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Clinical display, diagnostics and genetic implication of Novel Coronavirus (COVID-19) epidemic

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Abstract. – COVID-19 pandemic can cause irreparable damage to the involved society. This study aimed to provide a summary of the up-to-dated clinical display, diagnostics, molecular and genetic implications for COVID-19 infected patients.

In this review, 73 research articles published before 25 March 2020 were analyzed to better understand the clinical characteristics of patients and to introduce the available serological, hematology and molecular diagnostic methods. Apart from articles extracted from PubMed and Google Scholar, WHO (https://www.who.int/), NHC (National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/), NICE (National Institute for Health and Clinical Excellence, https://www.nice.org.uk/), CDC (Centers for Disease Control and Prevention, https://www.cdc.gov/), and National Administration of Traditional Chinese Medicine (http://www.satcm.gov.cn/) were also accessed to search for eligible studies. Papers published between January 1, 2020, and 25 March 2020 were searched in English and the terms "2019-nCoV, Covid-19, Clinical Characteristics OR manifestation, method of detection, COVID-19 Genome and molecular test" were used.

As the pandemic continues to evolve, there have been reports about the possibility of asymptomatic transmission of this newly emerged pneumonia virus. We highlighted the role of HLA haplotype in virus infection as HLA typing will provide susceptibility information for personalized prevention, diagnosis, and treatment in future studies. All the data in this article will assist researchers and clinicians to develop their clinical views regarding infected patients and to emphasize the origin of SARS-CoV-2 for diagnostics.

Key Words:
Diagnosis, Genetic, Epidemiology, Coronavirus, SARS-CoV-2, COVID-19, Detection, Clinical display, Novel coronavirus.

Introduction

Respiratory tract viral infection caused by viruses is deemed one of the most common diseases in human in the world. 2019-nCoV, divergent from SARS-CoV, belongs to the coronavirus family and emerged in December 2019 caused pneumonia outbreak in Wuhan, China[1]. The outbreak of this novel coronavirus disease (COVID-19) quickly spread all over China and to more than 184 other countries and territories worldwide[2]. This virus can cause the disease named coronavirus disease 2019 (COVID-19) resulting in multi-organ failures and has posed major threats to global public health[3-6]. At first, this virus named 2019 novel coronavirus (2019-nCoV); however, the International Committee of Taxonomy of Viruses (ICTV) attributed the name of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to this newly developed virus[7]. Based on varied clinical presentations in patients

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infected by COVID-19, the differentiation process of causative agents without presenting an accurate and efficient detection method is leading to false patient management and unnecessary use of antibiotics. Recommendation of the best diagnosis protocol is almost controversial because of overlapped clinical displays of involved patients.

Specimen Collection of Patients

In March 2020, the USA Centers for Disease Control and Prevention (CDC) published interim guidelines regarding the collection, handling, and testing of clinical specimens for the diagnosis of coronavirus disease 2019 (COVID-19). Accordingly, laboratories which test for COVID-19 virus should be strictly aligning with appropriate biosafety practices. Rapid collection and testing of appropriate specimens from suspect cases for COVID-19 is a priority for patient management, as well as outbreak control, and should be carried out by a laboratory expert[8].

According to the World Health Organization, recommended respiratory material should be selected from upper respiratory specimens such as Oropharyngeal (OP) and Nasopharyngeal (NP) swabs, Nasopharyngeal wash/aspirate and for lower respiratory specimens including Sputum, tracheal aspirate, bronchoalveolar lavage (BAL) fluid in patients with the severe condition and pleural fluid (Table I). Additional clinical specimens of COVID-19, as for other coronaviruses responsible for SARS and MERS can also be detected in blood and stool[9].

