan pandemi sürecinde her şüphelenilen hastaya bu testleri uygulamak yoğunluk nedeniyle kolay değildir. Başvuru sırasında rutin olarak uygulanan bazı basit testler ciddi veya daha sonra ciddi olabilecek vakaların hastaneye yatırılması konusunda fikir verebilir. Bu derleme, klinisyenlerin hastaların tanı ve tedavi süreçlerini yönetiminde, pandemi yoğunluğunun ortasında hızlı ve isabetli karar vermelerine yardımcı olmak için nonspesifik testler ve COVID-19'un Mikrobiyolojik testlerine yoğunlaşmıştır.

Anahtar kelimeler: RT-PCR, COVID-19, Tanı, Antikor Testi

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Corresponder Author: Abdurrahman Sarmış ***Correspondence Address*****:** Department of Microbiology, İstanbul Medeniyet University, Faculty of Medicine, İstanbul/TÜRKİYE. e-mail: asarmis@gmail.com

INTRODUCTION

The World Health Organization (WHO) was alerted by China Health Authority on December 31, 2019 because of pneumonia cases with unknown etiology in Wuhan City in Hubei Province in central China. WHO abbreviated the new coronavirus pathogen of these pneumonia cases as 2019-nCoV which was identified from throat swab sample of a patient (1). The Coronavirus Study Group renamed the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease was named coronavirus disease 2019 (COVID-19) by the WHO (2). The disease started to spread very fast and widely all over the World and WHO declared COVID-19 as a pandemic outbreak on 11th March 2020.

COVID-19 is highly contagious with a relatively high mortality rate (especially for patients with chronic diseases). Diagnosis of COVID-19 may take some time due to intensity of hospitals during pandemic outbreak; there are also diagnostic problems about test reliabilities. Some clinically suspicious cases may have negative test results. The aim of this review is to focus on laboratory diagnosis of COVID-19 with nonspecific routine tests and specific microbiological tests and give an idea about prognosis with some laboratory parameters to get medication and isolation precautions earlier.

DISCUSSION

Detection of SARS-CoV-2 genes by Real time reverse transcriptase polymerase chain reaction (RT-PCR) is very important, but it may take some time and could be expensive for some facilities. Meanwhile, routine tests such as hemogram, simple biochemistry tests may give an idea about the diagnosis and prognosis of COVID-19.

Hemogram

Hemogram (Complete Blood Count) is a very common, cheaper test that can be established easily. As we research scientific papers about hemogram results, National Centre for Infectious Disease (NCID) of Singapore report indicates that; 29.2% of 65 COVID-19 adult patients were presented with leukopenia (Only one of them with severe leukopenia), 36.9% of patients were presented with lymphopenia (19 with moderate, 5 with severe lymphopenia), and %20 of patients were presented with mild thrombocytopenia (Other were with normal platelet count) at the admission to the hospital. Patients stayed in ICU (Intensive Care Unit) had lower lymphocyte count and higher Absolute Neutrophil Count (ANC) and LDH values than Non- ICU patients. There

were no statistically difference at other hemogram parameters between ICU and non- ICU patients (3). Huang et al. (4) reported lymphopenia (defined as an absolute lymphocyte count <1.0 × 109/L) in 26 (63%) of patients and leukopenia (white blood cell count less than 4×10^9/L; ten [25%] of 40 patients).

G. Lippi et al. (5) performed an electronic research about laboratory abnormalities in patients with COVID-19 infection, they summarized these abnormalities as worse prognosis of disease:

– Increased white blood cell count

– Increased neutrophil count

– Decreased lymphocyte count

– Decreased albumin

– Increased lactate dehydrogenase (LDH)

– Increased alanine aminotransferase (ALT)

– Increased aspartate aminotransferase (AST)

– Increased total bilirubin

– Increased creatinine

– Increased cardiac troponin

– Increased D-dimer

– Increased prothrombin time (PT)

– Increased procalcitonin

– Increased C-reactive protein (CRP).

