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Review Article

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Features of Pathogens, Epidemiology, and Diagnosis of COVID-19 and Reliable Suggestion: Aiming to Solve the Confusion in Clinical Practice

Yangyi Jin and Hequan Li*

Department of Respiratory Diseases the First Affiliated Hospital of Zhejiang University School of Medicine Zhejiang University Hangzhou, China

***Corresponding author**
Li Hequan, Department of Respiratory Diseases, the First Affiliated Hospital of Zhejiang University School of Medicine, Zhejiang University, Hangzhou 310000 Zhejiang, China

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Abstract

In past several months, the worldwide outbreak of COVID-19 infection is unstoppable, which results in millions of people infected and thousands of people dead. However, there are still something controversial in the virus mechanism, the epidemiological feature and the diagnose criterion of COVID-19, while we also have some consensus. The structure of SARS-CoV-2, the pathogen of COVID-19, is roughly similar with other coronavirus such as SARS-CoV and bat-CoV, but by sequencing the amino acid, recombination of virus particles, and observation in Electron microscope, it is obvious to find the some slight difference between the protein of SARS-CoV-2 and others'. The spike protein of SARS-CoV-2 has a stronger ability to binding ACE2 than SARS-CoV. The carrier of SARS-CoV-2 is just as other respiratory viruses, like droplet, aerosol and surface, and the fecal-oral transmission is proved to be an efficient pathway. In clinical practice, the elder and the patients with comorbidity are more susceptible to infection and have poorer prognosis, while pediatric patients is the very opposite of it. Nucleic acid test represented by RT-PCR is a helpful method for diagnose, yet it has weaknesses of false negative in suspicious patient and resurgence in discharge patient. Serological and immunological test, reported not suitable for diagnose alone in early period, can be another reliable method that benefit the accuracy of diagnosis criterion when combined with RT-PCR.

Keywords: COVID-19, SARS-CoV-2, ACE2, Transmission, Diagnose, RT-PCR, False-negative, Resurgence, Antibody

Abbreviations

COVID-19: Coronavirus Disease 2019
CoV: Coronavirus
SARS: Severe Acute Respiratory Syndrome
MERS: Middle East Respiratory Syndrome
HCoV: Human Coronavirus
ARDS: Acute Respiratory Distress Syndrome
RT-PCR: Reverse Transcriptase–Polymerase Chain Reaction
NAT: Nucleic Acid Test
HE: Hemagglutinin-Esterase
ACE2: Angiotensin-Converting Enzyme
HNRNP a1: Heterogeneous Nuclear Ribonucleoprotein a1
RBD: Receptor-Binding Domain
TTSP: Trypsin or Trypsin-Like Protease
MHV: Murine Hepatitis Virus
DPP4/CD26: Dipeptidyl Peptidase 4
APN: Amino Peptidase N
CT: Computed Tomography
BNP: Brain Natriuretic Peptide
ELISA: Enzyme-Linked Immunosorbent Assay

Background

In December 2019, a group of unexplained pneumonia patients was observed in Wuhan, China. These patients have the common clinical features of fever, dry cough, and dyspnea, and ARDS, sepsis, etc. may develop in severe cases [1-5]. On December 29, a new coronavirus was detected as a pathogenic pathogen [3, 4, 6]. Later research validated that it is a coronavirus belonging to the genus of beta coronavirus, within the family of coronavirus. On February 11, 2020, WHO named this new coronavirus pneumonia "COVID-19" (Coronavirus Disease 2019). According to taxonomy and practice, the Coronavirus Research Group of the International Committee of Viral Taxonomy officially recognized the virus as a relative of SARS-CoV and renamed it SARS-CoV-2 [7]. By April 5, 2020, it has spread to 207 countries and regions. SARS-CoV-2 is responsible for lower respiratory tract infections and may cause acute respiratory distress syndrome (ARDS) [3, 4, 6]. Other human coronaviruses (HCoV 229E, NL63, OC43 and HKU1) are associated with upper respiratory tract infections and the common cold [8].

According to the public data of the World Health Organization, as of March 35, 2020, the number of COVID-19 diagnoses worldwide has reached 414,170 cases, of whom 18,440 deaths caused (Figure 1) [9]. At present, the epidemic situation in China has been controlled to a certain extent, and the cases reported outside of China have been> 90%. However, Western Europe, North America and some countries in South Asia have become the next popular area for the outbreak [10-13]. Considering the lack of Reverse transcriptase–polymerase chain reaction (RT-PCR) kit in many countries, the actual outbreak infection and the ratio of death will only be worse, which is still significantly lower than the mortality of severe acute respiratory syndrome (SARS) coronavirus infection (9.6%) in 2003 and Middle East respiratory syndrome (MERS) coronavirus infection (34.5%) in 2010 [14]. At present, there are still many confusions in the actual detection and treatment process, for instance, the lack of understanding about extraordinary transmission route, the absence of specific medicine and vaccine and the doubt about the value of tests other than nucleic acid test for diagnose. Obviously, it needs to pay more attention to research and provide more powerful evidence to clinical doctors. We will combine the previous research of other coronavirus and the clinical experience in the current diagnosis and treatment, especially the clinical experience from Chinese cases, and make a comprehensive review of Clinical epidemiology and diagnosis to, and discussed the hot spots of the next period of COVID-19 research and clinical practical strategies.