Autopsy of Deceased Cases by Forensic Medicine

Performing an autopsy on a patient with confirmed COVID-19 infection is sophisticated from both spiritually and physically aspects; however, it is fundamental for giving a forensic certificate to specify a mortality cause. Forensic medicine is legally responsible for performing the autopsy. The question of whether the autopsy of a dead body with an infectious disease should be performed is the issue of controversy[10]. The World Health Organization (WHO) developed Interim guidance on 24 March 2020 for the safe management of a dead body in patients with COVID-19 disease[11].

The presence of a manager who is aware of staff interacting with the dead bodies is highly needed. The specialists of forensic medicine should try the best to transfer the dead body to the mortuary area. It should be noted that there is no need to disinfect it; however, facilitating the process of burial setting is recommended. Additionally, there is no need to supply specific tools to transfer the dead body[10].

It is important to be vigilant of the dead body with an infectious disease at the time of expiration since the virus can be alive in the lung and other parts. Having mask and protection equipment is strongly recommended. Furthermore, air conditioners should be equipped to at least 160L/s/patient airflow or having negative pressure with at least 12 air changes per hour[10]. All parts of the autopsy room must be disinfected because the virus can remain active for 9 days[12].

Table I. Types of specimen collection.

Upper respiratory specimens
• Nasopharyngeal swab (NP)
• Oropharyngeal swab (OP)
• Nasopharyngeal wash/aspirate
Lower respiratory specimens
• Bronchoalveolar lavage (BAL) and Pleural fluid*
• Sputum
• Endotracheal aspirate
Stools, whole blood, urine and infectious material from autopsy in deceased cases

*Patients in severe condition.

Clinical and Physical Display of COVID-19 Patients

One of the key immediate clinical features used in detection is flu antigens. Based upon recent findings, routinely detected flu antigens are A, B, and H7N-subtypes. One factor for early rapid screening of flu is sampling of throat swabs; however, it has relatively high false-negative rate[7].

In addition to the fever which is still the typical symptom of 2019-nCoV infection[1,3,13], the 2019-nCoV infected cases have symptoms like fatigue, dry cough, dyspnea, etc., with or without nasal congestion, runny nose or other upper respiratory symptoms that also confirmed by the analysis of 262 infected cases to determine the clinical and epidemiological characteristics of COVID-19 in Beijing. They were categorized into severe and common group respectively, and the most common symptoms of illness onset reported were fever (82.1%), cough (45.8%), fatigue (26.3%), headache (6.5%), and dyspnea (6.9%, and severe cases with dyspnea 32.6%)[14]. In another retrospective study in Wuhan Jinyintan Hospital from Jan 1 to Jan 20, 2020[15], patients

had clinical manifestations of fever (83%), cough (82%), shortness of breath (31%), confusion (9%), headache (8%) sore throat (5%), rhinorrhea (4%), chest pain (2%), diarrhea (2%), nausea and vomiting (1%) and other minor symptoms including wheeze in lungs, weakened breath sounds, dullness in percussion, and increased or decreased tactile speech tremor and muscle ache (Figure 1). This study also showed that some patients developed acute respiratory distress syndrome and, among them, (11%) of patients exacerbated in a short period of time and died of multiple organ failure. Additionally, the 2019-nCoV infection is more likely to affect older males with additional disorders. Given all data extracted from Chinese hospitals[16], generalizing these clinical and physical symptoms statistics is somewhat controversial due to the varied nature of the genetic pool and therefore, different immune responses in each individual population. Table II illustrates the clinical display of COVID-19.

Clinical Display of Cardiovascular Disease (CVD) Combined with COVID-19

According to a study on 112 patients in the Western district of Union Hospital in Wuhan on their blood samples[17], COVID-19 patients combined with CVD were reported to have a higher risk of mortality and patients posed lower lymphocyte counts and a higher pulmonary CT, C-reactive protein CRP and procalcitonin (PCT) in blood samples[18]. This survey also indicates that contributing factors in the death of this group of patients include fulminant inflammation, lactic acid accumulation, and thrombotic events. He et al[19] have reported that severe or critically ill COVID-19 patients accompanied by myocardial injury are associated with a higher risk of in-hospital mortality. Additionally, Li et al[20] have announced that COVID-19 patients with previous cardiovascular metabolic diseases are at increased risk for developing a severe illness with COVID-19.

