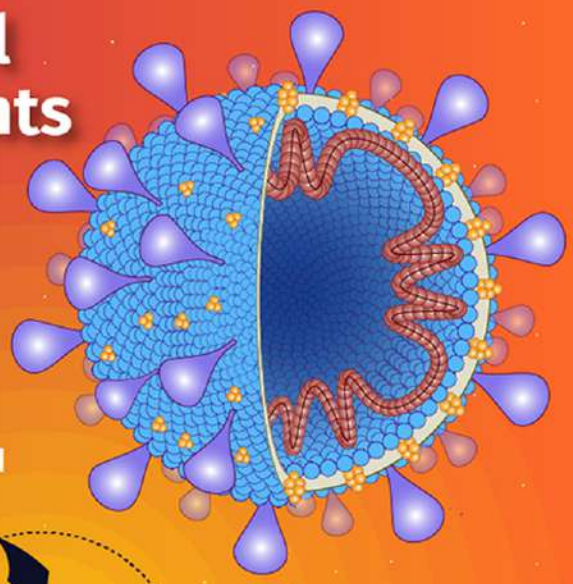
Chinese Medical Journal

2019 novel coronavirus disease (COVID-19) collection

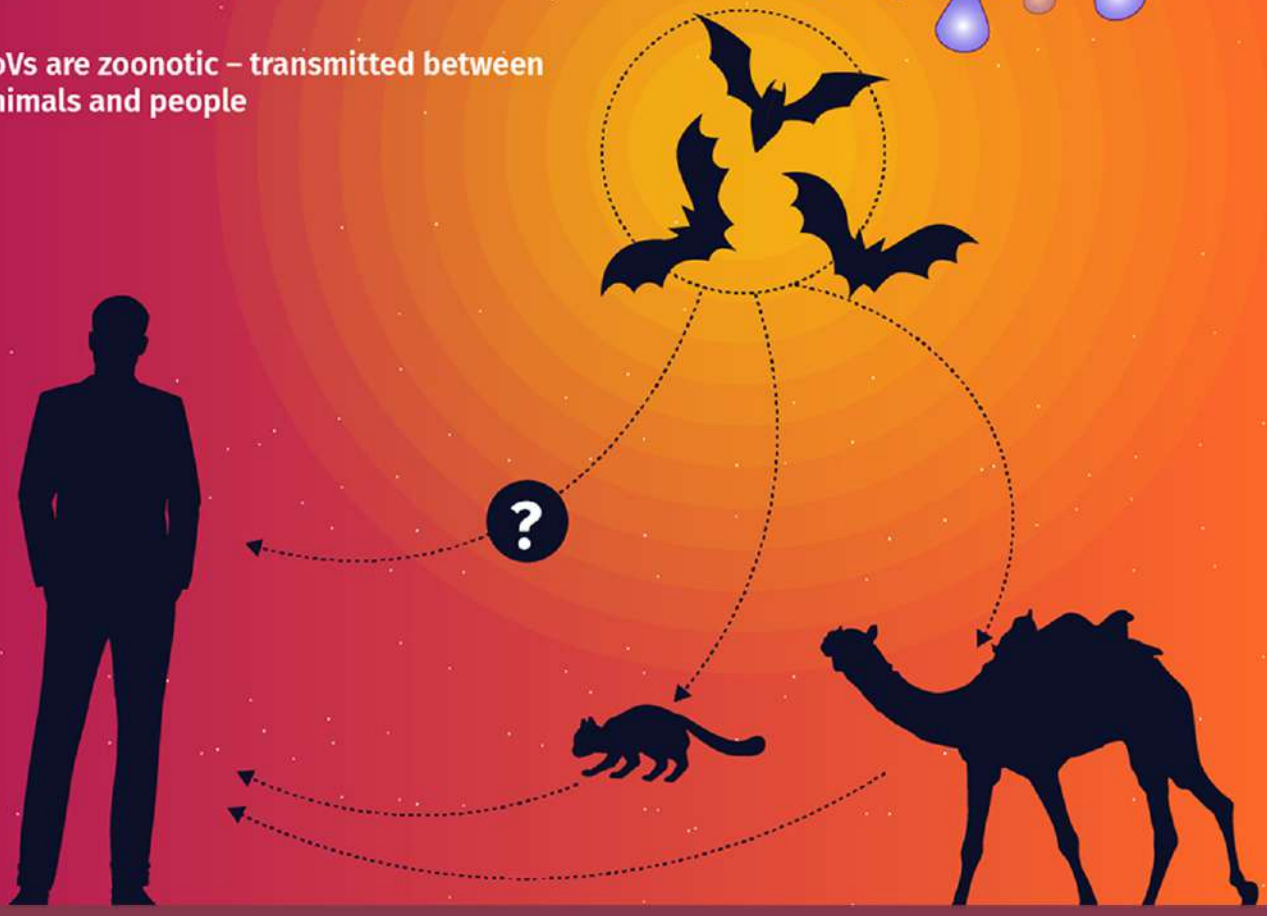
# Identification of a Novel Coronavirus from Patients with Pneumonia in Wuhan, China



## Coronaviruses (CoVs)

Enveloped viruses with a single positive-stranded RNA genome that infect humans as well as avian and other mammalian species

CoVs are zoonotic – transmitted between animals and people

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.....This list will be continuously updated

**Editorial**

# Science in the fight against the novel coronavirus disease

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In December 2019, a cluster of viral pneumonia cases of unknown origin emerged in Wuhan, Hubei Province of China. This emergency has attracted global concern, and the World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC). Joint efforts to identify the causative agent were undertaken by multidisciplinary task forces under the organization of the National Health Commission of the People's Republic of China, and a novel coronavirus, named 2019-nCoV by the WHO, was swiftly identified as the pathogen responsible for this contagious epidemic.

One of the teams involved in this task, Ren et al[1] from the Chinese Academy of

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Medical Sciences, reported their research on the identification of 2019-nCoV. They performed a metagenomic analysis of respiratory tract specimens obtained from five patients suffering from the pneumonia in question and identified the virus now known as 2019-nCoV as the causative agent. The virus was successfully isolated, and genomic sequencing showed that it belongs to the genus *Betacoronavirus*, which differs from that of previously known human coronaviruses. Their data showed that the 2019-nCoV viral genomes have about 79% homology to the genome of severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), about 52% homology to that of Middle East respiratory syndrome coronavirus (MERS-CoV), and about 87% homology to the genomes of two strains of bat-derived SARS-like coronavirus identified in Zhoushan in 2015. This evidence suggested that the isolated virus was a novel coronavirus. Similar results were published in parallel by a team from the Chinese Center for Disease Control and Prevention.[2] The key clinical symptoms of the disease are fever, dry cough, and fatigue, and patients also exhibited characteristic chest radiograph findings.[3] Studies also showed that the virus has strong human-to-human transmission capability.[4] The identification of the 2019-nCoV has laid the foundation for the diagnosis and treatment of patients, the formulation of prevention and control measures as well as the development of drugs and vaccines.

The novel coronavirus disease (COVID-19) is the most severe public health emergency since the outbreak of SARS in 2003. There are two main lines of combat against this public health threat: (1) control and prevention of the epidemic and (2) scientific research. For the effective control of the spread of a newly identified virus, we must first understand its infection and pathogenicity patterns, as quickly and as thoroughly as possible, to provide insights into the outbreak and develop targeted

prevention and control strategies.[5]

Genomic analyses indicate that 2019-nCoV may have originated from bats,[1,2] and current knowledge of other coronaviruses that infect humans, e.g., SARS-CoV and MERS-CoV, suggests that there may have been intermediate animal hosts.[6] Regarding epidemiology, most of the initial patients were exposed to the Huanan Seafood Market in Wuhan, but there were also individual cases that did not have a history of exposure. Tracing the source of the virus is of great importance for controlling the epidemic.

Polymerase chain reaction (PCR)-based diagnostic reagents have been rapidly developed based on available viral genome sequences, and these have served as important screening tools. Nevertheless, it is necessary to develop other types of diagnostic reagents, such as assays for antibodies and antigens, as PCR cannot detect the virus when it is present below a threshold level. The optimization of sample type and the time window selected for viral detection as well as the combination of different methods of diagnosis can improve diagnostic accuracy and decrease false negatives which can be an obstacle to the prevention of virus transmission. As it is currently the peak season for respiratory infectious diseases such as influenza, the development of rapid detection technology, improvement of the detection capabilities of primary medical institutions, and rapid examination of cases are of great importance for the timely isolation of patients and individuals who have had close contact with patients.

The clinical manifestation of COVID-19 is very complex, and four clinical phenotypes have been identified, i.e. mildly, commonly, severely, and critically ill patients.[7] Some cases are characterized by mild symptoms and close-to-normal body temperatures and some are asymptomatic carriers, but both symptomatic and

asymptomatic patients are contagious, which leads to difficulties in the timely identification of cases. Attention should be paid to the spectrum of disease severity and transmission modes to address questions such as how to identify the proportion of asymptomatic infections and whether a patient is contagious during the incubation period. Although a previous study showed that the overall mortality of the disease is about 2.3%,[8] but unregulated inflammatory responses and cytokine storms have been reported and the incidence of lymphopenia is also notable.[2] Insights into the pathological immune response are critical to understanding the pathogenesis of the disease and finding novel therapies to decrease mortality.

Past research into the pathogenic mechanism of SARS may help inform our understanding of 2019-nCoV, as studies have shown that the novel virus shares the angiotensin-converting enzyme 2 (ACE2) receptor with SARS-CoV.[9] In the pathogenesis of SARS, ACE2 contributes to lung injury and increases vascular permeability,[10] but the role of the receptor in the pathogenesis of COVID-19 still needs to be evaluated. As 2019-nCoV is an RNA virus that does not contain any proofreading mechanism during genome replication, it is prone to mutations; moreover, distinct viral subspecies have been identified within hosts.[11] Thus, it is necessary to investigate the biological characteristics and mutation trends of 2019-nCoV to assess viral transmissibility and pathogenesis.

Effective therapeutics and antivirals are urgently needed to decrease COVID-19 mortality. As specific therapies targeting 2019-nCoV are lacking, it may be useful to repurpose drugs already licensed for marketing or clinical trials to treat COVID-19 patients in an emergency response; researchers are actively working to identify such drugs. At the time of preparation of this manuscript, the Chinese Academy of Medical Sciences and the China-Japan Friendship Hospital had launched a multi-center,

randomized, double-blind, placebo-controlled clinical trial in Wuhan to test the effectiveness of remdesivir as an antiviral drug against 2019-nCoV,[12,13] and studies have already shown that chloroquine phosphate is an effective treatment for COVID-19.[14] Clinical trials are also underway to validate the effectiveness of various other licensed drugs against COVID-19.

Meanwhile, researchers are also assessing the effectiveness of treatment with serum samples from recovering patients. The development of neutralizing antibodies is underway, and efforts are also being made to develop a vaccine.

Scientific research is of vital importance for tackling emerging infectious diseases and developing effective intervention methods. The spread of infectious diseases is affected not only by the biological characteristics of the pathogen but also by various other factors such as politics, culture, economy, and the environment. Multidisciplinary research in biomedical, social, and environmental sciences is required to achieve a deeper understanding of disease transmission and develop more effective systems for emergency response.

In summary, strategies based on scientific evidence will be essential to curb the spread of the ongoing COVID-19 epidemic. As next steps, obtaining a comprehensive understanding of the epidemiological and clinical properties of the disease is critical for policy and decision making. We must also take full advantage of existing knowledge and experience to improve the diagnosis, treatment, prevention, and control of the disease and accelerate the development of drugs and vaccines to save lives.

***Conflicts of interest***

None.

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**Special Article**

# Voice from China: nomenclature of the novel coronavirus and related diseases

**Edited by:** Xiu-Yuan Hao and Pei-Fang Wei

Editor's Note:

Since early December 2019, the novel coronavirus has caused an outbreak of pneumonia that has claimed over 2000 lives, with more than 77,000 confirmed cases of infection by February 23, 2020 in China.[1] The World Health Organization (WHO) recently named the disease caused by this new coronavirus as "coronavirus disease 2019" (COVID-19). The International Committee on Taxonomy of Viruses (ICTV) has named the new virus "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2).[2] However, questions have been raised by different academic and professional bodies regarding whether the nomenclature is appropriate. In view of the lack of consensus, the *Chinese Medical Journal* invited Chinese scientists, epidemiologists, and virologists to submit their comments and provide references for establishing an acceptable nomenclature.

Gui-Zhen Wu, President of Asian-Pacific Biosafety Association, Chief Biosafety Specialist of Chinese Center for Disease Control and Prevention, Director of Biosafety Research Center in National Institute for Viral Disease Control and Prevention.

Comments：

Evidence from many aspects such as comparative genomics, viral origin, epidemiology, and clinical manifestations suggests that the coronavirus is a new kind of virus distinct from the severe acute respiratory syndrome coronavirus (SARS-CoV). According to scientific conventions, any new virus and associated disease can be named through expert consensus while taking into consideration existing public knowledge about the disease at the same time. Naming the new coronavirus SARS-CoV-2 is problematic. First, it may be misleading, especially for the general public who have a lack of virological knowledge. Second, the new name for the virus is not consistent with the disease name COVID-19. Third, the government and the general public may become complacent, believing that the

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disease caused by the new coronavirus, similar to the severe acute respiratory syndrome (SARS), will not recur after this outbreak. Therefore, the new coronavirus should have a unique name. I would like to suggest that the virus be named human coronavirus 2019 (HCoV-19) instead of SARS-CoV-2. The name HCoV-19 ensures that the new virus is distinct from SARS-CoV and is consistent with the name of the disease COVID-19.

Jian-Wei Wang, Professor and Vice President of Chinese Academy of Medical Sciences and Peking Union Medical College. He is also the Director of the Christophe Merieux Laboratory.

Comments：

An ICTV (The International Committee on Taxonomy of Viruses) work group suggested to name the new coronavirus SARS-CoV-2, which was temporarily referred to by WHO as “2019 novel coronavirus” (2019-nCoV). Discussions are needed for the nomenclature of the novel coronavirus until wide consensus reached by science community.

Currently, there are no standardized nomenclature guidelines for coronaviruses. Looking back in history, the first two human coronaviruses (HCoVs) were named after 229E and OC43. In recent years, two seasonal HCoVs have been named NL63 and HKU1 according to the country (e.g., NL = the Netherlands) or the organization (e.g., HKU = Hong Kong University) in which they were identified. The two highly pathogenic viruses, i.e., SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), were named primarily according to the key features of disease caused. [3]

The genomic sequences of the new coronavirus are distinct from those of SARS-CoV (approximately 79% identity).[4] Most individuals (approximately 80%) infected with 2019-nCoV develop mild or moderate symptoms and signs and asymptomatic individuals may also be infectious.[5,6] These may make it hard to control the epidemic. It is highly possible that COVID-19 will eventually evolve into a seasonal epidemic. Thus, the virus causing COVID-19 is largely different from SARS-CoV. To avoid conceptual confusion and considerable public fear associated with SARS, the new coronavirus should not be named SARS-CoV-2.

On the basis of the historical nomenclature procedure, I suggest to establish a standardized nomenclature process for coronaviruses. One approach may be to name the new coronavirus under the four existing genus, i.e. α, β, γ, and δ.[3] For example, the current new name suggested for HCoV-OC43 is betacoronavirus 1. Alternatively, the

new coronavirus can be named to reflect the WHO's naming of the disease (COVID-19), we can differentiate the new coronavirus according to the year of discovery, e.g., human coronavirus-19, which is similar to the naming of the influenza virus subtype A/H1N1/2009pdm.

Jian-Qing Xu, Professor at Institutes of Biomedical Sciences and Shanghai Public Health Clinical Center, Fudan University. Director of the Shanghai Institute of Emerging and Re-emerging Infectious Diseases and Director of the School of Translational Medicines at Shanghai Public Health Clinical Center, Fudan University.

Comments:

SARS-CoV-2, HCoV-19, or 2019 acute respiratory syndrome coronavirus (TARS-CoV): A new coronavirus emerged in Wuhan city, Hubei Province, China and then rapidly spilled over to many other parts of China and more than 20 other countries. Recently, the ICTV suggested that the new coronavirus be named SARS-CoV-2 because the genomic sequences of the two coronaviruses appear to be similar (approximately 80% homogeneity).[7,8] In addition, this new coronavirus and SARS-CoV of 2003 share similar pathogenesis, both primarily affecting the respiratory system.

This nomenclature is controversial. Several significant discrepancies between these two viruses should be noted. First, although both viruses may have originated from bats, the intermediate host involved is likely to be different, indicating that the transmission paths are distinct. Second, SARS-CoV results in more than 40% of patients developing severe acute respiratory syndrome (SARS) and 10% patient deaths.[9,10] In contrast, the new coronavirus only leads to SARS in approximately 10% of cases and 3% cases of death, respectively,[11-13] including all the cases from Hubei province. Third, treatment regimens or vaccines for SARS are not indicated for the new coronavirus. Altogether, there exist significant differences in transmission, pathogenesis, clinical treatment, and vaccine development between these two viruses.

Traditionally, human viruses in the same family or genus are typed based on serological tests, as the results help with the diagnosis of the infection, guide medications used for treatment, and assist in the development of a vaccine. In more recent times, genetic

sequence-based tests have advanced, and such genotyping tests have been able to provide a more rapid and precise typing approach than serological tests. Genotyping provides a new clinical diagnostic tool for directing the use of drugs and the development of vaccines. Based on genotyping, SARS-CoV-2 is unlikely to be significantly linked to SARS-CoV. Rather, SARS-CoV-2 may lead to the misunderstanding of the pathogenesis of the new coronavirus, which is less pathogenic compared with SARS-CoV.

Human coronavirus (HCoV) is commonly referred to coronaviruses of low pathogenicity, with human beings being their primary natural host, as exemplified by HCoV-OC43, 229E, NL63, and HKU1. In contrast, SARS-CoV and MERS-CoV are used to refer to coronaviruses that are highly pathogenic, with non-human animals as their primary natural host. When considering pathogenicity and natural hosts, the new coronavirus fits in between but shares more similarity with the latter. Hence, I would suggest the new coronavirus be referred to as the "2019 acute respiratory syndrome coronavirus" (TARS-CoV). TARS-CoV uniquely identifies the new coronavirus from SARS-CoV and is also different from human coronavirus. Accordingly, the disease caused by TARS-CoV should be named 2019 acute respiratory syndrome (TARS).

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## Original Article

# Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study

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# Abstract

**Background:** Human infections with zoonotic coronaviruses (CoVs), including severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS)-CoV, have raised great public health concern globally. Here, we report a novel bat-origin CoV causing severe and fatal pneumonia in humans.

**Methods:** We collected clinical data and bronchoalveolar lavage (BAL) specimens

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from five patients with severe pneumonia from Jin Yin-tan Hospital of Wuhan, Hubei province, China. Nucleic acids of the BAL were extracted and subjected to next-generation sequencing. Virus isolation was carried out, and maximum-likelihood phylogenetic trees were constructed.

**Results:** Five patients hospitalized from December 18 to December 29, 2019 presented with fever, cough, and dyspnea accompanied by complications of acute respiratory distress syndrome. Chest radiography revealed diffuse opacities and consolidation. One of these patients died. Sequence results revealed the presence of a previously unknown β-CoV strain in all five patients, with 99.8–99.9% nucleotide identities among the isolates. These isolates showed 79.0% nucleotide identity with the sequence of SARS-CoV (GenBank NC_004718) and 51.8% identity with the sequence of MERS-CoV (GenBank NC_019843). The virus is phylogenetically closest to a bat SARS-like CoV (SL-ZC45, GenBank MG772933) with 87.6–87.7% nucleotide identity, but is in a separate clade. Moreover, these viruses have a single intact open reading frame gene 8, as a further indicator of bat-origin CoVs. However, the amino acid sequence of the tentative receptor-binding domain resembles that of SARS-CoV, indicating that these viruses might use the same receptor.

**Conclusion:** A novel bat-borne CoV was identified that is associated with severe and fatal respiratory disease in humans.

**Keywords:** Bat-origin; Coronavirus; Zoonotic transmission; Pneumonia; Etiology; Next-generation sequencing

# Introduction

Coronaviruses (CoVs) are enveloped viruses with a single positive-stranded RNA genome (~26–32 kb in length). They belong to the subfamily *Orthocoronavirinae* under the family *Coronaviridae*, and are classified into four genera: *Alphacoronaviruses* (α), *Betacoronaviruses* (β), *Gammacoronaviruses* (γ), and *Deltacoronaviruses* (δ).[1,2] The viral genome normally encodes four structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as several non-structural proteins and multiple unique accessory proteins.[1,2]

CoVs infect humans and a variety of avian and mammalian species worldwide. There are six CoVs known to infect humans, including two α-CoVs (229E and NL63) and four β-CoVs (OC43, HKU1, severe acute respiratory syndrome [SARS]-CoV, and Middle East respiratory syndrome [MERS]-CoV).[1-4] All human CoVs are zoonotic as a distinguishing characteristic.[5] In particular, bats are regarded as a key reservoir of CoVs, and many human CoVs are believed to have originated from bats.[5,6] Since the beginning of this century, two zoonotic CoVs, SARS-CoV and MERS-CoV, have been identified to cause severe human diseases.[3,4,7] The outbreak of SARS-CoV in 2003 was responsible for 8,096 cases and 794 deaths worldwide.[8] Since its discovery in Middle Eastern countries in 2012, MERS-CoV has infected 2,260 people with a current case fatality rate of 35.5%.[9,10] These outbreaks have raised public health concerns of the potential for the emergence of another novel zoonotic CoV.

Here, we report a previously unknown bat-origin CoV causing severe and fatal pneumonia in five patients from Wuhan, China. Sequence results revealed that this virus, harboring a single open reading frame gene 8 (ORF8), is phylogenetically closest to bat SARS-like CoV, but is in a separate lineage. Furthermore, the amino acid sequence of the tentative receptor-binding domain (RBD) of this new CoV resembles that of SARS-CoV, indicating that they might use the same receptor. These findings highlight the urgent need for regular surveillance of the interspecies transmission of bat-origin CoV to human populations.

# Methods

### *Ethical approval*

This study was conducted in accordance with the *Declaration of Helsinki* and was approved by the National Health Commission of China and Ethics Commission of the Jin Yin-tan Hospital of Wuhan (No. KY-2020-01.01). The requirement for written

informed consent was waived given the context of emerging infectious diseases.

### *Clinical specimen and data collection*

Bronchoalveolar lavage fluid (BAL) samples were collected from five patients hospitalized with pneumonia in Jin Yin-tan Hospital of Wuhan, Wuhan, Hubei province, China from December 18 to 29, 2019. Information was gathered, including clinical data, demographic characteristics, underlying medical conditions, clinical signs and symptoms, chest radiographic findings, clinical laboratory testing results, traveling history, recent animal exposure, and outcomes. The data collected for the cases were deemed by the National Health Commission of the People's Republic of China as the contents of a public health outbreak investigation.

### *Genome sequencing*

Nucleic acids were extracted from 200 μl BAL of each sample with the Direct-zol RNA Miniprep kit (Zymo Research, Irvine, CA, USA) and Trizol LS (Thermo Fisher Scientific, Carlsbad, CA, USA) according to the manufacturer instructions in a biosafety III laboratory. A 50-μl elution was obtained from each sample. The DNA/RNA concentrations were measured by a Qubit Fluorometer (Thermo Fisher Scientific, Carlsbad, CA, USA). The sequencing library was constructed by a transposase-based methodology and sequenced on an Illumina Hiseq sequencing platform (Illumina, San Diego, CA, USA). At least 25 million single-end 76-bp reads were generated for each sample on the Illumina NextSeq platform. Quality control processes included removal of low-complexity reads by bbduk (entropy = 0.7, entropy -window = 50, entropy k = 5; version: January 25, 2018),[11] adapter trimming, low-quality reads removal, short reads removal by Trimmomatic (adapter: TruSeq3-SE.fa:2:30:6, LEADING: 3, TRAILING: 3, SLIDING WINDOW: 4:10, MINLEN: 70, version: 0.36),[12] host removal by bmtagger (using human genome GRCh38 and yh-specific sequences as reference),[13] and ribosomal reads removal by SortMeRNA (version: 2.1b).[14] Taxonomic assignment of the clean reads was performed with Kraken 2 against the reference databases, including archaea, bacteria, fungi, human, plasmid, protozoa, univec, and virus sequences (software 2.0.7-beta, database version: August 2, 2019).[15] A negative control sample was processed and sequenced in parallel for each sequencing run as a contamination control. The data were classified by simultaneous alignment to the microbial genome databases

comprising viruses, bacteria, fungi, and parasites after filtering of the adapters and human-origin reads. The sequences were confirmed by Sanger sequencing with specific primers and one-step real-time polymerase chain reaction (RT-PCR) Kit (Invitrogen, Carlsbad, CA, USA).

### *Phylogenetic analysis*

Multiple sequence alignment was performed with the ClustalW program using MEGA software (version 7.0.14). Phylogenetic trees were constructed by means of the maximum-likelihood method with MEGA software (version 7.0.14). The full-genome viral sequences were deposited in the dataset of Global Initiative on Sharing All Influenza Data (GISAISD, No. EPI_ISL_402123, EPI_ISL_403928-31) and the Genome Warehouse in National Genomics Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under Project ID PRJCA002165 that is publicly accessible at https://bigd.big.ac.cn/gwh as of January 2020.

### *Virus isolation*

The BAL specimens were inoculated onto Vero cells (ATCC, CCL-81). All cultures were observed daily for a cytopathic effect (CPE). Maintenance medium containing tosyl-phenylalanine chloromethyl-ketone (TPCK) enzyme at a final concentration of 1 μg/mL was replenished at day 4, and cultures were terminated 7 days after inoculation. The viral particles were negative stained with 1% solution of phosphotungstic acid (pH 7.0) and the morphology was characterized by using 120 kV TECNAI (Thermo Fisher Scientific, Hillsboro, OR, USA) electron microscopy and camera of Gatan832 (Gatan, Pleasanton, CA, United States). The culture supernatants of cells demonstrating CPE were mixed with paraformaldehyde, dried onto formvar/carbon-coated grids, and stained. Viral nucleic acids were confirmed by RT-PCR with specific primers [Supplementary Table 1].

### *Immunofluorescence assay*

Spot slides were prepared by applying 20 μl of the virus-infected or non-infected cell suspension onto 12-well Teflon-coated slides. The cells were fixed with 4% paraformaldehyde in 1× phosphate-buffered saline (PBS) for 30 min, washed three times with PBS, blocked, and stained with serum from a convalescent patient or serum from a healthy person for 30 min at 37°C at a dilution of 1:200. Goat

anti-human immunoglobulin G conjugated with fluorescein isothiocyanate was used as the secondary antibody (Jackson Immuno Research Laboratories, Inc., West Grove, PA, USA). Nuclei and the cytoplasm were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI) and Evans blue (Sigma-Aldrich, St. Louis, MO, USA). Fluorescent images were obtained and analyzed using laser-scanning confocal microscopy (Airyscan LSM880, Zeiss, Berlin, Germany).

# Results

### *Genereal information of patients*

Patient 1 was a 65-year-old man who reported a high fever and cough, with little sputum production, at the onset of illness. He had a continuous fever and developed severe shortness of breath 16 days later. He was a vendor at the Huanan Seafood Market, Wuhan, Hubei Province, China. Patient 2, a 49-year-old woman, presented with high fever and dry cough. Five days later, she developed dyspnea and was admitted to the hospital. She was also a worker in the Huanan Seafood Market. Patient 3 was a 52-year-old woman who did not report any market exposure. She was admitted to hospital because of fever, cough, and ground-glass opacity in the chest computed tomography (CT) scan. Patient 4 was a 41-year-old man who also presented with high fever and dry cough at the onset of the illness. He developed acute respiratory distress syndrome 7 days later. This patient had no known history of exposure to the Huanan Seafood Market. Patient 5, a 61-year-old man, was admitted to a local hospital with a 7-day history of fever, cough, and dyspnea. He also worked in the market.

With regards to medical history, Patient 4 had hypertension, and Patient 5 had chronic liver disease and abdominal myxoma, whereas none of the other patients had a record of underlying diseases. The demographic and clinical characteristics of the five patients are summarized in Table 1.

### *Novel CoV identification by next-generation sequencing*

The resultant clean reads accounted for 12.0–92.0% of the raw reads. Most of the reads could be successfully assigned. Notably, 80.3% of the reads mapped to the viral genome for sample from Patient 5 with the highest proportion of viral reads

among the five samples. Nearly all of the viral reads (97%) were classified as *Coronaviridae*. Similarly, in the other four patients, most of the viral reads were assigned to β-CoVs. Based on *de novo* assembly and careful curation, a consensus sequence of this CoV was obtained.

A substantial proportion of all sequencing reads mapped to the newly reported CoV genome (BWA mem, version: 0.7.12),[16] ranging from 71,883 (0.27% among all reads) in Patient 4 to 37,247,818 (85%) in Patient 5. In addition, very few reads mapped to known bacterial pathogens, including *Streptococcus*, *Acinetobacter baumannii*, and *Pseudomonas* [Figure 1A–E].

The reads mapping to CoVs were assembled, and their genome sequences were confirmed by Sanger sequencing. The nucleotide (nt) similarity among the obtained five whole-genome sequences was 99.8–99.9%. The full length of the obtained genome was 29,870 bp with a GC content of 37.99–38.02%. The genome organization, 5′-ORF1ab–S–E–M–N–3′, was similar to that of the most well-known bat SARS-like (SL)-CoV [Figure 2A]. In addition, unique accessory open reading frames (UA-ORFs) were identified that are characterized in the subgenus *Sarbecovirus*, encoding putative ORF3, ORF6, ORF7, and ORF8 proteins reading from the 5′-terminus to the 3′-terminus between the structural proteins [Figure 2A].

***Phylogenetic analysis***

Homology assessment showed that full-length viral genome sequences have 79.0% nt identity with that of SARS-CoV Tor2 (GenBank NC_004718), 51.8% with that of MERS-CoV (GenBank NC_019843), and 87.6–87.7% with those of bat SL-CoV ZC45 and ZXC21 (GenBank MG772933, MG772934), isolated from Chinese horseshoe bats (*Rhinolophus sinicus*) [Table 2], indicating that the novel CoVs are most similar to bat SL-CoVs.