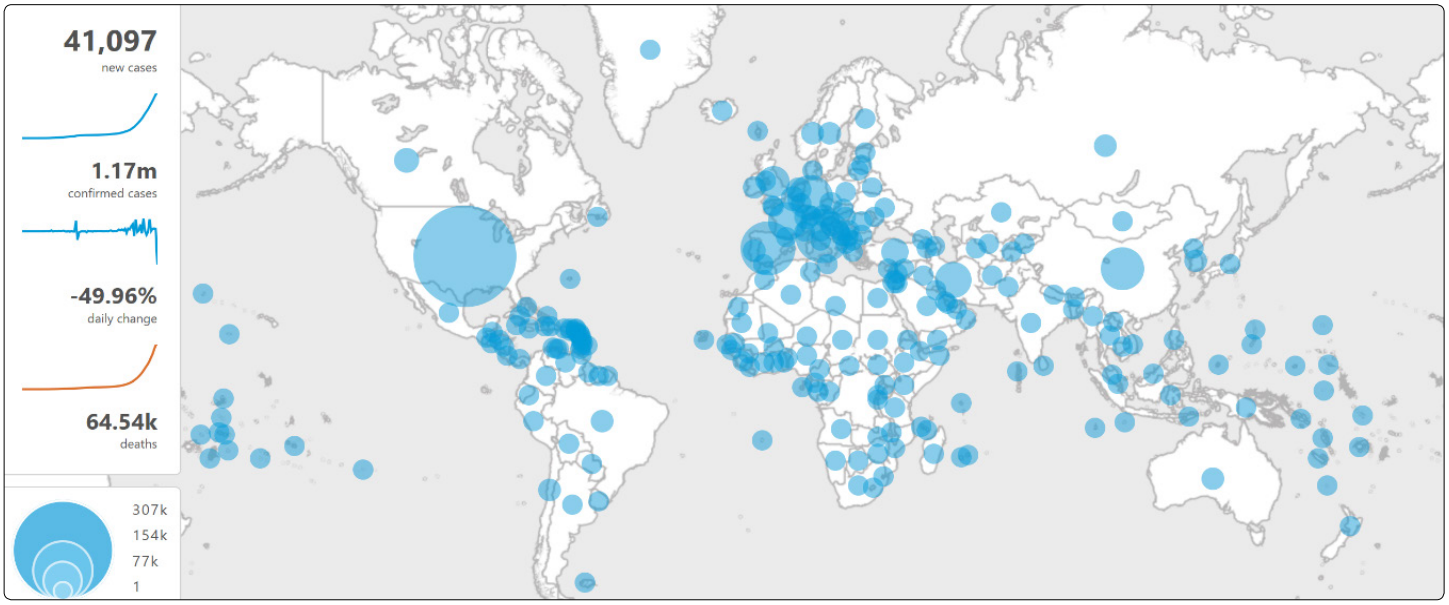

Figure 1: The Worldwide Outbreak of COVID-19

Main Text

The Structure of Coronavirus and Entry Mechanisms

The Structure of Coronavirus

Coronavirus, including SARS-COV-2, the criminal of recent COVID-19 global outbreak, are a positive-stranded RNA viruses family. According to phylogenetic tree, coronavirus family are classified into four main subgroup, named as α, β, γ, δ, and SARS-CoV-2 is beta coronavirus [15]. Like other coronaviruses, SARS-CoV-2 have spike protein that protrudes from the surface of the virus envelope under the electron microscope [16, 17]. The outer structure protein and the single-stranded positive RNA inside the protein capsid with the N protein fitting in constitute the main part of the virus [1, 18, 19]. Moreover, the surface of protein capsid is attached by hemagglutinin-esterases (HEs) [18]. The structural proteins in the protein capsid are mainly composed of spike protein (s protein), membrane protein (m protein), and envelope protein (e protein). The m protein constitutes the main shape of the outer structure protein, and the s protein and e protein are embedded in it. In addition, s protein act as spike-like protein protruding from the surface of the whole structure of the virus, playing an essential role in the process of virus infection.

The Spike protein, just as what it is called, is a highly glycosylated protein that can form homotrimeric spikes on the surface of virus particles and mediate the virus into host cells [18, 20-23]. Due to the slightly different amino acid sequence of spike protein, different coronaviruses have different affinity for different receptors of host cell [22-25]. It was reported during the SARS epidemic in 2003-2004 that the functional viral receptor of SARS-CoV was quickly identified using biochemical methods, unveil the fact purified S1 was proved to bind to human angiotensin-converting enzyme (ACE2) [26]. This interaction imply some novel finding about the biological preference of SARS-CoV, which is attached much significance of the mechanism of infection [27]. Furthermore, the protease of host cells cleave the spike protein in the process of some coronavirus replication, and spike protein exists in the form of two subunits (S1 and S2) on the virus particles [20]. However, the spike protein of other coronavirus like SARS-CoV, could remain intact on the virus particles, and finally cleaved by endocytic vesicles during virus entry [27].

The M protein is one of the most important proteins in the viral structure, whose content is the biggest in the virus protein coat [28]. The difference in abundancy may due to the fact that M protein gives the virus its shape and is critical together with E protein in orchestrating the assembly of the virus and in forming mature viral envelopes [29]. Also, the combination of membrane protein and spike protein is considered as a key event in the process of coronavirus assembly, and the former shows more critical.

The N protein is also named as the nucleoprotein capsid, binding to the viral RNA genome and forms the ribonucleoprotein core, which is necessary in the process of packaging the viral RNA into viral particles [30]. According to previous research, the nucleoprotein capsid of SARS-CoV can be specifically binding with heterogeneous nuclear ribonucleoprotein a1 (hnRNP a1) when virus is packaged in host cells, and directly regulate the whole process [31]. However, among different coronaviruses, the morphology and function of nucleoprotein capsid was greatly influenced in the different surroundings, and is very little conservative in evolution [32].

The envelope protein is the structural protein with the smallest molecular weight in the coronavirus. It usually shows a small amount of expression in the viral envelope and infected cells. It is related to many aspects of the virus life cycle, such as assembly, budding, envelope formation and pathogenesis [33]. Thanks to this situation, coronavirus lacking the envelope protein becomes a promising candidate for live vaccines.