Clinical Display of Liver and Kidney Injuries Combined with COVID-19

Several studies have demonstrated that 2-11% of COVID-19 patients had liver comorbidities, and also abnormal levels of alanine aminotransferase and aspartate aminotransferase (AST) were seen in 14-53% cases reported during disease progression. Further, liver injury was more widespread in severe cases compared to mild cases of COVID-19[21]. In addition to liver injuries, Cheng et al[22] have indicated that mortality rates of COVID-19 patients with acute kidney injury (AKI) were higher than other patients. Diao et al[23] suggested that the human kidney could be considered as a particular target for SARS-CoV-2 infection leading to AKI and viral dissemination

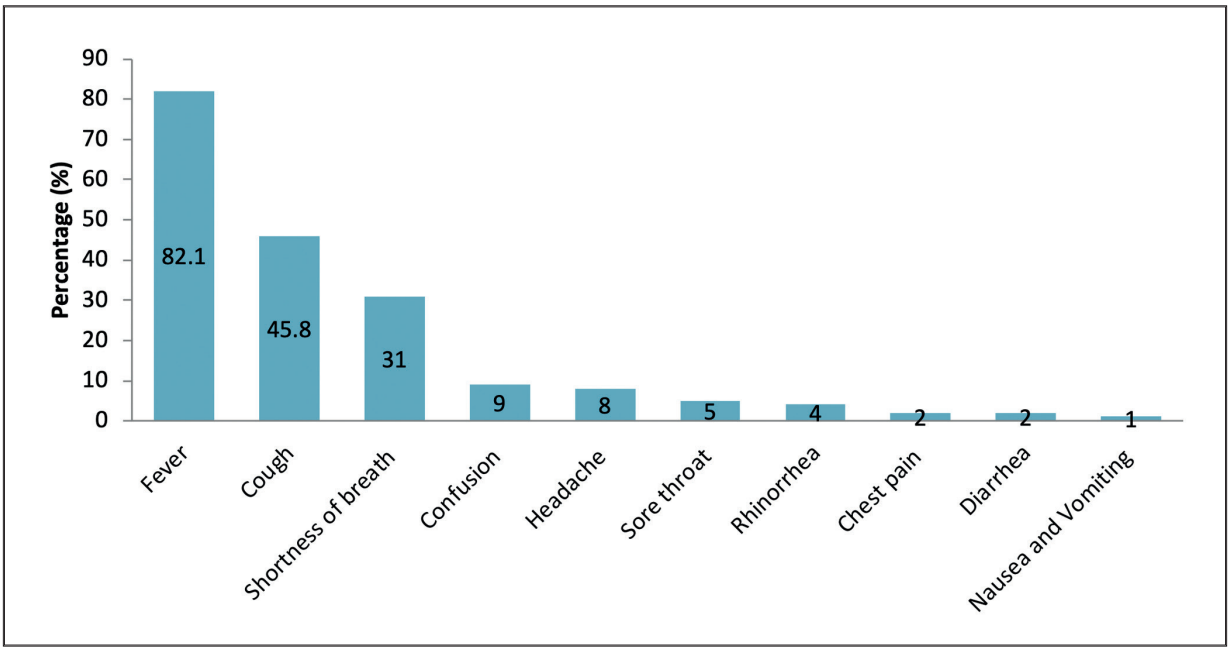

Figure 1. The most common symptoms of COVID-19 (11).

Table II. Clinical display of various conditions combined with COVID-19.

