Clinical and biochemical indexes of 11 COVID-19 patients and the genome sequence analysis of the tested SARS-CoV-2

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Abstract

BackgroundAt present, SARS-CoV-2 epidemic in the world rapidly spread. It is a serious global public health emergency.

MethodsHere we described the clinical characteristics of 11 SARS-CoV-2 infected patients hospitalized in the Meizhou People's Hospital. And viral genome sequences of SARS-CoV-2 from these patients were analyzed.

ResultsOf the 11 patients, six cases developed fever, nine cases developed cough, and two cases developed headache and chills. Four patients (36.4%) had underlying diseases. Pneumonia is the most common complication. The laboratory test results showed that there was no adult patients with increased LYM/LYM%. Most patients had normal total protein (TP) and albumin (ALB), but only two patients had decreased. Most patients had increased or normal levels of erythrocyte sedimentation rate (ESR), C reactive protein (CRP), activated partial thromboplastin time (APTT), fibrinogen (FIB), creatine kinase isoenzymes (CK-MB), and lactate dehydrogenase (LDH). Neutrophil (NEU) ($r=0.664$, $P=0.026$), CK-MB ($r=0.655$, $P=0.029$), blood urea nitrogen (BUN) ($r=0.682$, $P=0.021$) and SARS-CoV-2 virus cycle threshold (Ct) value were significantly correlated. Multiple sequence alignment (MSA) shows that we identified two different SNPs at positions 8781 and 28144, and have a completely linked genetic form of 8781C-28144T and 8781T-28144C.

ConclusionsThe reports of these 11 cases in our hospital will provide useful information for the diagnosis, treatment and drug development of SARS-CoV-2.

Background

In December 2019, a pneumonia caused by a novel coronavirus (2019-nCoV) first broke out in Wuhan, Hubei province, China. The novel coronavirus spread rapidly from person to person and spread, with confirmed cases appearing in a number of countries. On January 30, 2020, the World Health Organization (WHO) issued a statement declaring it is a global public health emergency[1]. The disease was later named as Coronavirus Disease 2019 (COVID-19) by the WHO[2], meanwhile 2019-nCoV was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) officially by the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV)[3].

In the past two decades, there have been two other virus epidemics caused by severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002-2003[4-6] and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012[7-10]. Based on the analysis of genome sequence, SARS-CoV-2, SARS-CoV and MERS-CoV belong to the β -coronavirus, the sequence similarity between SARS-CoV-2 and SARS-CoV is about 80%, and that between SARS-CoV and MERS-CoV is about 55%[11]. The virulence of SARS-CoV-2 is relatively weak compared with MERS-CoV and SARS-CoV[12], the virus spreads by human-to-human transmission and people are generally susceptible[13]. Since February 2020, SARS-CoV-2 is spreading around the world, causing a huge social and economic burden to various countries, and is being attracted the attention of the world.

Analysis of the clinical characteristics of confirmed COVID-19 patients is helpful to provide valuable information for the diagnosis and subsequent treatment of this disease. On the other hand, the viral genome sequences analysis is helpful for understanding of the mechanism of viral infection and the potential treatment options selection, and laying a foundation for the research and development of subsequent vaccines and drugs[11, 14-16].

In this study, we described the clinical characteristics of 11 patients with SARS-CoV-2 infected. All these patients were hospitalized in the Meizhou People's Hospital, Guangdong Province, China. Clinical characteristics and blood indexes of COVID-19 patients were recorded and examined in our hospital. The respiratory samples of patients were collected and real-time reverse transcription polymerase chain reaction (RT-PCR) was used to confirm SARS-CoV-2 infection. On the other hand, viral genome sequences of SARS-CoV-2 from these patients were analyzed. Our results should help physicians in the diagnosis, treatment of COVID-19 patients and drug development for SARS-CoV-2.

Materials And Methods

Patients

In this study, 11 COVID-19 inpatients of Meizhou People's Hospital from January to May 2020 were collected as subjects. This study was conducted on the basis of the Declaration of Helsinki and was supported by the Ethics Committee of the Meizhou *People's Hospital*.