Compared with bat SL-CoV ZC45, the novel CoVs showed 75.9%, 98.6%, 93.2–93.4%, and 91.1% nt identities in the S, E, M, and N genes, respectively. Overall, ORF1ab showed 89.0% nt identity between the novel CoVs and bat SL-CoV ZC45. Surprisingly, RNA-dependent RNA polymerase (RdRp), which is the most highly conserved sequence among different CoVs,[1,4] only showed 86.3–86.5% nt identities with bat SL-CoV ZC45. According to the International Committee on Taxonomy of Viruses criteria, a new CoV species could be defined if the nt identity is less than 90%

for the conserved RdRP sequence.[4] Thus, we considered that the novel CoVs should be classified as a new species under the subgenus *Sarbecovirus* of the genus *Betacoronavirus.*

The phylogenetic trees constructed with the sequences of the RdRp, S, and N genes, and the whole genome using a maximum-likelihood model showed that all five novel CoVs were closely related to bat SL-CoVs ZXC21 and ZC45, but in a separate evolutionary lineage under the subgenus *Sarbecovirus* [Figure 2B–E], which is consistent with the homology assessment results.

ORF3 and intact ORF8 gene regions were present in the novel CoVs, which are the characteristic features of bat-origin CoVs.[17,18] ORF3 of the novel CoVs showed 87.8% nt and 90.9% amino acid (aa) identities with bat SL-CoV ZC45, but less than 76.8% nt and 76.0% aa identities with the other members in the subgenus *Sarbecovirus*. In addition, ORF8 of the novel CoVs showed 88.5% and 94.2% nt and aa identity with bat SL-CoV ZC45, respectively, and less than 67.8% and 58.6% nt and aa identity, respectively, with other members of *Sarbecovirus*. These findings further indicated that the novel CoVs are of bat origin.

The RBD in the CoV S protein determines the host range.[19] The RBD aa sequences of the novel CoV showed several distinct features, including higher aa identities with those of SARS-CoV (73.8–74.8%) and human angiotensin-converting enzyme 2 (hACE2)-using SL-CoVs (76.4–76.9%) than those of SL-CoVs incapable of using hACE2 (61.5–64.1%). The novel CoV does not possess the deletions commonly found in the RBD of SL-CoVs incapable of using hACE2 as a receptor [Figure 2F].[20] In addition, the five critical aa residues interacting with hACE2 in SARS-CoV RBD (Y442, L472, N479, D480, T487) differ from the corresponding residues in the novel CoVs (L, F, Q, S, N), although these residues possess similar polarity.[20,21] These results suggested that the novel CoVs might still use hACE2 as the receptor.

### *Viral culture*

CPE were observed in 30% of Vero cells inoculated with the new CoV after two passages [Figure 3A]. The cells showed a round, refractive, and syncytium appearance. The Vero cells with CPE were further examined using negative-staining electron microscopy, demonstrating characteristic CoV particles with surface projections [Figure 3B]. Immunofluorescent assays of the culture of Vero cells showing CPE with the convalescent serum from patients showed green signals in the

cytoplasm, with no signals detected in wells containing control serum, indicating the presence of viral particles in the cells [Figure 3C].

### *Clinical features and outcomes of the patients*

The clinical features and laboratory test results of the five patients are summarized in Table 2. Fever, cough, and dyspnea were the most common symptoms. The white blood cell counts varied among these patients, but the lymphocyte counts were generally low. The alanine aminotransferase and serum creatine levels were normal or only slightly increased. Bilateral ground-glass opacities and consolidation were observed on chest radiography from two representative patients, Patient 2 based on aortic arch scan [Figure 4A] and pulmonary vein scan [Figure 4B] on day 10 after symptoms onset and Patient 5 taken on day 12 [Figure 4C] and 13 [Figure 4D] after symptoms onset.

Several complications were observed in these patients. Four of the five patients (except for Patient 3) developed acute respiratory distress syndrome requiring oxygen therapy, and two patients were given extracorporeal membrane oxygenation. Two patients (Patients 1 and 5) experienced secondary infections, and Patient 5 later developed septic shock as well as acute kidney injury, and ultimately died of multi-organ failure. Patient 3 was discharged on January 8, 2020 (day 17 after symptoms onset). The other three patients were still hospitalized at the time of manuscript preparation. The treatments for these patients were shown in Table 1.

## Discussion

In this study, we identified a previously unknown CoV from patients suffering from severe pneumonia. The whole-genome sequences of the viruses were obtained by a next-generation sequencing approach from all five patients, demonstrating overwhelmingly dominant viral reads in the BAL samples. Among the five novel CoV genome sequences, the nt identities reached up to 99.8–99.9%. The viruses successfully isolated from the patients could also be effectively recognized by serum from a convalescent patient. These findings primarily indicate that the novel CoV is associated with the pneumonia that developed in these patients. However, it remains to be determined whether this novel CoV is capable of causing similar diseases in experimental animals.

Sequence homology analysis of the viral genome showed that the CoV identified in this study is distinct from any of the known human CoVs, including SARS-CoV and MERS-CoV. The most closely related known viruses are two bat SL-CoVs (GenBank accession nos. MG772933, 772934) identified in 2005 in Zhoushan, Zhejiang, China, which is geographically distant from Wuhan;[22] however, the nt identities among the viruses are only 85.7–86.8%. Phylogenetic analysis showed that this virus forms a single clade. Collectively, these data indicate that this CoV should be considered a new species. The outbreak of SARS in 2003 largely boosted awareness of threats caused by emerging CoVs. Consequently, great efforts have been made to monitor novel emerging CoVs and to trace their origins so as to establish a risk assessment and alert system for preventing potential epidemics in the human population. Clarification of the coronavirome in animals, particularly in bats as a key reservoir of a wide range of CoVs, should be a priority for any task force.[23,24]

A few striking features of these novel CoVs indicated that they are of bat-origin. First, the genome sequences of the novel CoVs show high similarity with that of bat SL-CoV ZC45. Second, the phylogenetic analysis indicated that these viruses are evolutionarily close to bat SL-CoVs ZXC21 and ZC45. Third, all of these novel CoVs contain ORF3 and intact ORF8 gene regions, which are characteristic features of bat-origin CoVs. [17,20] Moreover, the aa sequences of the N-terminal domains (NTDs) of the novel CoVs were very similar to those of ZC45 and ZXC21, whereas the RBD of the novel CoV showed higher aa sequence identity to that of SARS-CoV than to those of ZC45 and ZXC21, suggesting that a recombination event might have occurred at the region between the NTD and RBD of the S gene, facilitating the interspecies transmission.

Owing to the lack of epidemic information at present, the transmission modes of the novel CoV remain obscure. It is notable that four of the five patients had a history of recent exposure to a seafood market in Wuhan. However, the origin of infection is unknown at the time of manuscript preparation. It is assumed that the zoonotic CoV jumped to humans through an intermediate host; for example, camel is suspected as the intermediate host of MERS-CoV, whereas the palm civet may have contributed to the interspecies transmission of SARS-CoV to humans.[25,26] Bat CoVs may evolve to adapt to using humans as a host during their circulation in a mammalian host, thereby enabling them to effectively infect humans.[26] However, two of our patients did not have a history of exposure to the seafood market. Therefore, further investigation will

be needed to determine the potential of multiple infection sources responsible for this uncommon outbreak.

One of the most striking and concerning features of this virus is its ability to cause severe respiratory syndrome. The disease progressed rapidly with a major presentation of lower respiratory pathology. Notably, no obvious upper respiratory tract symptoms such as a sore throat and rhinorrhea were present in these patients. Therefore, further exploration is needed on the distribution of the viral receptor in the organs to potentially account for pathogenesis development. In addition, the possibility of unrecognized mild infections or subclinical infections should be clarified, as identification of such infections is critical to control spread of the disease. Development of serological assays would be largely beneficial to detect such types of infection at the population level.

In conclusion, we identified a novel bat-borne CoV associated with a severe and fatal respiratory disease in humans. The emergence of this virus poses a potential threat to public health. Therefore, clarification of the source and transmission mode of these infections is urgently needed to prevent a potential epidemic.

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***Conflicts of interest***

None.

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**Figure legends**

**Figure 1:** Microbial species profiles (proportions) in bronchoalveolar lavage fluid specimens from Patient 1 (A), Patient 2 (B), Patient 3 (C), Patient 4 (D), and Patient 5 (E) analyzed by deep sequencing.

**Figure 2:** Characteristics of viral genes. The schematic diagram of the novel coronavirus (CoV)'s genome (A). Phylogenic analysis of viral whole genome (B), spike (C), nucleocapsid (D) and RNA-dependent RNA polymerase (E) genes. The novel CoV identified and closely related viruses are in red in the phylogenetic trees. Other outgroup viruses from public database are shown in blue. Evolutionary distances were calculated with the maximum likelihood method. Amino acid sequence of the putative receptor binding domain (RBD) of IPBCAMS-WH-01/2019 compared with strains of severe acute respiratory syndrome coronavirus (SARS-CoV) and bat SARS-like CoV (F).

**Figure 3:** Viral isolation and identification. The untreated control (left) and cytopathic effect (right) on Vero cells visualized with an inverted microscope (A, original magnification ×20). Viral particles are negative stained with 1% solution of phosphotungstic acid and observed by using electron microscopy analysis (B, scale

bar 200 nm). Immunofluorescent assays with convalescent serum and anti-human IgG conjugated FITC show signals (green) in cytoplasma in Vero cells. The Nuclei and cytoplasma were counterstained with 4′,6′-diamidino-2-phenylindole (DAPI, blue) and Evans blue (red), respectively (C, scale bar 20 μm).

**Figure 4:** Computed tomographic chest radiographs of Patient 2, obtained on day 10 from illness onset at aortic arch (A) and pulmonary vein (B) scan demonstrating bilateral ground-glass opacity and consolidation, and Patient 5 on day 12 (C) and 13 (D) after illness onset demonstrating white lungs.

**Table 1: Demographic, epidemiologic, clinical presentations, and treatment of the patients.**

| Characteristics | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 |
|---|---|---|---|---|---|
| Age, year | 65 | 49 | 52 | 41 | 61 |
| Gender | Male | Female | Female | Male | Male |
| Huanan Seafood Market exposure | Yes | Yes | No | No | Yes |
| Smoking | No | No | No | No | No |
| Underlying conditions | No | No | No | Hypertension | Chronic liver disease, myxoma of abdominal cavity |
| Date of illness onset | Dec 15, 2019 | Dec 22, 2019 | Dec 22, 2019 | Dec 16, 2019 | Dec 20, 2019 |
| Date of admission | Dec 18, 2019 | Dec 27, 2019 | Dec 29, 2019 | Dec 22, 2019 | Dec 27, 2019 |
| Admission to ICU | Dec 22, 2019 | Dec 29, 2019 | Dec 29, 2019 | Dec 30, 2019 | Dec 28, 2019 |
| Sampling date | Dec 24, 2019 | Dec 30, 2019 | Dec 30, 2019 | Dec 30, 2019 | Jan 1, 2020 |
| **Signs and Symptoms** | | | | | |
| Cough | Yes | Yes | Yes | Yes | Yes |
| Sputum production | Yes | No | No | No | No |
| Dyspnea (onset date) | Dec 31, 2019 | Dec 27, 2019 | Not available | Dec 22, 2019 | Dec 27, 2019 |
| Fever | Yes | Yes | Yes | Yes | Yes |
| Muscle pain/Fatigue | No | No | Yes | Yes | Yes |
| Headache | No | No | Yes | No | No |
| Diarrhea | No | No | No | No | No |
| Highest temperature, °C | 39.3 | 38.5 | 37.5 | 39 | N.A. |
| **Laboratory tests on admission** | | | | | |
| Number of white blood cells, $\times 10^9$ /L | 11.9 | 8.3 | 2.4 | 6.6 | 17.9 |
| Number of neutrophils, $\times 10^9$ /L | 11.6 | 7.6 | 2.0 | 5.0 | 16.2 |

| | | | | | |
|---|---|---|---|---|---|
| Number of lymphocytes, $\times 10^9$ /L | 0.2 | 0.44 | 0.3 | 0.98 | 1.2 |
| Number of platelets, $\times 10^9$ /L | 92 | 273 | 140 | 129 | 315 |
| Prothrombin time, S | 12 | 12.5 | 12.3 | 11.2 | 28.4 |
| APTT, S | 25.5 | 25.2 | 28.5 | 26.9 | 105.2 |
| D-dimer, mg/L | 40.7 | 0.21 | 0.65 | 1.31 | 20.6 |
| ALT, U/L | 50 | 45 | 19 | 29 | 35 |
| Serum potassium, mmol/L | 5.0 | 3.8 | 4.0 | 5.4 | 4.8 |
| Serum creatine, μmol/L | 53.1 | 42.6 | 54.5 | 85.1 | 106.6 |
| Procalcitonin, ng/mL | 1.46 | <0.05 | < 0.05 | <0.05 | 0.69 |
| **Pathogen testing** | | | | | |
| Coronavirus reads proportion, % | 39.9 | 73.1 | 13.6 | 1.6 | 80.3 |
| **Imaging** | | | | | |
| Bilateral GGO | Yes | Yes | Yes | Yes | Yes |
| Consolidation | Yes | Yes | No | Yes | Yes |
| **Treatment** | | | | | |
| Oxygen therapy | Mechanical ventilation | HFNC | Nasal cannula | Non-invasive ventilation | Mechanical ventilation |
| ECMO (initiation date) | Jan 6, 2020 | No | No | No | Jan 2, 2020 |
| Antibiotic therapy | Yes | Yes | Yes | Yes | Yes |
| CRRT | No | No | No | No | Yes |
| **Complications** | | | | | |
| ARDS (onset date) | Dec 22, 2019 | Dec 29, 2019 | No | Dec 23, 2019 | Dec 27, 2019 |
| Septic shock (onset date) | No | No | No | No | Dec 31, 2019 |
| AKI (onset date) | No | No | No | No | Dec 31, 2019 |

| | | | | | |
|---|---|---|---|---|---|
| Secondary infections | Yes | No | No | No | Yes |
| **Outcome*** | Hospitalized | Hospitalized | Discharged | Hospitalized | Death |

*Outcome data as of January 11, 2020.

ICU: Intensive Care Unit; WBC: White blood cell; APTT: Activated partial thromboplastin time; ALT: Alanine aminotransferase; HFNC: High-flow nasal cannula; GGO: Ground-glass opacity; ECMO: Extracorporeal membrane oxygenation; CRRT: Continuous renal replacement therapy; ARDS: Acute respiratory distress syndrome; AKI: Acute kidney injury.

**Table 2: Location and size of the putative proteins of the representative strain, IPBCAMS-WH-01/2019.**

| Gene region | Location* | Size, aa | GC content, % | Similarity, % (nt/aa) | |
|---|---|---|---|---|---|
| | | | | SARS-Cov | bat-SL-ZC45 |
| Whole genome | NA | NA | 38.02 | 79.0 | 87.7 |
| 5′UTR | 1–265 | NA | 44.53 | 88.7 | 93.5 |
| nsp1 | 266–805 | 180 | 48.52 | 82.2/84.4 | 93.1/95.5 |
| nsp2 | 806–2719 | 638 | 39.86 | 68.3/68.3 | 92.2/95.2 |
| nsp3 (PLpro) | 2720–8554 | 1945 | 35.94 | 72.7/75.8 | 91/93.8 |
| nsp4 | 8555–10,054 | 500 | 36.47 | 74.8/79.8 | 90.2/96.6 |
| nsp5 (3CLpro) | 10,055–10,972 | 306 | 38.13 | 83.6/96.0 | 92.1/99.0 |
| nsp6 | 10,973–11,842 | 290 | 36.32 | 79/87.2 | 91.7/97.9 |
| nsp7 | 11,843–12,091 | 83 | 38.15 | 79.9/98.7 | 82.7/100 |
| nsp8 | 12,092–12,685 | 198 | 38.38 | 86.1/97.4 | 87.2/97.4 |
| nsp9 | 12,686–13,024 | 113 | 40.12 | 84.9/97.3 | 86.7/97.3 |
| nsp10 | 13,025–13,441 | 139 | 42.44 | 88.2/97.1 | 90.8/97.1 |
| nsp12 (RdRp) | 13,442–13,468<br>13,468–16,236 | 932 | 37.28 | 88.5/96.3 | 86.5/95.9 |
| nsp13 (Hel) | 16,237–18,039 | 601 | 38.16 | 88.4/99.8 | 87.9/99.3 |
| nsp14 (ExoN) | 18,040–19,620 | 527 | 38.27 | 83.3/95.0 | 82.9/94.4 |
| nsp15 | 19,621–20,658 | 346 | 34.01 | 82/88.7 | 82.7/89.0 |
| nsp16 | 20,659–21,552 | 298 | 35.46 | 85.1/93.2 | 89.5/97.9 |
| S | 21,563–25,384 | 1274 | 37.31 | 72.3/75.5 | 75.9/80.4 |
| 3 | 25,393–26,220 | 276 | 39.49 | 75.3/72† | 87.8/90.9 |
| E | 26,245–26,472 | 76 | 38.16 | 93.5/94.7 | 98.6/100 |

| | | | | | |
|---|---|---|---|---|---|
| M | 26,523–27,191 | 223 | 42.6 | 85.2/90.5 | 93.4/98.6 |
| 6 | 27,202–27,387 | 62 | 27.96 | 73.9/66.6 | 95.1/93.4 |
| 7a | 27,394–27,759 | 122 | 38.25 | 82.1/85.2 | 88.7/87.6[‡] |
| 7b | 27,756–27,887 | 43 | 31.06 | 82.0/79.5 | NA |
| 8 | 27,894–28,259 | 122 | 35.79 | NA | 88.5/94.2 |
| N | 28,274–29,533 | 420 | 47.2 | 88.1/90.5 | 91.1/94.2 |
| 9b | 28,284–28,577 | 97 | 49.66 | 88.5/72.4 | 89.1/73.1 |
| 3′UTR | 29,534–29,870 | | 40.36 | 95.2 | 93.2 |

[*]Representative strain, IPBCAMS-WH-01/2019, no. EPI_ISL_402123. [†]Compared with 3a of SARS-CoV. [‡]Compared with 7a of bat-SL-ZC45. aa: amino acids; nt: nucleotide; SARS-CoV: severe acute respiratory syndrome coronavirus; SL: SARS-like; UTR: untranslated regions; nsp: non structural protein; NA: not applicable.

Figure 1

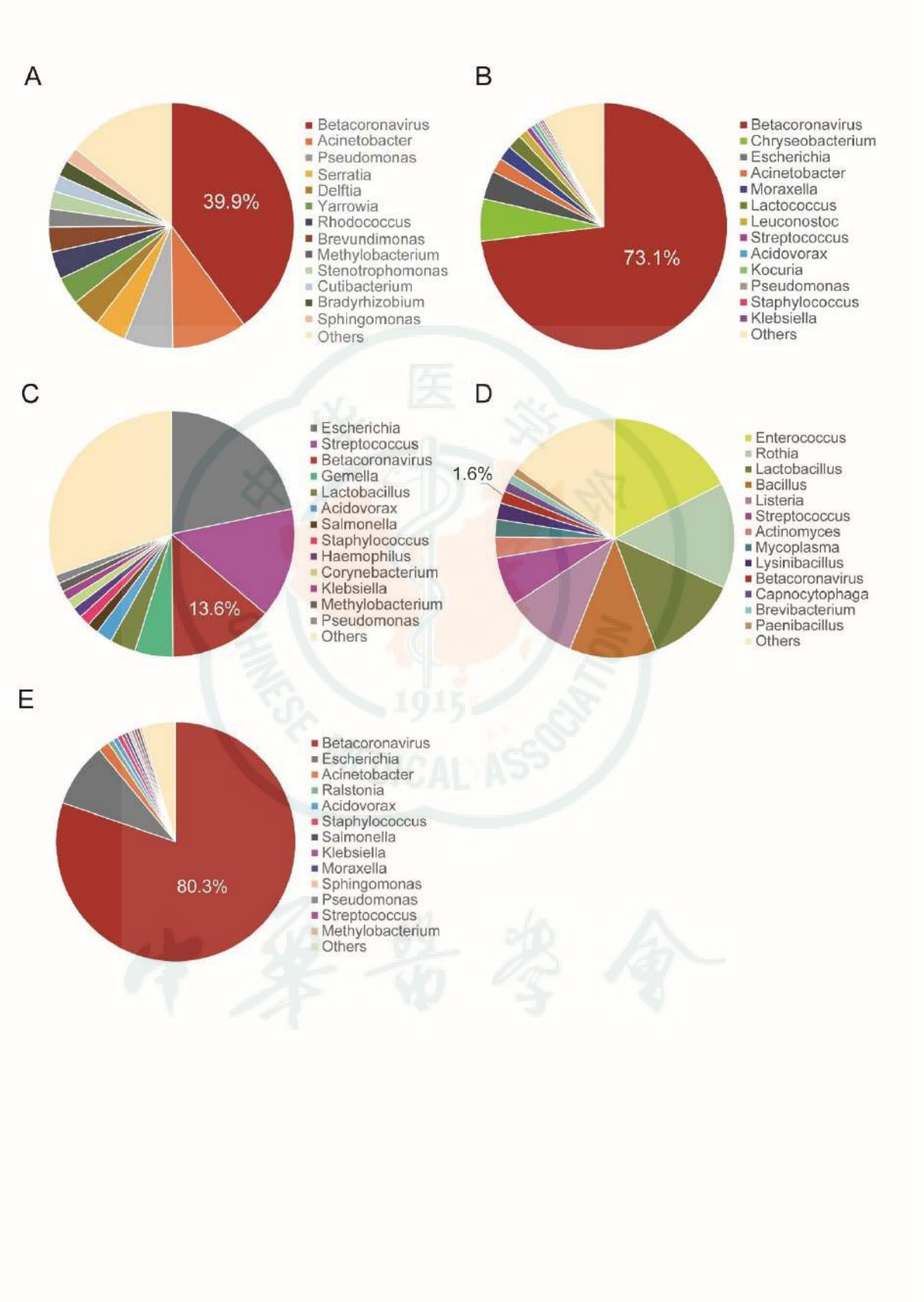



Figure 2

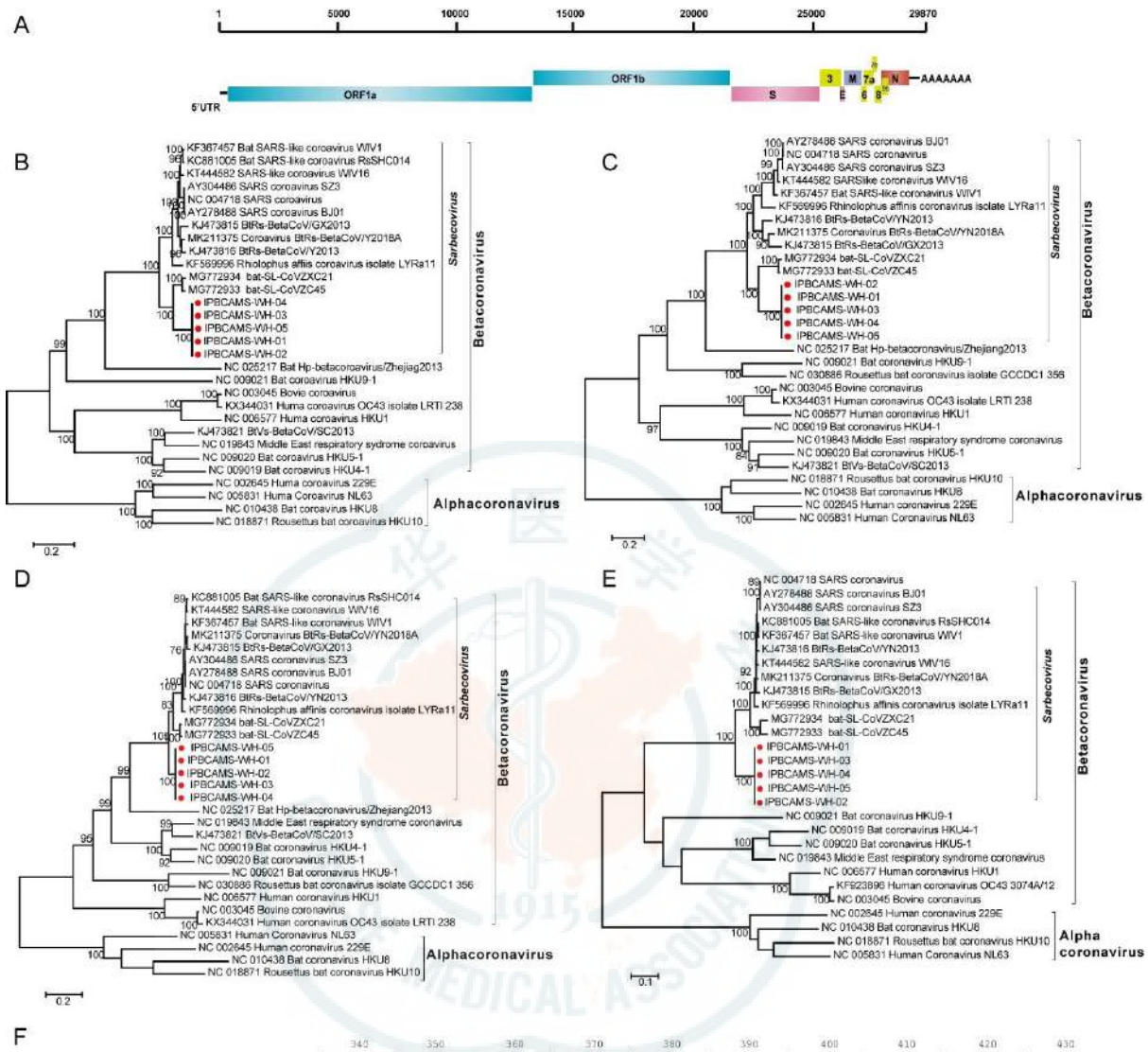

A
1
5000
10000
15000
20000
25000
29870
5'UTR
ORF1a
ORF1b
S
E
M
N
AAAAAAA
B
C
D
E
Sarbecovirus
Betacoronavirus
Alphacoronavirus
Alpha coronavirus
IPBCAMS-WH-01
IPBCAMS-WH-02
IPBCAMS-WH-03
IPBCAMS-WH-04
IPBCAMS-WH-05
NC_004718 SARS-CoV
AY278488 SARS-CoVBJ01
KF367457 BatSLCoVWIV1
KT444582 BatSLCoVWIV16
KY417146 BatSLCoVRs4231
KY417144 BatSLCoVRs4084
MG772933 BatSLCoVZC45
MG772934 BatSLCoVZXC21
KJ473813 BatSLCoVSX2013
KJ473812 BatSLCoVHeB2013
KJ473816 BatSLCoVYN2013
KJ473815 BatSLCoVGX2013
MK211375 BatSLCoVYN2018A
KF569996 RaLYRa11
F