Interestingly, lymphopenia is very rare in infants according to Henry et al. (6) study. They reported in only 3% (n=2) of 66 cases. The reason is unknown, lymphopenia may not occur due to the relative immaturity of infants' immune system and differences in their immune response compared to adults (7).

Leukocytosis is not very common in COVID-19 patients, it may be seen more due to bacterial infections and superinfections in severe patients (11.4%) compared to mild to moderate patients (4.8%) (8).

Neutrophilia is not studied widely in recent literature, the available data suggest that neutrophilia may indicate cytokine storm and hyperinflammatory state of COVID-19 (8). Neutrophilia is also seen at superimposed bacterial infection at ICU patients during hospitalization (3).

Another interesting criteria indicating prognosis could be Neutrophil/ Lymphocyte ratio. Jianhong Fu et al. (9) reported that NLR and D-Dimer levels distinguished the severe COVID-19 cases from the mild/ moderate on different days. NLR and D-dimer levels stayed higher in severe cases. Neutrophilia was also seen more in ICU patients at Singapore study (3) lymphocytopenia was also observed in severe cases at different studies (3,5), as a consequence this ratio seems logical to indicate worse prognosis.

Thrombocytopenia is also another observed laboratory parameter in severe COVID-19 cases, identified in up to 57.7% of patients vs 31.6% of patients with less significant COVID-19 symptoms (10). Mo et al. (11) found lower levels of platelets in severe cases (p=0.049). Salamanna et al. (12) submit that altered platelet levels may indicate the need for aggressive treatment and understanding platelet functions on disease will help the management of the different stages of disease much better.

Another interesting research about diagnosis of COVID-19 is performed by Wu et al. (13); they developed a web assistant which gives the percentage of probability of COVID-19 diagnosis according to 2 routine hematological and 9 biochemical parameters, which can help for early treatment and isolation, and the link is here:
http://lishuyan.lzu.edu.cn/COVID2019_2/
The problem about this research that it is a preprint and has not been peer- reviewed yet.

Last parameter to check from the hemogram test is eosinophil count. Zhang et al (14) found eosinopenia at 52.9 % and lymphopenia at 75.4 % of 140 hospitalized confirmed COVID-19 patients. They concluded eosinopenia and lymphopenia co-existence may be an indicator for worse prognosis.

Microbiological Tests

Real Time PCR Test

The preferred initial specific test to diagnose COVID-19 is Nucleic acid amplification testing (NAAT), most commonly with a reverse-transcription polymerase chain reaction (RT-PCR) assay, to detect SARS-CoV-2 RNA from the upper respiratory tract samples (15). In some testing protocols, viral antigen tests are preferred initially, but the problem is lower sensitivity of antigen tests than NAATs, and negative antigen tests need confirmation by NAAT test (16).

There are conflicts about anatomical regions for sampling between some health authorities; but the most commonly advised sampling method is Nasopharyngeal swab specimen collection performed by a healthcare professional. Fang et al. (17) studied 51 SARS-CoV-2 RT-PCR confirmed (mainly on throat swab) hospitalized cases, 15 patients (29 %) had a negative initial test and only were diagnosed by serial testing. RT-PCR from throat swabs assisted to diagnose 71% of patients initially. Lee et al. (18) found out RT-PCR from nasopharyngeal swab diagnosed 89% of 70 patients initially.

Another study about sampling anatomical site performed by Wang et al. (19) indicates best performance of RT-PCR from

bronchoalveolar lavage (95 %, 14 of 15 specimens) and sputum (72 %, 72 of 104 specimens) while oropharyngeal swab had 32 % (126 of 398 specimens) positive rate. They studied oropharyngeal swab tests instead of nasopharyngeal tests at an early stage of pandemic outbreak. Oropharyngeal sampling is easier than nasopharyngeal for patients, but the sensitivity of the former is lower. A study about comparing nasopharyngeal and oropharyngeal samples found out nasopharyngeal samples had almost two fold more positive results than oropharyngeal samples (66% versus 34%) (20).