Data collection and analysis of clinical findings

Clinical information, including complete blood counts, blood biochemistry was collected at the earliest time possible after admission. The main information including initial symptoms (fever, cough, headache, myalgia, chill, vomiting and diarrhea), underlying diseases, complications (pneumonia, ARDS, liver, kidney, heart function) and therapeutic drugs (antiviral agents, corticosteroid and immunoglobulin) were collected. We collected the first results of complete blood counts and blood biochemistry of each patient upon admission.

Respiratory samples collection and nucleic acid test

Respiratory specimens (including pharyngeal swabs, nasal swabs, sputum, bronchial lavage fluid, alveolar lavage fluid, etc.) were collected on admission. Nasopharyngeal swabs were sampled and placed in test tubes containing the cell preservation solution. Sputum specimens were collected in spiral plastic tubes containing sputum digestive juices. The lavage fluid obtained by puncture was collected in the screw collector. The samples should be tested as soon as possible. The samples can be stored at 4°C within 24 hours. Samples should be stored at -70°C if cannot be detected within 24 hours, but avoid repeated freezing and thawing of samples. Viral RNAs were extracted using the Liferiver RNA extraction kit, and real-time RT-PCR was performed using the primers and probes targeting the ORF1ab, N and E genes of SARS-CoV-2 by Novel Coronavirus (2019-nCoV) real-time RT-PCR kit (Liferiver Bio-Tech Co., Ltd., Shanghai, China), which certified by the National Medical Products Administration (NMPA) of China. Accordance with the manufacturer's instructions, if the Ct value is

less than 43.0, the corresponding gene (ORF1ab, N, E gene) is positive, when the Ct value is more than 43.0 or there is no Ct value, the corresponding gene is negative. The specimens are considered SARS-CoV-2 positive if ORF1ab gene (+)-N gene (+)-E gene (+). If only one target is repeated positive and the remaining two are negative, the result is reported as indeterminate and a fresh sample should be collected from that patient. When ORF1ab gene (-), N gene (-) and E gene (-), the specimens are considered SARS-CoV-2 negative.

Viral genome sequence analysis

The SARS-CoV-2 viral genome sequence is 29.9 kb length, including ten open reading frames (ORFs), which code for helper proteins and structural proteins. The first ORF (ORF1ab) accounts for about 71% of the entire genome. The four major structural proteins are spike glycoprotein (S), envelope protein (E), matrix protein (M), and nucleocapsid protein (N).

Respiratory samples were collected and viral RNAs were extracted from 11 COVID-19 inpatients in Meizhou People's Hospital. The viral RNA was reverse-transcribed. 38 pairs of primers were used to amplify the full-length sequence of the virus gene. The 20 μ l PCR reaction mix contained 10 μ l 2 $\times$ TransTaq® High Fidelity (HiFi) PCR SuperMix II (Transgen, Beijing, China), 0.5 μ l of each primer (10 μ M) and 2 μ l template RNA. Amplification was performed as follows: 95°C for 5 min, followed by 35 cycles consisting of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min in Bio-Rad S1000 machine. Sequences analyzed by Sanger sequencing on an ABI 3500 Genetic Analyzer (Applied Biosystems, USA). These sequencing results were compared with thirty-four SARS-CoV-2 genomic sequences in China using DNAMAN software, which were collected on 26th March 2020 from GenBank database.

Statistical analysis

SPSS statistical software version 21.0 was used for data analysis. The Spearman rank correlation coefficient and Pearson's Correlation were used for correlation analysis between two groups with variables. A value of $P < 0.05$ was considered as statistically significant.

Results

Eleven COVID-19 inpatients in Meizhou People's Hospital and confirmed to be infected with SARS-CoV-2 by Meizhou Center for Disease Control and Prevention (CDC) from January to May 2020. Of the 11 patients, six cases developed fever, ten cases developed cough, and two cases developed headache and chills. There were five patients with fever and cough at the same time, five patients with cough only, and one patient with fever only. Four patients (36.4%) had underlying diseases, including hypertension, diabetes, coronary heart disease and fatty liver. Pneumonia was the most common complication. In addition, one case (Case 4) developed acute respiratory distress (ARDS) and respiratory failure. All patients were treated with anti-viral therapy (Arbidol, Lopinavir/litonavir, Prezista, Ribavirin and Interferon), and 1 patient was treated with non-invasive mechanical ventilation. Cases 4 and 6 were treated with corticosteroids. Cases 1, 2, 3, 4, 5, 6, 7, 8 and 9 were treated with immunoglobulin (Table 1).