Condition	Comment
Cardiovascular disease (CVD)	• Higher risk of mortality • Lower lymphocyte counts • Higher pulmonary CT, C-reactive protein CRP and procalcitonin (PCT) in blood samples • Increased risk for developing a severe illness with COVID-19 in patients with previous cardiovascular metabolic diseases
Liver injury	• Abnormal levels of alanine aminotransferase and aspartate aminotransferase (AST) • Common in severe cases
Kidney injury	• Higher risk of mortality • Proteinuria and hematuria • SARS-CoV-2 infection leading to AKI
Lung	• Bilateral lung involvement • Alveolar exudative inflammation • Interstitial inflammation • Alveolar epithelium proliferation • Hyaline membrane formation
Diabetes	• The uncertain effect on mortality
Neurologic	• Hysgeusia • Hyposmia • Anosmia
Pregnant women	• Similarity of clinical presentation in pregnant women with SARS-CoV-2 infection in the last trimester pregnancy to non-pregnant patients • No morphological changes in placenta

in the body[23]. Likewise, another study showed that about 40% of patients admitted to the hospital had proteinuria and hematuria[24].

Clinical Display of Lungs Combined With COVID-19

The various studies have demonstrated that the lungs of most patients with novel coronavirus were involved bilaterally and pathological features comprising the alveolar exudative inflammation and interstitial inflammation, alveolar epithelium proliferation and hyaline membrane formation have been observed[25,26].

Clinical Display of Diabetes Mellitus Patients With Coronavirus Infection

Results of different studies about the role of diabetes or raised blood glucose as a risk factor leading to death in COVID-19 patients are controversial. For instance, a report of 72,314 cases of COVID-19 published by the Chinese Centre for Disease Control and Prevention indicated that the case-fatality rate was raised among COVID-19 patients with diabetes (7.3%)[27]. Although, in another study, diabetes has not been identified as a risk factor for disease severity and progression in 140 patients with COVID-19 in Wuhan, China[28].

Clinical Display of Pregnant Women With New Coronavirus Infection

To investigate the clinical characteristics and placental pathology of 2019-nCoV infection in pregnancy, as well as the assessment of intrauterine vertical transmission potential of this virus, Chen et al[16] performed a study on three pregnant cases. The clinical manifestations of pregnant women with 2019-nCoV infection in the last trimester pregnancy were similar to those of non-pregnant patients, and no severe detrimental pregnancy outcome was found in the 3 case studies. Furthermore, no morphological changes related to infection in the three placentas were observed. Accordingly, there was no evidence for intrauterine vertical transmission of 2019-nCoV in the three observed infected women in their late pregnancy[29].

COVID-19 Diagnostics

Methods of Detection

Imaging Examination (CT Imaging)

For both initial evaluation and follow-up, due to the primary involvement of the respiratory system, chest CT is strongly recommended in sus-

pected COVID-19 cases. In the intermediate to advanced stages of the disease, chest radiographs may show a progression of features of acute respiratory distress syndrome (ARDS). In contrast, in early stages, chest radiographs characterized by a low diagnostic value, while CT findings may be present even before symptom onset. According to several studies on myriads of cases, CT findings have proven to have a high diagnostic value in a number of cases with an initial false-negative reverse transcription-polymerase chain reaction (RTPCR) screening test[30].

Hematology and Serology Tests

Data extracted from the SARS epidemic prove that serological responses, including viral-specific IgM and IgG, can allow for serologic diagnosis[31,32]. It was shown that patients with 2019-nCov pneumonia also had similar acute serological responses and enzyme-linked immunoassay (ELISA) for specific IgM and IgG antibodies as a conventional serological assay provide a high-yield alternative, and it can be utilized as a uniform test for all suspected patients and can facilitate more complete identification of infected cases. In spite of the fact that the nucleocapsid protein can serve as a sensitive antigen, other 2019-nCoV-specific antigens or epitopes should be explored for use in the serology assay, thus, the use of the whole N protein as the antigen for the serological assay can lead to potentially specificity and sensitivity issues[33-37].