As mentioned above, hemagglutinin-esterase exists on the surface of certain beta coronavirus. It seems that it is a hemagglutinin similar to the hemagglutinin of influenza virus (which binds sialic acid to the glycoprotein on the host cell surface) and has acetyl esterase activity, which could enhance the ability of the entry and pathogenesis of coronaviruses [34]. What's more, in HCoV (β1CoV) OC43, HE-mediated receptor binding was specific against and finally lost through progressive accumulation of mutations in the HE lectin domain [35]. This indicates that hemagglutinin-esterase's receptor-binding domain (RBD) and structure are not very conservative in evolution, and other reports have mentioned this.

The Mechanism of Coronavirus Entry

Spike protein is the viral protein that mediates the entry of coronavirus into host cells, classified as viral membrane fusion proteins I including typical influenza virus hemagglutinin (HA) and retroviral envelope proteins [20, 36]. Virus fusion proteins can be formed as trimers, and each monomer is usually divided into two domains, one is a receptor-binding domain and the other is a fusion domain. In the process of different coronavirus invasion, spike protein's function is mostly similar, which is binding to the receptor on the cell surface, mediating the contact between the virus and surface of host cells, and mediating the fusion of the viral envelope into the cell membrane [37]. Thus, the spike protein is cleaved into the s1 domain and the s2 domain by enzymes on the cell membrane as mentioned above, of whom the former mediates the binding to the host cell receptor and the latter mediates the fusion between the viral membrane and the host cell membrane required for coronavirus to enter the host cells [20, 38]. Yet, because of the more or less differences in the s protein of different coronaviruses, the protein amino acid sequence corresponding to the spike protein cleavage and hydrolase is also different, which may determine the virus's Affinity to receptors on the cell surface, and then affects the propensity of the virus-infected tissue cells and the invasiveness of its infection. For instance, the cellular proteases furin, cathepsin L and TMPRSS2 can activate MERS and may cleave the S protein at two distinct sites, termed S1/S2 and S2' [39]. The S1 / S2 cleavage point of SARS-CoV-2 is the same as SARS-CoV, and previous studies have made it clear that the SARS-CoV S1 / S2 site is cleaved by Cathepsin L after receptor binding and during the entry of viruses. But the activation process of proteases is quite complicated and may depend on the cell type to a great extent [37, 40]. Previous studies have suggested that the pathway mediated by trypsin or trypsin-like protease (TTSP) is mainly related to the virus infecting respiratory epithelial cells [40]. We believe that the spike protein of SARS-CoV S is cleaved by cathepsin L or TMPRSS2 at the S2 'site of different cell positions during viral entry, one of which is located at the junction of the S1 and S2 subunits and the other is located Upstream of the first fusion peptide [27, 40]. Recently several studies have showed that the cathepsin L and TMPRSS2 promote the entry of SARS-CoV, and increase inflammatory responses in the affected cells [41-43]. The novel SARS-CoV-2 have clinical symptoms and invade tissues similar to SARS-CoV, and the structure of its spike protein has been confirmed by electron microscope [44]. However, previous study found that, unlike SARS-CoV, SARS-CoV-2 has a furin protease-like protease cleavage site (RRAR ↓ SV), located at S1 / S2 site (AYT ↓ M). The N terminal does not exist in SARS-CoV, which means that it can be cleaved by furin [45]. In other coronaviruses, the inhibition of specific proteases can inhibit the infection of host cells, which also suggests specific enzyme inhibitors for furin proteases as the direction of future drug research possibly.

As involved above, The S1 domain is a key part of coronavirus that mediates the binding to the host cell receptor, it is a kind of protein that directly binds to the host cell membrane surface receptor. For example, the RBD region in SARS-CoV and MERS-CoV is located at the C-terminus of the S1 subunit and at the N-terminus of the S1 subunit in murine hepatitis virus (MHV) [24-47]. The corresponding affinity of coronavirus is also consistent with the interaction of S protein with receptors on host cells, and several cell receptors are described as coronavirus receptors. For example, ACE2 has been identified as the receptor for SARS-CoV and SHC014-CoV(a coronavirus spread in Chinese horseshoe bats), and dipeptidyl peptidase 4 (DPP4, also named as CD26) is also identified as MERS-CoV receptors [26, 48, 49]. As for newly emerged SARS-CoV-2, a consensus among scientists revealed that its RBD is also binding with ACE2 on the surface of the host cells, and mediates virus entry. At present, a number of teams have sequenced and observed under electron

microscope the RBD region of spike protein in SARS-COV-2 [22, 50-55]. A study reports a compare of the RBD of the s protein of SARS-CoV and SARS-CoV-2, showing that RBD of the two viruses share 72% identity in amino acid sequences, and the affinity between SARS-CoV-2 and ACE2 is higher than comparative [22, 54] . Yan, R mixed the recombinantly expressed and purified RBD-mFc of SARS-CoV-2 and ACE2-B0AT1 complex, and obtained the 3D EM reconstruction of the ternary complex [53]. It indicates that RBD is mainly recognized through the polar residues extracellular peptidase domain of ACE2. These findings provide crucial insights into the molecular basis of coronavirus recognition and infection. Still, there is also some evidence that SARS-CoV-2 enter the host cells by ACE2 receptor-independent infection pathway. It is suggested that the SARS-CoV specific human monoclonal antibody CR3022 that does not overlap with the ACE2 binding site can effectively bind to the RBD of SARS-CoV-2, yet some of the SARS-CoV specific neutralizing antibodies (eg m396, CR3014) against the SARS-CoV ACE2 binding site failed to bind the spike protein of SARS-CoV [56]. What's more, A study have mentioned that the spike protein of SARS-CoV-2 may also interact with dipeptidyl peptidase 4 [44]. However, some teams suggest that SARS-CoV-2 does not use other cell surface receptors such as aminopeptidase n (APN) and DPP-4 [55]. These contradictory evidences imply that the mechanism of SARS-CoV-2 infection is not exactly the same as SARS-CoV's, and it needs to be clarified later.