Complete blood count and blood biochemistry were performed at the earliest time of admission for each patient. The laboratory test results showed that lymphocytopenia (LYM) and lymphocytic percentage (LYM%) were decreased or normal among adults, there was no adult patients with increased LYM/LYM%. Most patients had normal total protein (TP) and albumin (ALB), but only two patients had decreased. Most patients had increased or normal levels of erythrocyte sedimentation rate (ESR), C reactive protein (CRP), activated partial thromboplastin time (APTT), fibrinogen (FIB), creatine kinase isoenzymes (CK-MB), and lactate dehydrogenase (LDH), there was no patients with decreased ESR, CRP, APTT, FIB, CK-MB, LDH level (Table 2).

SARS-CoV-2 virus real-time PCR cycle threshold (Ct) value is reciprocal to virus load. It can indirectly reflect the severity of the infection. Spearman method was used to further analyze the correlation between Ct value (viral load) of SARS-CoV-2 virus and biochemical and clinical indicators. It was found that NEU ($r = 0.664$, $P = 0.026$), CK-MB ($r = 0.655$, $P = 0.029$), BUN ($r = 0.682$, $P = 0.021$) and SARS-CoV-2 viral load were significantly correlated (Figure 1).

According to previous research, the SARS-CoV-2 viral genome sequence was 29.9 kb long, including ten ORFs. The first ORF (ORF1ab) accounts for about 71% of the entire genome, while the remaining ORF codes for helper proteins and structural proteins. The four major structural proteins are spike glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N) [17] (Figure 2A). We identified two different SNPs at positions 8781 and 28144 (based on the reference genome NC_045512.2, SARS-CoV-2 isolate Wuhan-Hu-1) according to multiple sequence alignment (MSA). The two most variable located in the ORF1ab and in ORF8. Position 8781 located in ORF1ab gene, appears either T (U) or C variation. It causes a synonymous mutation of amino acids at position 2839 (p.Ser2839Ser). It is likely not to introduce phenotypical differences between the different strains. In addition, position 28144 located in ORF8 gene and appears either T (U) or C variation. It causes a Ser/Leu change in amino acid 9214 (p.Ser9214Leu), which Serine is a polar amino acid and Leucine is nonpolar. This may cause conformational changes in amino acid peptide chains, given that Serine is a polar amino acid, and Leucine is nonpolar. According to the sequences of the 34 viruses we analyzed, if it was a T base at position 8781, it must be a C base at position 28144. In contrast, if there is a C base at position 8781, there must be a T base at position 28144 (Figure 2B). The two SNPs were found to maybe have a completely linked genetic form of 8781C-28144T and 8781T-28144C.

We sequenced the virus of the first patient (case 1) admitted to our hospital using Sanger sequencing and the sequencing result was compared with the sequences from GenBank. The results suggested that case 1 also had variation at positions 8781 and 28144. We sequenced virus samples from other ten COVID-19 patients admitted to our hospital for two different SNPs. The results showed that case 1, 2, 6, 7, 8 and 9 was T base at position 8781 and C base at position 28144, and the C base at position 8781 and the T base at position 28144 for cases 3, 4, 5, 10 and 11 (Figure 3A). We also attached the Sanger sequencing results (Figure 3B). These sequences have been deposited to the GenBank database. And GenBank accession numbers are MT856370, MT856692, MT872190, MT872188, MT872187, MT872189, MT510727, MT860726, MT510728, MT872199, and MT872198 for position 8781. GenBank accession numbers are MT856370, MT856443, MT860461, MT860462, MT860463, MT860464, MT510727, MT856477, MT510728, MT860465, and MT860469 for position 28144.

Discussion

We report eleven COVID-19 patients admitted to Meizhou people's hospital. Consistent with other reports[18-20], the most common symptoms are fever and cough, and diarrhea is rare. Cases 1, 2, 3 and 5 have been living in Wuhan for a long time and have all come to Meizhou since late January. The son of case 4 arrived in Meizhou from Shenzhen on January 20. Cases 7, 8 and 9 (the daughter of case 7 (mother) and case 8 (father)) have been living in Nanchang city, Jiangxi Province for a long time. Cases 7 and 8 arrived in Wuhan on January 15, 2020 and returned to Nanchang on January 17, 2020. Cases 7, 8 and 9 arrived in Meizhou from Nanchang on January 25, 2020 (Figure 4). Three family clusters were identified. The sister and mother of case 1 were also COVID-19 patients (admitted to other designated hospitals). The mother of case 5 was also a COVID-19 patient (admitted to other designated hospitals). Cases 7, 8 and 9 are from a family.