Test performance of RT-PCR during illness

Kucirka et al. (21) studied the performance of RT-PCR tests at different stages of COVID-19. They found out that over the 4 days of infection before the typical time of symptom onset (day 5), the probability of a false-negative result in an infected person decreases from 100% on day 1 to 67% on day 4. On the day of symptom onset, the median false-negative rate was 38%. This decreased to 20% on day 8 (3 days after symptom onset) then began to increase again, from 21% on day 9 to 66% on day 21.

In contrast, Furukawa et al. (22) indicated presymptomatic or asymptomatic persons carrying high virus load at RT-PCR tests with lower Cycle threshold (Ct) values. The lower Ct values indicate the higher viral load on RT-PCR results (23). This situation is accepted as evidence supporting the transmission of SARS-CoV-2 before symptom onset. But these data are limited due to lack of viral culture which indicates the viability of the virus.

Serologic Tests

Detection antibodies to SARS-CoV-2 antigens (Especially to nucleocapsid or spike protein) is crucial to decide about active immunity against COVID-19. The main problem about antibody tests is about specificity. The CDC (Centers for Disease Control and Prevention) of USA recommends using assays that have been granted emergency use authorization and optimizing the positive predictive value of the tests by choosing tests with high specificity (≥99.5 percent); or advices to apply two different antibody assay tests for one person. IgA-based antibody tests are not recommended. Advised tests are for detection of IgG, IgM and IgG, or total antibody (24).

Guo et al. (25) studied samples of confirmed and suspected cases, and found out the median duration of IgM and IgA antibody detection were 5 days (IQR 3-6), while IgG was detected on 14 days (IQR 10-18) after symptom onset, with a positive

rate of 85.4%, 92.7% and 77.9% respectively. The detection efficiency by IgM ELISA is higher than that of qPCR method after 5.5 days of symptom onset. The positive detection rate is significantly increased (98.6%) when combining IgM ELISA assay with PCR for each patient compared with a single qPCR test (51.9%). Combining RT-PCR and ELISA antibody test is very important to diagnose, and also FDA does not recommend using antibody test solely to diagnose or exclude COVID-19. Also, CDC does not recommend serologic test results to be used to make decisions about returning persons to the workplace and grouping persons residing in or being admitted to congregate settings, such as schools, dormitories, or correctional facilities (24).

Zhao et al. (26) also found combining RT-PCR and antibody detection tests significantly improved the sensitivity of pathogenic diagnosis for COVID-19, even in the early phase of 1-week since onset.

Serologic screening with validated tests for a wide population is a need to provide more information about the disease activity and immunity status of society (16).

Antigen tests

Antigen tests of SARS-CoV-2 are commercially available, but not very commonly performed due to lower sensitivity than NAATs. A negative antigen test should be confirmed using a sensitive NAAT if the clinical suspicion is high. Many countries gave a try at the first stage of the pandemic outbreak, but gave up due to lower sensitivity and lower cost-effectiveness than NAAT.

Viral culture

Viral culture is advised only for research purposes due to biosafety causes. Viral culture is not performed routinely at laboratories for diagnosis.

CONCLUSION

Fast and accurate diagnosis of COVID-19 is very important to start medication and get isolation precautions to prevent transmission. NAATs with RT-PCR assays from nasopharyngeal swabs could be preferred initially, and it would be better to combine with ELISA antibody tests concurrently if available. Hemogram and some biochemical tests may give ideas quickly about management of patients to hospitalize, take into ICU or send home. Especially increased neutrophil count, D-Dimer, LDH levels and decreased lymphocyte count may give ideas about bad prognosis, so clinicians should be alerted. Researchers can study more about the effect of routine tests on diagnosis of COVID-19 and some formulations may help clinicians

to make fast and accurate decisions during pandemic outbreak.

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