Epidemiology of SARS-CoV-2

The Source of Infection of Sars-Cov-2

Since evidence of infection has been found in both humans and animals, this new appearance of COVID-19, which is kind of different from SARS and MERS, is defined as zoonotic disease [8, 49-59]. So what is the source of infection of COVID-19?

SARS-CoV-2 comes from nature rather than humans as the first infection host, which is very similar to SARS. Recent research indicates that wild animals may be the intermediate host of SARS-CoV-2. Multiple studies have shown that by extracting and sequencing coronaviruses from individuals from bats, and pangolins, their RNA sequences or spike protein amino acid sequences were found to be same as those from patients. This suggests that these animals may be the original source or intermediate host of the virus [52, 57-62]. Liu, Z. that turtles may also be potential intermediate hosts by comparing the binding affinity of s protein RBD and ACE2 in different animal hosts [63]. But Li, X. identified a unique peptide insertion in the human SARS-CoV-2 virus by analyzing the virus data sets of SARS-related coronavirus isolates from pangolins and bats, yet the coronavirus carried by the pangolin has no RRAR motif, inferring that the human SARS-CoV-2 is not directly from the pangolin. There used to be a view that snakes are also potential intermediate hosts, but a recent study have refuted it [63]. It has reintroduced the analysis on larger data sets through bioinformatics methods and databases; the previous evidence is not as powerful as we used to think.

After the outbreak of an unexpected COVID-19 infection in Wuhan, December 2019, some researchers followed a 6-person family, and 5 of them stayed in Wuhan and had contact with others from the end of December 2019 to the beginning of January 2020. Then they were diagnosed as COVID-19, and the last person was also diagnosed as COVID-19 later without a history of contact with the infected area [64, 65]. This may be the earliest evidence that direct human-to-human transmission has been observed in COVID-19. Subsequently, other countries and regions have reported the situation about the human-to-human transmission [66]. However, most of the research mentioned above is a source of infection with clear symptoms. There is a case report mentioned that a family member in Shanghai has returned from the epidemic area, and the suspected patient in the incubation period has transmitted the SARS-CoV-2 to another person with limited mobility without any contact history of high-risk area [67]. This situation is not in accordance with the clinical experience at the time of the SARS outbreak, which is that only in the symptomatic period would it be contagious [68]. Sporadically, reports of many cases have also appeared throughout the world, and a similar conclusion has been drawn that potential infection without any symptom exists [4, 69-72].

The transmission route of SARS-CoV-2

Droplet Transmission

As many respiratory viruses, SARS-CoV-2 can be transmitted directly from person to person through respiratory droplets. It has been reported that live virus is present in the saliva of infected persons [73, 74]. Furthermore, some researchers compared the viral load of nasal mucosa in patients with symptomatic diagnosis and asymptomatic patients, and found it make no significant difference, thus this founding also corroborates the identity of the source of infection in asymptomatic or latent patients [75]. However, it is still unclear whether there is still potential ability for spreading infection after the symptoms disappear [76]. Scott, S. E. launch a community experiment reported a positive outcome up to 18 days of patients after the diagnosis of COVID-19, but mild or asymptomatic patients after non-intimate contact or using protective measures recommended by CDC will not be infected. It also reminds us the role of daily protective equipment such as masks in cutting off transmission route.

Aerosol transmission

Aerosol transmission is currently recognized as an important pathway of transmission for COVID-19, which used to be neglected at the very beginning of this outbreak. Aerosol transmission has proved to be effective about SARS, MERS and other respiratory viruses [77-79]. Ones and Brosseau proposed that when the pathogen meets the following three conditions, it is considered to be transmitted through the aerosol route: (1) the infected person can produce the pathogen aerosol; (2) the pathogen can survive in the environment for a period of time; (3) The aerosol can reach the target tissue and cause infection. Researchers quantitatively evaluated the biological rationality of the pathogen aerosol transmission route from these three conditions, and scored and calculated the total score based on the strength of the evidence

level (weak = 1 point, medium = 2 points, strong = 3 points) [80]. When the total score is ≥6 points, it is considered that the pathogen can be transmitted by aerosol. In the early days of the COVID-19 outbreak in the Wuhan area, the Ministry of Health of China issued the original diagnosis and treatment guideline, in which the possibility of aerosol transmission was still "not confirmed". With the emergence of a series of epidemiological and clinical evidence, preventing aerosol transmission has been added to the current guideline [73, 75]. Moreover, a team analysis the ability of SARS-CoV-2 to form an aerosol infection in a simulated atomized environment, and the results suggest that SARS-CoV-2 can survive in the aerosol for several hours [81].

Fecal-Oral Transmission

Fecal-oral transmission is currently a popular area for research. Several studies did SARS-CoV-2 nucleic acid detection to the feces of patients with different conditions, and all have obtained the results that confirming the existence of viral nucleic acid shedding, which was believed that the same diagnostic efficacy comparing to pharyngeal swab test [82-87]. Among them, wang.w and his team observed biologically active SARS-CoV-2 in feces, and some studies reported a long period of fecal virus nucleic acid shedding observed during the disease recovery period, and the positive rate is higher than pharyngeal swab [82, 84, 85, 87]. Some researchers believe the higher amount of SARS-CoV-2 viral RNA and even live virus in the feces of patients may be due to the expression of ACE2, the high affinity receptor towards the virus, in the gastrointestinal tract, testis and kidney [54]. However, there is no clinical case report that can prove that fecal mouth transmission can spread SARS-CoV-2 without other pathways.