Five of the 11 patients had no symptoms of fever upon admission. Patients without fever are easily overlooked, increasing the risk of transmission[13, 21]. It is important to determine the epidemiological history of a patient in clinical practice. The laboratory test results showed that LYM and LYM% were decreased or normal among adults, there was no adult patients with increased LYM/LYM%. Most patients had normal or decreased levels of TP and ALB. Most patients had normal or increased levels of CRP and LDH, there was no patients with decreased CRP and LDH level. Our results are consistent with those reported in previous studies[19, 22, 23]. In addition, we found that increased erythrocyte sedimentation rate (ESR), activated partial thromboplastin time (APTT), increased fibrinogen (FIB) and creatine kinase isoenzymes (CK-MB) were also laboratory abnormalities in some patients. Our results are consistent with some research reports[24, 25].

Our study found that NEU ($r=0.664$, $P=0.026$), CK-MB ($r=0.655$, $P=0.029$), BUN ($r=0.682$, $P=0.021$) and Ct value were significantly correlated. That is to say, NEU and CK-MB were negatively correlated with the SARS-CoV-2 viral load, and BUN was positively correlated with the SARS-CoV-2 viral load. Therefore, the combination of low NEU, CK-MB and high BUN concentration may indicate higher viral load and greater risk of transmission in patients with SARS-CoV-2 infection upon admission. A study has shown that CRP, ALB, LYM (%), LYM and NEU were highly correlated to the Ct value[22]. We hope that the reports of these 11 cases in our hospital will provide useful information for the diagnosis and treatment of COVID-19.

We identified two different SNPs at positions 8781 and 28144, one is synonymous mutation (position 8781) in the ORF1ab locus, and the other as a missense mutation (position 28144) in ORF8. The mutation of position 28144 causes a Ser/Leu change, which is predicted to be affecting the structural disorder of the protein[17]. In addition, it was a T base at position 8781, it must be a C base at position 28144. In contrast, if there is a C base at position 8781, there must be a T base at position 28144. The two SNPs were found to have a completely linked genetic form of 8781C-28144T and 8781T-28144C. According to the whole-genome molecular evolution analysis of SARS-CoV-2, it is found that there are two subtypes (L subtype and S subtype)[26]. The difference between the two subtypes lies in the position 28144 of the viral RNA genome, where L subtype is the T base (Leucine), and S subtype is the C base (Serine). It is speculated that there may be some differences in the transmission capacity and the severity of disease between L subtype and S subtype, among which L type is more common in the early stage of the outbreak in Wuhan. Among them, L subtype may be more infectious. To date, most COVID-19 patients have been infected with only one of these subtypes[26]. In our study, cases 3, 4, 5, 10 and 11 have been infected with L subtype SARS-CoV-2, only cases 3 and 5 have lived or visited Wuhan. And the severity of these patients with L subtype was not significantly different from that of other patients.

The Novel Coronavirus Pneumonia (NCP) Protocol Trial Version three to Trial Version seven released by the National Health Commission of the People's Republic of China provide some reference drugs for the treatment of COVID-19[27]. The Trial Version 3 proposes the atomized inhalation of alpha interferon and recommends the use of lopinavir/ritonavir for treatment. The Trial Version four suggested that severe patients can be treated with intestinal microecological modulators and convalescent plasma treatment. In the Trial Version five, ribavirin (4 g/dose for first day in adults, 1.2 g/dose for the next day and once every 8 hours, or 8 mg/kg/dose, once every 8 hours) was recommended. The Trial Version six recommended chloroquine phosphate (500 mg/dose, 2 times/d for adults, not exceeding 10 d) and Arbidol (200 mg/dose, 3 times/d for adults, not exceeding 10 d).