Figure 3

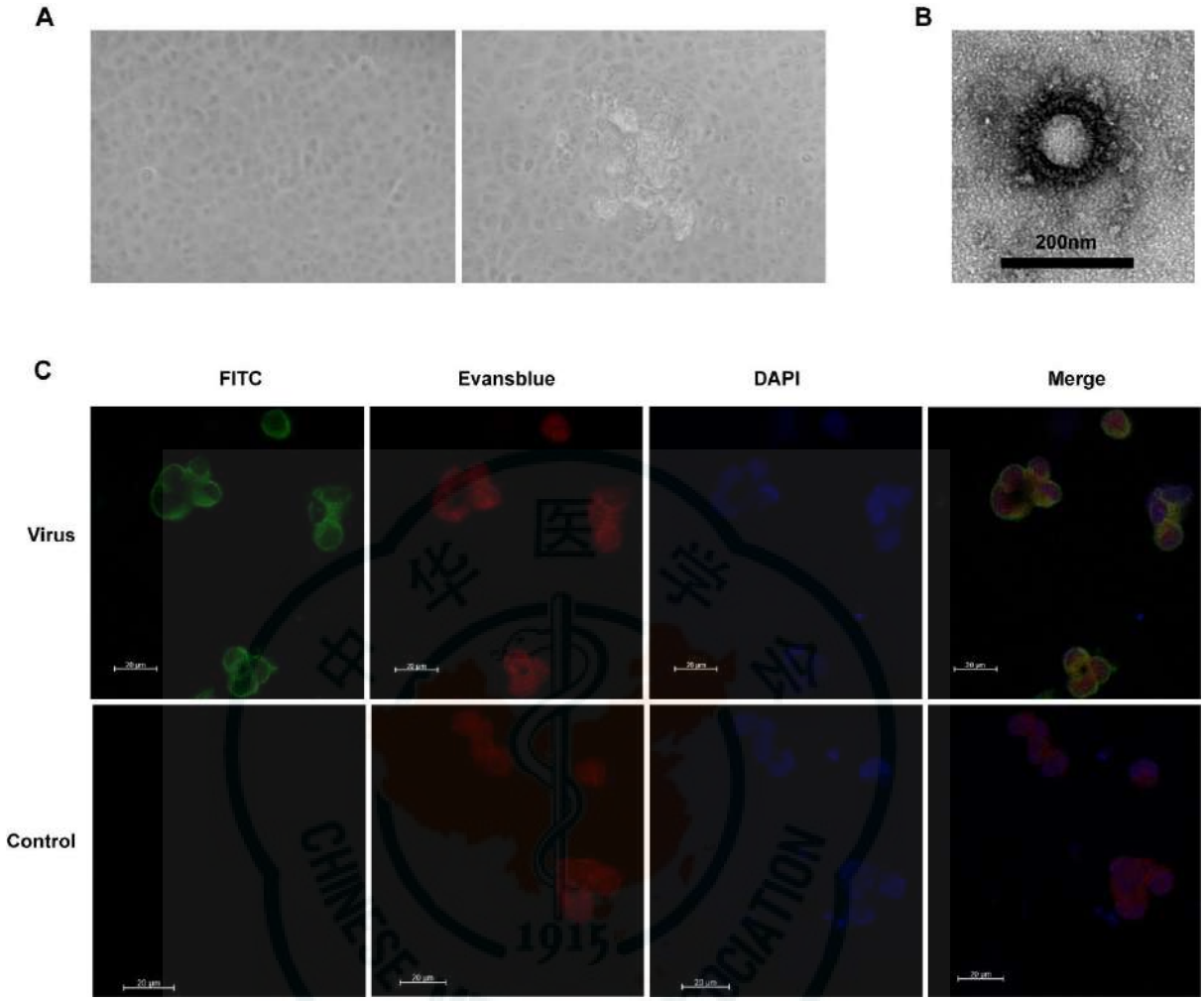
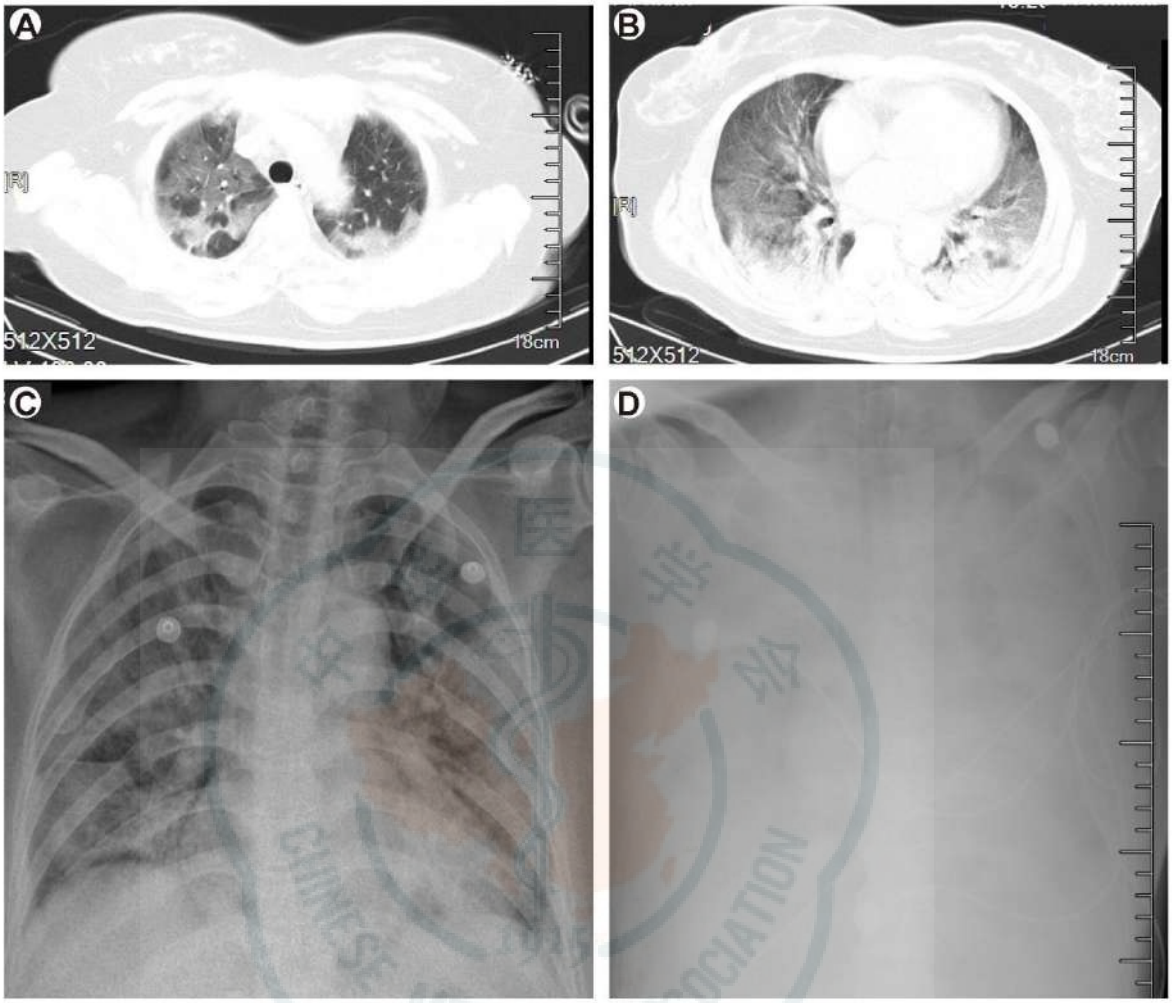



Figure 4



**Supplementary Table 1: Primers used for whole genome amplification of 2019 novel coronavirus.**

| Primer code | Sequence (5'-3') | Start | Stop | Targeted gene region |
|---|---|---|---|---|
| 5'RACE-1 | AGTGCACTCACGCAGTAT | 113 | 130 | UTR |
| 5'RACE-2 | CTGTCGTTGACAGGACACGAGTAA | 147 | 170 | |
| 5'RACE-3 | CGATCATCAGCACATCTAGG | 218 | 237 | |
| B1 | F: TGTCGTTGACAGGACACGAG | 148 | 167 | ORF1a |
| | R: TAACAAAATCGCCCGTCTGC | 1277 | 1258 | |
| B2 | F: GTCTATCCAGTTGCGTCACCA | 1172 | 1192 | ORF1a |
| | R: TCCACCGACAATTTCACAAGC | 2251 | 2231 | |
| B3 | F: AACCCGTCCTTGATTGGCTT | 2139 | 2158 | ORF1a |
| | R: TGATTGTCCTCACTGCCGTC | 3258 | 3239 | |
| B4 | F: GCCACTTCTGCTGCTCTTCA | 3155 | 3174 | ORF1a |
| | R: TTTCAGTAGTGCCACCAGCC | 4199 | 4180 | |
| B5 | F: GGGTGATGTTGTTCAAGAGGG | 4126 | 4146 | ORF1a |
| | R: AGCCTCAACACGTAGAGTGTC | 5158 | 5138 | |
| B6 | F: GGACAACAGTTTGGTCCAACTTA | 5036 | 5058 | ORF1a |
| | R: AGCTTGCGTTTGGATATGGTTG | 6038 | 6017 | |
| B7 | F: CAAAGTCCTCAGAATACAAAGGTCC | 5826 | 5850 | ORF1a |
| | R: GCCTCTAGACAAAATTTACCGACA | 6903 | 6880 | |
| B8 | F: ACGCGCAGGGAATGGATAAT | 6360 | 6379 | ORF1a |
| | R: ACCATAGCTGAAATCGGGGC | 7407 | 7388 | |
| B9 | F: TGGATTGGCTGCAATCATGC | 7288 | 7307 | ORF1a |
| | R: ATGACGCGCACTACAGTCAA | 8359 | 8340 | |
| B10 | F: TGCAACTGCAGAAGCTGAAC | 8104 | 8123 | ORF1a |
| | R: TTTCACAAGTGCCGTGCCTA | 9236 | 9217 | |
| B11 | F: TCAGCTTGTGTTTTGGCTGC | 8996 | 9015 | ORF1a |
| | R: CGTCATCAAGCCAAAGACCG | 10157 | 10138 | |
| B12 | F: GCTGCTTGTTGTCATCTCGC | 9950 | 9969 | ORF1a |
| | R: CAACCAGTGGTGTGTACCCT | 11014 | 10995 | |
| B13 | F: CCTCTTTCTGCTCAAACTGGAA | 10808 | 10829 | ORF1a |
| | R: GTGTAACTGGACACATTGAGCC | 11950 | 11929 | |
| B14 | F: TCACAGGGACTACTCCCACC | 11738 | 11757 | ORF1a |
| | R: TGTAGTACCGGCAGCACAAG | 12742 | 12723 | |
| B15 | F: AGGGCCAATTCTGCTGTCAA | 12659 | 12678 | ORF1a, RdRP |
| | R: AGACGAGGTCTGCCATTGTG | 13825 | 13806 | |
| B16 | F: GTGGGGGACAACCAATCACT | 13122 | 13141 | RdRP |
| | R: CAGGAACTCCACTACCTGGC | 14137 | 14118 | |
| B17 | F: TGATGCCATGCGAAATGCTG | 14019 | 14038 | RdRP |
| | R: GCTACGGTGCGAGCTCTATT | 15113 | 15094 | |
| B18 | F: ACCAAGTCATCGTCAACAACCT | 14912 | 14933 | RdRP |
| | R: ATCTACAAAACAGCCGGCCC | 15975 | 15956 | |

| | | | | |
|---|---|---|---|---|
| B19 | F: TGTTGGACTGAGACTGACCTT | 15835 | 15855 | RdRP, ORF1b |
| | R: CGGTAAACAACAGCATCACCA | 16871 | 16851 | |
| B20 | F: TGCAGCAGAAACGCTCAAAG | 16635 | 16654 | ORF1b |
| | R: CTCCAAGCAGGGTTACGTGT | 17756 | 17737 | |
| B21 | F: TGTTCCTCGGAACTTGTCGG | 17543 | 17562 | ORF1b |
| | R: CAACTCAAAGCCATGTGCCC | 18615 | 18596 | |
| B22 | F: TAGTGCTAAACCACCGCCTG | 18447 | 18466 | ORF1b |
| | R: TGTCTACAGACAGCACCACC | 19496 | 19477 | |
| B23 | F: CATGCATTCCACACACCAGC | 19309 | 19328 | ORF1b |
| | R: TGAACCTGTTTGCGCATCTG | 20481 | 20462 | |
| B24 | F: AGGCTATGCCTTCGAACATATC | 20304 | 20325 | ORF1b |
| | R: CGCGTGGTTTGCCAAGATAA | 21307 | 21288 | |
| B25 | F: CTTGGAGGTTCCGTGGCTAT | 21145 | 21164 | S |
| | R: AAACCCTGAGGGAGATCACG | 22221 | 22202 | |
| B26 | F: TATCTTGGCAAACCACGCGA | 21289 | 21308 | S |
| | R: ACCAGCTGTCCAACCTGAAG | 22345 | 22326 | |
| B27 | F: CCCTCAGGGTTTTTCGGCTT | 22210 | 22229 | S |
| | R: CTGTGGATCACGGACAGCAT | 23302 | 23283 | |
| B28 | F: CCAGCAACTGTTTGTGGACC | 23123 | 23142 | S |
| | R: GTGGCAAAACAGTAAGGCCG | 24149 | 24130 | |
| B29 | F: ACTTGCAGATGCTGGCTTCA | 24043 | 24062 | S |
| | R: CTCATTGAGGCGGTCAATTTCT | 25126 | 25105 | |
| B30 | F: TGATTTAGGTGACATCTCTGGCA | 25054 | 25076 | S |
| | R: ACAACTCCGGATGAACCGTC | 26159 | 26140 | |
| B31 | F: GCTGGCTTGATTGCCATAGT | 25226 | 25245 | S, ORF3 |
| | R: ACAATCGAAGCGCAGTAAGG | 26362 | 26343 | |
| B32 | F: ACGGTTCATCCGGAGTTGTT | 26141 | 26160 | E |
| | R: TAGTACCGTTGGAATCTGCCAT | 26544 | 26523 | |
| B33 | F: TCGATTGTGTGCGTACTGCT | 26355 | 26374 | M |
| | R: AGCTCACAAGTAGCGAGTGT | 27443 | 27424 | |
| B34 | F: ACAGTCGCTACAGGATTGGC | 27109 | 27128 | M, ORF6, 7, 8 |
| | R: CCCACTGCGTTCTCCATTCT | 28375 | 28356 | |
| B35 | F: GAATTGTGCGTGGATGAGGC | 28068 | 28087 | N |
| | R: CGTTCCCGAAGGTGTGACTT | 29260 | 29241 | |
| B36 | F: CTACGCAGAAGGGAGCAGAG | 28786 | 28805 | N, ORF9b |
| | R: TAGGCAGCTCTCCCTAGCAT | 29790 | 29771 | |
| 3'RACE-1 | GTCTACTCTTGTGCAGAATG | 29601 | 29620 | UTR |
| 3'RACE-2 | GAGAGCTGCCTATATGGAAG | 29779 | 29798 | |

Reference sequence IPBCAMS-WH-01/2019, no. EPI_ISL_402123. RACE: Rapid amplification of cDNA end. UTR: Untranslated Regions; ORF: Open reading frame; S: spike; E: envelope; M: membrane; N: nucleocapsid.

**Original Article**

# Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province

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**Edited by:** Xiu-Yuan Hao and Pei-Fang Wei

## Abstract

**Background:** A novel coronavirus (2019-nCoV) causing an outbreak of pneumonia in Wuhan, Hubei province of China was isolated in January 2020. This study aims to investigate its epidemiological history, and analyzed the clinical characteristics, treatment regimens and prognosis of patients infected with 2019-nCoV during this outbreak.

**Methods:** Clinical data from 137 2019-nCoV-infected patients admitted to the respiratory departments of nine tertiary hospitals in Hubei province from December 30, 2019 to January 24, 2020 were collected, including general status, clinical

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manifestations, laboratory test results, imaging characteristics, and treatment regimens.

**Results:** None of the 137 patients (61 males, 76 females, aged 20–83 years, mean age 55 ± 16 years) had a definite history of exposure to Huanan Seafood Wholesale Market. Major initial symptoms included fever (112/137, 81.8%), coughing (66/137, 48.2%), and muscle pain or fatigue (44/137, 32.1%), with other, less typical initial symptoms observed at low frequency, including heart palpitations, diarrhea, and headache. Nearly 80% of the patients had normal or decreased white blood cell counts, and 72.3% (99/137) had lymphocytopenia. Lung involvement was present in all cases, with most chest computed tomography scans showing lesions in multiple lung lobes, some of which were dense; ground-glass opacity co-existed with consolidation shadows or cord-like shadows. Given the lack of effective drugs, treatment focused on symptomatic and respiratory support. Immunoglobulin G was delivered to some critically ill patients according to their condition. Systemic corticosteroid treatment did not show significant benefits. Notably, early respiratory support facilitated disease recovery and improved prognosis. The risk of death was primarily associated with age, underlying chronic diseases, and median interval from the appearance of initial symptoms to dyspnea.

**Conclusions:** The majority of patients with 2019-nCoV coronavirus pneumonia present with fever as the first symptom, and most of them still showed typical manifestations of viral pneumonia on chest imaging. Middle-aged and elderly patients with underlying comorbidities are susceptible to respiratory failure and may have a poorer prognosis.

**Keywords**: Human–human transmission; Hubei province; Coronavirus; novel coronavirus (2019-nCoV); Clinical characteristics; Treatment

## Introduction

In late 2019, Wuhan in Hubei province, China became the focus of the world owing to an outbreak of pneumonia with unknown etiology. A pathogen was successfully isolated on January 12, 2020 and named the 2019 novel coronavirus (2019-nCoV).[1] As of 6:00 p.m. Beijing time on January 26, 2020, there are more than 2000 confirmed cases worldwide, with over half from Hubei province. Here, we report the clinical manifestations, laboratory test results, imaging characteristics, and treatment regimen of 2019-nCoV-infected patients admitted to several tertiary hospitals in Hubei to provide a basis for further specification of the diagnosis and treatment protocol for this disease.

## Methods

### *Ethics*

This study was conducted in accordance with the *Declaration of Helsinki*. Informed consent was waived because of the retrospective nature of the study and the analysis used anonymous clinical data.

***Patients and inclusion criteria***

Data were analyzed from 137 patients admitted to the respiratory departments identified to be nucleic acid-positive for 2019-nCoV in nine tertiary hospitals in Hubei province from December 30, 2019 to January 24, 2020. Of these patients, 61 were male and 76 were female, with an age range of 20 to 83 years and a mean age of 55 ± 16 years. The inclusion procedures and criteria were as follows. Suspected cases were screened out according to the diagnosis and treatment protocol for novel coronavirus (CoV) pneumonia.[2] Once selected, respiratory tract secretions and other samples were acquired for real-time fluorescence reverse transcription-polymerase chain reaction (RT-PCR). Patients tested positive for the nucleic acids of this CoV were identified as confirmed cases and enrolled in the study.

***Sample collection and pathogen identification***

After admission to the hospital, respiratory tract samples including sputum and nasopharyngeal swabs were collected from the patients, which were tested for influena, avian influenza, respiratory syncytial virus, adenovirus, parainfluenza virus, severe acute respiratory syndrome (SARS)-CoV, and Middle East respiratory syndrome (MERS)-CoV, along with routine bacterial, fungal, and pathogenic microorganism tests. Respiratory samples were employed for real-time fluorescence RT-PCR to detect the presence of 2019-nCoV. Total RNA was extracted using TRIzol according to the manufacturer's instructions. Real-time PCR was performed as the instructions by used the 2019-nCoV (ORF1ab/N) nucleic acid detection kit (Bio-germ, Shanghai, China).The following primers and probe for CoV envelope genes were used: forward primer 5′-TCAGAATGCCAATCTCCCCAAC-3', reverse primer 5′-AAAGGTCCACCCGATACATTGA-3′, and probe 5′-CY5-CTAGTTACACTAGCCATCCTTACTGC-3′ BHQ1. The amplification conditions were 50°C for 15 min and 95°C for 3 min, followed by 45 cycles of 95°C for 15 s and 60°C for 30 s.

***Data collection***

Basic information (age, gender, smoking history, and comorbidities) was collected for each patient. In addition, epidemiological histories were taken, including (1) long-time vendors, employees, and workers at Huanan Seafood Wholesale Market as of December 1, 2019; (2) staff who conducted 3 h or more work in processing, sale, slaughter, treatment, and transportation activities at Huanan Seafood Wholesale Market 2 weeks prior to disease onset as of December 1, 2019; (3) definite history of contact with birds or wildlife at Huanan Seafood Wholesale Market 2 weeks prior to disease onset as of December 1, 2019; (4) close contact with individuals suspected or confirmed to be infected by the virus, such as common transport or staying in the same room. Clinical manifestations were recorded (fever,

coughing/expectoration/myalgia or fatigue/hemoptysis/headache/heart palpitations/diarrhea/dyspnea, etc.), along with disease condition changes. Laboratory test results were compiled, including standard blood counts (absolute white blood cells and lymphocytes), blood biochemistry (alanine transaminase, aspartate transaminase, creatine kinase, creatinine), coagulation function, procalcitonin, C-reactive protein, erythrocyte sedimentation rate, and myocardial enzyme spectrum. Additional data collected included medical imaging, treatment regimens (antiviral, antibacterial, systemic corticosteroid, immunoglobulin G, respiratory support), and prognosis (recovered and discharged, inpatient treatment, or death).

### *Data analysis*

Continuous data are expressed as medians and ranges, and categorical data are presented as counts and percentages.

## Results

### *General information*

More than half of confirmed cases included in this study were from Wuhan (69/137, 50.4%). According to epidemiological histories, patients admitted to hospitals in other cities of Hubei province predominantly originated from Wuhan [Figure 1].

Basic characteristics of the study population are summarized in Table 1. The median and mean ages of the patients were 57 years and 55 years, respectively, suggesting that middle-aged and elderly people are more susceptible to infection, whereas healthy, young adult patients were less susceptible. In addition, none of the 137 patients included in this study had a clear history of exposure to Wuhan Huanan Seafood Wholesale Market, suggesting that the virus is capable of secondary or tertiary transmission. This highlights the extreme likelihood of human–human transmission, which aligns with the current national epidemic situation. Moreover, critically ill patients more frequently had an underlying comorbid systemic disease, resulting in a poor prognosis.

### *Clinical manifestations*

As shown in Table 2, the majority of the patients showed an initial symptom of fever, nearly half of whom developed a high fever. By contrast, one-quarter of the patients did not develop fever, alerting to the need for caution of atypical cases. In addition to fever, the major symptoms were coughing and myalgia or fatigue. Some patients developed initial symptoms in the cardiovascular system, digestive system, and nervous system, which increased the difficulty of diagnosis. The median interval from the onset of initial symptoms to dyspnea or significant symptom aggravation was 7 days, ranging between 1 day (i.e., appearance of acute respiratory distress syndrome) up to 20 days, which was consistent with previous reports.[3] Nearly 20% of the

patients showed comorbidities with respect to dysfunction of other organs, primarily renal impairment. Patients with underlying cardiovascular diseases often demonstrated comorbid heart failure. Since respiratory support was administered to most of the patients upon admission, oxygen saturation could be maintained at above 90% as indicated by pulse oximetry monitoring.

### *Laboratory tests and imaging examinations*

A definitive diagnosis of 2019-nCoV was acquired by real-time fluorescence RT-PCR. As shown in Table 3, nearly 80% of the patients had normal or decreased white blood cell counts, and 72.3% (99/137) of the patients developed lymphocytopenia, consistent with the main characteristic of viral infection. The lung images of most patients showed abnormal characteristics that involved both of the lungs in most cases (116/137, 84.7%). Figure 2 shows representative lung images of a patient in which lesions developed in multiple lobes, most of which were dense, and ground-glass opacity co-existed with consolidation or cord-like shadows.

### *Treatment regimen and prognosis*

At present, there are no drugs available that can target CoVs. Therefore, treatment was focused on symptomatic and respiratory support [Table 4]. Although most patients received empirical antibiotic therapy (119/137, 86.9%), for patients with rapid progression (median interval from onset of initial symptoms to dyspnea less than 3 days), it was recommended that respiratory support (119/137, 86.9%) should be administered as soon as possible, and that γ-immunoglobulin should be administered based on the patient's condition. Although clear medical evidence was not available, intravenous methylprednisolone (30–80 mg/day) was still provided to some patients (40/137, 29.2%) who either suffered from persistent high fevers that did not subside or showed significant short-term disease progression determined by imaging results. Based on the mechanism of action of the drug, it was expected that systemic corticosteroid treatment could inhibit a cytokine storm and promote the absorption of exudative lesions. However, this treatment neither significantly shortened the disease course nor improved the prognosis.

For example, the images in Figure 3 are from a patient who was treated with 40 mg intravenously (iv) every day (qd) methylprednisolone after admission. On day 6 of treatment, review of the lung computed tomography (CT) scan showed significant lesion progression and the patient ultimately died, indicating that lung changes caused by the novel CoV were not inhibited by corticosteroid as was expected.

None of the patients is currently on invasive respiratory support and extracorporeal membrane oxygenation (ECMO). This is likely because the patients included in this study were predominantly admitted to the respiratory department and therefore

relevant data from the intensive care unit and other departments are insufficient, which could lead to a biased conclusion. Sixteen of the 137 (11.7%) patients eventually died from complications of this infection. This is a higher mortality rate than previously reported (24/549),[4] which might be attributed to the fact that these were all inpatients with more critical conditions than most other patients infected in this outbreak.

## Discussion

CoV, as an enveloped RNA virus that is ubiquitous in humans, other mammals, and birds, can cause respiratory, digestive, liver and nervous system disorders.[5,6] To date, six CoVs have been known to cause human infection.[7] Among them, two zoonotic viruses, SARS-CoV and MERS-CoV, were responsible for serious outbreaks: in China in 2002–2003[8,9] and in the Middle East in 2012,[10] respectively. In addition, cross-species infection and occasional spillover events may lead to the cyclical emergence of new CoVs.[11] In December 2019, a "pneumonia of unknown etiology" appeared in Wuhan in Hubei province, the cause of which has since been confirmed as the 2019-nCoV. Of particular concern, our observations found that 137 patients had no clear history of exposure to the Wuhan Huanan seafood market, suggesting a strong likelihood of significant human-to-human transmission of the disease. At the meantime, the rapid increase in cases across the country so far confirms the virus's human-to-human transmission.

The rapid spread of 2019-nCoV is reminiscent of the SARS outbreak in China in 2003. Although more widespread transmission of the SARS virus was successfully prevented, owing to inadequate experience with outbreaks of this nature at the time, SARS-CoV still caused more than 8000 infections leading to 774 deaths.[12] The typical clinical manifestations of SARS included sudden fever (usually >38°C) accompanied by chills or headache, myalgia, fatigue, and coughing. Some patients also developed significant respiratory distress within a short period. Laboratory tests showed either a normal or decreased peripheral white blood cell count. Imaging showed focal or patch-like shadows or reticular exudates, although this did rapidly progress to diffuse consolidations in some patients.[13] In the present cohort of patients, the typical initial symptoms of 2019-nCoV were primarily fever, most of which were high fevers that occurred within several days and were not alleviated by routine anti-infective drugs. However, some middle-aged and elderly patients with underlying comorbidities only developed a moderate, low, or no fever during the disease course. In addition, some patients suffered from fatigue and dry cough on disease onset.

Nevertheless, these atypical initial symptoms deserve similar attention with the more common symptoms. For example, there have been reports of patients with novel CoV pneumonia presenting with diarrhea as the initial symptom of disease onset.[14] Some patients presented with elevated troponin levels and myocarditis.[15] Others developed headache, myalgia, and other symptoms similar to those of influenza.[14] It should be emphasized that the author observed that some patients in the outpatient clinic even

had no obvious symptoms, or came to see the doctor only with "discomfort". Moreover, a previous study reported that one asymptomatic patient was diagnosed as 2019-nCoV;[16] therefore, the presence of asymptomatic carriers requires due attention, and relevant contacts should be tracked and isolated as soon as possible. In this study, routine peripheral blood tests showed either normal or decreased white blood cell counts and lymphocytopenia, as well as elevated C-reactive protein and erythrocyte sedimentation rates, all of which are generally consistent with previous reports of patients with novel CoV pneumonia.[3] In these patients, early-stage lung CT scans mostly showed multiple, small patch-like shadows and interstitial changes, which were more obvious in the extrapulmonary region. These shadows subsequently progressed to multiple ground-glass opacities in both lungs, along with infiltration shadows with a “large white lung” observed in more severe cases. The median interval from the onset of initial symptoms to dyspnea was 7 days. In some severe cases, the disease rapidly progressed to acute respiratory distress syndrome, septic shock, refractory metabolic acidosis, and coagulation disorder, eventually leading to death.

Based on present data, the mortality rate of 2019-nCoV is lower than that of SARS.[12,17] Moreover, there are some other notable epidemiological differences between the two outbreaks. For example, most of the critically ill SARS patients were young adults who required invasive ventilation for treatment, whereas most of the critically ill patients included in this study were middle-aged and elderly people. This age difference may have contributed to the poor prognosis in some cases. Thus, the proportion of young adult patients with SARS with moderate and severe disease was substantially higher than that of young adult patients with the novel CoV. However, this is not a reason to not take this new threat seriously. Although the mortality caused by the novel CoV is seemingly lower than that of SARS, its incubation period may be longer, producing a much larger number of potential asymptomatic carriers. This possibility imposes further pressure on securing a definitive diagnosis of the disease and containment of its transmission.

Based on the experience and lessons learned from the SARS and MERS outbreaks, the treatment site and protocol for confirmed cases of novel CoV pneumonia are decided by disease severity: patients with mild symptoms (i.e., coughing, low-grade fever, runny nose, and asymptomatic sore throat) are quarantined at home, whereas patients with moderate or severe disease are hospitalized for treatment. Our study cohort included only patients who were already critically ill. As there is currently no effective drug against the novel CoV, symptomatic treatment and respiratory support were provided. Since the large-dose glucocorticoids used in the treatment of SARS resulted in serious adverse reactions[18,19] but did not effectively decrease the mortality rate of CoV infection,[20-22] we treated patients with low-dose (30–80 mg/day) and short-term (3–5 days) methylpredisolone to alleviate the pulmonary exudates and inhibit a systemic cytokine storm. Unfortunately, this treatment did not provide significant benefits. As an alternative, intravenous injection with γ-immunoglobulin can be offered; however, more clinical data are required to determine the efficacy of

this treatment. In the middle and late stages of the disease, patients often develop additional bacterial or even fungal infections; therefore, careful attention must be paid to the rational use of antibiotics. In addition to the aforementioned treatments, respiratory support should be provided as early as possible. In this cohort, different types of oxygen therapy were administered according to each patient's pulse oximetry oxygen saturation and oxygenation index. For most patients, early non-invasive ventilation could promote positive outcomes. Alternatively, for critically ill patients, invasive ventilation or even extracorporeal membrane oxygenation (ECMO) should be considered. To date, ECMO has been successfully used to resuscitate one critically ill patient infected with this new CoV. Several drugs have already been tested against the novel CoV and relevant clinical observation studies have been initiated with encouraging preliminary results. In particular, research on anti-coronaviral drugs and vaccines should be a continuous priority. None of these currently tested treatments was used in our cohort of patients, and thus their efficacy remains unknown.

Our study has some limitations. For example, all of the patients included in this study were inpatients and our sample size was relatively small. We did not collect and analyze data from outpatients with mild symptoms or from suspected patients under home observation and awaiting a definitive diagnosis. This may result in some bias in our general understanding of the disease. In addition, since most of the patients in this study were admitted to the respiratory departments of some tertiary hospitals in Hubei province, data from the intensive care units and other departments are insufficient, which can similarly lead to a biased understanding of the disease. On this basis, a broader and larger study is necessary in the immediate future.

In conclusion, by analyzing 137 confirmed cases of infection with the 2019-nCoV in some tertiary hospitals in Hubei province, we preliminarily identified major clinical characteristics and corresponding treatment principles for the disease. However, there is still a large gap in our understanding of the origin, epidemiology, and persistence of human transmission of this disease. Therefore, continuous monitoring and tracing is required to secure an in-depth understanding of the disease, thereby providing an improved evidentiary basis for standardizing the diagnosis and treatment of 2019-nCoV.

### *Conflicts of interest*

None.