Contaminated surfaces transmission

Based on previous research, some virus such as SARS-CoV, MERS-CoV or endemic HCoV could persist on inanimate surfaces like glass, plastic or metal for up to 9 days with biological activity [88]. In experiments related to SARS-CoV-2, the results suggest that, compared with SARS-CoV, the virus can survive for several days on an inanimate surface and keep the ability of infection [81]. However, similar to fecal-oral transmission, there are no case reports about precise epidemiological investigations to prove this ability. It is obviously difficult to launch a report at this topic, as we can hardly separate contaminated surfaces transmission from droplet and aerosol.

Other pathways for transmission

As there are news reports that a newborn baby was diagnosed as COVID-19, it was inferred whether this is a possibility of the vertical transmission of mother and child [89-93]. Recent retrospective studies showed that after following up to hundreds of cases, no evidence of intrauterine infection was found, and only a very small number of infants born were diagnosed as COVID-19 after their mothers [92]. Further researchers have reported that the placenta samples left after pregnancy with COVID-19 had a negative outcome of nucleic acid test [93]. On the other hand, it is worth nothing that nosocomial transmission is becoming more and more unstoppable. A recent retrospective study indicate that, in Wuhan alone, as of February 12, a total of 1,716 health workers were infected, accounting for 3.84% of the total local cases [94]. Droplets and aerosols from hospital-infected patients, body fluids that are often in direct or indirect contact, and equipment or environmental surfaces contaminated by patients may all be carriers of SARS-CoV-2 [95]. A lot of respiratory treatments for critically ill patients are deemed as high-risk factors for nosocomial transmission, such as intubation, manual ventilation by resuscitator, noninvasive ventilation, high-flow nasal cannula, bronchoscopy examination, suction and patient transportation. This fact force us to pay more attention to the separation of clean areas and contaminated areas, and do personal protection work as possible as we can.

The Susceptible Population of SARS-CoV-2

The susceptible population of SARS-CoV-2 can be analyzed from various cases and clinical data statistics since the COVID-19 epidemic. As of March 20, thousands of COVID-19 cases have been reported worldwide, as shown in Table 1 [4, 5, 12, 85, 96-108]. As we can see, the susceptible population of COVID-19 can cover persons with all ages, including a minimum of 2 months and a maximum of 79 years old.

Table 1: The Clinical Features of Patients with COVID-19: Retrospective Studies

reference	the number of case	target type of population	area	sex ratio	History of smoking (%)	Patients with Comorbidity (%)	elder (%) (y≥65)	Death (%)	item evaluated in patients	summary of outcomes
Huang, Chaolin[4].	41	covid-19 inpatients	Wuhan, china	2.70		13 (32)	-		clinical symptoms, laboratory medicine	Compared with non-ICU patients, ICU patients had higher plasma levels of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα.
Tian, S.[96]	262	covid-19 inpatients	Beijing, china	0.94	-	-	48 (18.3)	3 (0.9)	clinical symptoms, History of contact, clinical outcomes	Provided the ratio of the COVID-19 infection on the severe cases to the mild, asymptomatic and non-pneumonia cases in Beijing. Population was generally susceptible, and with a relatively low fatality rate.

Liu, W.	78	COVID-19 inpatients likely to be sicker	Wuhan, china	1.00	5 (6.4)	29 (37.1)	-	0	clinical symptoms, laboratory medicine, history of Comorbidity	The patients in the progression group were significantly older and had a significantly higher proportion of patients with a history of smoking. Several factors led to the progression of COVID-19 pneumonia like age, history of smoking, maximum body temperature on admission, respiratory failure, albumin, C-reactive protein.
Xia, W.[108]	20	pediatric inpatients with COVID-19	Wuhan, china		-	7 (35)	-	0	clinical symptoms, laboratory medicine, radiographic findings, History of Comorbidity	Procalcitonin elevation and consolidation with surrounding halo signs were common in pediatric patients which were different from adults. It is suggested that underlying coinfection may be more common in pediatrics, and the consolidation with surrounding halo sign which is considered as a typical sign in pediatric patients.
Wang,[85] Wenling	205	covid-19 inpatients	Beijing, china	2.13	-	-	-	-	RT-PCR	Bronchoalveolar lavage fluid specimens showed the highest positive rates, followed by sputum, nasal swabs, fibrobronchoscope brush biopsy, pharyngeal swabs, feces, and blood. None of the 72 urine specimens tested positive
Wu, Chaomin[99]	201	severe covid-19 patients	Wuhan, china	1.75	-	93 (46.3)	40 (19.9)	44 (21.9)	clinical symptoms, laboratory medicine, History of Comorbidity	In those who developed ARDS, compared with those who did not, usually had comorbidities such as hypertension. Risk factors associated with the development of ARDS and progression from ARDS to death included older age, neutrophilia, and organ and coagulation dysfunction. High fever (≥39 °C) was associated with higher likelihood of ARDS development and lower likelihood of death .
Qian, G. Q.[100]	91	covid-19 inpatients	Zhejiang, china	0.67		26 (28.6)	19 (20.9)	0	clinical symptoms, laboratory medicine, History of contact,	Social activity cluster, family cluster and travel by airplane were how COVID-19 patients get transmitted and could be rapidly diagnosed COVID-19 in Zhejiang.
Zheng, F.[107]	25	pediatric inpatients with COVID-19	Wuhan, china	1.27	-	2(8)	-	0	clinical symptoms, laboratory medicine, History of contact,	It was concluded that children were susceptible to COVID-19 like adults, while the clinical presentations and outcomes were more favorable in children. However, children less than 3 years old accounted for majority cases and critical cases lied in this age group, which demanded extra attentions during home caring and hospitalization treatment.