In view of the characteristics of SARS-CoV-2, such as long incubation period, strong infectivity and general susceptibility of the population, there are currently no specific drug/drugs that can better treat the COVID-19 caused by SARS-CoV-2. Therefore, there is great significance to increase the recognition of clinical features, biochemical indexes of COVID-19 patients and the molecular biology level of SARS-CoV-2 and to search for potential drug/drugs with inhibitory effect on this virus.

Conclusions

We described the clinical characteristics of 11 SARS-CoV-2 infected patients. The correlation between blood biochemical markers and viral load was analyzed. We identified two different SNPs, one of which is responsible for the serine/leucine variation of the viral ORF8 coding protein. The reports of these 11 cases in our hospital will provide useful information for the diagnosis, treatment and drug development of SARS-CoV-2.

Abbreviations

COVID-19, Coronavirus Disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; MERS-CoV, Middle East respiratory syndrome coronavirus; WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MONO, monocyte; EO, eosinophil; BASO, basophil; ESR, erythrocyte sedimentation rate; PCO₂, partial pressure of carbon dioxide; PO₂, partial pressure of oxygen; PCT, procalcitonin; CRP, C-reactive protein; TP, total protein; ALB, albumin; GLB, globulin; PA, prealbumin; PT, prothrombin time; INR, international normalized ratio; TT, thrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; CK, creatine kinase; CK-MB, creatine kinase isoenzymes; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CREA, creatinine; UA, uric acid.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-sen University (Approved on April 17, 2020). Due to this emergency public health event, the written informed consents were abandoned and oral consents were obtained from patients included in this study.

Consent for publication

Not applicable.

Availability of data and materials

Sequences information obtained from the 11 patients may be found online in the GenBank database.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Zhixiong Zhong and Xuemin Guo designed the study. Zhikang Yu, Heming Wu and Qingyan Huang performed the experiments. Heming Wu collected clinical data. Heming Wu and Zhikang Yu analyzed the data. Heming Wu and Zhikang Yu prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

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Tables

Table 1
Epidemiological and clinical features of hospitalized COVID-19 patients.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Cumulative
Age*	20–30	20–30	40–50	50–60	20–30	40–50	20–30	20–30	< 5	4
Sex	Male	Male	Male	Male	Female	Male	Female	Male	Female	Male
Initial symptoms										
Fever	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	Male
Cough	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Headache	No	No	Yes	No	Yes	No	No	No	No	Male
Myalgia	No	No	No	No	No	No	No	No	No	Male
Chill	No	No	Yes	Yes	No	No	No	No	No	Male
Nausea or vomiting	No	No	No	No	No	No	No	No	No	Male
Diarrhea	No	No	No	No	No	No	No	No	No	Male
Underlying diseases										
Chronic heart disease	No	No	No	No	No	Yes	No	No	No	Male
Chronic lung disease	No	No	No	No	No	No	No	No	No	Male
Chronic renal disease	No	No	No	No	No	No	No	No	No	Male
Chronic liver disease	No	No	No	No	No	No	No	No	No	Male
Diabetes	No	No	No	Yes	No	Yes	No	No	No	Male
Hypertension	No	No	Yes	Yes	No	No	No	No	No	Male
Cancer	No	No	No	No	No	No	No	No	No	Male
Complications										
Pneumonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
ARDS	No	No	No	Yes	No	No	No	No	No	Male
Severe ARDS	No	No	No	No	No	No	No	No	No	Male
Respiratory failure	No	No	No	Yes	No	No	No	No	No	Male
Hepatic insufficiency	No	No	No	No	No	No	No	No	No	Male
Renal insufficiency	No	No	No	No	No	No	No	No	No	Male
Cardiac failure	No	No	No	No	No	No	No	No	No	Male
Shock	No	No	No	No	No	No	No	No	No	Male
Treatment										

*, To protect patients' privacy, we provide ages as age-ranges.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	C
Antiviral agents	Arbidol	Lopnave/litonwe	Lopnave/litonwe	Arbidol	Arbidol	Arbidol	Arbidol	Arbidol	Lopnave/litonwe	A
	Lopnave/litonwe			Lopnave/litonwe	Lopnave/litonwe	Lopnave/litonwe	Lopnave/litonwe	Prezista	Ribavirin	L
	Prezista			Prezista	Prezista	Prezista	Prezista		Interferon	F
Corticosteroid	No	Yes	No	Yes	No	No	No	No	No	N
Non-invasive mechanical ventilation	No	No	No	Yes	No	Yes	No	No	No	N
Immunoglobulin	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N

*, To protect patients' privacy, we provide ages as age-ranges.