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**Table 1: Demographic and general characteristics of 2019- novel**

**coronavirus-infected patients included in the study (*N* = 137).**

| Items | *n* | Percentage, % |
|---|---|---|
| Age, years | 57 (20–83) | - |
| Gender | | |
| Male | 61 | 44.5 |
| Female | 76 | 55.5 |
| History of exposure to Huanan Seafood Wholesale Market | 0 | - |
| Comorbidity | 27 | 19.7 |
| Hypertension | 13 | 9.5 |
| Cardiovascular disease | 10 | 7.3 |
| Chronic obstructive pulmonary disease | 2 | 1.5 |
| Diabetes | 14 | 10.2 |
| Malignancy | 2 | 1.5 |
| Other chronic diseases | 24 | 17.5 |

**Table 2: Clinical manifestations and physical signs of 2019- novel coronavirus-infected patients (*N* = 137).**

| Items | *n* | Percentage, % |
|---|---|---|
| Initial symptom | | |
| Fever | 112 | 81.8 |
| <37.3°C | 28 | 25.0 |
| 37.3–38°C | 29 | 25.9 |
| 38.1–39°C | 35 | 31.3 |
| >39°C | 20 | 17.7 |
| Cough | 66 | 48.2 |
| Myalgia or fatigue | 44 | 32.1 |
| Expectoration | 6 | 4.4 |
| Hemoptysis | 7 | 5.1 |
| Headache | 13 | 9.5 |

| | | |
|---|---|---|
| Diarrhea | 11 | 8.0 |
| Heart palpitations | 10 | 7.3 |
| Dyspnea | 26 | 19.0 |
| Interval from disease onset to dyspnea or significant symptom aggravation, days, median (range) | 7 (1–20) | - |
| Comorbid organ dysfunction | 26 | 18.9 |
| Blood oxygen saturation, % | 94 (89–98) | - |

**Table 3: Laboratory test and imaging data for 2019- novel coronavirus-infected patients (*N*=137).**

| Items | *n* | Percentage, % |
|---|---|---|
| White blood cell count, $\times 10^9$/L | | |
| <4 | 51 | 37.2 |
| 4–10 | 60 | 43.8 |
| >10 | 26 | 19.0 |
| Lymphocyte count, $\times 10^9$/L | | |
| <1.0 | 99 | 72.3 |
| >1.0 | 38 | 27.7 |
| White blood cell count/lymphocyte count | 7.6 (2.2–26.8) | - |
| Elevated C-reactive protein | 115 | 83.9 |
| Positive etiology | 137 | 100 |
| Bilateral lung involvement in lung computed tomography scan | 116 | 84.7 |

| | | |
|---|---|---|
| Early stage (multiple patch-like shadows) | 36 | 31.0 |
| Middle stage (ground-glass opacity in both lungs) | 55 | 47.4 |
| Late stage (consolidation shadow) | 25 | 21.6 |

**Table 4: Treatment regimen and prognosis of 2019- novel coronavirus-infected patients (*N* = 137).**

| Items | *n* | Percentage, % |
|---|---|---|
| Treatment | | |
| Antiviral treatment | 105 | 76.6 |
| Antibacterial treatment | 119 | 86.9 |
| Systemic corticosteroid treatment | 40 | 29.2 |
| Human γ-immunoglobulin | 44 | 32.1 |
| Respiratory support | 119 | 86.9 |
| Nasal cannula | 85 | 62.0 |
| Non-invasive ventilation | 34 | 24.8 |
| Prognosis | | |
| Improved and discharged | 44 | 32.1 |
| Inpatient treatment | 77 | 56.2 |
| Death | 16 | 11.7 |

Figure lendgends:

**Figure 1:** Regional distribution of 2019-nCoV-infected patients included in the study.

**Figure 2:** Chest CT images of a 47-year-old female patient upon admission, who had symptoms of fever for 7 days and post-exertional shortness of breath for 2 days (A and B). Transverse chest CT images showed the bilateral multiple lobular and subsegmental areas of consolidation.

**Figure 3:** Lung computed tomography (CT) images of a 66-year-old male patient admitted for symptoms of high fever for 2 days and dyspnea for 1 day (A-C). After admission on January 8, 2020, 40 mg intravenously (iv) every day (qd) methylprednisolone was administered, but the subsequent CT review 6 days later showed significant disease progression (D-F).

## Original Article

# Distribution of the COVID-19 Epidemic and Correlation with Population Emigration from Wuhan, China

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**Abstract**

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**Background**

The ongoing new coronavirus pneumonia (Corona Virus Disease 2019,COVID-19) outbreak is spreading in China, but it has not yet reached its peak. Five million people emigrated from Wuhan before lockdown, potentially representing a source of virus infection. Determining case distribution and its correlation with population emigration from Wuhan in the early stage of the epidemic is of great importance for early warning and for the prevention of future outbreaks.

**Methods**

The official case report on the COVID-19 epidemic was collected as of January 30, 2020. Time and location information on COVID-19 cases was extracted and analyzed using ArcGIS and WinBUGS software. Data on population migration from Wuhan City and Hubei province were extracted from Baidu Qianxi, and their correlation with the number of cases was analyzed.

**Results**

The COVID-19 confirmed and death cases in Hubei province accounted for 59.91% (5806/9692) and 95.77% (204/213) of the total cases in China respectively. Hot spot provinces included Sichuan and Yunnan, which are adjacent to Hubei. The time risk of Hubei province on the following day was 1.960 times that on the previous day. The number of cases in some cities was relatively low, but the time risk appeared to be continuously rising. The correlation coefficient between the provincial number of cases and emigration from Wuhan was up to 0.943. The lockdown of 17 cities in Hubei province and the implementation of nationwide control measures efficiently prevented an exponential growth in the number of cases.

**Conclusions**

The population that emigrated from Wuhan was the main infection source in other cities and provinces. Some cities with a low number of cases showed a rapid increase in case load. Owing to the upcoming Spring Festival return wave, understanding the risk trends in different regions is crucial to ensure preparedness at both the individual and organization levels and to prevent new outbreaks.

**Keywords:** COVID-19; 2019-nCoV; Temporal; Spatial; Distribution; Outbreak

## Introduction

Emerging infectious diseases are a major challenge in the 21st century. In recent years, worldwide outbreaks of Ebola and Middle East Respiratory Syndrome (MERS) caused great health and economic losses.[1,2] The ongoing new coronavirus pneumonia (Corona Virus Disease 2019,COVID-19) outbreak is becoming a global public health problem. The COVID-19 outbreak is highly similar to the severe acute respiratory syndrome (SARS) outbreak that occurred in 2003; both outbreaks were caused by new coronaviruses during time periods overlapping with the Chinese Spring Festival.[3] On December 31, 2019, the Wuhan Municipal Health Committee reported 27 cases of pneumonia with an unknown cause, and many cases were traced to the Wuhan Southern China Seafood Market, which was subsequently closed on January 1, 2020.[4] On January 7, 2020, laboratory tests showed that the pathogen causing the previously unexplained pneumonia was a new type of coronavirus; this pneumonia was then officially named COVID-19 by the World Health Organization (WHO). [5, 6] The COVID-19 outbreak started in Wuhan and spread rapidly to other provinces and countries. [7, 8] As of January 30, 2020, a total of 34 provinces and regions in China had reported 9692 cases, and nearly all imported cases were derived from Wuhan in Hubei province.[9, 10]

COVID-19 has been defined as a class B infectious disease but has been managed as a class A infectious disease by the Chinese government. Daily case reports are being released, and any omission or concealment is punishable by law. Currently, the number of cases is still increasing, and the epidemic has not yet reached its peak; however, the situation differs from province to province. Information on the temporal and spatial distributions of cases is important for

developing targeted treatment and prevention strategies. Because the return peak of Spring Festival travel is approaching, information on the possible changes in the incidence of COVID-19 in different cities will help in better preparation for disease prevention and management. Therefore, in this study, we investigated the temporal and spatial distributions of the early COVID-19 epidemic to reveal the dynamic changes and trends in reported cases. These results will provide valuable information for disease prevention at both the individual and organization levels.

## Methods

### Collection of case data

All officially reported confirmed and suspected cases of COVID-19 and related deaths were collected from the official website of health departments or articles citing their reports. Case data were imported into Microsoft Excel (Microsoft Corporation, Redmond, USA) and analyzed.

### Temporal and spatial distribution and risk analysis

The national and Hubei province shapefiles were used for ArcGIS (Environmental Systems Research Institute, Redlands, USA) analysis. The map was linked to an Excel file containing time and location information. Location data were available for 34 provinces of China and 17 prefecture level cities of Hubei Province. The time span was from January 16 to January 30, 2020. The COVID-19 risk analysis was based on the Bayesian space–time model of the WinBUGS (Microsoft Corporation, Redmond, USA) software.[11, 12] The model was divided into three levels:

(1) Data model

The statistical data on low incidence were assumed to follow a Poisson distribution for the

parameters $n_i$ and $\mu_{it}$: $y_{it} \sim$ Poiss ($n_i\mu_{it}$), where the Hubei province $y_{it}$ was i (1, ..., 17) cities with t (1, ..., 15) days number of cases occurring during the day, and the nationwide $y_{it}$ was the number of cases occurring in t (1, ..., 15) days in i (1, ..., 34) provinces. We assumed that there was no change in the number of people at risk in each city during the study period, such that $n_i$ was the number of people at risk in the town (i), and $\mu_{it}$ was the corresponding disease risk in the city (t) per day (i).

(2) Process model

$\mu_{it}$'s logarithmic transformation of disease risk allows the relative risk to be expressed as a linear combination of spatial, temporal, and spatiotemporal interaction components. The mathematical expression is shown in equation (2).

$$\log(\mu_{it}) = \alpha + s_i + (b_{0t}^{*} + \upsilon_t) + b_{1i}t^{*} + \varepsilon_{it} \quad (2),$$

where α is the fixed effect of the overall relative risk in the entire study area within 11 days, and $t^{*}$ = t-5.5 is the time span relative to the intermediate time point. In this model, the risk of disease is broken down into three parts: spatial change, temporal change, and space–time interaction; $s_i$ is a component of spatial variability, describing the urban disease risk relative to the risk in the entire study region over an 11-day observation period; $b_{0t}^{*}+\upsilon_t$ is the change over time, which represents the overall trend of disease risk in the entire study area relative to that on the medium-term observation day, including the linear trend $b_{0t}^{*}$ and the time random effect $\upsilon_t$; $b_0$ is the time coefficient, representing the time trend in the study area; and $b_{1i}t^{*}$ allows each city to have different time-varying trends and is part of the spatiotemporal interaction. Relative to $b_0$, it represents the trend of local change in each city based on $b_0$; $\varepsilon_{it}$ is used to explain local changes

that cannot be explained by spatiotemporal random effects.[13]

(3) Parametric model

According to the Besag York and Molliè (BYM) model,[14] a spatial structure effect is defined by a prior conditional autoregressive (CAR) structure. In this process, a spatial adjacency weight matrix needs to be defined. If adjacent, the weight $w_{ij} = 1$; otherwise the weight $w_{ij} = 0$, and the special $w_{ij} = 0$. Similarly, $b_{1i}$ is also assumed to follow BYM characteristics. For the time structure effect $\upsilon_t$, a conditional autoregressive process is used, and the adjacency weight matrix in time is defined. For the over-discrete parameter $\varepsilon_{it}$, according to Gelman, the normal distribution with a mean value of 0 and a variance of $\sigma^2\varepsilon$, is generally assumed and the variance of each parameter obeys Gamma (a, b).[15] Based on this model, through the spatial component $s_i$ and its posterior probability, high- or low-risk cities (identified based on the average risk [α] in the entire study area) can be identified. By calculating the probability that spatial relative risk exp ($s_i$) is greater than 1, regions can be divided into five categories: those with probability >0.8, 0.6–0.8, 0.4–0.6, 0.2–0.4, and <0.2 are defined as hot spots, secondary hot spots, warm-spots, sub-cold spots, and cold-spots, respectively. Similarly, based on the probability threshold, the differences in these regions can be identified considering the trend over time. Further, based on the probability that exp ($b_{1i}$) is greater than 1, regions can be divided into five categories: cities with an incidence risk probability greater than 0.8 show a trend for a rapid change in risk relative to the overall change, and those with an incidence risk probability between 0.6 and 0.8 show a trend for a greater change in the incidence risk than the overall change. A value between 0.4 and 0.6 indicates that the change in the occurrence risk is the same as the overall risk change; 0.2 to 0.4, that the trend of

change in disease risk is lower than the overall risk change; and less than 0.2, that the trend of change in disease risk is much lower than the overall risk.

Statistical analysis

Population migration data were collected from the Baidu website (http://qianxi.baidu.com/). Data on emigration from Wuhan City and Hubei province to other cities and provinces were extracted and edited with Microsoft Excel for Windows (Microsoft Corporation, Redmond, USA) . Emigration intensity was calculated using the migration index multiplied by the migration proportion in the province or city. Correlation analysis was performed using IBM SPSS Statistics software (International Business Machines Corporation, Armonk, USA) (version 22). *P* values less than 0.05 were considered statistically significant. Pearson correlation coefficients greater than 0.2 were considered indicators of a positive correlation.

**Results**

To obtain a general profile of the case distribution, we first analyzed all the available cases during this COVID-19 outbreak.[16] As shown in Figure 1A, the number of cases remained stable from January 11 to January 15, 2020, and the number of new and cumulative cases increased rapidly after January 16. The first death was reported on January 10, and the number of deaths began to increase rapidly from January 17 onwards, with the cumulative number of deaths reaching 213 on January 30 [Figure 1B].[6]After the nucleic acid assay became available, suspected cases waiting for laboratory confirmation could be diagnosed rapidly.[17] After January 19, the number of suspected cases increased rapidly, and about 40–50% of these suspected cases were then confirmed [Figure 1C]. Before January 19, the number of severe cases remained low,

but they increased steadily from January 20 onwards [Figure 1D]. Because Wuhan is the capital city of Hubei province and the virus spread throughout the province quickly, we also analyzed the changes in number of cases in Hubei province. On January 9, 41 cases were first reported, and by January 30, 5806 cases had been reported, accounting for 59.91% (5806/9692) of the total cases in China [Figure 1E]. The cumulative number of deaths in Hubei province was 204, accounting for 95.77% (204/213) of the total deaths in China [Figure 1F]. These data indicated that both the incidence and mortality of COVID-19 disease were the highest in Hubei province.[18]

Before January 16, cases were mainly reported in Hubei province. From January 17 onward, the outbreak spread to many provinces and the number of cases increased rapidly. Therefore, our spatial and temporal analyses used data from January 17 to January 30, 2020. The location of each case was extracted from official reports and mapped onto the national map at the city level using ArcGIS. Of the 362 cities, 307 (84.8%,307/362) had reported cases. In general, the core outbreak area, Wuhan, and its surrounding cities had the highest number of cases, followed by cities with a high population that are transportation hubs. Spatial distribution was then analyzed with a Bayesian model using WinBUGS. After nearly 100,000 iterations, the model converged successfully. After the model converged, it was iterated another 110,000 times to obtain parameter estimations. Generally, a ratio close to 1 indicates that the two chain iterative sequences are close, and that the model has a good convergence and is stable [Figure 2A]. Using the established model and parameters, hot and cold spots were identified. The results showed that Sichuan, Yunnan, Guizhou, Hainan, and Taiwan were hot spots, and Inner Mongolia, Gansu, Ningxia, Qinghai, Xinjiang, Chongqing, Hunan, and Guangxi were secondary hot spots. Generally, hot spots

clustered in the midwest, and cold spots clustered in the southeast [Figure 2B].

The overall temporal trend was calculated using the time risk model ($\exp(b_{0t}*+v_t)$), which described the general incidence risk according to time between January 16 and January 30, 2020. Through the analysis, $b_0$ was estimated to be 0.4604, that is, the disease risk on the following day was found to be approximately 1.585 times higher than that on the previous day. The relative risk according to time increased steadily from January 20 onward and the upward trend continued as of January 30 [Figure 2C], indicating that the number of cases nationwide is on the rise. As shown in Figure 2D, Heilongjiang, Hebei, Beijing, Tianjin, Xinjiang, Ningxia, Jiangsu, Hunan, Taiwan, and Hainan showed a faster increase in the number of cases than was observed overall in the country. The increase in the number of cases in Jilin, Liaoning, Shaanxi, Guangxi, and Fujian provinces also occurred relatively fast [Supplymentary Table 1]. The increase in other provinces was consistent with or lower than the overall national trend [Figure 2D].

Since Hubei province had the highest number of cases, we analyzed the temporal and spatial distribution in different cities of Hubei province. Wuhan had the highest number of cases, followed by Huanggang and Xiaogan city. Suizhou, Jingmen, and Xianning were part of the second group with a high number of cases. The spatial convergence analysis had 100,000 iterations [Figure 3A]. Hot spots were identified in the east regions and cold spots were identified in the west regions [Figure 3B]. The overall temporal trend in the change in the number of cases was calculated using the model. The average time trend coefficient $b_0$ was estimated to be 0.6727, indicating the time risk (occurrence probability in time) on the following day was 1.960 times higher than that on the previous day, suggesting that the daily number of cases in Hubei Province

is on the rise [Figure 3C]. Xiangyang, Suizhou, Yichang, and Ezhou showed the highest increase rates, and Shiyan, Shenlongjia, Xiaogan and Huangshi showed relatively high increase rates [Figure 3D]. Other cities had a growth slower than the overall growth in the province [Supplymentary Table 2]. The increase rate in Hubei province (1.960) was higher than that in the whole country (1.585), indicating that the rate of increase in Hubei province was significantly higher than that in other provinces in China.

The outbreak started from Wuhan, and nearly all early cases were derived from this city, which is located in Hubei province. Because the outbreak occurred just before the Spring Festival, large-scale population migration during this period influenced the subsequent epidemic. From January 1 to January 23, 2020, the population that migrated out of Wuhan city and Hubei province increased steadily, peaking on January 21 and 22 [Figure 4A]. Wuhan city was under lockdown on Jan 23, and after that, population migration was greatly inhibited. As observed in 2019, high population migration occurred on January 31; the timely city lockdown prevented a subsequent outbreak burst. We analyzed the migration into and out of Wuhan city and Hubei province. The top targets for emigration included Henan and Hunan provinces [Figure 4B]. More people migrated out of Wuhan than into the city [Figure 4C]. To analyze the correlation between the number of cases and the emigration in Wuhan city and Hubei province, population migration data were collected from Baidu Qianxi. The correlation coefficient between the provincial number of cases and emigration from Hubei province was 0.719 [Figure 4D]. The correlation coefficient between the provincial number of cases and emigration from Wuhan increased to 0.943, with the highest coefficient of 0.996 observed between Wuhan and other cities of Hubei provinces [Figure

4E and 4F; Supplementary Tables 3 and 4]. These data strongly indicated that the number of cases was highly related to population emigration from Wuhan. Although we do not know the exact number of people emigrating from Wuhan, five million is an astonishing number, considering that each individual may be a potential virus carrier. If no control measures were implemented, the number of cases would exponentially increase. Of the five million emigrants, 74.22% emigrated to other cities of Hubei province [Supplymentary Table 3]. Fortunately, 17 cities of Hubei province were under lockdown from January 23 to 26 [Supplymentary Table 5]. After the lockdown of Wuhan and other cities of Hubei province, outbreak bursts were prevented, and the number of cases increased steadily but did not show exponential growth.

Because the outbreak duration overlaps with the Spring Festival transport waves, large-scale migration will be a strong determinant of the characteristics of this outbreak. We analyzed the migration in the three days before the Spring Festival. The top 50 cities from where emigration occurred before the Spring Festival were mainly located in the south and east of China, with Beijing, Shenzhen, Shanghai, and Guangzhou showing the highest emigration, accounting for over 15% of the migration population [Supplymentary Figure 1]. However, cities with high immigration were relatively scattered. Chongqing experienced the highest immigration, accounting for 1.50% of the total number of immigrants [Figure 4]. As immigrants will be traveling back to work after the Spring Festival, the cities showing high “emigration” may be at a high risk of another wave of new cases owing to the return of the migrants.

**Discussion**

COVID-19 is causing great public health and economic losses in China. The number of cases

has increased rapidly, with over 70% coming from Hubei province[16, 19]. As of January 30, the number of cases has exceeded the total number of cases of the SARS-CoV outbreak.[20] Until February 15, 2020, the cumulative number of confirmed cases was 70533, nearly 10 times that noted during the SARS outbreak. Prevention and control of the outbreak has required concerted action from the whole population of China. Although all individuals have participated in the campaign against the outbreak, people in areas with a low number of cases assumed that they were safe from the disease. Therefore, awareness of high-risk regions is important for preparing individuals, particularly in regions with low incidence. Further, it must be noted that five million persons emigrated from Wuhan to all over the country[21]. We do not know exactly how many of them are virus carriers, and it is impossible to track and diagnose them all. Evidence from previous cases showed that asymptomatic patients in the incubation period are also infectious, making it a greater challenge to track virus carriers. Therefore, isolation at home and less contact with others is the most efficient measure to prevent infection and transmission. To reduce transmission, the Spring Festival holiday has been extended from January 31 to February 2. The opening time for all schools and universities has been delayed ,and online teaching programs have been launched.. Factories have been required to delay resumption or allow work from home.

We analyzed the temporal and spatial distribution of reported cases. In general, the number of cases is still on the rise. For Hubei Province, which has the highest number of cases and deaths, the growth trend is relatively stable. Conversely, in other hot spots, the number of cases was not very high, but the growth continued. Hence, these areas should be closely monitored[22]. It is particularly noteworthy that the cities with the fastest change in temporal risk, such as Chongqing,

have large population movements and rapid temporal risk. If they are not strictly monitored, there may be more outbreaks. In order to prevent disease outbreaks caused by the return travel wave after the Spring Festival, the country has extended the Spring Festival holiday.

Correlation analysis showed that early incidence was closely related to the emigration waves from Wuhan, that is, the higher the migrating population index, the larger was the number of cases. This also proved that the first generation of cases in each province mainly came from Wuhan. However, with the progress of the epidemic, migrants are spreading the virus to other people and are becoming an important source of local community transmission. Therefore, it is necessary to strictly implement isolation and related control measures in accordance with the guidelines. Particularly, control measures must be taken to prevent the spread of diseases in communities, which is crucial to prevent a large-scale outbreak.

Very soon, many company staff will return to their workplaces. Because many enterprises in China are labor-intensive, with large populations, human-to-human transmission is extremely easy. Therefore, workers need to meet requirements for isolation after returning to the city and use personal protection at work to prevent clustered outbreaks. At present, there have been several reports of employee infections caused by resumption of work; these represent a warning for all enterprises. Super megacities such as Guangzhou, Shenzhen, and Shanghai, which have the largest number of migrant workers, need to be prepared for this.

From February 16, the number of new cases began to decrease, but the epidemic did not stop completely. Therefore, we must act together to stop the spread of the disease. At present, the state has adopted mobility control measures to encourage people to avoid going to public places and

wear masks when going out to reduce the risk of human-to-human transmission. We believe that with the joint efforts made by everyone, the number of cases and losses will be kept to a minimum.

**Acknowledgement**

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**Conflicts of Interest**

None

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## Figure legends

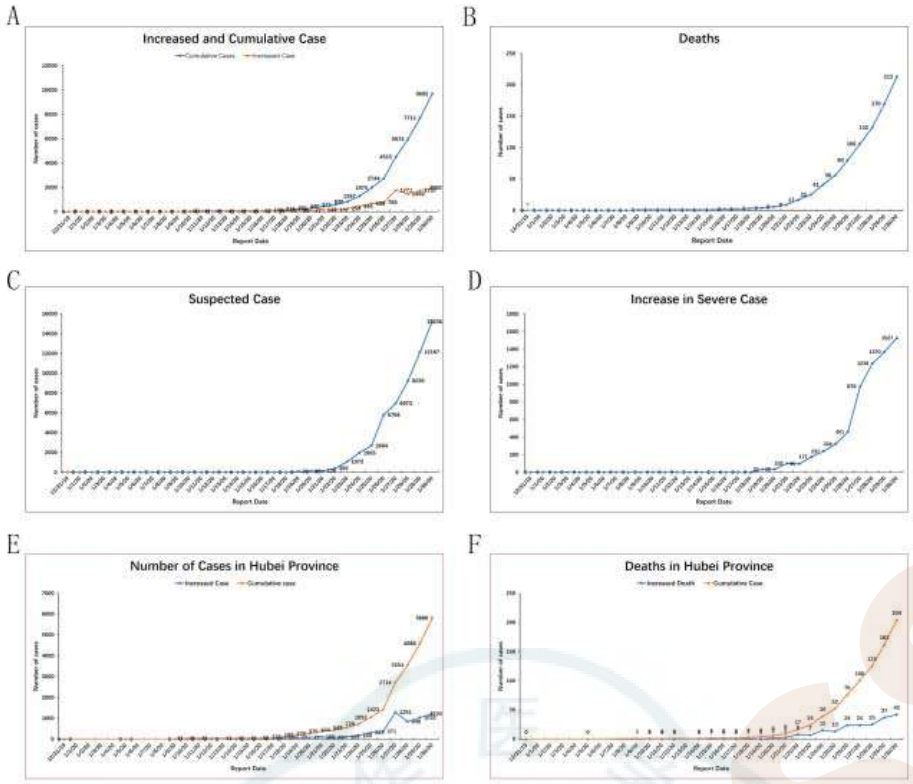


Figure 1 Daily changes of Corona Virus Disease 2019,cases in China

A, Number of the increased and cumulative cases, B, number of death case, C, suspected cases , and D,increase in severe cases. E, Number of the increased and cumulative cases in Hubei Province; F, Number of the increased and cumulative death case in Hubei province.

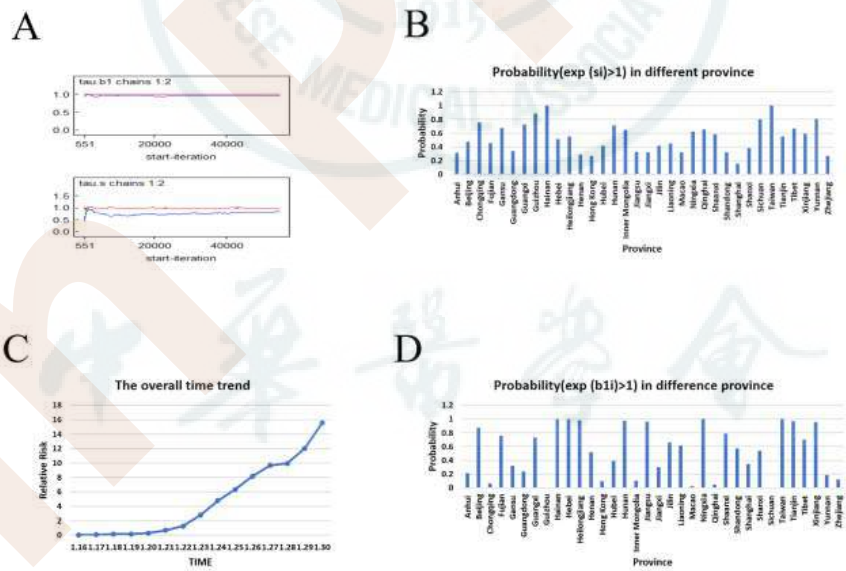


Figure 2 Nationwide distribution of Corona Virus Disease 2019 cases and change in trends across Provinces in China

A, Model convergence analysis; B, Hot spots and cold spots of case distribution; C, Overall trendline of relative risk with time; D, Time risk probability of different provinces.

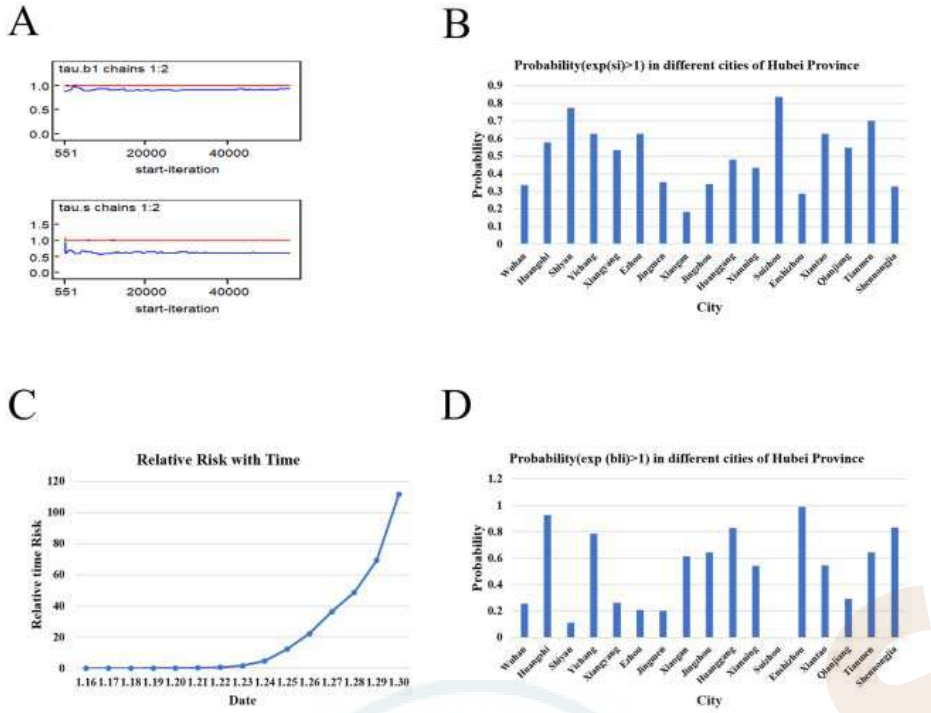


Figure 3 Distribution of Corona Virus Disease 2019, cases and change in trends of cities of Hubei Province

A, Model convergence analysis of case distribution; B, Hot spots and cold spots of case distribution; C, Trendline of relative risk with time; D, Time risk probability of different cities of Hubei Province.

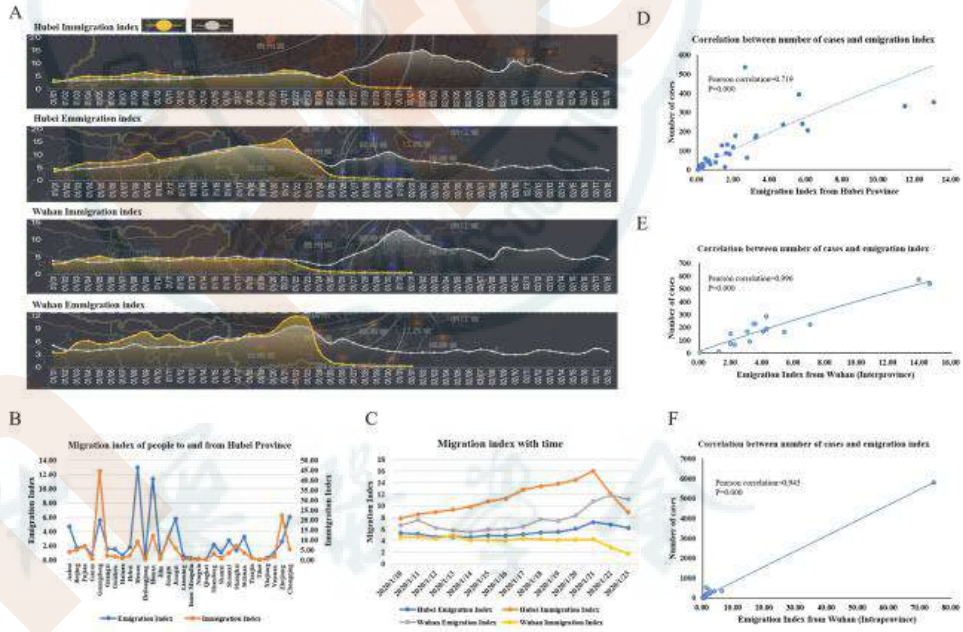


Figure 4 Correlation between migration index and the number of cases

A, Migration index indicating the movement of people to and from Wuhan city and Hubei Province during spring festival. (Yellow, 2020; Gray, 2019). B, Emigration and immigration index of people to and from Hubei Province; C, Emigration and immigration index of people to and from Wuhan City and Hubei Province from Jan 10 to 23, 2020; D, Correlation between the

number of cases and emigration index of people from Hubei Province; E, Correlation between the number of cases and emigration index of people from Wuhan city (interprovince migration); F, Correlation between the number of cases and emigration index of people from Wuhan city (intraprovince migration).