Deng, Y.[5]	109	fatal cases of COVID-19 inpatients	Wuhan, china	2.03	-	79 (72.5)	-	109 (100)	clinical symptoms and complications, laboratory medicine, History of contact, treatment	Compared to the recovered group, more patients in the death group exhibited characteristics of advanced age, pre-existing comorbidities, dyspnea, oxygen saturation decrease, increased WBC count, decreased lymphocytes, and elevated CRP levels. More patients in the death groups had complications such as ARDS, acute cardiac injury, acute kidney injury, shock, and DIC.
Sun, Y.[101]	54	covid-19 patients	Singapore	1.16	-	5 (9.3)	-	0	clinical symptoms, laboratory medicine, History of contact,	Rapidly ascertainable clinical and laboratory data could identify individuals at high risk of COVID-19 and enable prioritization of PCR-testing and containment efforts. Basic laboratory test results were crucial to prediction models.
Shi, S.[102]	416	covid-19 inpatients	Wuhan, china	0.97	-	316(76.0)	-	57 (13.7)	clinical symptoms and complications, laboratory medicine, History of Comorbidity	Higher leukocyte counts, levels of C-reactive protein, creatinine kinase-myocardial band, procalcitonin, myohemoglobin, N-terminal pro-B-type natriuretic peptide, aspartate aminotransferase, high-sensitivity troponin I, and creatinine is the characterization of patients with cardiac injury than the opposed. Complications were more common in patients with cardiac injury than those without cardiac injury. Patients with cardiac injury had higher mortality than the opposite.
CDC COVID-19 Response Team[12]	2449	covid-19 patients and inpatients	Kim county, US	-	-	-	759 (31%)	-	age	31% of cases, 45% of hospitalizations, 53% of ICU admissions, and 80% of deaths associated with COVID-19 were among adults aged ≥65 years with the highest percentage of severe outcomes among persons aged ≥85 years. In contrast, no ICU admissions or deaths were reported among persons aged ≤19 years.
McMichael, T. M.[103]	129	covid-19 patients and inpatients	Seattle, US	0.54	-	58(45)	-	-	clinical symptoms, history of Comorbidity	The findings in this report suggest that once COVID-19 has been introduced into a long-term care facility, it has the potential to result in high attack rates among residents, staff members, and visitors.
Chen, T.[104]	113	fatal cases of COVID-19 inpatients	Wuhan, china	2.77	9 (8)	71 (63)	94 (83)	113 (100)	clinical symptoms and complications, laboratory medicine, History of Comorbidity, treatment	Chronic hypertension and other cardiovascular comorbidities were more frequent among deceased patients than recovered patients. The median time from disease onset to death in deceased patients was 16 days. Concentrations of ALT, AST, Cr, CK, LDH, cardiac troponin I, BNP, and D-dimer were markedly higher in deceased patients than in recovered patients. Patients with cardiovascular comorbidity were more likely to develop cardiac complications.

Guan, W. J.[105]	1590	covid-19 patients	multi-center, china	1.32	111(7)	399 (25.1%)	-	50 (3.1)	clinical symptoms, history of Comorbidity	Among laboratory-confirmed cases of Covid-19, patients with any comorbidity yielded poorer clinical outcomes than those without. A greater number of comorbidities also correlated with poorer clinical outcomes. The HR was 1.79 (95%CI 1.16-2.77) among patients with at least one comorbidity and 2.59 (95%CI 1.61-4.17) among patients with two or more comorbidities.
Bhatraju, P. K.[106]	24	patients admitted to the intensive care unit (ICU) with COVID-19	Seattle, US	1.70	5 (22)	-	-	12 (50)	clinical symptoms	The most common reasons for admission to the ICU were hypoxemic respiratory failure leading to mechanical ventilation, hypotension requiring vasopressor treatment, or both. Mortality among these critically ill patients was high

The specific population has some differences in the susceptibility and prognosis of COVID-19. Several studies have reported that children diagnosed with COVID-19 (<13 years old) generally have milder symptoms, lower proportion of severe patients and better prognosis than adults [107-109]. Besides, it was reported that elevated procalcitonin and surrounding halo signs in computed tomography (CT) images are very common in pediatric patients, which is different from the adults [108]. Otherwise, it was shown in most studies that elder people are account for a large proportion of all cases, and the age of patients in progressive cases is significantly greater than the recover and stable groups [97]. It is widely acknowledged that the incidence of cardiovascular disease is high, which also lead to the upregulation of ACE2. This may be one of possible explanations about the situation.

We can also find an interesting tendency that among COVID-19 infected patients, the incidence of chronic diseases is higher than that of the normal population. Several studies have counted the prevalence of chronic diseases in the cases they collected [4, 97, 100-108]. At the same time, different researchers separately analyzed the progression cases and death cases, and counted the proportion of chronic diseases [5, 99, 104, 109]. The results suggest that suffering from chronic diseases and comorbidities may be a marker of infection aggravation and poor prognosis with COVID-19.

Notably, smoking may also be one of the marker of COVID-19 pneumonia progression. Vardavas, C. I. conducted a systematic review of the smoking cases in the published case reports, suggesting that in the situation of a large number of cases, patients with poor prognosis have a higher smoking rate than others and in their multivariate logistic regression analysis, smoking history is a risk factor for disease progression [110]. Although the above results are not adjusted for other factors that may affect disease progression, this also imply a potential worsening factor for smoking. Recently, WHO announced the suggestion about the risk of smoking during COVID-19 outbreak that smokers are likely to be more vulnerable to COVID-19 as the act of smoking, and induce lung disease or reduced lung capacity which would greatly increase risk of serious illness [111].