Table 2 Clinical characteristics and laboratory results of hospitalized COVID-19 patients.

	Normal range	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11
WBC($\times 10^9/L$)	3.5-9.5	4	3.7	3.6	3.4	4.8	3.9	6.6	7.1	10	3.4	5.7
NEU (%)	40.0-75.0	47.5	84.6	77.2	79.3	59.9	61	63.1	65.4	33.5	59.8	47.4
LYM (%)	20.0-50.0	38.3	9.7	14.3	17.5	28.3	28	29.4	26.4	56.6	35.4	45.3
MONO (%)	3-10	13	5	8	3	11	11	6	7	8	5	7
EO (%)	0-8	1	0	1	0	0	0	2	1	1	0	1
BASO (%)	0.00-1.00	1.1	0.3	0.2	0.3	0.3	0.1	0.2	0.3	0.4	0	0
NEU($\times 10^9/L$)	1.8-6.3	1.9	3.1	2.8	2.7	2.9	2.4	4.2	4.6	3.3	2	2.7
LYM($\times 10^9/L$)	1.1-3.2	1.5	0.4	0.5	0.6	1.4	1.1	1.9	1.9	5.7	1.2	2.6
MONO($\times 10^9/L$)	0.1-0.6	0.5	0.2	0.3	0.1	0.5	0.4	0.4	0.5	0.8	0.2	0.4
EO($\times 10^9/L$)	0.0-0.5	0	0	0	0	0	0	0.1	0.1	0.1	0	0
BASO($\times 10^9/L$)	0.00-0.06	0.04	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.04	0	0
ESR(mm/h)	0-15	6	6	28	25	37	36	30	1	NA	2	4
PCO ₂ (mm/Hg)	34.90-44.90	40.2	27.7	34.2	33.9	39.3	29.7	36.4	NA	27.6	46.8	31
PO ₂ (mm/Hg)	83.20-108.00	85.2	27.7	104.5	78.7	105.8	77.4	185.2	NA	127.2	107.6	91.4
SO ₂ (%)	83.20-108.00	93.6	213.3	98.6	93.1	98.3	93.2	99.9	NA	99.4	98.3	95.1
PCT(ng/mL)	0.00-0.05	<0.05	<0.05	0.05	0.06	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
CRP(mg/L)	0.00-6.00	1.21	8.83	19.77	46.62	10.51	22.46	0.83	2.09	1.53	3.63	3.1
TP(g/L)	65.0-85.0	79.1	68.4	76.7	62.4	67.5	62.4	65.9	70.6	75.3	69.7	72.6
ALB(g/L)	40.0-55.0	44.9	41.5	46.1	37.7	40.1	32.3	40	40.6	40.6	40.2	40.5
GLB(g/L)	20.0-40.0	34.2	26.9	30.6	24.7	27.4	30.1	25.9	30	34.7	29.5	32.1
A/G	1.20-2.40	1.31	1.54	1.51	1.53	1.46	1.07	1.54	1.35	1.17	1.36	1.26
PA(mg/L)	100.00-400.00	187	134.2	211.4	111	153.8	112.3	281.2	273.9	211.7	254.8	348.5
PT(s)	11.0-14.5	14	13	12.3	12.8	12.7	13.3	13.5	13	NA	12.2	12.2
INR	0.80-1.20	1.1	1	0.93	0.98	0.97	1.03	1.05	1	NA	0.92	0.92
TT(s)	14.0-21.0	16.9	16.4	17	17.5	16.1	19.4	17	18.2	NA	17.1	16.9
APTT(s)	28.0-43.0	45.8	38.6	38.4	43.6	40.4	34	34.8	33.4	NA	34.7	34.2
FIB(g/L)	2.00-4.00	3.05	3.9	5.09	4.48	3.99	3.85	2.74	2.68	NA	3.35	4.07
D-Dimer(mg/L)	0.00-0.50	0.22	0.57	0.41	0.31	0.26	0.25	0.22	0.42	NA	0.22	0.27
CK(U/L)	20-173	88	91	60	127	46	399	62	90	15	51	251
CK-MB(U/L)	0.0-25.0	4.7	50.6	12.7	8.7	15.5	34.3	14.2	13.1	14.9	10.3	15.9
LDH(U/L)	114-240	148	373	202	168	168	230	155	154	278	159	171
TCO ₂ (mmol/L)	20.00-30.00	28.1	20.5	31.4	24.5	24.9	24.3	22.7	31	22.3	36.7	29.4
BUN(mmol/L)	2.90-8.20	4.15	3.46	4.65	4.92	2.99	3.93	3.52	3.21	5.56	3.79	5.74
CREA(μ mol/L)	44.0-97.0	66	51.2	65.6	84	54.4	83.9	46.6	84.4	24.2	70.3	97
UA(μ mol/L)	120.0-420.0	396.2	222	412.4	217.6	287.8	201	301.6	380.2	432.1	308.2	529.4
Ct value of PCR	≤ 45	24.30	37.97	25.04	29.71	40.87	35.93	37.35	39.81	33.71	27.74	31.51