A

| Rank | Name | Percent |
|---|---|---|
| 1 | Beijing | 4.10% |
| 2 | Shenzhen | 3.77% |
| 3 | Shanghai | 3.66% |
| 4 | Chengdu | 3.58% |
| 5 | Guangzhou | 3.38% |
| 6 | Suzhou | 2.57% |
| 7 | Dongguan | 2.23% |
| 8 | Zhengzhou | 2.13% |
| 9 | Wuhan | 2.03% |
| 10 | Xi'an | 1.92% |

B

| Rank | Name | Percent |
|---|---|---|
| 1 | Chongqing | 1.50% |
| 2 | Chengdu | 1.17% |
| 3 | Beijing | 1.01% |
| 4 | Guangzhou | 1.00% |
| 5 | Zhoukou | 0.94% |
| 6 | Xi'an | 0.85% |
| 7 | Fuyang | 0.79% |
| 8 | Maoming | 0.78% |
| 9 | Yancheng | 0.78% |
| 10 | Xuzhou | 0.78% |

C

| Rank | Name | Percent |
|---|---|---|
| 1 | Beijing | 4.33% |
| 2 | Chengdu | 4.10% |
| 3 | Shanghai | 3.55% |
| 4 | Shenzhen | 3.22% |
| 5 | Guangzhou | 3.12% |
| 6 | Suzhou | 2.32% |
| 7 | Xi'an | 2.26% |
| 8 | Zhengzhou | 2.22% |
| 9 | Wuhan | 2.01% |
| 10 | Changsha | 1.94% |

D

| Rank | Name | Percent |
|---|---|---|
| 1 | Chongqing | 1.43% |
| 2 | Chengdu | 1.16% |
| 3 | Zhoukou | 0.93% |
| 4 | Guangzhou | 0.88% |
| 5 | Beijing | 0.87% |
| 6 | Yancheng | 0.83% |
| 7 | Fuyang | 0.77% |
| 8 | Xi'an | 0.77% |
| 9 | Nanchong | 0.75% |
| 10 | Xuzhou | 0.74% |

E

| Rank | Name | Percent |
|---|---|---|
| 1 | Chengdu | 4.17% |
| 2 | Beijing | 4.15% |
| 3 | Shanghai | 3.79% |
| 4 | Guangzhou | 2.96% |
| 5 | Shenzhen | 2.94% |
| 6 | Xi'an | 2.46% |
| 7 | Zhengzhou | 2.22% |
| 8 | Suzhou | 2.20% |
| 9 | Nanjing | 1.83% |
| 10 | Chongqing | 1.70% |

F

| Rank | Name | Percent |
|---|---|---|
| 1 | Chongqing | 1.50% |
| 2 | Chengdu | 1.15% |
| 3 | Zhoukou | 0.99% |
| 4 | Yancheng | 0.92% |
| 5 | Guangzhou | 0.83% |
| 6 | Beijing | 0.82% |
| 7 | Xianyang | 0.80% |
| 8 | Nanchong | 0.75% |
| 9 | Shangqiu | 0.74% |
| 10 | Fuyang | 0.74% |

Figure S1 Top cities with the highest migrants before Spring Festival

A-B, Top cities with highest migrant on Jan 22, 2020; C-D, Top cities with highest migrant on Jan 23, 2020; E-F, Top cities with highest migrant on Jan 24, 2020.;A,C,E, Top 10 cities with high export migrant; B,D,F,Top 10 cities with high import migrants.

**Supplementary Table**

Table 1 Analysis results of WinBUGS software in different provinces of China

| Province | Probability(exp ($s_i$)>1) | Probability(exp ($b_{1i}$)>1) |
|---|---|---|
| Beijing | 0.4769 | 0.8696 |
| Shanxi | 0.385 | 0.5388 |
| Liaoning | 0.4512 | 0.6182 |
| Jilin | 0.4155 | 0.664 |
| Shanghai | 0.154 | 0.3449 |
| Tianjin | 0.556 | 0.9665 |
| Hebei | 0.5157 | 0.993 |
| Heilongjiang | 0.5525 | 0.9842 |
| Jiangsu | 0.3301 | 0.9655 |
| Guizhou | 0.8839 | 5.05E-04 |
| Hunan | 0.7131 | 0.9753 |
| Ningxia | 0.6267 | 0.9979 |
| Hainan | 1 | 1 |
| Shaanxi | 0.5823 | 0.7892 |
| Guangdong | 0.3432 | 0.2368 |
| Guangxi | 0.7244 | 0.7338 |
| Gansu | 0.6757 | 0.3203 |

| | | |
|---|---|---|
| Inner Mongolia | 0.6481 | 0.0999 |
| Qinghai | 0.6557 | 0.04762 |
| Jiangxi | 0.323 | 0.3025 |
| Anhui | 0.3144 | 0.2089 |
| Zhejiang | 0.2715 | 0.1229 |
| Henan | 0.2924 | 0.5172 |
| Shandong | 0.3202 | 0.5701 |
| Tibet | 0.6693 | 0.7019 |
| Hubei | 0.4193 | 0.3886 |
| Yunnan | 0.8116 | 0.184 |
| Xinjiang | 0.5939 | 0.9551 |
| Chongqing | 0.7554 | 0.06471 |
| Sichuan | 0.8052 | 0.005132 |
| Hong Kong | 0.2732 | 0.09537 |
| Macao | 0.3195 | 0.02594 |
| Taiwan | 1 | 1 |
| Fujian | 0.4593 | 0.7565 |

Data of Table 1 came from the operation results of the WinBUGS software.

Table 2 Analysis results of WinBUGS software in different cities of Hubei Province

| City | Probability(exp ($s_i$)>1) | Probability(exp ($b_{1i}$)>1) |
|---|---|---|
| Wuhan | 0.3363 | 0.2563 |
| Huangshi | 0.5783 | 0.9291 |
| Shiyan | 0.7753 | 0.1089 |
| Yichang | 0.626 | 0.7855 |
| Xiangyang | 0.5343 | 0.2616 |
| Ezhou | 0.6285 | 0.2083 |
| Jingmen | 0.352 | 0.2025 |
| Xiaogan | 0.184 | 0.6151 |
| Jingzhou | 0.3427 | 0.644 |
| Huanggang | 0.4802 | 0.8308 |
| Xianning | 0.435 | 0.5423 |
| Suizhou | 0.836 | 1.36E-05 |
| Enshizhou | 0.288 | 0.9887 |
| Xiantao | 0.628 | 0.5455 |
| Qianjiang | 0.5476 | 0.2918 |
| Tianmen | 0.7013 | 0.6441 |
| Shennongjia | 0.3286 | 0.8336 |

Data of Table 2 came from the operation results of the WinBUGS software.

Table 3 Emigration index and number of confirmed cases in different cities of Hubei Province

| City | Emigration index | Confirmed,*n* |
|---|---|---|
| Xiaogan | 14.63 | 541 |
| Huanggang | 13.92 | 573 |
| Jingzhou | 7.03 | 221 |
| Xianning | 5.38 | 166 |
| Ezhou | 4.25 | 189 |
| Xiangyang | 4.24 | 286 |
| Yellowstone | 4.05 | 168 |
| Jingmen | 3.53 | 227 |
| Suizhou | 3.42 | 228 |
| Xiantao | 3.17 | 90 |
| Yichang | 3.03 | 167 |
| Tianmen | 2.24 | 67 |
| Shiyan | 1.99 | 150 |
| Enshi | 1.95 | 75 |
| Qianjiang | 1.23 | 12 |
| Shennongjia | 0.04 | 7 |

Data of Table 3 came from Baidu Qianxi and the official website of health departments.

Table 4 Emigration index and number of confirmed cases in different provinces of China

| Province | Emigration index | Confirmed, *n* |
|---|---|---|
| Tibet | 0.04 | 1 |
| Qinghai | 0.06 | 8 |
| Ningxia | 0.09 | 21 |
| Tianjin | 0.16 | 31 |
| Jilin | 0.18 | 14 |
| Inner Mongolia | 0.19 | 20 |
| Xinjiang | 0.20 | 17 |
| Heilongjiang | 0.30 | 59 |
| Liaoning | 0.35 | 45 |
| Gansu | 0.37 | 29 |
| Hainan | 0.40 | 49 |
| Yunnan | 0.57 | 76 |
| Guizhou | 0.60 | 15 |
| Shanxi | 0.64 | 39 |
| Shanghai | 0.72 | 128 |
| Shaanxi | 0.77 | 63 |
| Guangxi | 0.85 | 87 |
| Beijing | 0.94 | 132 |
| Fujian | 0.97 | 120 |

| | | |
|---|---|---|
| Hebei | 1.01 | 82 |
| Zhejiang | 1.15 | 537 |
| Shandong | 1.18 | 178 |
| Sichuan | 1.33 | 177 |
| Chongqing | 1.36 | 206 |
| Jiangsu | 1.57 | 168 |
| Guangdong | 2.04 | 393 |
| Jiangxi | 2.28 | 240 |
| Anhui | 2.44 | 237 |
| Hunan | 3.74 | 332 |
| Henan | 6.11 | 352 |
| Hubei | 74.22 | 5806 |

Data of Table 4 came from Baidu Qianxi and the official website of health departments.

Table 5 Lockdown time of different cities in Hubei Province

| City | Lock down date |
|---|---|
| Wuhan | 23-Jan-20 |
| Ezhou | 23-Jan-20 |
| Xiantao | 23-Jan-20 |
| Zhijiang | 23-Jan-20 |
| Qianjiang | 23-Jan-20 |
| Huanggang | 23-Jan-20 |
| Enshi | 24-Jan-20 |
| Jingmen | 24-Jan-20 |
| Xianning | 24-Jan-20 |
| Chibi | 24-Jan-20 |
| Yellowstone | 24-Jan-20 |
| Jingzhou | 24-Jan-20 |
| Dangyang | 24-Jan-20 |
| Xiaogan | 24-Jan-20 |
| Suizhou | 24-Jan-20 |
| Shiyan | 25-Jan-20 |
| Yichang City | 25-Jan-20 |
| Xiangyang | 26-Jan-20 |
| Shennongjia | 26-Jan-20 |

Original Article

# Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease

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## Abstract

**Background:** Since early December 2019, the 2019 novel coronavirus disease (COVID-19) has caused pneumonia epidemic in Wuhan, Hubei province of China. This study aims to investigate the factors affecting the progression of pneumonia in COVID-19 patients. Associated results will be used to evaluate the prognosis and to find the optimal treatment regimens for COVID-19 pneumonia.

**Methods:** Patients tested positive for the COVID-19 based on nucleic acid detection

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were included in this study. Patients were admitted to three tertiary hospitals in Wuhan between December 30, 2019, and January 15, 2020. Individual data, laboratory indices, imaging characteristics, and clinical data were collected, and statistical analysis was performed. Based on clinical typing results, the patients were divided into a progression group or an improvement/stabilization group. Continuous variables were analyzed using independent samples *t*-test or Mann-Whitney *U* test. Categorical variables were analyzed using chi-squared test or Fisher's exact test. Logistic regression analysis was performed to explore the risk factors for disease progression.

**Results:** Seventy-eight patients with COVID-19-induced pneumonia met the inclusion criteria and were included in this study. Efficacy evaluation at 2 weeks after hospitalization indicated that 11 patients (14.1%) had deteriorated, and 67 patients (85.9%) had improved/stabilized. The patients in the progression group were significantly older than those in the disease improvement/stabilization group (66 [51, 70] years *vs.* 37 [32, 41] years, $U$ =4.932, $P$ =0.001). The progression group had a significantly higher proportion of patients with a history of smoking than the improvement/stabilization group (27.3% *vs.* 3.0%, $\chi^2$=9.291, $P$ =0.018). For all the 78 patients, fever was the most common initial symptom, and the maximum body temperature at admission was significantly higher in the progression group than in the improvement/stabilization group (38.2 [37.8, 38.6]℃ *vs.* 37.5 [37.0, 38.4]℃, $U$=2.057, $P$ =0.027). Moreover, the proportion of patients with respiratory failure (54.5% *vs.* 20.9%, $\chi^2$=5.611, $P$=0.028) and respiratory rate (34 [18, 48] breaths/min *vs.* 24 [16, 60] breaths/min, $U$=4.030, $P$=0.004) were significantly higher in the progression group than in the improvement/stabilization group. C-reactive protein was significantly elevated in the progression group compared to the improvement/stabilization group (38.9 [14.3, 64.8] mg/L *vs.* 10.6 [1.9, 33.1] mg/L, $U$=1.315, $P$=0.024). Albumin was significantly lower in the progression group than in the improvement/stabilization group (36.62±6.60 g/L *vs.* 41.27±4.55 g/L, $U$ =2.843, $P$ =0.006). Patients in the progression group were more likely to receive high-level respiratory support than in the improvement/stabilization group ($\chi^2$=16.01, $P$=0.001). Multivariate logistic analysis indicated that age (OR, 8.546; 95% CI: 1.628–44.864; $P$ = 0.011), history of smoking

(OR, 14.285; 95% CI: 1.577–25.000; $P$ = 0.018), maximum body temperature at admission (OR, 8.999; 95% CI: 1.036-78.147, $P$ = 0.046), respiratory failure (OR, 8.772, 95% CI: 1.942–40.000; $P$ = 0.016), albumin (OR, 7.353, 95% CI: 1.098–50.000; $P$ = 0.003) and C-reactive protein (OR, 10.530; 95% CI: 1.224–34.701, $P$ = 0.028) were risk factors for disease progression.

**Conclusions:** Several factors that led to the progression of COVID-19 pneumonia were identified, including age, history of smoking, maximum body temperature on admission, respiratory failure, albumin, C-reactive protein. These results can be used to further enhance the ability of management of COVID-19 pneumonia.

**Keywords:** 2019 novel coronavirus disease (COVID-19); Disease outcome; Predictors

## Introduction

Since December 2019, unexplained pneumonia has been successively identified in several patients with a history of exposure to the Huanan seafood market, in multiple hospitals in the city of Wuhan, Hubei Province, China. These patients have now been confirmed as acute respiratory infection (i.e., pneumonia) caused by a novel coronavirus.[1,2] Clinical investigation of confirmed cases and cases under observation has shown that the number of patients with no history of exposure to the Huanan Seafood Market has been rapidly increasing. As of February 1, 2020, there were 14,380 confirmed cases of 2019 novel coronavirus disease (COVID-19) in China.[3]

A previous study found that highest temperature, dyspnea, respiratory rate, white blood cell count, neutrophil count, lymphocyte count, D-dimer, albumin, procalcitonin were risk factors for ICU care in patients with COVID-19.[4] Therefore, it is absolutely necessary to evaluate the possible factors affecting the progression of disease in COVID-19 patients. We investigated factors affecting the outcomes of COVID-19 patients to evaluate the prognosis and further improve the treatment of patients with COVID-19 associated pneumonia with the hope of reducing mortality.

## Methods

***Ethical approval***

This study was conducted in accordance with the *Declaration of Helsinki*. The requirement for written informed consent was waived given the context of emerging infectious diseases.

***Subjects***

Patients included in the study had been diagnosed with COVID-19 associated pneumonia between December 30, 2019, and January 15, 2020, and hospitalized at one of three tertiary hospitals in Wuhan for over two weeks. Specific inclusion criteria were: (1) patients with confirmed diagnosis from a positive test result for COVID-19 nucleic acids by real-time fluorescence reverse transcription-polymerase chain reaction (RT-PCR) according to the "Diagnosis and Treatment Protocol for Novel Coronavirus Infection-Induced Pneumonia version 4 (trial)"[5]; (2) patients who had been hospitalized for over two weeks when preparing the manuscript, died while hospitalized, or had recovered and been discharged.

***Evaluation of conditions***

All patients were evaluated and clinically typed upon admission, according to the "Diagnosis and Treatment Protocol for Novel Coronavirus Infection-Induced Pneumonia version 4 (trial)".[5] Specific clinical types included: (1) common: fever, respiratory tract infection symptoms, and so on, with imaging indicating pneumonia; (2) severe (any of the following conditions): I, respiratory distress, respiratory rate (RR) ≥30 breaths/min; II, oxygen saturation ≤93% at rest; III, partial pressure of oxygen ($PaO_2$)/fraction of inspired oxygen ($FiO_2$) ≤300 mmHg (1 mmHg = 0.133 kPa); (3) critical (any of the following conditions): I, respiratory failure and a requirement for mechanical ventilation; II, shock; III, concomitant failure of other organs and requirement for intensive care unit (ICU) monitoring and treatment.

In addition to clinical typing, laboratory indices of all patients were measured, the details of which are listed in the biochemical test section.

After two weeks of hospitalization, disease evaluation and clinical typing were performed on all patients according to the "Diagnosis and Treatment Protocol for Novel Coronavirus Infection-Induced Pneumonia version 4 (trial)".[5] All patients were di-

vided into a progression group or an improvement/stabilization group based on clinical typing results. Specific criteria were as follows: (1) progression group: common-type changed to severe- or critical-type, or death; severe-type changed to critical-type or death; critical-type progressed to death. (2) improvement/stabilization group: common-, severe-, and critical-types remained unchanged; severe-type changed to common-type; critical-type changed to severe- or common-type.

***Specimen collection, etiology, and biochemical tests***

COVID-19 was detected by real-time fluorescence RT-PCR of samples collected by using nasopharyngeal swabs. Influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus, parainfluenza virus, chlamydia, and mycoplasma were detected by collecting body fluid (nasopharyngeal swabs and sputum) samples. Relevant laboratory indicators were tested with conventional methods, including routine blood tests (white blood cell [WBC], lymphocytes, neutrophils, platelets), liver and kidney function (alanine transaminase [ALT], aspartate aminotransferase [AST], creatinine and albumin), infection indices (procalcitonin and C-reactive protein), D-dimer, and partial pressure of oxygen.

***Data collection***

The personal data and clinical data of patients included in the study were collected. Personal data included sex, age, epidemiological history, history of smoking, and comorbidities [e.g., chronic obstructive pulmonary disease (COPD), cancer, hypertension and/or diabetes]. Clinical data included initial symptoms, clinical presentation, vital signs, therapeutic drug-use, respiratory support, and disease outcomes.

***Statistical analysis***

Categorical variables were presented as numbers (percentages) and analyzed using chi-squared test or Fisher's exact test. Continuous variables with normal distribution were expressed as mean±standard deviation and analyzed using independent samples *t*-test, while those with skewed distribution were shown as median ($Q_1$, $Q_3$) and analyzed using Mann-Whitney *U* test. Univariate and multivariate logistic regression analysis were adopted to identify risk factors of disease progression. All variables from the univariate analysis with a *P* value <0.1 were entered into a forward-stepwise

multivariate logistic regression analysis. SPSS software version 25.0 (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. A two-sided $P < 0.05$ was considered statistically significant.

## Results

### *General characteristics and clinical presentations*

In the present study, 78 patients with COVID-19 associated pneumonia included 39 males and 39 females. No patient had a history of exposure to the Huanan Seafood Market. The median age ($Q_1$, $Q_3$) was 38 (33, 57) years, and only 15 patients were aged ≥60 years (19.2%). Among the 78 patients, there were 70 patients with the common-type (89.7%) and 8 patients with the severe-type (10.3%). Re-examination after two weeks of hospitalization showed that among the 70 patients with the common-type symptoms, there were 8 patients with progression and 62 patients with improvement/stabilization. Among the 8 patients with the severe-type symptoms, 3 patients showed progression (including 2 deaths) and 5 showed improvement/stabilization. A total of 11 patients (14.1%) were included in the progression group, and 67 patients (85.9%) were included in the improvement/stabilization group. The patients in the progression group were significantly older than those in the improvement/stabilization group (66 [51, 70] *vs*. 37 [32, 41], $U$ =4.932, $P$ =0.001). This study suggested that the progression group had a significantly higher proportion of patients with a history of smoking than the improvement/stabilization group (27.3% *vs*. 3.0%, $\chi^2$=9.291, $P$ =0.018). Twenty patients (25.6%) had comorbidities, of which hypertension was the most common. There was no significant difference in sex between the two groups ($P > 0.05$). There was no significant difference in any comorbidity including hypertension, diabetes, chronic obstructive pulmonary disease (COPD), cancer, and others between the two groups (all $P > 0.05$). Fever was the primary initial symptom. Fifty-seven patients (73.1%) sought treatment for fever, and 37.3°C–38.0°C was the most commonly observed maximum body temperature in 31 patients (39.7%). The maximum body temperature at admission was significantly higher in the progression group than in the improvement/stabilization group (38.2

[37.8, 38.6]℃ *vs*. 37.5 [37.0, 38.4]℃, $U$=2.057, $P$=0.027). A total of 20 of the 78 patients (25.6%) developed respiratory failure, with the proportion of respiratory failure being significantly higher in the progression group than in the improvement/stabilization group (54.5% *vs*. 20.9%, $\chi^2$=5.611, $P$ =0.028). The median respiratory rate of the 78 patients with COVID-19 was 24 breaths/min, and the respiratory rate in the progression group was significantly higher than in the improvement/stabilization group (34 [18, 48] breaths/min *vs*. 24 [16, 60] breaths/min, $U$=4.030, $P$=0.004). There were no significant differences in blood oxygen saturation, or heart rate between the two groups (both $P > 0.05$). Eight (10.3%) of the 78 patients with COVID-19 were severely ill, and the proportions of severely ill patients were not significantly different between the two groups (27.3% *vs*. 7.5%, $\chi^2$=13.480, $P > 0.05$) [Table 1].

*Laboratory indices and imaging characteristics*

In this study, relevant laboratory indices of the 78 patients with COVID-19 were determined at the time of admission. These results showed that C-reactive protein was significantly elevated in the progression group compared to the improvement/stabilization group (38.9 [14.3, 64.8] mg/L *vs*. 10.6 [1.9, 33.1] mg/L, $U$ =1.315, $P$ =0.024). Albumin was significantly decreased in the progression group compared to the improvement/stabilization group (36.62±6.60 g/L *vs*. 41.27±4.55 g/L, $U$=2.843, $P$ =0.006). There were no significant differences in D-dimer, WBC, lymphocytes, neutrophils, platelets, ALT, AST, creatinine, erythrocyte sedimentation rate, procalcitonin, partial pressure of oxygen, and extent and characteristics of lesions on CT scan between the two groups (all $P > 0.05$) [Table 2]. No patients had other concomitant microbial infection.

*Treatment*

Among the 78 hospitalized patients, the most common treatment was a combination of antivirals/antibacterials with glucocorticoids (45/78, 57.7%). The most commonly used antiviral drug was ribavirin, and the most commonly used antibacterial drugs

were cephalosporins or quinolone antibiotics. The median glucocorticoids dose was 40 (20, 40) mg intravenously (iv) every day (qd) and there was no significant difference between the two groups (60 [40, 80] mg qd iv *vs*. 40 [20, 40] mg qd iv, $U$=4.713, $P$=0.075). The proportions of patients using different drug protocols including antivirals/antibacterials, antivirals/antibacterials + glucocorticoids, antivirals/antibacterials + gamma globulin, antivirals/antibacterial + thymosins, and antivirals/antibacterials + lopinavir between improvement/stabilization group and progression group were not significantly different ($P$>0.05). All hospitalized patients had some degree of hypoxia. Nasal cannula was the most common form of respiratory support (71/78, 91.0%), followed by continuous noninvasive positive pressure ventilation. The progression group typically had more severe hypoxia and was significantly more likely to receive higher levels of respiratory support compared to the improvement/stabilization group ($\chi^2$=16.01, $P$ =0.001) [Table 3].

*Risk factors for disease progression in COVID-19 patients*

The results of univariate logistic analysis found that age (odds ratio [OR], 10.575; 95% confidence interval [CI]: 2.095–53.386; $P$ = 0.004), history of smoking (OR, 12.187; 95% CI: 1.762–84.306; $P$ = 0.011), maximum body temperature at admission (OR, 9.709; 95% CI: 1.176–83.330; $P$ = 0.035), respiratory failure (OR, 8.021; 95% CI: 2.022–31.821; $P$ = 0.003), severe illness (OR, 4.651; 95% CI: 0.930–23.250; $P$ = 0.061), albumin (OR, 12.536; 95% CI: 2.409–65.233; $P$ = 0.003), creatinine (OR, 6.800; 95% CI: 1.424–32.470; $P$ = 0.016), procalcitonin (OR, 3.831; 95% CI: 0.551–27.027; $P$ = 0.071), C-reactive protein (OR, 5.988; 95% CI: 1.179–30.30; $P$ = 0.031) were significantly associated with the disease progression. Furthermore, the multivariate logistic analysis indicated that age (OR, 8.546; 95% CI: 1.628–44.864; $P$ = 0.011), history of smoking (OR, 14.285; 95% CI: 1.577–25.000; $P$ = 0.018), maximum body temperature at admission (OR, 8.999; 95% CI: 1.036–78.147, P = 0.046), respiratory failure (OR, 8.772, 95% CI: 1.942–40.000; $P$ =0.016), albumin (OR, 7.353, 95% CI: 1.098–50.000; $P$ =0.003) and C-reactive protein (OR, 10.53; 95% CI: 1.224–34.701, $P$ = 0.028) were risk factor for disease progression [Table 4].

## Discussion

Coronavirus is a highly contagious pathogen found in several domestic animals, pets, and humans, causing a variety of acute and chronic diseases.[6] Currently, six coronaviruses are known to infect humans, including 229E and NLR6 in the α-genus. The β-genus comprises OC43, HKU1, Middle East respiratory syndrome-related coronavirus (MERSr-CoV),[7] and severe acute respiratory syndrome-related coronavirus (SARSr-CoV).[8] Coronavirus has gradually become a popular topic of research in the field of virology because of the outbreak of SARSr-CoV in 2003 and MERSr-CoV in 2012.[9] The current outbreak is due to a novel coronavirus in the β-genus, which was isolated from the lower respiratory tract in patients with unexplained pneumonia, in Wuhan, China.[10,11] Currently, the source and pathogenesis of the COVID-19 remain unclear, and there are no uniform diagnostic and treatment standards. Unfortunately, in certain patients, the disease progresses rapidly, and respiratory failure can occur within a short time, even leading to death. Therefore, we investigated the disease outcomes and factors affecting the outcomes of patients with COVID-19 pneumonia at three tertiary hospitals in Wuhan to provide a theoretical basis for improving hospitals' efforts to effectively treat patients with COVID-19 pneumonia.

The present study included 78 patients diagnosed with the COVID-19. All patients were evaluated for therapeutic efficacy after at least two weeks of hospitalization. These results indicated progression in 11 patients (14.1%) and improvement/stabilization in 67 patients (85.9%). 80.8% of the patients were younger than 60 years, and the median age of the patients was 38 (33, 57) years, which suggest that middle-aged people are susceptible to COVID-19. Also, the age of patients in the progression group was significantly higher than that in the improvement/stabilization group, and multivariate logistic analysis indicated that higher age was a risk factor for disease progression. Elderly individuals are physically frail and are likely to have several comorbidities, which not only increases the risk of pneumonia[12] but also affects their prognosis[13]. the assessment of comorbidities is an essential component in determining the prognosis of several diseases, especially pneumonia.[14] Probably be-

cause of the small sample size, there was no significant difference in any comorbidity including hypertension, diabetes, COPD, cancer, and others between the two groups. The potential impact of comorbidities on the disease outcomes of patients with COVID-19 pneumonia requires further observation and research. The proportion of patients with a history of smoking was significantly higher in the progression group compared to the improvement/stabilization group, suggesting that smoking is associated with disease progression.

A significant symptom of SARS is a body temperature above 38°C for over two weeks. Additionally, 60% of patients diagnosed with MERS presented with fever.[15] In the present study, 73.1% of patients with COVID-19 sought treatment for the fever. The results showed that the maximum body temperature at admission in the progression group was significantly higher than in the improvement/stabilization group, and multivariate logistic models indicated that higher temperature was a risk factor for disease progression. Therefore, patients presenting with a high fever, long fever duration, and rapid fever progression should be monitored more closely during clinical diagnosis and treatment in order to avoid complications associated with high fevers, which lead to poor prognosis.

Vital signs are essential indicators for assessing the current symptoms of patients. Respiratory system indices, such as respiratory rate and whether respiratory failure occurred, are particularly crucial for assessing the condition severity in patients with COVID-19. The present study found that the median respiratory rate of 78 patients with COVID-19 pneumonia was 24 breaths/min, which was higher than the normal respiratory rate (12-20 breaths/min). The respiratory rate and proportion of patients with respiratory failure in the progression group were significantly higher than in the improvement/stabilization group. Abnormal respiratory indices can directly reflect the extent of lung invasion and multivariate logistic models revealed that respiratory failure was a risk factor for disease progression. Therefore, respiratory indices should be one of the top priorities in the efficacy evaluation.