Autopsy material including lung tissue is highly recommended in case of deceased patients, while in surviving patients, paired serum, both acute and convalescent, can be useful to retrospectively define cases. Most recently, it was proved that in the early stage of the disease, the total number of lymphocyte count is highly decreased, with decreased or increased or normal monocytes. When the absolute value of lymphocyte is less than 0.8 × 109/L, or the numbers of CD4 and CD8 T cells are significantly decreased, high attention is required generally emphasizing a recheck for the blood changes after 3 days. To highlight the role of cytokine, Chen et al[36] carried out a study on 29 patients with 2019 novel coronavirus and they showed an increased level of expression in IL-10, IL-2R, and IL-6 in serum. These results hallmark the important role of interleukins as a marker for prediction of the severity of the 2019-nCoV pneumonia and the prognosis of patients[38].

There are other serological indicators for the probability of 2019-nCoV infection, including myoglobin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Procalcitonin (PCT), liver and kidney function, myocardial enzyme, lactate, D-dimer, coagulation image, inflammatory factors (TNF - α, interleukin (IL-6, IL-10), complement, anti-acid staining, urine routine test, and blood gas analysis. Blood gas analysis is helpful to determine the oxygenation of moderately to severe infected patients. Combining this analysis with the observation of increased lactic acid provides the screen of patients with high-risk of oxygenation disorder. Some infected patients have increased D-dimer with microthrombotic formation and frequent clotting disorders in blood vessels, increased liver enzymes, Erythrocyte sedimentation rate ESR, muscle enzyme and myoglobin. To detect whether there was bacterial infection in the lung, the detection of CRP and PCT is of prominent value. Detection of other inflammatory factors may help to a fast evaluation of the immune status of patients[39,40]. Table III demonstrates a summary of provisional available testing for the management of patients with confirmed coronavirus (COVID-19) disease.

Genetics of SARS-CoV-2

Notable Features of the SARS-CoV-2 Genome

The SARS-CoV-2 genome contains two flanking untranslated regions and a single long open reading frame encoding a polyprotein including replicase complex (orf1ab), S gene, E gene, M gene, and N gene. According to a recently published study in Nature on the origin of SARS-CoV-2[39], there are two prominent genome characteristics attributed to SARS-CoV-2. This virus holds a structure that makes this virus optimum for binding to the human receptor Angiotensin-converting enzyme, ACE2. Furthermore, polybasic cleavage site and the three adjacent predicted O-linked glycans of SARS-CoV-2 structure were not previously seen in lineage B betacoronavirus family. However, in the newest computational study performed[41], it was shown that the receptor binding domain RBD sequence available in this new version of virus does not bind ideally to ACE2 and this high-affinity binding of SARS-CoV-2 spike protein to human ACE2 is most likely the result of natural selection which rules out the role of human manipulation for creation of this virus.

Table III. Techniques for diagnosis of COVID-19 patients.

CT Scan	
Hematology and serology tests	• Complete blood count (CBC) • Viral specific IgM and IgG antibodies • Whole N protein • Level of expression in IL-2R, IL-6 IL-7, IL-10, GCSF, IP10, MCP1, MIP1A and TNF-α* • Prothrombin time • LDH • Amylase and D-dimer • ALT • C-reactive protein (CRP) • Procalcitonin (PCT) • Muscle enzyme and myoglobin • Myocardial enzyme • Lactate • Complement • Creatine kinase • ESR
Autopsy material	
Molecular Test	• Rapid diagnostic tests (RDT) • Mesa's test (updated 25 March) • Coronavirus test: UK to make 15-minute at-home kits available 'within days50. • Nucleic acid amplification tests & point-of-care tests (POCT) • RT-PCR and rRT-PCR • Loop-mediated isothermal amplification (LAMP) • Clustered regularly interspaced short palindromic repeats (CRISPR) • Multiplex respiratory virus infection assays • Shotgun metagenomics sequencing, NGS

*ICU patients had higher plasma levels of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNF-α.