WBC, white blood cell; NEU, neutrophil; LYM, lymphocyte; MONO, monocyte; EO, eosinophil; BASO, basophil; ESR, erythrocyte sedimentation rate; PCO₂, partial pressure of carbon dioxide; PO₂, partial pressure of oxygen; PCT, procalcitonin; CRP, C-reactive protein; TP, total protein; ALB, albumin; GLB, globulin; PA, prealbumin; PT, prothrombin time; INR, international normalized ratio; TT, thrombin time; APTT, activated partial thromboplastin time; FIB, fibrinogen; CK, creatine kinase; CK-MB, creatine kinase isoenzymes; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; CREA, creatinine; UA, uric acid; Ct, cycle threshold; PCR, polymerase chain reaction; NA, not available.

Figures

Figure 1

The Ct value of virus are highly correlated with clinical and laboratory manifestations in SARS-CoV-2-infected patients. The Ct value of virus is highly correlated with NEU, CK-MB and BUN in 11 SARS-CoV-2-infected patients. Spearman rank correlation analysis (r) and P values are provided in each graph.

Figure 2

Variability within 34 SARS-CoV-2 full genomic sequences in China from GenBank. A, location of major structural protein encoding genes (accessory protein ORFs, S=Spike protein, E=Envelope protein, M=Membrane protein, N=Nucleocapsid protein) of SARS-CoV-2. B, The two most variable locations in the genome, in the ORF1ab (left) and in ORF8 (right) derived from the multiple sequence alignment (MSA) of all genomes.

[illegible]