Diagnosis Method of Covid-19 and its Value

Normal Laboratory Medicine

Laboratory medicine has the characteristics of convenience and speed, and is less restricted by the technical level of hospital laboratories. Especially the sudden appearance of COVID-19 leaves most hospitals unprepared. In ordinary non-critically ill patients, due to the selection of samples and various biases in various research, the value of many laboratory markers is still controversial. In the diagnosis of COVID-19, for example, some researchers pointed out that only a few patients present with lymphopenia and elevated concentration of procalcitonin, while another researcher raise an opposed view that lymphopenia accounted for more than half [4, 100]. Another report from Singapore also suggested that the lymphocytes of COVID-19 patients after diagnosis were significantly reduced compared with the control group [101]. There used to be a kind of view that renal insufficiency is common in COVID-19 patients with the appearance of some preprint and news, while this situation does not exist in some reliable clinical researches [4, 102-112]. On the contrary, most of clinical research draw the same conclusion that the serum concentration of C-reactive protein will elevate in patients, yet make no difference to diagnosis [113]. Zhao, D. compared with COVID-19 patients and other common pneumonia patients with similar exposure history in epidemic areas. The former has abnormal laboratory tests, including AST, ALT, γ-GT, LDH and α-HBDH, which suggest potential diagnostic value.

Now the hotspots area for research is changing. Through the Observation of normal laboratory indicators in special populations, it is feasible to evaluate the prognosis of patients, especially the markers of possible progression of the disease [97, 114]. There were some retrospective studies of progressive or severe cases indicate that these patients usually have higher level of CRP and lower albumin than patients in the moderate group, and alanine aminotransferase, Lactate dehydrogenase, ferritin, D-dimer, IL-

2R, IL-6, IL-10 and TNF-α levels are also higher than normal [114]. Similarly, Huang, C. suggested that the serum concentration of IL-2, IL-7, IL-10, GSCF, IP10, MCP1, MIP1A and TNFα in ICU patients are higher than those in non-ICU patients [4]. This implies the existence of an inflammatory cytokine storm during the progress of COVID-19. In addition, Wu.C. Considered that neutropenia, coagulation dysfunction and elevated level of D-dimer, are the risk factors for ARDS inpatients. Deng, Yan, Chen, T. collected clinical data of 109 and 113 of fatal cases respectively, and they had a significant increasing count of white blood cell, decreasing count of lymphocytes and increased CRP levels compared with the recovery group [5, 99, 110]. It is also accompanied by significant differences in the concentration of creatinine, troponin, and brain natriuretic peptide (BNP). The above research suggests that some laboratory tests may have more important significance for prognostic evaluation rather than diagnosis and screening.

PCR-based Etiology Test

According to previous clinical experience, the gold standard for the diagnosis of respiratory virus infection is usually nucleic acid test (NAT) [115-119]. This is widely used in diagnosis of COVID-19, making a great difference in identifying patients, especially some asymptomatic patients. In the meanwhile, what kind of specimens are extracted will also affect the diagnostic value of nucleic acid test. Wang, W. analysis the RT-PCR outcomes of specimens from 205 COVID-19 patients and found that the order of the positive rate of the specimens from high to low was bronchopulmonary lavage fluid, sputum, nasal swab, fiberoptic bronchoscopy brush biopsy, pharyngeal swab, feces, and blood, and no positive in urine. Similarly, Qiu, L. reported that the virus was not found in the vaginal fluid in 10 severe female patients [84-89, 115]. Zhang, Wei, Ling, Yun and Zhang, J. separately reported that 2019-nCoV is also found in anal swabs and blood. In the late period of infection, the positive rate of anal swabs is higher than those of the pharyngeal swab. Zou, L. also reached a similar conclusion through clinical data analysis and his team also found that all specimens with viral RNA detectable in serum were collected in severe case [116-118]. Whereas, the viral load of nasal mucosa moderate patients and asymptomatic patients was almost the same [75]. Some researchers reported that the viral load of the specimens obtained in the early and progressive period after the onset of symptoms is the highest, which decreases with time [74, 116].

Nevertheless, nucleic acid testing is not reliable at any condition. There are some situations that can break the current view that the outcome of RT-PCR is the only criterion in diagnosis.

False Negative in Suspicious Patient

Xie, X. reported that 5 patients were negative for RT-PCR in the first test, but had typical CT features sign consistent with COVID-19, and after repeated swab tests it was finally confirmed that these patients were diagnosed with COVID-19 [117]. To, K. K. reported a case in which SARS-CoV-2 was first detected in saliva 25 days after the onset of symptoms [74]. These cases mentioned indicates that for persons who are clinically highly suspected of COVID-19 infection but negative for RT-PCR screening, a combination of repeated swab tests and CT image may be imperative for the final diagnosis. Lippi, G. proposed that RT-PCR has a series of systematic biases and human errors, such as insufficient transportation, incorrect storage procedures, sample contamination and testing of patients receiving antiretroviral therapy [118]. Some medical problems may also impair the accuracy of the RT-PCR, including testing outside the diagnostic window, effective virus reorganization, use of tests that have not been fully validated, insufficient uniformity, instrument malfunctions, and other specific technical issues. Therefore, when the RT-PCR test is negative, it should be combined with clinical symptoms and chest CT for comprehensive diagnosis. At present, in the latest version of the guideline in China, nucleic acid detection is no longer the only criterion, but it is plausible to take account into many other factors and markers for diagnosis.

Resurgence in Discharge Patient

News and case reports from various places across the country reveal the fact that a few number of patients were re-hospitalized and had re-positive nucleic acid test again for RT-PCR after being discharged. For example, Xing, Y. reported that two asymptomatic medical staffs re-examined after being discharged from hospital [119]. Other case reports indicated the similar phenomenon mentioned above [120-122]. It is likely for SARS-CoV-2 pathogen to come from the environmental source or the inside of original patients themselves. But it still remains to be uncertain that why the antibody against SARS-CoV-2 pathogen didn't work, whether the virus has already mutated in vivo, and whether the recurrent patients have ability to transmit virus to others. Some researchers infer that probably the recurrence may attribute to the use of glucocorticoid, which drives immunity to decrease and prolongs the period with active SARS-CoV-2 pathogen [123]. Meanwhile, Jin, X. and his colleagues report that in the long-term use of glucocorticoid in GI patients with COVID-19, the proportion of severe cases and critical cases are significantly higher than the control group without GI, hence bioinformatic methods show the m6 Methylation mutation of SARS-CoV-2, which change the binding ability to ACE2 [124]. All the possibility referred above deserve to further virological research.