The Role of the nsp2 and nsp3 in COVID-19 Pathogenesis

In a homology modeling study performed by SwissModel and HHPred servers, the transmembrane helical segments in coronavirus ORF1ab nonstructural protein 2 (nsp2) and nsp3 were tested and the results showed that both stabilizing and destabilizing mutation of the endosome-associated-protein-like domain of the nsp2 protein and nsp3 can explain for the high ability of contagious in COVID-2019[42].

HLA Haplotypes and SARS-CoV-2 Infection

One important factor in genetic susceptibility to infectious diseases is the major-histocompatibility-complex antigen loci (HLA). During co-evaluation with pathogens, selective HLA-loci variability results from selective pressure. Immunologists have found that different HLA haplotypes are associated with distinct disease susceptibilities. It is mostly due to T-cell antigen receptors with their ability to recognize the conformational structure of the antigen-binding-grove with the associated antigen peptides[43]. Accordingly, it seems advantageous to have HLA molecules with increased binding specificities to the SARS-CoV-2 virus peptides on the cell surface of antigen-presenting cells and identification of dominant alleles will be conducive for the development of detection kits. Therefore, it is recommended to study HLA haplotypes to see if specific HLA loci either class I or II induce protective immunity in the population.

Nucleic Acid Detection of SARS-CoV-2

There are updated diagnostic methods including rapid diagnostic tests (RDT), NAATs, multiplex respiratory virus infection assays, CRISPR, and metagenomics NGS, and the time required for RT-PCR, CRISPR and metagenomics NGS diagnostic tests is about 3, 2 and 24 h, respectively[44].

Various culture-independent nucleic acid amplification tests (NAATs), as well as point-of-care tests (POCT), have contributed to the diagnosis of unexplained pneumonia. These techniques are including polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), and clustered regularly interspaced short palindromic repeats (CRISPR), etc. Laboratories participating in the evaluation regularly use the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), as well as QIAGEN One-Step RT-PCR Kit with the specific cycling conditions and concentrations. All SARS-CoV-2 commercially available or in development tests for the diagnosis of COVID-19 is listed on this website (https://www.finddx.org/covid-19/pipeline/).

The current nucleic acid amplification test methods mainly targeted the open reading frames of the replicase complex (orf1ab), S, E, M and N genes. For primer designing two sequence regions (ORF1b and N) that are highly conserved among sarbecovirus, are often selected for primer and probe designs Chu et al[37] showed that N gene assay is about 10 times more sensitive than the ORF-1b gene assay in detecting positive clinical specimens. Although the virus (SARS-Cov-2) nucleic acid RT-PCR test has become the standard method for diagnosis of this infection, these test kits have many limitations. Apart from contamination and technical problem, the false-negative rates have been reported due to high genetic diversity of the SARS-CoV-2 genome, it is therefore difficult to deduce a meaningful evaluation of what proportion of symptomatic cases are infected and Shotgun metagenomics sequencing is suggested for confirmation in some negative NAATs cases[45,46].

Shotgun metagenomics sequencing (mNGS) including short-read and long-read sequencing could obtain genomic data from both known and novel pathogens. The first full genomic sequence of this coronavirus was released on January 10, 2020. Many public access databases for deposition of genetic sequence data are available, including GISAID. The next-generation sequencing (NGS), and electron microscope technology play a role in the early diagnosis, and it can tell about the possible mutation; however, because of the scarcity of information regarding this new virus, the combination of RT-PCR, CRISPR, and mNGS can assure clinical diagnosis for COVID-19[45-49].

Conclusions

Currently, there is a big challenge regarding reporting asymptomatically infected cases or very mild cases of infection who are not tested for viral RNA and they compose a large group of patients. Therefore, the true rate of infection in the population still remains unknown.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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