28144

COVID-19_WUHAN-HU-1_CHN.SEQ

Case 1. SEQ

Case 2. SEQ

Case 3. SEQ

Case 4. SEQ

Case 5. SEQ

Case 6. SEQ

Case 7. SEQ

Case 8. SEQ

Case 9. SEQ

Case 10. SEQ

Case 11. SEQ

Figure 1. Sanger sequencing chromatograms of the *hprt* gene. The top panel shows the sequencing results for the patient, and the bottom panel shows the results for the control. The chromatograms are aligned to the reference sequence, with positions 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637, 639, 641, 643, 645, 647, 649, 651, 653, 655, 657, 659, 661, 663, 665, 667, 669, 671, 673, 675, 677, 679, 681, 683, 685, 687, 689, 691, 693, 695, 697, 699, 701, 703, 705, 707, 709, 711, 713, 715, 717, 719, 721, 723, 725, 727, 729, 731, 733, 735, 737, 739, 741, 743, 745, 747, 749, 751, 753, 755, 757, 759, 761, 763, 765, 767, 769, 771, 773, 775, 777, 779, 781, 783, 785, 787, 789, 791, 793, 795, 797, 799, 801, 803, 805, 807, 809, 811, 813, 815, 817, 819, 821, 823, 825, 827, 829, 831, 833, 835, 837, 839, 841, 843, 845, 847, 849, 851, 853, 855, 857, 859, 861, 863, 865, 867, 869, 871, 873, 875, 877, 879, 881, 883, 885, 887, 889, 891, 893, 895, 897, 899, 901, 903, 905, 907, 909, 911, 913, 915, 917, 919, 921, 923, 925, 927, 929, 931, 933, 935, 937, 939, 941, 943, 945, 947, 949, 951, 953, 955, 957, 959, 961, 963, 965, 967, 969, 971, 973, 975, 977, 979, 981, 983, 985, 987, 989, 991, 993, 995, 997, 999, 1001, 1003, 1005, 1007, 1009, 1011, 1013, 1015, 1017, 1019, 1021, 1023, 1025, 1027, 1029, 1031, 1033, 1035, 1037, 1039, 1041, 1043, 1045, 1047, 1049, 1051, 1053, 1055, 1057, 1059, 1061, 1063, 1065, 1067, 1069, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1115, 1117, 1119, 1121, 1123, 1125, 1127, 1129, 1131, 1133, 1135, 1137, 1139, 1141, 1143, 1145, 1147, 1149, 1151, 1153, 1155, 1157, 1159, 1161, 1163, 1165, 1167, 1169, 1171, 1173, 1175, 1177, 1179, 1181, 1183, 1185, 1187, 1189, 1191, 1193, 1195, 1197, 1199, 1201, 1203, 1205, 1207, 1209, 1211, 1213, 1215, 1217, 1219, 1221, 1223, 1225, 1227, 1229, 1231, 1233, 1235, 1237, 1239, 1241, 1243, 1245, 1247, 1249, 1251, 1253, 1255, 1257, 1259, 1261, 1263, 1265, 1267, 1269, 1271, 1273, 1275, 1277, 1279, 1281, 1283, 1285, 1287, 1289, 1291, 1293, 1295, 1297, 1299, 1301, 1303, 1305, 1307, 1309, 1311, 1313, 1315, 1317, 1319, 1321, 1323, 1325, 1327, 1329, 1331, 1333, 1335, 1337, 1339, 1341, 1343, 1345, 1347, 1349, 1351, 1353, 1355, 1357, 1359, 1361, 1363, 1365, 1367, 1369, 1371, 1373, 1375, 1377, 1379, 1381, 1383, 1385, 1387, 1389, 1391, 1393, 1395, 1397, 1399, 1401, 1403, 1405, 1407, 1409, 1411, 1413, 1415, 1417, 1419, 1421, 1423, 1425, 1427, 1429, 1431, 1433, 1435, 1437, 1439, 1441, 1443, 1445, 1447, 1449, 1451, 1453, 1455, 1457, 1459, 1461, 1463, 1465, 1467, 1469, 1471, 1473, 1475, 1477, 1479, 1481, 1483, 1485, 1487, 1489, 1491, 1493, 1495, 1497, 1499, 1501, 1503, 1505, 1507, 1509, 1511, 1513, 1515, 1517, 1519, 1521, 1523, 1525, 1527, 1529, 1531, 1533, 1535, 1537, 1539, 1541, 1543, 1545, 1547, 1549, 1551, 1553, 1555, 1557, 1559, 1561, 1563, 1565, 1567, 1569, 1571, 1573, 1575, 1577, 1579, 1581, 1583, 1585, 1587, 1589, 1591, 1593, 1595, 1597, 1599, 1601, 1603, 1605, 1607, 1609, 1611, 1613, 1615, 1617, 1619, 1621, 1623, 1625, 1627, 1629, 1631, 1633, 1635, 1637, 1639, 1641, 1643, 1645, 1647, 1649, 1651, 1653, 1655, 1657, 1659, 1661, 1663, 1665, 1667, 1669, 1671, 1673, 1675, 1677, 1679, 1681, 1683, 1685, 1687, 1689, 1691, 1693, 1695, 1697, 1699, 1701, 1703, 1705, 1707, 1709, 1711, 1713, 1715, 1717, 1719, 1721, 1723, 1725, 1727, 1729, 1731, 1733, 1735, 1737, 1739, 1741, 1743, 1745, 1747, 1749, 1751, 1753, 1755, 1757, 1759, 1761, 1763, 1765, 1767, 1769, 1771, 1773, 1775, 1777, 1779, 1781, 1783, 1785, 1787, 1789, 1791, 1793, 1795, 1797,

Figure 3

Sequencing results of positions 8781 and 28144 from SARS-CoV-2 in 11 patients. A, The two most variable locations in the genome derived from the multiple sequence alignment (MSA). B, Sanger sequencing results of the two most variable locations.

Figure 4

A timeline of events in human cases with COVID-19.