The present study suggests that elevated C-reactive protein, and decreased albumin are factors associated with poor prognosis of COVID-19 infection. Albumin is the

most intuitive index of the nutritional status of the body. When albumin decreases, the body loses resistance to the virus, leading to disease progression.[16] Elevated C-reactive protein is an important inflammatory index in addition to abnormal blood coagulation function. Close monitoring of dynamic changes in these indices has a significant proactive effect on understanding changes in the patient's condition. In addition, studies have shown that lymphocytes are the main target cells of viral infections.[17] Viral infections in the human body primarily involve damage to the immune system, which presents as decrease in the absolute number of lymphocytes.[18] The present study did not find these indices significant for assessing the outcome of COVID-19 patients, and their correlation requires further investigation. This study included CT scan characteristics of patients with COVID-19 pneumonia for analysis and suggested that the extent and characteristics of the lesion had no statistical significance on disease outcomes. However, the use of CT scans at earlier stages for disease assessment is still of great significance for early detection, early diagnosis, and improved prognosis.

Appropriate antibiotic treatment can be administered to prevent secondary infection in critical type viral pneumonia.[19] We analyzed the diagnosis and treatment protocols of patients with COVID-19 pneumonia, and results suggested that some patients undergoing antiviral treatment were also proactively undergoing antibacterial treatment. Whether viral pneumonia should be treated with glucocorticoids has been controversial. Some researchers believe that the use of glucocorticoids in viral pneumonia can easily aggravate the disease and increase the risk of secondary infections, leading to an increase in mortality, thus advocating against the use of glucocorticoids.[20] Other studies have suggested that the use of an appropriate dose of glucocorticoids at early stages could inhibit the elevated secretion of inflammatory cytokines due to excessive activation of immune cells because of the viral infection, thereby preventing continued exacerbation of lung injury.[21] We found that the combination of antivirals, antibacterials, and glucocorticoids had the highest use rate in the treatment of COVID-19 pneumonia. Moreover, other researchers have suggested using thymosin and gamma globulin during the early stages of infection to improve patient immunity. In addition,

current ongoing related studies suggest that COVID-19 and HIV have structural similarities. Thus, certain researchers have proposed that the anti-HIV drug, lopinavir, may play a role in inhibiting COVID-19. In this study, a comparison of efficacy of antivirals/antibacterials, antivirals/antibacterials + glucocorticoids, antivirals/antibacterials + gamma globulin, antivirals/antibacterial + thymosins, and antivirals/antibacterials + lopinavir was performed. The results did not suggest that drug protocols affected disease outcomes. Therefore, further studies should include more drugs for the treatment of COVID-19. COVID-19 pneumonia is characterized by an acute onset and rapid progression. Therefore, the early use of glucocorticoids with proactive antiviral and antibacterial treatment after comprehensive evaluation may block the inflammatory cascade caused by severe viral infections and prevent acute inflammation. The lung damage caused by such infections can further progress to acute respiratory distress syndrome. Respiratory support is an essential treatment for patients with severe viral infections. The present study revealed that all patients with COVID-19 were treated with respiratory support, and the majority of patients were administered nasal cannula oxygen and continuous positive air pressure. The progression group was significantly more likely to receive higher levels of respiratory support. No patients in this study were treated with invasive ventilation and ECMO due to the refusal of the patients' family. Treating patients with severe viral pneumonia, timely application of glucocorticoids and respiratory support therapy is essential, in combination with personalized treatment specific to each patient.

There were a few limitations for this observational study. CT scan imaging has delayed scanning time, which may introduce bias in our results. In addition, a relatively small sample size was included in this study, which may lead to biased results. Thus, a multi-center large-scale study with additional researchers is required. Currently, the best diagnostic and treatment protocols for COVID-19 are still being investigated. Early diagnosis and dynamic monitoring of prognostic factors are essential for improving the ability to treat the COVID-19.

***Conflicts of interest***

None.

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**Table 1: Demographic data and clinical presentations of COVID-19 patients.**

| Items | Total (*n*=78) | Improvement/ stabilization (*n*=67) | Progression (*n*=11) | Statistics | *P* |
|---|---|---|---|---|---|
| Age, years | 38 (33, 57) | 37 (32, 41) | 66 (51, 70) | 4.932* | 0.001 |
| Male | 39 (50.0) | 32 (47.8) | 7 (63.6) | 0.953† | 0.517 |
| History of smoking | 5 (6.4) | 2 (3.0) | 3 (27.3) | 9.291† | 0.018 |
| Comorbidity | | | | | |
| Hypertension | 8 (40.0) | 6 (9.0) | 2 (18.2) | 0.874† | 0.318 |
| Diabetes | 5 (25.0) | 3 (4.5) | 2 (18.2) | 2.958† | 0.143 |
| COPD | 2 (10.0) | 1 (1.5) | 1 (9.1) | 2.184† | 0.264 |
| Cancer | 4 (20.0) | 2 (3.0) | 2 (18.2) | 4.485† | 0.093 |
| Others | 10 (50.0) | 7 (10.4) | 3 (27.3) | 2.393† | 0.144 |
| History of exposure to | 0 | 0 | 0 | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Huanan seafood market | | | | | |
| Initial symptom | | | | | |
| Maximum body temperature on admission, °C | 37.8 (37.1, 38.2) | 37.5 (37.0, 38.4) | 38.2 (37.8, 38.6) | 2.057* | 0.027 |
| Cough | 34 (43.6) | 30 (44.8) | 4 (36.4) | 0.272† | 0.748 |
| Respiratory failure | 20 (25.6) | 14 (20.9) | 6 (54.5) | 5.611† | 0.028 |
| Respiratory rate, breaths/min | 24 (16, 60) | 24 (16, 60) | 34 (18, 48) | 4.030* | 0.004 |
| Blood oxygen saturation, % | 96 (50, 99) | 96 (50, 99) | 95 (89, 98) | 0.086* | 0.436 |
| Heart rate, beats/min | 94 (68, 130) | 94 (70, 112) | 100 (68, 130) | 0.073* | 0.737 |
| Severe illness | 8 (10.3) | 5 (7.5) | 3 (27.3) | 13.480† | 0.079 |

Data were shown as median ($Q_1$, $Q_3$) or *n* (%). *$U$ values; †$\chi^2$ values. COVID-19: 2019 novel coronavirus disease; COPD: chronic obstructive pulmonary disease; -: not applicable.

**Table 2: Laboratory indices and imaging characteristics of COVID-19 patients.**

| Items | Total (*n*=78) | Improvement/stabilization (*n*=67) | Progression (*n*=11) | Statistics | *P* |
|---|---|---|---|---|---|
| D-dimer, ng/mL | 0.42 (0.20, 1.08) | 0.39 (0.20, 1.07) | 0.56 (0.21, 6.84) | 1.282* | 0.501 |
| Albumin, g/L | 40.47±5.21 | 41.27±4.55 | 36.62±6.60 | 2.843† | 0.006 |
| WBC, $\times10^9$/L | 5.31±2.63 | 5.18±1.63 | 6.08±2.56 | 1.057† | 0.294 |
| Lymphocytes, $\times10^9$/L | 0.98 (0.61, 1.35) | 1.00 (0.68, 1.39) | 0.53 (0.30, 1.15) | 0.458* | 0.075 |
| Neutrophils, $\times10^9$/L | 3.11 (2.25, 4.82) | 2.94 (2.20, 4.60) | 4.69 (2.96, 7.06) | 1.562* | 0.059 |
| Platelets, $\times10^9$/L | 169.10±57.26 | 173.20±55.37 | 143.90±64.81 | 1.589† | 0.116 |
| ALT, U/L | 18.1 (13.7, 30.7) | 18.5 (12.5, 27.7) | 17.4 (13.9, 43.9) | 0.301* | 0.776 |
| AST, U/L | 20.5 (13.8, 33.5) | 20.0 (13.9, 30.9) | 21.6 (12.0, 45.6) | 2.006* | 0.788 |
| Creatinine, μmol/L | 65.10 (51.20, 80.40) | 71.75 (48.78, 114.80) | 64.50 (51.45, 78.95) | 2.528* | 0.385 |
| Erythrocyte sedimentation rate, mm/1 h | 31 (17, 43) | 31 (11, 40) | 30 (22, 52) | 0.155* | 0.794 |
| Procalcitonin, ng/mL | 0.06 (0.04, 0.10) | 0.06 (0.04, 0.09) | 0.12 (0.05, 0.49) | 0.388* | 0.195 |
| C-reactive protein, mg/L | 11.1 (2.3, 36.0) | 10.6 (1.9, 33.1) | 38.9 (14.3, 64.8) | 1.315* | 0.024 |

| | | | | | |
|---|---|---|---|---|---|
| Partial pressure of oxygen, % | 56 (48, 79) | 56 (51, 78) | 55 (41, 76) | 0.199* | 0.666 |
| CT characteristics at initial diagnosis | | | | | |
| Extent | | | | 0.786‡ | 0.782 |
| Unilateral lung involvement | 23 (29.5) | 20 (29.9) | 3 (27.3) | | |
| Bilateral lung involvement | 45 (57.7) | 38 (56.7) | 7 (63.6) | | |
| Lesion characteristics | | | | 2.705‡ | 0.100 |
| Multifocal opacity | 44 (56.4) | 40 (59.7) | 4 (36.4) | | |
| Bilateral lung ground glass opacity | 13 (16.7) | 10 (14.9) | 3 (27.3) | | |
| Other concomitant microbial infection | 0 | 0 | 0 | - | - |

Data were shown as mean±standard deviation, median ($Q_1$, $Q_3$), or *n* (%). *$U$ value; †$t$ test; ‡$\chi^2$ value. COVID-19: 2019 novel coronavirus disease; WBC: white blood cell; ALT: alanine transaminase; AST: aspartate aminotransferase; CT: computed tomography; -: not applicable.

**Table 3: Treatment for COVID-19 patients.**

| Items | Total (*n*=78) | Improvement/stabilization (*n*=67) | Progression (*n*=11) | Statistics | *p* |
|---|---|---|---|---|---|
| Respiratory support | | | | 16.01* | 0.001 |
| Nasal cannula | 71 (91.0) | 64 (95.5) | 7 (63.6) | | |
| High-flow nasal cannula | 2 (2.6) | 0 | 2 (18.2) | | |
| NPPV | 5 (6.4) | 3 (4.5) | 2 (18.2) | | |
| Invasive ventilation | 0 | 0 | 0 | | |
| ECMO | 0 | 0 | 0 | | |
| Treatment protocol | | | | 4.267* | 0.371 |
| Antiviral/antibacterial | 12 (15.4) | 10 (14.9) | 2 (18.2) | | |
| Antiviral/antibacterial + lopinavir | 24 (30.8) | 21 (31.3) | 3 (27.3) | | |
| Antiviral/antibacterial + gamma globulin | 14 (17.9) | 9 (13.4) | 5 (45.5) | | |
| Antiviral/antibacterial + thymosin | 24 (30.8) | 21 (31.3) | 3 (27.3) | | |
| Antiviral/antibacterial + | 45 | 38 (56.7) | 7 (63.6) | | |

| | | | | | |
|---|---|---|---|---|---|
| glucocorticoids | (58.0) | | | | |
| Glucocorticoids dose, mg qd iv | 40 (20, 40) | 40 (20, 40) | 60 (40, 80) | 4.713† | 0.075 |

The data were presented as median ($Q_1$, $Q_3$) or *n* (%). *$\chi^2$ value; †*U* value. COVID-19: 2019 novel coronavirus disease; NPPV: Noninvasive positive pressure ventilation; ECMO: Extracorporeal membrane oxygenation; qd: every day; iv: intravenously.

**Table 4: Logistic analysis results of risk factors for disease progression (*n* = 78).**

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| **Variables** | **OR** | **95% CI** | ***P*** | **OR** | **95% CI** | ***P*** |
| Age (≥60 years *vs.* <60 years) | 10.575 | 2.095–53.386 | 0.004 | 8.546 | 1.628–44.864 | 0.011 |
| Sex (male *vs.* female) | 1.914 | 0.512–7.156 | 0.335 | | | |
| History of smoking (yes *vs.* no) | 12.187 | 1.762–84.306 | 0.011 | 14.285 | 1.577–25.000 | 0.018 |
| Hypertension (yes *vs.* no) | 2.259 | 0.394–12.958 | 0.360 | | | |
| Diabetes (yes *vs.* no) | 4.741 | 0.695–32.350 | 0.112 | | | |
| Maximum body temperature at admission (≥37.3°C *vs.* < 37.3°C) | 9.709 | 1.176–83.330 | 0.035 | 8.999 | 1.036–78.147 | 0.046 |
| Cough (yes *vs.* no) | 1.063 | 0.295–3.834 | 0.925 | | | |
| Respiratory failure (yes *vs.* no) | 8.021 | 2.022–31.821 | 0.003 | 8.772 | 1.942–40.000 | 0.016 |
| Respiratory rate (>20 breaths/min *vs.* ≤20 breaths/min) | 3.952 | 0.473–33.333 | 0.204 | | | |
| Blood oxygen saturation (≤93% *vs.* >93%) | 2.259 | 0.394–12.958 | 0.360 | | | |
| Heart rate (≥100 beats/min *vs.* <100 beats/min) | 1.536 | 0.315–7.519 | 0.596 | | | |
| Severe illness (yes *vs.* no) | 4.651 | 0.930–23.250 | 0.061 | 2.524 | 0.339–18.784 | 0.336 |
| D-dimer (>1 μg/mL *vs.* ≤1 μg/mL) | 1.799 | 0.363–8.928 | 0.742 | | | |
| Albumin (<40 g/L *vs.* ≥40 g/L) | 12.536 | 2.409–65.233 | 0.003 | 7.353 | 1.098–50.000 | 0.003 |
| WBC (≥$4\times10^9$/L *vs.* <$4\times10^9$/L) | 1.488 | 0.163–2.770 | 0.582 | | | |
| Lymphocytes (<$1.1\times10^9$/L *vs.* ≥$1.1\times10^9$/L) | 1.600 | 0.167–2.336 | 0.485 | | | |
| Neutrophils (≥$1.8\times10^9$/L *vs.* <$1.8\times10^9$/L) | 2.037 | 0.236–17.544 | 0.518 | | | |
| Platelets (<$100\times10^9$/L *vs.* ≥$100\times10^9$/L) | 2.259 | 0.394–12.958 | 0.360 | | | |
| ALT (>50 U/L *vs.* ≤50 U/L) | 1.032 | 0.231–2.148 | 0.923 | | | |
| AST (>40 U/L *vs.* ≤40 U/L) | 2.088 | 0.443–9.901 | 0.352 | | | |
| Creatinine (>111 μmol/L *vs.* ≤111 μmol/L) | 6.800 | 1.424–32.470 | 0.016 | 0.713 | 0.021–1.350 | 0.062 |
| Erythrocyte sedimentation rate (>15 mm/1 h *vs.* ≤15 mm/1 h) | 2.625 | 0.574–12.048 | 0.213 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Procalcitonin (≥0.5 ng/mL *vs.* <0.5 ng/mL) | 3.831 | 0.551–27.027 | 0.071 | 0.174 | 0.012–2.353 | 0.696 |
| C-reactive protein (>8.2 mg/L *vs.* 8.2 mg/L) | 5.988 | 1.179–30.30 | 0.031 | 10.53 | 1.224–34.701 | 0.028 |
| Partial pressure of oxygen (<60% *vs.* ≥60%) | 1.742 | 0.167–3.312 | 0.535 | | | |

OR: Odds Ratio; CI: Confidence interval; WBC: White blood cell; ALT: Alanine transaminase; AST: Aspartate aminotransferase.

**Original Article**

# Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients

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**Abstract**

**Background:** A patient's infectivity is determined by the presence of the virus in different body fluids, secretions, and excreta. The persistence and clearance of viral RNA from different specimens of patients with 2019 novel coronavirus disease (COVID-19) remain unclear. This study analyzed the clearance time and factors influencing 2019 novel coronavirus (2019-nCoV) RNA in different samples from patients with COVID-19, providing further evidence to improve the management of patients during convalescence.

**Methods:** The clinical data and laboratory test results of convalescent patients with COVID-19 who were admitted to from January 20, 2020 to February 10, 2020 were collected retrospectively. The reverse transcription polymerase chain reaction (RT-PCR) results for patients' oropharyngeal swab, stool, urine, and serum samples were collected and analyzed. Convalescent patients refer to recovered non-febrile patients without respiratory symptoms who had two successive (minimum 24 h sampling interval) negative RT-PCR results for viral RNA from oropharyngeal swabs. The effects of cluster of differentiation 4 (CD4)+ T lymphocytes, inflammatory indicators, and glucocorticoid treatment on viral nucleic acid clearance were analyzed.

**Results:** In the 292 confirmed cases, 66 patients recovered after treatment and were included in our study. In total, 28 (42.4%) women and 38 men (57.6%) with a median age of 44.0 (34.0–62.0) years were analyzed. After in-hospital treatment, patients' inflammatory indicators decreased with improved clinical condition. The median time from the onset of symptoms to first negative RT-PCR results for oropharyngeal swabs in convalescent patients was 9.5 (6.0–11.0) days. By February 10, 2020, 11 convalescent patients (16.7%) still tested positive for viral RNA from stool specimens and the other 55 patients' stool specimens were negative for 2019-nCoV following a median duration of 11.0 (9.0–16.0) days after symptom onset. Among these 55 patients, 43 had a longer duration until stool specimens were negative for viral RNA than for throat swabs, with a median delay of 2.0 (1.0–4.0) days. Results for only four (6.9%) urine samples were positive for viral nucleic acid out of 58 cases; viral RNA was still present in three patients' urine specimens after throat swabs were negative. Using a multiple linear regression model ($F$=2.669, $P$=0.044, and adjusted $R^2$=0.122), the analysis showed that the CD4+ T lymphocyte count may help predict the duration of viral RNA detection in patients' stools ($t$=-2.699, $P$=0.010). The duration of viral RNA detection from oropharyngeal swabs and fecal samples in the

glucocorticoid treatment group was longer than that in the non-glucocorticoid treatment group (15 days vs 8.0 days, respectively; $t$=2.550, $P$=0.013) and the duration of viral RNA detection in fecal samples in the glucocorticoid treatment group was longer than that in the non-glucocorticoid treatment group (20 days vs 11 days, respectively; $t$=4.631, $P$ <0.001). There was no statistically significant difference in inflammatory indicators between patients with positive fecal viral RNA test results and those with negative results ($P$ >0.05).

**Conclusions:**

In brief, as the clearance of viral RNA in patients' stools was delayed compared to that in oropharyngeal swabs, it is important to identify viral RNA in feces during convalescence. Because of the delayed clearance of viral RNA in the glucocorticoid treatment group, glucocorticoids are not recommended in the treatment of COVID-19, especially for mild disease. The duration of RNA detection may relate to host cell immunity.

**Keywords:** COVID-19; 2019-nCoV; nucleic acid detection; glucocorticoid

## Introduction

In January 2020, a new coronavirus was confirmed as the cause of unexplained pneumonia in a group of patients from Wuhan, Hubei, and was subsequently named the 2019 novel coronavirus (2019-nCoV).[1] Due to increasing numbers of cases reported out of China, the World Health Organization (WHO) announced on January 30 that the emerging new coronavirus pneumonia epidemic constituted a "PHEIC" (public health emergency of international concern).[2] Up until February 11, 2020, there were 44,653 confirmed 2019 novel coronavirus disease (COVID-19) cases

reported in China and 395 cases in 24 other countries.[3-4] The transmission capacity of 2019-nCoV was underestimated at first. Initial studies showed its regeneration number, R0, was 2.2–2.9,[5-7] meaning that each infector could transmit to another 2.2 to 2.9 people. Recently a novel study revealed that the R0 of 2019-nCoV is 3.77 based on clinical and epidemiological data from nearly 8866 patients in 30 provinces,[8] which is higher than the R0 of severe acute respiratory syndrome coronavirus (R0, 2–3).[9]

The patient's infectivity is determined by the presence of the virus in different body fluids, secretions, and excreta. All patients with positive viral RNA detection need to be isolated. As mentioned in the "Diagnosis and Treatment Scheme of New Coronavirus Infected Pneumonia" (trial version 5), only after the relief of symptoms and two successive (minimum 24 h sampling interval) negative viral nucleic acid results for respiratory specimens, the isolated cases can be disisolation. However, the persistence and clearance of viral RNA in different specimens of COVID-19 patients remains unclear. In this study, viral RNA detection was performed on throat swabs, and stool, urine, and serum specimens, which were analyzed based on different clinical conditions and lab results, in order to figure out the clearance time of the virus and factors which may influence this.

## Methods

### *Ethical approval*

This retrospective study was approved by the Shanghai Public Health Clinical Center Ethics Committee (No. YJ-2020-S015-01) and was exempted from the need for informed consent from patients.

### *Subjects*

From January 20, 2020 to February 10, 2020, all confirmed patients with COVID-19 in the Shanghai region were admitted to the Shanghai Public Health Clinical Center. The convalescent patients refer to recovered non-febrile patients without respiratory symptoms who had two successive (minimum 24 h

sampling interval) negative reverse transcription polymerase chain reaction (RT-PCR) results for viral RNA from oropharyngeal swabs.

### *Clinical measures*

Clinical data and lab results were recorded at admission and last fecal viral RNA test, including sex, age, cluster of differentiation 4 (CD4)+ T lymphocyte counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and procalcitonin (PCT). The glucocorticoid treatment group included any patients who were ever treated with glucocorticoids, such as prednisolone or dexamethasone. The feces-positive and feces-negative groups were classified based on the detection of viral RNA in patients' feces. The clinical conditions and lab results of study subjects, together with the viral RNA results from different specimens (oropharyngeal swab, stool, urine, and serum) from each day were collected retrospectively.

### *Detection of viral RNA in COVID-19*

A magnetic bead-method nucleic acid extraction kit was applied in a fully automated nucleic acid extraction instrument (Master Biotechnology, China). The total RNA was extracted from a 200-μl sample and dual fluorescence PCR (Applied Biosystems 7500 Real-Time PCR Systems, USA) was performed according to the manufacturer's instructions.

### *Statistical analysis*

Statistical analyses were performed using SPSS 20.0 (International Business Machines Corporation, IBM, USA). Normally distributed continuous variables are summarized as the mean and standard deviation, and non-normally distributed data are recorded as median and interquartile range (IQR) as appropriate. Categorical variables are expressed as counts and percentages for each category. The *t*-tests or Wilcoxon rank-sum tests were applied to test differences between two groups; Fisher's exact tests or Chi-square tests were used for categorical variables. Multiple linear regression was applied to

determine the relationship between outcomes and the exploratory factor. $P$ <0.05 was considered significant.

## Results

### *Demographics and laboratory exanimation results*

From January 20, 2020 to February 10, 2020, 292 patients with COVID-19 were admitted to the Shanghai Public Health Clinical Center. Sixty-six convalescent patients were included in our study. In total, 28 (42.4%) women and 38 men (57.6%) with a median age of 44.0 (34.0–62.0) years were analyzed. The oldest patient was 78 years old and the youngest was 16 years old. There was no difference in sex or age between those with or without glucocorticoid treatment ($\chi^2$=0.342, $P$=0.599; $t$=1.059, $P$=0.294). On admission, the average high level of ESR was 70.0 (25.5–90.0) mm/h, high-sensitivity CRP was 8.4 (1.6–20.3) mg/L, and PCT was 0.03 (0.02–0.05) ng/mL, which decreased to 44.0 (29.5–81.3) mm/h, 0.5 (0.5–2.1) mg/L, and 0.02 (0.02–0.02) ng/mL, respectively, upon treatment [Table 1].

### *Virus RNA detection in different samples from patients with COVID-19*

The median time from the onset of symptoms to first negative RT-PCR results for oropharyngeal swabs of convalescent patients was 9.5 (6.0–11.0) days with improvement in symptoms such as fever, cough, and dyspnea. This time varied greatly between patients, ranging from 2 to 22 days. The RT-PCR for viral RNA was performed using stool, urine, and blood specimens during convalescence. Until the end of the observation period (February 10, 2020), 11 convalescent patients (16.7%) still tested positive for viral RNA in stool specimens. The other 55 patients' stool specimens were negative for 2019-nCoV following a median duration of 11.0 (9.0–16.0) days. Twelve patients; (21.8%) viral RNA in oropharyngeal swabs or fecal samples was negative at the same time; 78.2% (43/55) of cases had longer duration until stool specimens were negative for viral RNA than throat swabs, with a median delay of 2.0 (1.0–4.0) days. Viral nucleic acid was found in urine in only four (6.9%) patients out of 58 cases; viral RNA was present in urine specimens after throat swabs were negative. Fourteen serum specimens were tested for 2019-nCoV and none of them showed positive results [Table 1].

***Factors related to virus clearance***

An analysis of the correlation between the absolute values of CD4+ T lymphocytes, C-reactive protein, red blood cell sedimentation rate, procalcitonin, and the time of detoxification for feces during convalescence was performed. Using a multiple linear regression model ($F$=2.699, $P$=0.044, and adjusted $R^2$=0.122), the analysis showed that the CD4+ T lymphocyte count may help predict the duration of viral RNA detection in patient stool samples ($t$=-2.699, $P$=0.010;Table 2).

During hospitalization, five patients received glucocorticoid treatment. The duration of viral RNA detection in throat swabs and fecal samples in the glucocorticoid treatment group was longer than that in the non-glucocorticoid treatment group (15 days vs. 8.0 days, $t$ =2.550, $P$=0.013; 20 days vs. 11 days $t$=4.631, $P$ <0.001, respectively). We further analyzed the differences in the results of the last tests for inflammatory indicators upon positive results for viral RNA in fecal samples. There was no statistically significant difference in inflammatory indicators between patients with positive and negative fecal viral RNA test results ($P$ >0.05) [Table 3].

**Discussion**

The novel coronavirus was firstly identified in respiratory specimens from patients with COVID-19 and viral nucleic acids were subsequently detected in patients' stool, urine, and gastrointestinal mucosa.[10-12] Recently a neonatal infection was reported, indicating the possibility of fecal-oral and vertical transmission from mother to child, in addition to the currently confirmed droplet transmission and direct contact transmission. In this study we found that the viral RNA can be detected in the stool of 81.8% (54/66) patients, even in those with negative results from throat swabs. The continuous detection of viral nucleic acids in feces suggests that the virus may be transmitted through the digestive tract or re-transmitted through aerosols containing viruses. Therefore, it is necessary urgently to standardize the stool transport process of COVID-19 patients to reduce risk of further transmission. Moreover, viral RNA detection in fecal samples should be applied regularly in patients with COVID-19, even during the recovery period. Transmission by urine or blood may occur less frequently because of the low rate of positive findings in patients.

Glucocorticoids have been widely used in the treatment of severe acute respiratory syndrome and Middle East respiratory syndrome, and are now also used in conjunction with other drugs to treat patients infected with 2019-nCoV. However, in the published clinical management opinions for the COVID-19, the application of glucocorticoids is not recommended unless there are other indications.[13-14] The use of glucocorticoids may delay the clearance of viral nucleic acids in patients and should be avoided during viral replication. Some bias exists in our study because the patients in the glucocorticoid treatment group had more severe disease and lower CD4+ T lymphocytes counts. Our point is that mild patients are not recommended glucocorticoid treatment, which may delay virus clearance. The randomized controlled double-blind experiments with expanded sample sizes will help clarify this issue. T cell immunity may play an important role in 2019-nCoV infection. The absolute values of CD4+ T lymphocytes, C-reactive protein, erythrocyte sedimentation rate, and procalcitonin measured upon admission were analyzed with respect to virus clearance. The lower the absolute value of CD4+ T lymphocytes before treatment, the longer duration of virus clearance. The relationship between the fecal viral RNA results and inflammatory indicators of patients were analyzed and no statistical difference in erythrocyte sedimentation rate, C-reactive protein, or procalcitonin during rehabilitation was found.

In brief, as the clearance of viral RNA from patients' stools was delayed compared to that from oropharyngeal swabs, it is important to detect the viral RNA in feces during convalescence. Because of the delayed clearance of viral RNA in the glucocorticoid treatment group, glucocorticoids are not recommended in the treatment of COVID-19, especially for mild disease. The duration of RNA detection may be related to host cell immunity.

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**Conflict of Interest**

None.

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**Table 1** Clearance time of viral RNA with or without glucocorticoid treatment

| Parameters | All patients (*n*=66) | GC treatment (*n*=5) | No GC treatment (*n*=61) | *Statistics* | *P* |
|---|---|---|---|---|---|
| **Gender** | | | | $\chi^2$=0.342 | *0.559* |
| **Female** | 38 | 4 | 34 | | |
| **Male** | 28 | 1 | 27 | | |
| **Age (years)** | 44.0 (34.0–62.0) | 51.0 (44.0–68.8) | 41.0 (34.0–61.3) | *t* =1.059 | *0.294* |
| **Pharyngeal swab virus nucleic acid negative time (days)*** | 9.5.0 (6.0–11.0) | 15.0 (9.8–16.8) | 8.0 (6.0–11.0) | *t* =2.550 | ***0.013*** |
| **Fecal virus nucleic acid negative time (days) †** | 11.0 (9.0–16.0) | 20.0 (17.5–22.5) | 11.0 (9.0–14.0) | *t* =4.631 | ***<0.001*** |
| **Feces after throat swab virus nucleic acid negative time (days) ‡** | 2.0 (1.0–4.0) | 8.0 (2.25–11.0) | 2.0 (1.0–3.0) | *t* =1.983 | 0.115 |

Values are presented as ***n* or** median (interquartile range, IQR); GC: Glucocorticoid. *Pharyngeal swab virus nucleic acid negative time: the time from when the patient has fever and respiratory symptoms to the first negative nucleic acid test of the throat swab. †Fecal virus nucleic acid negative time: the time from the onset of symptoms to when the fecal virus nucleic acid test is first negative during the recovery period. ‡Feces after throat swab virus nucleic acid negative time: the time between the first negative nucleic acid test of the throat swab and that of the fecal sample.