Serological and Immunological Test

The serological and immunological test mainly means to the detection of antibodies against SARS-CoV-2 nucleocapsid protein (rN) and spike protein (rS) by enzyme-linked immunosorbent assay (ELISA), which can reflect the strength immune response towards specific antigen in vivo. Several studies reported high positive rates of detection of IgG and IgM targeting these two proteins in non-early infection patients, in which Li, Z. reported the overall sensitivity of serum antibody diagnosis was 88.66%, and the specificity was 90.63% through the analysis to 397 patients diagnosed by RT-PCR and 128 non-patients [74, 84, 125-128]. Moreover, Zhao, J. indicated combining PCR and antibody detection can significantly improve the sensitivity of COVID-19 pathogenic diagnosis ($p < 0.001$), even in the early period after

onset of symptoms (p = 0.007) [128]. This reminds us of potential value of serological test towards SARS-CoV-2 specific antibody, and significance of combined PCR and antibody detection for diagnosis. However, the studies above mentioned are all for patients with non-early COVID-19 infections [129]. Recently, Cassaniti, I. launched an experiment based on the person initially registering in emergency room registration, and he found that 38 cases were detected COVID-19 positive by real-time RT-PCR, of which only 7/38 (18.4%) of IgM and / or IgG. Serological test were positive or weak positive, inferring the sensitivity of IgM / IgG rapid detection was only 18.4%, and specificity was 91.7%. This indicates that immunological examination may not be suitable for early diagnosis alone.

Notable Hotspots for Later Research

At present, COVID-19 is still in a global outbreak. Therefore, it is necessary to further improve the research work in this field. Undoubtedly, the perfecting of diagnostic criterion and the development of drugs and vaccines are very important. For susceptible people, the elderly and patients with basic diseases have the higher risk for COVID-19, and poorer prognosis when diagnosed, which is the opposite of pediatric patients. However, the reason of children's mild symptom is still unclear. Some researchers implied that there might be other viruses limiting the growth of SARS-CoV2 through direct virus-to-virus interaction and competition in the lungs and respiratory mucosa of children, which is common in pediatric patients [109]. Moreover, it was mentioned that elevated procalcitonin and surrounding halo signs in CT images were very common in pediatric patients [96], suggesting that the potential co-infection of SARS-CoV-2 and other pathogen in pediatric patients is possible. On the other hand, another view for children with mild COVID-19 infection is related to the difference in the expression of ACE2 receptor necessary for SARS-CoV-2 infection. This receptor prefers to be expressed in the airways, lungs, and intestines, but not on immune cells [54, 130], and it need more clinical and pathogenic evidence to support this view.

As for the pathway of transmission, whether regarding the nucleic acid shedding in fecal as a criterion of discharge remains to be a controversy. And it is widely accepted that there are no vertical transmission available in pregnant patients with COVID-19. Nevertheless, Dong, L. and Zeng, H. recently reported several infant patients born from pregnant patients with COVID-19 in different hospitals at the same time, and these newborns have negative RT-PCR tests in pharyngeal swabs while the count of specific IgG and IgM to SARS-CoV-2 [130, 131]. Also, the laboratory test of inflammation and liver injury indirectly support the possibility of vertical transmission in these cases. Undoubtedly, due to its large molecular structure, IgM cannot pass through the placenta. So the existence of the cases above force us to rethink the possibility of vertical transmission.

The diagnostic criterion based on RT-PCR is effective in clinical practice, but RT-RCP also has problems such as false negatives and recurrence after turning negative. At present, there are few recurrent cases reported, and it is still unclear whether it has the ability to retransmit. In case of the above situation, serological and immunological test can be another reliable method that benefit the accuracy of diagnosis criteria based on RT-PCR. However, antibody testing is usually of little significance in the screening, so how to definite the diagnosis window of immunological examination, and what is the earliest time when the immunological examination has acceptable diagnostic value? There is still a lack of similar research. With more cases being reported, we believe this will be the next focus of future research.

Conclusion

Nowadays, the outbreak of newly discovered COVID-19 appear in China and rapidly spread to other countries, which reminds medical staff lots of confusion in research and development of medicine and clinical practice. Similar as SARS-CoV and bat-CoV, SARS-CoV-2, the pathogen of COVID-19, has a typical structure of coronavirus while the spike protein decide the entry mechanism of virus into host cells. The spike protein of SARS-CoV-2 shows a higher affinity to ACE2 than SARS-CoV while they share a certain degree identity in amino acid sequences, and SARS-CoV-2 also has other potential ACE2-independent pathway in the entry process into host cells. The COVID-19 patients without obvious symptom were reported actively shedding virus and is likely to cause another emergence. Besides droplets, aerosol and contact, the possibility of fecal-oral transmission was accepted for researchers, while the vertical transmission still cannot be confirmed. The elderly and patients with basic diseases have the higher risk and poorer prognosis for infection. Laboratory medicine doesn't work in screening, but could make a difference in the evaluation of prognosis. RT-PCR is effective for filter patients with COVID-19 from the suspected. However, the problem about false negative cannot be ignored in clinical practice. What's more, resurgence reported maybe is not an individual case in discharge patients. Detection for antibody isn't suitable for early diagnosis alone, but can be another reliable method that benefit the accuracy of diagnosis criteria based on RT-PCR.

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