**Table 2** Multiple linear regression analysis of immune and inflammatory parameters with respect to virus clearance

| **ANOVA** | | | | | |
|---|---|---|---|---|---|
| **Source of variation** | **df** | **ss** | **ms** | ***F*** | ***P*** |
| **Regression** | 4 | 228.509 | 57.127 | 2.669 | 0.044 |
| **Residual** | 44 | 941.614 | 21.400 | | |
| **Total** | 48 | 1170.122 | | | |
| Multiple Linear Regression | | | | | |
| | **Coefficient** | **Std. Error** | **Beta** | ***t*** | ***P*** |
| **(Constant)** | 15.883 | 2.281 | | 6.965 | |
| **CD4+ T lymphocyte counts (cell/μL)** | -0.009 | 0.003 | -0.445 | -2.699 | ***0.010*** |
| **ESR (mm/h)** | 0.019 | 0.019 | 0.153 | 1.006 | 0.320 |
| **CRP (mg/L)** | -0.027 | 0.045 | -0.102 | -0.589 | 0.559 |
| **PCT (ng/mL)** | -6.278 | 15.607 | -0.064 | -0.402 | 0.689 |
| **$R^2$=0.195** | **Adj $R^2$=0.122** | | | | |

Adj $R^2$:Adjusted $R^2$, ANOVA: Analysis of variance; df:degrees of freedom; ms:mean squares; ss:sum of squares; CD4:Cluster of differentiation 4; ESR:Erythrocyte sedimentation rate; CRP:C-reactive protein, PCT:Procalcitonin

**Table 3** Viral RNA analysis of stool samples with the inflammatory indicators of patients

| Parameters | **Stool test-positive (*n*=11)** | **Stool test-negative (*n*=55)** | ***t*** | ***P*** |
|---|---|---|---|---|
| **ESR (mm/h)** | 82.0 (36.8–90.8) | 40.5 (28.0–79.0) | -1.307 | 0.196 |
| **CRP (mg/L)** | 1.02 (0.5–2.9) | 0.5 (0.5–1.2) | -0.132 | 0.896 |
| **PCT (ng/mL)** | 0.02 (0.02–0.02) | 0.02 (0.02–0.02) | 1.041 | 0.302 |

Values are presented as median (interquartile range, IQR); ESR:Erythrocyte sedimentation rate; CRP:C-reactive protein; PCT:Procalcitonin

**Perspective**

# Non-invasive respiratory support for patients with novel coronavirus pneumonia: clinical efficacy and reduction in risk of infection transmission

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Pneumonia caused by a novel coronavirus known as 2019 novel coronavirus disease (COVID-19)[1] appeared in Wuhan, China in December 2019, and approximately 15–30% of patients developed acute respiratory distress syndrome (ARDS) within a short period of time.[2,3] To reduce respiratory symptoms and improve prognosis, respiratory support is the most important means of life support,[1] and noninvasive respiratory support systems,[2] including various conventional oxygen therapies, non-invasive positive pressure ventilation (NPPV), and high-flow nasal cannula (HFNC), are most commonly used. However, their efficacy and safety remain unclear, and whether they increase the risk of aerosol dispersion and disease transmission is particularly controversial.[4,5] Given that there are many similarities between COVID-19 pneumonia and Severe Acute Respiratory Syndrome (SARS)

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and Middle East Respiratory Syndrome (MERS),[6] this study primarily discusses clinical indications and provides details regarding the prevention of nosocomial infections during NPPV and HFNC treatment of COVID-19 pneumonia based on previous clinical data on the use of NPPV for SARS and MERS and our experience with the treatment of COVID-19 pneumonia.

**Clinical efficacy of NPPV:** NPPV can reduce the rate of tracheal intubation; therefore, theoretically, it can significantly reduce the risk of infection of medical personnel during tracheal intubation and artificial airway management for COVID-19 pneumonia patients.[7] A recent retrospective epidemiological study of 99 COVID-19 pneumonia patients in China[2] revealed that NPPV is the most commonly used mechanical ventilation method for acute respiratory failure. The rates of using non-invasive and invasive mechanical ventilation are 13% and 4%, respectively; however, the efficacies of these ventilation methods need to be further investigated. There are little clinical data on NPPV for SARS,[4,5] of which most are small-sample, single-center retrospective studies from China, and the NPPV failure rate is approximately 20–40%. Cheung *et al*[8] in their study of 20 Hong Kong patients with SARS and acute respiratory failure (oxygen flow >6 L/min, percutaneous oxygen saturation [$SpO_2$] 93–96%) revealed that NPPV could prevent tracheal intubation in 70% of patients and significantly reduce the time spent in the intensive care unit (ICU). Reports on NPPV for the treatment of MERS are also limited.[9] Because the degree of lung and extrapulmonary injuries in patients with MERS are significantly higher than that in patients without MERS,[9] the failure rate of NPPV is relatively high (60–70%). In addition, current evidence and clinical guideline[10] do not recommend NPPV for treating acute hypoxic respiratory failure and pandemic viral illness. Therefore, we believe that NPPV should currently not be used as a first-line treatment to correct respiratory failure in patients with COVID-19 pneumonia. For strictly selected early-stage patients with mild-to-moderate (partial pressure of arterial oxygen [PaO2]/fraction of inspired oxygen [$FiO_2$] > 200 mmHg) hypoxic respiratory failure and especially for units with limited numbers of invasive ventilators, it is recommended that

NPPV be attempted for short periods of time (1–2 hours)[1,8,11] and to intubate immediately if no improvement is observed. In addition, early-stage identification of high-risk factors (shock, metabolic acidosis; multiple organ failure; $PaO_2/FiO_2$ ≤175 mmHg at 1 h after NPPV treatment; severe hypoxemia with $PaO_2/FiO_2$ ≤147 mmHg; Simplified Acute Physiology Score II [SAPS II] >34; tidal volume [VT] >9.5 mL/kg; elevated $PaCO_2$; respiratory rate >30 breaths/min) for NPPV failure in the treatment of hypoxic respiratory failure can improve the safety of NPPV treatment.[11] NPPV should be avoided in patients with hemodynamic instability, multiple organ failure, disorders of consciousness, or mucus drainage disorders.[1]

**NPPV aerosol dispersion and disease transmission problems:** Notably, NPPV can lead to aerosol transmission during use. *In vitro* simulation experiments have shown that NPPV can lead to the dispersion of exhaled aerosols within 1 m of patients. In addition, the dispersion range increases with increased air leakage and increased inspiratory pressure,[12,13] such that the WHO considers NPPV to be an important form of aerosol transmission in patient wards. However, clinical studies on the use of NPPV for SARS did not clearly demonstrate that NPPV increases the risk of infection transmission between infected patients and medical staffs.[4,8] Conversely, NPPV masks may also reduce aerosol exhalation during coughing and talking.[4,5] Recent studies have shown that NPPV is a low-risk airborne route with good interface fitting.[1,14] Therefore, it is still unclear whether NPPV increases the risk of aerosol diffusion and disease transmission, especially with respect to transmission to medical personnel.[4] The use of NPPV for COVID-19 pneumonia still requires strict control of the medical environment and vigilance and monitoring of the infection risk to medical personnel. Table 1 shows specific prevention and control measures for preventing aerosol production and disease transmission in patient wards during NPPV.[7,15]

**Clinical efficacy of HFNC:** HFNC is a new form of noninvasive respiratory support[16] that can be adjusted to a maximum gas flow of 60–80 L/min and a $FiO_2$ of 0.21–1.0. No clinical data exist regarding the use of HFNC for SARS, MERS, or COVID-19, and the clinical

efficacy of HFNC needs to be further investigated. However, for patients with non-infectious mild-to-moderate hypoxic respiratory failure, compared with conventional oxygen therapy, HFNC can reduce the rate of tracheal intubation and mortality.[17] Therefore, HFNC treatment for COVID-19 pneumonia can be attempted when hypoxemia cannot be treated using conventional oxygen therapy devices, NPPV cannot be tolerated, or in the following situations[1]: mild-to-moderate hypoxemia (100 mmHg ≤ $PaO_2/FiO_2$ < 300 mmHg); no indications for emergency tracheal intubation; and relatively stable vital signs. HFNC should be avoided in patients with hemodynamic instability, multiple organ failure, or disorders of consciousness. The therapeutic response should be closely monitored (1–2 hours) after HFNC treatment. The patient should be switched to noninvasive or invasive positive pressure ventilation if the following conditions persist: respiratory rate >30 breaths/minute; $SpO_2$ <88 – 90%; paradoxical breathing and/or continuous assisted respiratory muscle activity; pH <7.35; or partial pressure of arterial carbon dioxide ($PaCO_2$) >45 mmHg.[18]

**Nosocomial infection prevention and control:** To prevent and control the nosocomial infection during HFNC therapy, we provide the following suggestion based on our experience: (1) disposable, single-use high-flow nasal plugs and tubing should be used during HFNC treatment; (2) patients should be instructed to breathe with the mouth closed as much as possible while wearing surgical masks or oxygen mask; (3) condensation in the circuit should be cleaned in a timely manner to avoid production of aerosols caused by high flow gas and condensed water entering the nasal cavity, stimulating coughing in patients; (4) recent evidence shows that the dispersion distance of exhaled gases during HFNC treatment is limited, and the risk of airborne transmission is low.[14,19] However, loose connections between HFNC and nasal plugs significantly increases the dispersion distance of exhaled gases (from 172 mm to 620 mm).[14,19] Therefore, attention should be paid to correct the positioning and wearing of high-flow nasal plugs.

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***Conflicts of interest***

None.

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**Table 1: Nosocomial infection prevention and control measures during non-invasive positive pressure ventilation treatment of 2019 novel coronavirus disease (COVID-2019).**

| Items | Prevention and control measures |
|---|---|
| Treatment environment and medical personnel | Negative-pressure single patient rooms as much as possible |
| | At least 1 m of separation between patient beds |
| | Minimize number of entries by medical personnel and others |
| | Strict use of personal protective equipment (PPE) when entering patient rooms |
| | Strict monitoring of whether medical personnel exhibit symptoms of infection |
| Non-invasive positive pressure ventilator | Viral/bacterial filter (effective rate 99.9997): placed between face mask and respiratory valve (single-limb circuit non-invasive ventilator) or between respiratory support and respiratory outlet (double-limb circuit non-invasive ventilator) |
| | Double-limb circuit non-invasive ventilators should be more effective in preventing aerosol diffusion |
| | Helmets are superior to other non-invasive connection methods in reducing aerosol production |
| | Avoid using nose masks |
| | Avoid using non-invasive connection methods with the respiratory valve on the face mask |
| | Timely replacement of the ventilator air filter |
| Connections and parameter settings | Minimize turning the ventilator on and off |
| | Minimize air leakage (<25 L/min) |
| | Minimize airway pressure (e.g., inspiratory pressure <10 $cmH_2O$) |
| | Appropriate use of sedatives and analgesics (e.g., dexmedetomidine, sufentanil), reducing respiratory drive and minute ventilation |
| | Appropriate use of cough suppressants, and preventing frequent coughing |

**Perspective**

# Extracorporeal membrane oxygenation support in 2019 novel coronavirus disease: indications, timing, and implementation

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The 2019 novel coronavirus disease (COVID-19) has spread rapidly across Hubei province and dispersed to all regions in China owing to its person-to-person transmission and strong invasiveness targeting the lower respiratory tract.[1] By the end of February 15, 2020, more than 68,000 cases of COVID-19 pneumonia had been confirmed in China, including over 1,600 fatalities.[2] Most infected patients who developed COVID-19 pneumonia suffered from only mild symptoms and then completely recovered. However, in some patients, the phenotype may rapidly progress to acute respiratory distress syndrome (ARDS) and multi-organ failure. The initial clinical data, collected in Jinyintan Hospital, Wuhan, showed that ARDS was reported in 12 (29%) among 41 confirmed patients.[3] Among the 41 patients, 13

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patients received medical care in the intensive care unit (ICU), 4 patients were provided invasive mechanical ventilations, whereas for 2 others, extracorporeal membrane oxygenation (ECMO) treatment was applied. Finally, 6 of the 41 patients died. The clinical data of 99 confirmed patients from the same hospital demonstrated that 17 in 99 patients developed ARDS; among them, 3 received ECMO treatment, and 11 died.[4] Another study reported that 22 in 138 cases (16%) developed into ARDS and were admitted into the ICU, of which 4 received ECMO.[5]

# Rationale

ECMO use has been increasing in severe respiratory and/or cardiac failure despite implementation of conventional care. This technology has been proven valuable in treating viral pneumonia during the pandemic influenza A H1N1 in 2009.[6] The epidemics caused by the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 led to a fatality rate of up to 34.4%.[7] The therapeutic effect of ECMO should be considered in MERS, whose causes of death during the epidemics were predominantly refractory hypoxemia and multi-organ failure, similar to COVID-19. Alshahrani MS *et al*[8] reported 35 MERS-CoV infected patients who were critically ill with refractory hypoxemia (partial pressure of arterial oxygen [$PaO_2$]/fraction of inspired oxygen [$FiO_2$] <100 mm Hg), of which 17 had received venous-venous ECMO (VV-ECMO). Compared with that in patients receiving only conventional respiratory care, the fatality of those who had received ECMO was significantly lower (100% *vs*. 65%). Because the evidence for recovering from COVID-19 with ECMO is extremely limited so far, we can learn from the previous experiences in the treatment of similar severe viral pneumonia cases through retrospective literature review and data analysis.

# Indications

Considering the potential reversibility of COVID-19, it is essential to integrate recent

recommendations in severe viral pneumonia therapy.[9,10] An experiential strategy, which is summed from the guidelines on ARDS management, is suggested for critically ill COVID-19 patients rescued with ECMO. Implementation of ECMO should be suggested when the standard conventional respiratory care (lung-protective mechanical ventilation strategy, with tidal volume (Vt) ≤ 6 ml/kg maintaining plateau pressure < 30 cm $H_2O$ and positive end-expiratory pressure (PEEP) ≥ 10 cm $H_2O$; use of lung recruitment maneuver, prone positioning, neuromuscular blockade, and sedation) fails to correct respiratory failure.[11] The indications for ECMO should be followed: (1) $PaO_2/FiO_2$ < 100 mm Hg, or alveolar-arterial gradient of the partial pressure of oxygen [P(A-a) $O_2$] > 600 mm Hg; (2) ventilator frequency < 35 breath per minute (bpm), pH < 7.2 with the plateau pressure > 30 cm $H_2O$; (3) Age < 65 years; (4) mechanical ventilation < 7 days. Alternatively, based on the standard care of the ECMO to Rescue Lung Injury in Severe ARDS (EOLIA) trial,[12] ECMO should be considered if the patients meet one of the following criteria: (1) $PaO_2/FiO_2$ < 50 mm Hg, more than 3 hours; (2) $PaO_2/FiO_2$ < 80 mm Hg, more than 6 hours; (3) arterial blood pH < 7.25 and $PaCO_2$ > 60 mm Hg, more than 6 hours. Studies have confirmed that early implementation of ECMO ($PaO_2/FiO_2$ between 100–150 mm Hg) in ARDS can be advantageous. It is proven to minimize respiratory driven pressure, to inhibit pulmonary and systemic inflammation, and to reduce severe dysfunction of lung and extrapulmonary organs.[13,14] Early "awake ECMO" treatment may be considered in the group of younger patients without extrapulmonary organ disorder or serious co-infection, who are expected to gain more benefits.[15,16]

# Protocol

Owing to the infectivity of 2019-nCoV, ECMO poses a high risk when it is performed for COVID-19 patients, which might produce various body fluid splashes, including airway secretions, blood, and others. Therefore, standardized protocols and protective measures should be reevaluated for implementation and management of ECMO for COVID-19 patients. To minimize the risk of nosocomial infections in

medical staff and to reduce ECMO-related complications, we recommend the following precautions while performing ECMO in COVID-19 patients:

(1) Patients should be placed in an independent area in the ICU under negative pressure; alternatively, adequate ventilation is to be ensured even when negative pressure cannot be applied.

(2) To avoid unnecessary entries and exits, all supplies, including surgical instruments, consumables, medications, and blood products should be carefully inspected, and the number of staff should be restricted in the independent area.

(3) All staff should be supplied with protection for biosafety level 3 and if necessary, comprehensive airway protective devices such as positive pressure medical protective hoods should be supplied.

(4) A bed-side ultrasound device is essential to evaluate vascular conditions, to monitor cardiopulmonary interaction and assess hemodynamic status. Ultrasound imaging offers incomparable convenience and advantages over any other imaging techniques.

(5) Catheterization is recommended to be guided by ultrasound, with the bed unit elevated to an optimal position to facilitate the operation.

(6) Dual-lumen catheter for the jugular vein is the best choice because of its advantages in operation and later rehabilitation. Our recommendation to the China Food and Drug Administration (CFDA) is to approve its use in the mainland of China as soon as possible.

(7) Vein-vein extra corporeal membrane oxygenation (VV-ECMO) should be considered the primary mode; however, since myocarditis is reported as a common complication associated with H1N1 influenza A and MERS-CoV viral infections,[17-20] a heart-assisted mode of veno-arterial ECMO (VA-ECMO) should be considered in this group of patients.[21]

Recognized as a highly skilled and high-risk operation, ECMO is frequently

demanded in the rescue of COVID-19 patients. We call for an action to establish more ECMO centers in affected cities with numerous COVID-19 cases, especially in Hubei province. Expert ECMO teams should be organized for immediate and professional rescue. A standard ICU single room is recommended, and daily care by ICU-specialized nursing teams should be established to avoid lethal complications. All ECMO-related equipment and consumables should be distributed or deployed by a centralized department.

Key knowledge gaps about ECMO include the need for more actionable data linking to the novel disease. More information is needed on the pathophysiology and effective treatment of COVID-19 patients. Each ECMO team will face new serious challenges in this battle. Information collected from the practice of ECMO for severe COVID-19 must be compiled and shared. We call for the creation of recommendable ECMO procedures and the rescue of severe and critically ill COVID-19 patients.

***Conflicts of interests***

None.

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**Perspective**

**Six weeks into the 2019 coronavirus disease (COVID-19) outbreak- it is time to consider strategies to impede the emergence of new zoonotic infections**

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Coronaviruses have in the past been known to be the etiologic agents of mild upper respiratory infections in humans, similar to the ubiquitous and relatively benign “common cold”-type upper respiratory illnesses induced by the human rhinoviruses in adults and children. Subsequent to the severe acute respiratory syndrome (SARS) outbreak in China 2003, and the Middle East respiratory syndrome (MERS) outbreak in the Middle East in 2012, global concerns regarding the pathogenicity and epidemic/pandemic potential of novel human coronaviruses began to emerge, with some experts predicting that novel coronaviruses could likely again cross the species barrier and present humans with future pandemic-potential infections [1]. These concerns have proven prescient with the emergence, late in 2019, of the 2019 coronavirus disease (COVID-19) or novel coronavirus pneumonia (NCP) outbreak, caused by SARS-CoV-2 (previously known as 2019-nCoV), in Wuhan, China.

A significantly large variety of coronavirus species cause a diverse range of diseases in domesticated and wild mammals and birds, and these animals may also be carriers of and reservoirs for coronaviruses [2]. Six coronavirus species had, prior to the 08th January 2020, been known to cause disease in humans. Four species are endemic in human populations, and cause



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mild common cold symptoms in immunocompetent humans. The two remaining species, SARS-CoV and MERS-CoV, are zoonotic in origin, and their infection of humans may have fatal outcomes. SARS-CoV-2 is the seventh coronavirus species that is now known to infect humans, is also zoonotic in origin, and is the causative organism for the current viral pneumonia epidemic in China.

Both SARS-CoV and MERS-CoV are believed to have originated from bats, with common masked civets and dromedary camels respectively being intermediary hosts [3]. SARS-like coronaviruses have been isolated from Chinese horseshoe bats, and may attach to and utilize the angiotensin converting enzyme 2 (ACE2) receptor in human lower respiratory tract cells to gain entry into these cells, thus facilitating transmission to, and initiating infection in, humans [4]. The genomic sequence of SARS-CoV-2 is strikingly similar to that of SARS-like coronaviruses found in bats, and phylogenetic data from recent genomic studies on bat-associated coronaviruses and SARS-CoV-2 suggest that bats are the natural reservoir for coronaviruses in general, and SARS-CoV-2 in particular [5]. It has been postulated that the reservoir for SARS-CoV-2 is the Chinese horseshoe bat, which is known to host SARS-like coronaviruses. It is now hypothesized that one of the reservoir coronavirus species in bats crossed the species barrier to an intermediate mammal host (presumed to be a masked civet) sold at the wet market at the epicenter of the current epidemic, with subsequent mutation and transmission to humans, initiating the present epidemic of COVID-19.

It has been noted that the two previously known human coronaviruses causing epidemic disease and spread, SARS-CoV and MERS-CoV, had a relatively low rate of spread from an individual infected patient (an index referred to as its basic reproductive number- $R_0$). The $R_0$ of SARS was estimated to be around 3, meaning that on average, each infected patient is presumed to spread the virus to 3 other individuals [6]. It is currently estimated that the $R_0$ for SARS-CoV-2 is between 2.2 and 2.7 [6, 7]. However, approximately 10% of individuals infected with SARS-CoV and MERS-CoV were associated with a phenomenon referred to as “super spreading”, associated with an $R_0$ >10 [8]. Wide transmission and spread of SARS-CoV and MERS-CoV occurred to a large extent by means of super-spreading events [8]. Human super spreaders for SARS-CoV-2 have not

been identified thus far in limited epidemiological studies conducted in the past six weeks of the outbreak [6]. However, clinicians and researchers should be acutely aware of the likelihood for the potential existence of such transmitters of SARS-CoV-2 infection in the general population, and of the means to identify and isolate such individuals expeditiously in order to prevent a reduction of the current epidemic doubling time of approximately 7 days, and to limit viral transmission and spread [7]. A compelling mathematical modeling study done by researchers at Hong Kong University indicates, despite limitations to their study, that these figures may not be a fair representation of the actual scale of the SARS-CoV-2 outbreak in China. Their figures estimated that the total number of infected individuals in greater Wuhan alone on the 25th January 2020 was 75,815 (95% confidence interval [CI] 37,304–130,330) persons, and that if there was no reduction in viral transmissibility, the epidemic in Wuhan would peak at around April 2020 [7]. Ominously, a further mathematical model, proposed by Tang *et al*, [9] suggests that the basic reproductive number for SARS-CoV-2 might be as high as 6.47.

The putative zoonotic origin of SARS-CoV-2, and the zoonotic origins of the SARS and MERS epidemics, brings into sharp focus the existence of unregulated wet markets in China, trading in live wild game, game meat and game products. Zoonotic origins for emerging viral infections are not new, with AIDS, Ebola, influenza viruses, SARS, MERS, and a multitude of other viral illnesses all crossing the species barrier and causing devastating illness in humans, at enormous economic and human cost [10]. The presence and availability of markets that trade in wild animals for human consumption, and for purchase as pets, greatly increases the potential for viral infections originating from these reservoir animals to jump to human populations. The complete ban on market trading and sale of wild game meat in China on 26th January 2020 will help prevent zoonotic transmission of SARS-CoV-2 in the current epidemic and, to a certain degree, help prevent emergence of new zoonotic infections. Further social and cultural changes regarding wild game trading and consumption is required in China and worldwide, to prevent scenarios where regular emergence of zoonotic infections becomes commonplace, with their inevitably attendant economic and human costs. It is estimated that the SARS epidemic cost the global economy approximately $US 54 billion in 2003 alone. The 2015 MERS outbreak in the Republic of Korea resulted in a $US 2.6 billion loss for the South Korean tourism industry alone. The 2014 outbreak

of Ebola in Guinea, Liberia and Sierra Leone costed their already lean economies approximately $US 300 million. The human and economic costs of the SARS-CoV-2 outbreak to the global economy will, without doubt, be scrupulously studied after the present outbreak ends, and the global economic costs will be immense, and the human cost, agonizing. Each preventable zoonotic outbreak costs the country of origin and the world vast amounts of money and resources, and an inestimable cost in human lives, and if emerging zoonotic outbreaks can be prevented by severely limiting human exposure to wild animals and their trade, then effective measures to ensure that this occurs should be implemented by regulatory government authorities globally as soon as it is practicable.

It is clearly apparent that the work done thus far in the quest to contain the current SARS-CoV-2 outbreak is massive, focused and resolute. It is also abundantly evident that a large quantum of work remains to be done in order for the current public health effort to be successful in containing the present outbreak. Managing this requires international cooperation using traditional and proven public health strategies that ultimately succeeded inthe SARS epidemic. It is, however, inevitable that new zoonotic infections will emerge in the future. It is therefore an urgent priority for local and international health and wildlife regulatory authorities to structure and implement robust control mechanisms that effectively reduce human exposure to wild game meat and their products. In contrast to Africa, the consumption of wild game meat in Asia is not generally motivated by poverty, hunger or starvation. The common motivations for the human consumption of wild game meat in Asia are for their purported medicinal value, and the supposed health-enhancing effects of certain varieties of wild game meat, or their products. Specific rare and exotic Asian and other international wild game and their products, are also consumed and offered to guests and influential persons in an effort project status, prestige and wealth, depending on the rarity of the animal involved. There is also the existence of wildlife trafficking between Asia and other regions of the world, which has created an international supply and demand chain, with savvy wildlife entrepreneurs marketing wild game meat and products as “traditional specialties”, in their effort to boost sales. The existence of local and international wildlife trade for meat and animal products needs urgent and decisive change. It is fervently hoped that the steadfast efforts by China, in partnership with the international community, will reap positive results with

respect to SARS-CoV-2 control in the future weeks and months. Additionally, urgent international attention to and curtailment of the hitherto unregulated and commonplace trade in wild game, meat and products is essential if a repeat of the human and economic loss, and public fear and social disruption wreaked by the current SARS-CoV-2 outbreak is to be avoided in the future.

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**Conflict of Interest**

None

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**Types：Perspective**

# From SARS-CoV to 2019-nCoV Outbreak: Similarities in the Early Epidemics and Prediction of Future Trends

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Emerging infectious diseases represent a serious threat for human public health worldwide[1,2]. The 2019 novel coronavirus (2019-nCoV) caused a pneumonia outbreak originating in Wuhan, China, which is spreading around the country and has affected 32 provinces and regions of China as of January 27, 2020[3,4]. Countries outside China, including Japan, the United States, Thailand, and South Korea, have also reported cases imported from other countries [5]. With the joint efforts of Chinese scientists, health workers, and related

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departments, the pathogen causing this epidemic was quickly identified as a new type of coronavirus, 10 days after the first official report. After confirming the pathogen, specific detection methods were rapidly developed, with improvement in etiological diagnosis. As of January 22, 2020, it has been confirmed that the new coronavirus came from wild bats and belonged to group 2b of the beta coronavirus, which includes severe acute respiratory syndrome-associated coronavirus (SARS-CoV)[6]. Although 2019-nCoV and SARS-CoV belong to the same subgroup of beta coronaviruses, the similarity at the genome level is only 80%[7,8]. , meaning that the new virus is genetically different from SARS-CoV [Supplementary Figure 1A]. Rapid discovery of the causative agent and development of diagnostic reagents demonstrated that technology has greatly improved in the 17 years since the SARS outbreak. However, no effective antiviral medication or vaccines are available for this new virus, and many of its aspects remain to be explored. Similar to the SARS outbreak, this outbreak also occurred during the spring festival, the most important of the Chinese traditional festivals, when 3 billion people travel throughout the country[9]. This unexpectedly provides beneficial conditions for the transmission of this highly infectious disease and correspondingly poses great challenges for the prevention and control of the outbreak.

Although technology has greatly improved since the 2003 SARS outbreak, the basic laws and characteristics of the occurrence and development of infectious diseases have not fundamentally changed[10]. Therefore, the epidemic laws and characteristics of the SARS outbreak and the painful lessons we learned in responding to the epidemic are of great value currently and in the future. Due to concerns about controlling the impact of the epidemic and the relatively less developed information exchange tools of that time, the early epidemics and characteristics of the early SARS cases were not reported. However, as we had participated in the epidemiological investigations of early SARS cases in 2003, we had collected important data about the early stages of the outbreak. Using these valuable data, we analyzed the characteristics of the early SARS cases and the progression of the outbreak. By comparing the epidemic situations of the two outbreaks, we found some strikingly similar characteristics and trends, providing lessons for better responses to the present and future epidemics.

On January 2, 2003, a hospital in Heyuan city, Guangdong Province, reported two strange cases of severe pneumonia, which were then transferred to a larger hospital for further treatment. Several days later, seven

medical staff members in the department that treated these patients developed symptoms. Retrospective investigation found that a hospital in Foshan had treated a similar case on November 25, 2002 [Figure 1A]. This patient developed symptoms on November 16, 2002, and subsequently, five family members also developed symptoms. This indicated that SARS-CoV emerged with high human-to-human transmission capability, characterized by familial and medical staff infections[11,12]. An investigation of family clustering identified 35 clusters involving 105 patients, in families with two or more family members in Guangzhou. The largest cluster was derived from a female patient. A total of 91 persons were infected due to visiting or nursing the female patient, and two of these people died[13] [Figure 1B]. This indicated that the super virus spreader emerged at the earliest stage of the outbreak, confirming the high infection capability of the virus[14,15]. Subsequent case investigations also showed that SARS-CoV had the capability to multiply and continuously undergo human-to-human transmission [Figure 1C]; at least four generations of cases were identified from one original patient. Among the clusters of cases, healthcare workers were common victims[16]. As of April 13, 2003, a total of 48 medical institutions had medical staff with SARS-CoV infection, and 33 medical institutions in Guangzhou reported a total of 283 cases. The incidence among medical staff in the respiratory care department of a university affiliated hospital in Guangzhou was 61.7% (29/47), *i.e.*, more than half of the medical staff were infected while treating their patients[17].

As for the 2019-nCoV outbreak, the first patient with unexplained pneumonia was identified on December 12, 2019. On December 31, 2019, 27 cases of viral pneumonia were officially announced; seven of these patients were in a severe condition[18]. Respiratory infectious diseases, including influenza, SARS, and Middle East respiratory syndrome (MERS), were screened for and excluded[19]. On Jan 3, 2020, only 1 week later, a new type of coronavirus was discovered. The identification of pathogenic nucleic acids was completed on January 10[20], and on January 12, the World Health Organization officially named the new coronavirus the "2019 novel coronavirus."(news) It took less than 10 days from the first official announcement to the identification of the pathogen. In contrast to that of SARS-CoV, the discovery of human-to-human transmission of 2019-nCoV came relatively late. On Dec 31, 2019, 27 confirmed pneumonia cases were officially reported, no human-to-human transmission case was identified[18]. On Jan 19, 2020, a cluster of cases, including 15 healthcare workers, were confirmed to have been infected via patients, confirming that

2019-nCoV also has human-to-human transmission capability[21].. Based on these results, it was concluded that 2019-nCoV also has high human-to-human transmission capability. It remains unclear whether earlier cases also showed this capability, and if so, how many victims were not identified. The close contacts of these unidentified patients might act as new infection sources and could become super-spreaders.

The incidence and development process of the SARS outbreak has valuable implications for the 2019-nCoV outbreak. After discovering the earliest case identified on Nov 16, 2002, the incidence remained low until Jan 2, 2003. The peak of the incidence was observed between Jan 3 and Feb 4, 2003, and the number of cases accounted for 54.7% of the total cases(Wikipedia). According to the case numbers and the developmental characteristics, the SARS epidemic can be roughly divided into four stages: stage 1, from Nov 16, 2002 to Jan 31, 2003; stage 2, from Feb 1 to Mar 2, 2003; stage 3, from Mar 3 to Apr 2; and stage 4, after Apr 4 [Figure 1D]. Coincidentally, the SARS outbreak duration also coincided with the Chinese spring festival. Each year, the Chinese government launches a 40-day spring festival transport support system, and during this period, billions of people migrate around China. In 2003, the spring festival transport period started from Jan 17 to Feb 25, 2003 and coincided with the peak incidence [Figure 1D, purple box]. The spring festival travel period in 2020 started from Jan 10 to Feb 18, which coincided with the rapid increase in 2019-nCoV cases between Jan 10 and 22, 2020 [Figure 1D, red box]. Both outbreaks happened in the winter, when the two provinces have similar climate patterns suitable for virus survival and spread. Temperature and weather are risk factors of natural infectious diseases, and those in Wuhan and Guangzhou seem to be suitable for disease transmission. Given previous trends, this is unlikely to be the incidence peak of this new virus outbreak. The daily counts of 2019-nCoVcases were higher than the daily counts of SARS cases during its peak in 2003, implying a possibly higher number of cumulative cases[10]. We analyzed the transportation between different and large cities. High frequency transportation is mainly distributed among megacities [Figure 1E]. The highest ranked cities include Beijing, Guangzhou, and Shanghai[22]. Wuhan, the outbreak center, has a population of 10 million and is also a major hub of the spring festival transportation network[23]. The predicted number of passengers traveling during the 2020 spring festival is 3.11 billion, 1.7 times the total number in 2003 (1.82 billion) [Figure 1F]. This large-scale migration has brought favorable conditions for disease spread that are difficult to control.

Because we are now in the early stage of the outbreak, we must be prepared for subsequent larger-scale

outbreaks and predict the scale of the outbreak. Since 2019-nCoV is highly similar to SARS-CoV, some important characteristics of SARS-CoV could be used for this prediction. By combining the reported daily counts of 2019-nCoV cases and data from the SARS outbreak, we constructed a logistic model and predicted the incidence of 2019-nCoV over time. During the 2003 SARS outbreak, a total of 8000 cases were reported[24]. With this data and the present situation, we predict that the cumulative number of 2019-nCoV cases might be 60,000–70,000. Logistic models were fitted to these data, and the cumulative and daily counts of 2019-nCoV cases were predicted. As shown in Supplementary Figure 1B &1C, we also calculated the time needed to reach the peak of incidence under different scenarios. Setting the upper limit of cumulative incidence (K) to 50,000, 60,000, or 70,000, the end date of incidences will be in 56 days (Mar 6, 2020), 60 days (Mar 10, 2020), or 62 days (Mar 12, 2020), respectively.

Using valuable epidemiological data from the SARS outbreak, we systematically evaluated and compared the characteristics of the 2019-nCoV and SARS-CoV outbreaks. The two outbreaks share many similarities, and the ongoing 2019-nCoV outbreak situation seems to be a repetition of the SARS-CoV outbreak situation. Fortunately, the Chinese government is implementing many efficient measures, including shutting down public transportation in Wuhan and other cities, reducing population migration, and encouraging personal protection such as mask-wearing. With these measures, case numbers could be reduced significantly. However, due to the lack of awareness regarding the human-to-human transmission capability of 2019-nCoV in the early stages, there is a possibility that super-spreaders exist[25]. These super-spreaders may be distributed in different places and are difficult to track. This represents the most important problem for this outbreak.

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**Conflicts of Interest**

None

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**Figure 1**

A. Human-to-human transmission was observed in the first identified cases.

B. In the „one-to-X‟human-to-human transmission clusters, 91 persons were infected via the super spreader.

C. The „n-x-y‟continuous human-to-human transmission cluster. Up to four generations of transmitted patients were observed in this cluster.

D. The two outbreaks happened around the Chinese spring festival, when billions of people travel between different locations. Red box, spring festival duration of 2020 (Jan 10 to Feb 18, 2020); Purple box, spring festival duration of 2003 (Jan 17 to Feb 25, 2003); the first official reports of the outbreaks were given in Jan 2, 2003, and Dec 27, 2019.

E. Population movement network during the spring festivals of 2003, 2019, and 2020.

F. Passenger flow surge during the spring festivals of 2003, 2019, and 2020. The total passenger numbers are 1.81, 2.91, and 3.11 billion for 2003, 2019, and 2020, respectively. The spring festival transport data for 2020 was predicted.

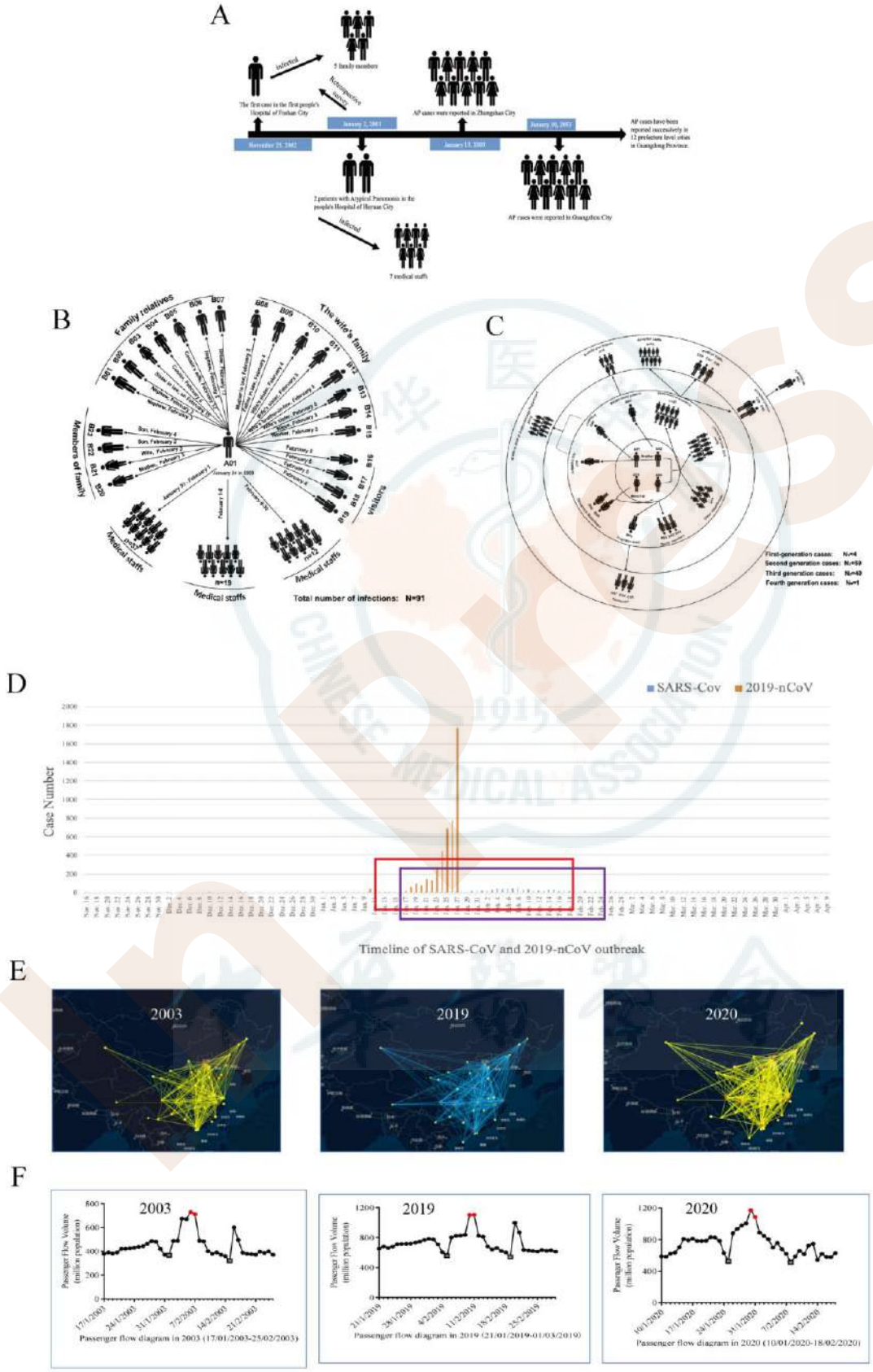

A
B
C
D
E
F
Family relatives
The wife's family
Members of family
Visitors
Medical staffs
Total number of infections: N=91
SARS-Cov
2019-nCoV
Case Number
Timeline of SARS-CoV and 2019-nCoV outbreak
2003
2019
2020
Passenger Flow Volume (million population)
Passenger flow diagram in 2003 (17/01/2003-25/02/2003)
Passenger flow diagram in 2019 (21/01/2019-01/03/2019)
Passenger flow diagram in 2020 (10/01/2020-18/02/2020)

**Figure S1**

A. Basic epidemiological characteristics of the 2019-nCoV and SARS-CoV outbreaks.*the Chinese annual spring festival travel surge was from Jan 17 to Feb 25 in 2003 (1.8 billion passengers) and from Jan 10 to Feb 18 in 2020 (estimated number of passengers, 3.1 billion). B-C. Fitting logistic models to the dynamics of global infection cases of SARS-CoV-2. Prediction results of cumulative cases and daily cases with upper limits of cumulative cases of 50,000, 60,000, and 70,000. t=0 denotes Jan 11, 2020.

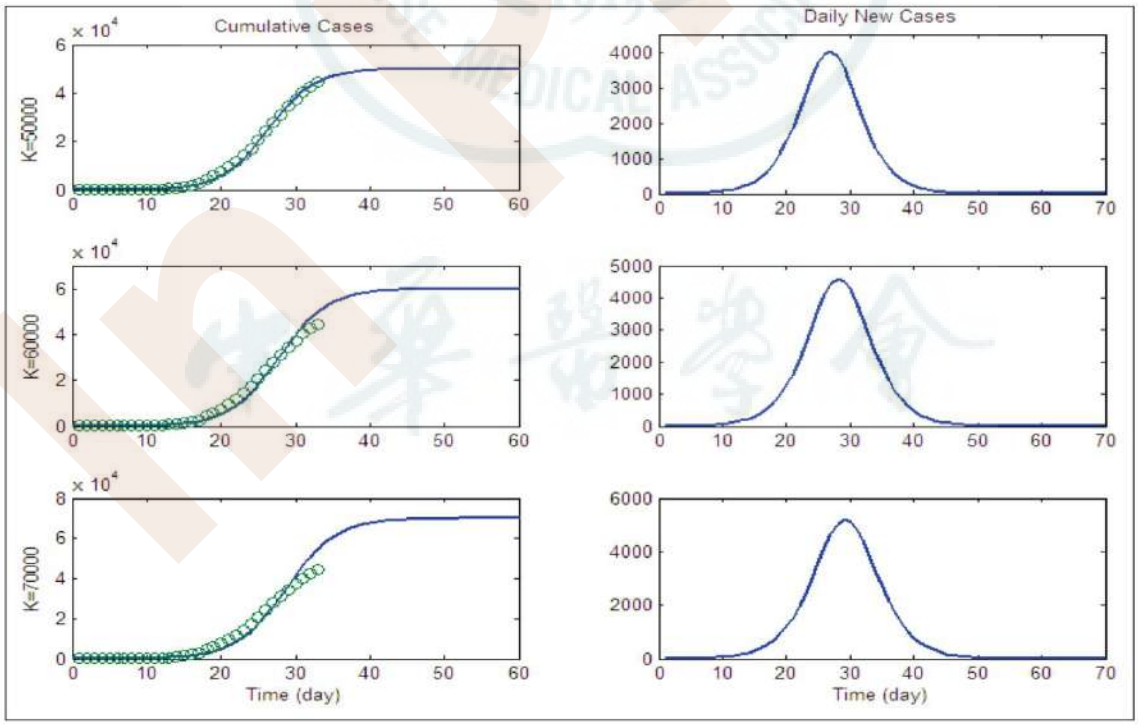
A

| Characteristics | 2019-nCoV | SARS |
|---|---|---|
| Lineage | Beta-coronavirus Group 2b, with >70% genetic similarity | |
| Source | Bat (Chrysanthemum) | Civet and bat |
| Latent period | 2-14 days | 2-7 days |
| Human-to-human transmission | Confirmed | |
| Duration time* | On-going since Dec 31, 2019 | Nov 16, 2002 to July 2003 |
| Cases | 913 known cases with 17 fatalities (as Jan 23, 2020) | 8, 098 cases with 774 fatalities |
| Current status | Mainly reported in Wuhan city. Imported cases are identified in other regions of China and foreign countries, including U.S.. | No reported new cases since 2004 |
| Symptom | Fever, cough and shortness of breath | |
| Treatment | No effective antiviral medication or protective vaccine | |
| Prevention | Reduce unnecessary exposure, hand and respiratory hygiene and safe food practices | |

B

| K | a | r | $R^2$ | *F* | *P* | $t_{end}$ (days) | $t_{end}$ (date) |
|---|---|---|---|---|---|---|---|
| 50000 | 8.459 | 0.322 | 0.986 | 2186.0 | 0.001 | 56 | Mar 6, 2020 |
| 60000 | 8.481 | 0.306 | 0.981 | 1616.1 | 0.001 | 60 | Mar 10, 2020 |
| 70000 | 8.559 | 0.298 | 0.978 | 1346.7 | 0.001 | 62 | Mar 12, 2020 |

C



## Viewpoint

# Personal knowledge on novel coronavirus pneumonia

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The epidemiology of novel coronavirus (2019-nCoV) infection in some patients is unclear, and the incubation period of the virus can last for 2 weeks, even longer. During the period of latent infection or the period of incubation following infection, the disease may be infectious. As in cases of influenza, some patients develop only upper respiratory tract infection, whereas others with a severe form of the disease develop pneumonia. Patients may not have fever, mild cough, or apparent respiratory symptoms, and headache or gastrointestinal symptoms may be present. Some patients show insidious onset and slow progression, and do not appear to be sick. Thus, they may not receive attention or be identified. Some patients with severe disease or critical illness may present with moderate to low-grade fever, but apparent fever may also be absent.

Even though several clinical studies have assessed the use of

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corticosteroids in acute respiratory distress syndrome (ARDS) and severe viral pneumonia, it remains unclear whether corticosteroids treatment can decrease mortality and improve patients' outcomes.

In ARDS, corticosteroids are believed to antagonize certain pathophysiological processes, including hyperinflammation, excessive cell proliferation, and aberrant collagen deposition.[1] However, evidence-based clinical research gives us other insights. As for severe acute respiratory syndrome (SARS), a retrospective study revealed that patients receiving corticosteroids treatment had poorer outcomes, such as higher risk of intensive care unit admission and higher mortality, even though they were younger and had fewer underlying diseases.[2] Furthermore, it was found that corticosteroids did not improve mortality and could delay viral nucleic acid clearance in Middle East Respiratory Syndrome (MERS), which is also caused by a coronavirus.[3] A large meta-analysis which included 16 studies on influenza A virus subtype H1N1 infection showed that corticosteroids increased mortality.[4]

In contrast, other studies reported that short-term treatment with corticosteroids may decrease the risk of acute respiratory distress syndrome (ARDS) and shorten the length of the disease in patients with severe community-acquired pneumonia.[5] In addition, the use of corticosteroids in ARDS caused by *Pneumocystis carinii* pneumonia has obtained widespread acceptance, as it can improve oxygenation and

patients' outcomes.[6] At present, the World Health Organization does not recommend routinely applying systemic corticosteroids for the treatment of viral pneumonia or ARDS, except in clinical trials.[7] However, the 5th edition of "Novel Coronavirus Pneumonia Diagnosis and Treatment Protocol" recommended short-term (3–5 days) treatment with corticosteroids for severe and critical cases should be based on the comprehensive assessment of patients' dyspnea level and the progression observed on chest imaging, with the dose not exceeding a methylprednisolone equivalent dose of 1–2 mg/kg per day.[8] Currently, there is insufficient evidence of the value of corticosteroids in the treatment of novel coronavirus pneumonia (NCP), and further high-quality randomized controlled trials (RCTs) are warranted.

Despite the numerous RCTs on ARDS in the last 30 years, there has been no significant reduction in ARDS mortality. ARDS caused by 2019-nCoV appears to be more severe than that observed routinely. In this outbreak of NCP, the majority of critically ill patients have been aged 50 years and above, with a large number of them aged 70–80 years. These patients often had underlying diseases such as hypertension, diabetes, and coronary heart diseases, with some having multiple underlying diseases.

Our previous clinical experience and observations indicated that many patients with severe illness receiving high-flow nasal cannula (HFNC) oxygen therapy or non-invasive ventilation (NIV) (fraction of inspired

oxygen [$FiO_2$] of 1.0) have oxygenation indexes (partial pressure of arterial oxygen [$PaO_2$]/$FiO_2$) below 150 mmHg or even lower than 100 mmHg. We observed that such oxygenation support was required for a longer time, indicating that the hypoxic duration in these patients was longer. Extended durations of hypoxia can cause irreversible organ damage. Even with the subsequent use of invasive ventilation or extracorporeal membrane oxygenation (ECMO), the rate of successful resuscitation in such patients remains very low. Therefore, we suggest that patients with an oxygenation index below 150 mmHg after being treated with non-invasive ventilation for 2 hours with an $FiO_2$ of 1.0 or a relatively high $FiO_2$ should receive endotracheal intubation as soon as possible to enable invasive ventilation. WHO' interim guidance also suggested that HFNC and NIV should only be used in selected patients with hypoxemic respiratory failure, and patients treated with either HFNC or NIV should be closely monitored for clinical deterioration.[7]

If oxygenation index remains below 100 mmHg after invasive ventilation for 24 hours with high positive end-expiratory pressure (PEEP) in prone position, ECMO should be used promptly. This is consistent with the recommendations of Chinese Society of Extracorporeal Life Support.[9] The 5th edition of "Novel Coronavirus Pneumonia Diagnosis and Treatment Protocol" also recommended that endotracheal intubation and invasive mechanical ventilation should be performed promptly if the

condition does not improve or even deteriorate within a short period of time (1 to 2 hours) when using HFNC or NIV, and in case invasive mechanical ventilation in prone position is ineffective, ECMO should be performed at the earliest if possible.[8]

The use of personal experiences to guide treatment is not recommended.

Supportive treatment remains the mainstay for NCP. Respiratory support ensures that the patient is not hypoxic and also protects other organs. There are currently no effective antiviral drugs, and antimicrobial drugs should be administered strictly and rationally. Shuanghuanglian and similar drugs have demonstrated inhibitory effects against the virus in *in vitro* experiments. However, their clinical effects are unknown, and clinical experiments are required to demonstrate their efficacy.

**Conflicts of interest**

None.

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**Rapid report**

# Single-cell RNA sequencing data suggest a role for angiotensin-converting enzyme 2 in kidney impairment in patients infected with 2019-nCoV

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**Conflict of interest**

None.

The World Health Organization has recently declared the novel coronavirus (2019-nCoV) a global public health emergency. Huang et al.[1] reported acute kidney injury (AKI) in 7% of the 41 patients infected with 2019-nCoV, this value was even higher (up to 31%) among intensive-care patients. Furthermore, Li et al.[2] reported that plasma creatinine levels increased in 11 of 59 patients with 2019-nCoV infection, suggesting that kidney function was probably impaired when the disease progressed. Guan et al.[3] reported that plasma creatinine level increased in 4.3% of severely diseased patients. 2019-nCoV is a highly contagious pathogen that predominantly causes pneumonic symptoms. To date, infection by this virus has caused tens of thousands of fatalities, and hundreds of thousands of people have been isolated as a preventive measure. Although respiratory failure has been associated with the highest mortality, the lungs were not the only organs involved. Hoffmann et al.[4] reported that 2019-nCoV and SARS-CoV share a common receptor angiotensin-converting enzyme 2 (ACE2) that is required to enter target cells, and cellular protease transmembrane protease serine 2 (TMPRSS2) can cleave and activate the spike protein of 2019-nCoV for membrane fusion. We investigated whether ACE2 and TMPRSS2 were expressed in kidney cells using precision-technology single-cell RNA sequencing.

Single-cell RNA sequencing data were acquired from the Gene Expression Omnibus (GEO) database and from the Kidney Interactive Transcriptomics (KIT) database

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(http://humphreyslab.com/SingleCell/). Original sequence data were downloaded from the GEO database for further analyses (accession numbers GSE131685, GSE112570, GSE109564, and GSE114156), and immunohistochemical staining results were acquired from the Human Protein Atlas (http://www.proteinatlas.org).

R software (version 3.6.1, https://www.r-project.org/) and the Seurat package (version 3.1, https://satijalab. org/seurat/) were used for the single-cell RNA sequencing data processing.

In order to investigate whether ACE2 was expressed in a specific cell type in human kidneys, published single-cell RNA sequencing data were downloaded from the GEO and KIT databases. Kidney samples assigned the GEO accession numbers GSE109564 and GSE114156 originated from a healthy donor, and 4487 cells were retained for further analysis after quality control. Kidney samples under accession number GSE131685 originated from para-carcinoma tissue of three patients with tumors, and 23,366 cells were retained for further analysis after quality control; data from four samples were combined for further analysis. Fetal kidney samples originated from embryos of 8–18 weeks, and 7343 cells were retained for further analysis after quality control. ACE2 was mainly expressed in proximal tubule cells in cases under the accession numbers GSE109564 and GSE114156 (Figure 1A). Accordingly, ACE2 was found to be expressed predominantly in tubular precursors of the kidney of the fatal case (Supplemental Figure 1). Similarly, in GSE131685, ACE2 was also expressed mainly in proximal tubule cells (Figure 1B). TMPRSS2 was predominantly expressed in the loop of Henle and in the collecting duct in GSE109564 and GSE114156 (Supplemental Figure 1). Single-cell RNA sequencing of fetal and adult kidney samples revealed that ACE2 was mainly expressed in tubule cells.

After verifying ACE2 expression in specific kidney cell types at RNA level, we investigated whether this was consistent at a protein level using the Human Protein Atlas. Interestingly, ACE2 was found to be expressed in several human organs such as the intestines, adrenal gland, gallbladder, and in the kidneys, and it was highly expressed in the urogenital and digestive systems. ACE2 was highly expressed in the glandular cells of the intestine and gallbladder (Figure 1C). As 2019-nCoV preferably occurs in the lungs, we tested whether ACE2 was also expressed in lung tissue; however, we found that ACE2 showed only low expression levels in normal lungs, and only some positive staining was observed in lung macrophages (Figure 1C). Therefore, whether ACE2 levels would increase due to 2019-nCoV infection requires further investigation. Consistent with single-cell RNA sequencing data, ACE2 was predominantly expressed in the proximal tubules (Figure 1C).

Our results showed that ACE2 and TMPRSS2 were expressed in the human kidney, indicating that the kidney is a potential target organ of 2019-nCoV. These findings may suggest that antibodies or biological inhibitors targeting virus proteins such as spike protein, the ACE2 receptor, or protease TMPRSS2 could potentially be part of therapeutic strategies.

Among patients infected with SARS-CoV, 6.7% (36/536) exhibited AKI with a median duration of 20 days (from 5 to 48 days) despite normal plasma creatinine levels at the first clinical presentation, and those who experienced AKI eventually suffered extremely high mortality of up

to 91.7% (33/36)[5]. Middle East respiratory syndrome-related coronavirus (MERS-CoV) has also been found in 26.7% (8/30) of the patients with AKI, and the mean and median durations until occurrence of AKI from symptom onset were 18 and 16 days, respectively. The receptor of MERS-CoV, DPP4, is also expressed in kidney cells such as tubule cells and podocytes. Furthermore, tubules are often found to be severely damaged during AKI caused by various reasons. High expression of the coronavirus receptors ACE2 and DPP4 in kidney tubule cells suggests that the kidney is at high risk of coronavirus infection.

Thus, there is an urgent need to develop specific drugs that target coronavirus receptors so as to prevent kidney damage. Moreover, kidney functions in patients infected with 2019-nCoV should be monitored frequently, particularly in patients with increased levels of plasma creatinine. Early interventions, including continuous renal replacement therapies, should be applied as early as possible to preserve kidney function in patients who show signs of kidney failure such as increased concentrations of urine protein, blood urea nitrogen, or plasma creatinine.

**Acknowledgement**

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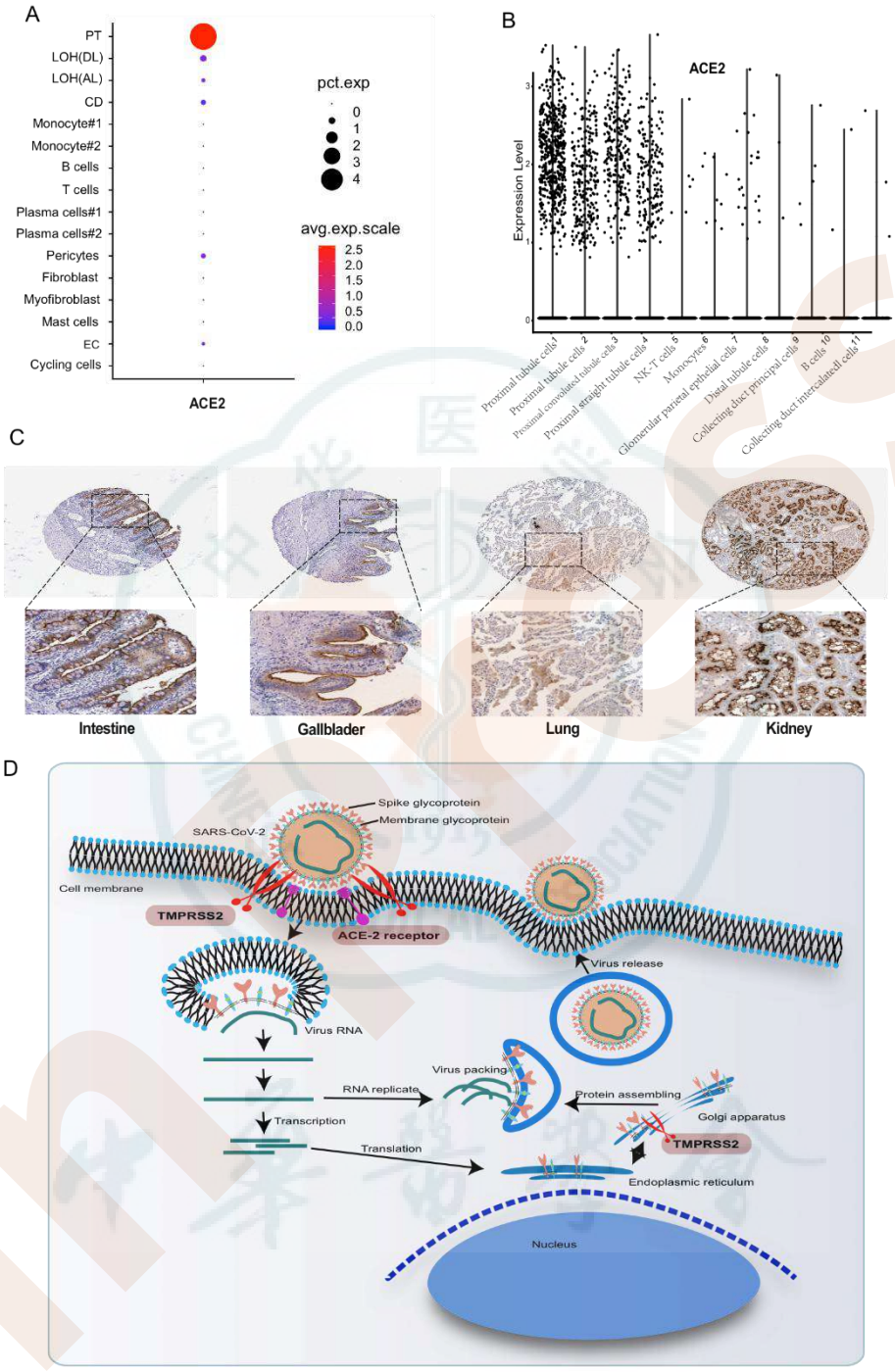
**Figure legend**

**Figure 1.** (A) Expression of ACE2 in different cell clusters of cases with accession numbers GSE109564 and GSE114156. (B) Expression of ACE2 in different cell clusters of accession number GSE131685. (C) Immunohistochemical staining of ACE2 in human organs. (D) Illustration of 2019-nCoV entering the target cell. ACE2: Angiotensin-converting enzyme 2.


A
PT
LOH(DL)
LOH(AL)
CD
Monocyte#1
Monocyte#2
B cells
T cells
Plasma cells#1
Plasma cells#2
Pericytes
Fibroblast
Myofibroblast
Mast cells
EC
Cycling cells
ACE2
pct.exp
0
1
2
3
4
avg.exp.scale
2.5
2.0
1.5
1.0
0.5
0.0
B
ACE2
Expression Level
Proximal tubule cells 1
Proximal tubule cells 2
Proximal convoluted tubule cells 3
Proximal straight tubule cells 4
NK-T cells 5
Monocytes 6
Glomerular parietal epithelial cells 7
Distal tubule cells 8
Collecting duct principal cells 9
B cells 10
Collecting duct intercalated cells 11
C
Intestine
Gallbladder
Lung
Kidney
D
Spike glycoprotein
Membrane glycoprotein
SARS-CoV-2
Cell membrane
TMPRSS2
ACE-2 receptor
Virus release
Virus RNA
Virus packing
RNA replicate
Protein assembling
Golgi apparatus
Transcription
Translation
TMPRSS2
Endoplasmic reticulum
Nucleus

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