

Comparison and Clinical Characteristics of COVID-19 in Different Time Period in Wuhan, China

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Research article

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Abstract

Background SARS-CoV-2 is making deadly impact on the human lives all over the world. Therefore, we aimed to analyze the changes involved in clinical characteristics of COVID-19 patients over time.

Methods We conducted a retrospective study to compare the patients whose onset of illness in January with the patients whose onset of illness in February in Wuhan, China.

Results Among enrolled 896 patients, the median age was 60 years (47-69 years), 685 (76.5%) were categorized into group A (patients with illness onset in January), and 211 (23.5%) were categorized into group B (patients with illness onset in February). Compared with group B, group A had a higher rate of fever ($p < 0.001$), the lower rate of asymptomatic ($p < 0.001$). Group A had a higher incidence of neutrophilia ($p = 0.043$), elevated D-dimer ($p < 0.001$), increased LDH ($p = 0.002$), but lower incidence of normal CT scan ($p = 0.001$). CD3 cells ($p = 0.015$) and CD4 cells ($p = 0.04$) count significantly reduced in group A. Critical patients decreased ($p = 0.005$) and mild patients increased ($p = 0.001$) in group B. The fatality rate significantly decreased in group B ($p = 0.028$).

Conclusions The condition of patients with onset of illness in January were more serious than patients with onset of illness in February. It indicates that virulence showed reducing effect, but more basic research is required to support the hypothesis.

Introduction

A novel coronavirus disease (COVID-19) outbreak has been sweeping all over the world. It has been a huge threat to human life and health which exerted pressure on our socio-economic development. However, since the outbreak began in January, during the month of March, it was well controlled in Wuhan, China. As of May 6, 2020, the cumulative confirmed cases with COVID-19 were 50333, whereas, the death toll stood at 3869 in Wuhan [1].

The SARS-CoV-2 is a coronavirus. Until now, there were two outbreaks caused by coronaviruses, i.e. SARS in 2002, and MERS in 2012 [2]. The SARS outbreak led to report of about 8098 cases, whereas, the death stood at 774, and the mortality rate was 9% [3]. While, MERS led to infected cases of about 2519, whereas, 866 people died, and the mortality rate was about 34.4% [4]. Previous studies have shown different pathogenicity of SARS and MERS in a different time period. Compared to early stage, in the late stage of the outbreak, SARS-CoV showed reduced replicative ability and decreased virulence for host adaptation in vitro independent of the cell system [5, 6]. MERS-CoV was probably more sensitive to IFN-I (type I interferon) treatment at the later time of outbreak [7, 8]. Whereas, Ebola virus which belonged to a strain of late-outbreak, exhibited reduced virulence and prolonged survival in experimental animals [9].

Since December 2019, strict measures were taken to prevent crowd gathering, self-segregation was conformed to curb the spread of SARS-CoV-2 in Wuhan city. The influx of medical teams provided support for the treatment. These efforts ultimately resulted in obtaining the phase achievements. On April

4, 2020, no new cases were diagnosed with the COVID-19 in Wuhan city [10]. Previous studies have shown that the SARS-CoV and MERS-CoV showed reduced pathogenicity overtime. However, it was unclear whether the pathogenicity of SARS-CoV-2 would also degrade over time. Therefore, we compared clinical characteristics and outcome of COVID-19 patients in the different period for analyzing the change of pathogenicity of SARS-CoV-2.

Methods

Study population

We administered 1347 patients who admitted to the Renmin Hospital of Wuhan University from 30th January 2020 to until they got discharged before 31st March 2020. However, we excluded the patients as follows: 167 patients who were transferred to Fangcang shelter hospital around 9th February for missing outcome information, whereas, 156 patients who were transferred from Fangcang shelter hospital in early March for without initial data, 44 patients with constant negative SARS- CoV-2 test results in pharyngeal swab samples, and 84 patients for missing essential data. Therefore, we finally considered 896 patients diagnosed with COVID-19 (Fig. 1). Oral informed consent was obtained from all the patients. This retrospective study was approved by the Medical Ethics Committee of Renmin Hospital of Wuhan University, Wuhan, China. (No.WDRY2020-K019).

Procedures

Trained physicians and medical students reviewed demographics, clinical characteristics, radiologic and laboratory findings, treatment, and clinical outcomes from electronic medical records. Symptoms appearing before admission were also recorded. Vital signs and disease severity status were recorded during the admissions. The results of the blood test and radiologic findings after admission were analyzed. The most intense level of oxygen support during hospitalization was recorded. During the whole study period, patients were protected for confidentiality. The nasopharyngeal swab samples of patients were collected during admission and hospital stay if needed. The double nucleic acid detection kit (Bio-Germ, Shanghai, China) was used to detect SARS-CoV-2 in the open reading frame 1ab (ORF1ab) / nucleocapsid protein (N) gene. Nearly all patients received routine blood test, coagulation, biochemical tests and computed tomography.

Definitions

The COVID-19 was defined based on the positive results of nasopharyngeal swab samples at any one time during clinical course plus any one point including: fever or respiratory symptoms and bilateral or multiple ground-glass opacity in CT scan. The severity of COVID-19 was defined based on the Chinese National for COVID-19 Diagnosis and Treatment Guidelines version-7 [11]: Mild types were showed with only slight clinical symptoms. Common types were presented with both slight clinical symptoms and an abnormal CT scan. Severe types were exhibited with dyspnea, respiratory rate ≥ 30 times/min, SpO₂

(percutaneous oxygen saturation) $\leq 93\%$, and oxygenation index ($\text{PaO}_2/\text{FiO}_2$) ≤ 300 mmHg. The critical type patients were presented with respiratory failure, shock or multiple organ failure which ultimately required mechanical ventilation and intensive care. Fever was defined with not less than 37.3°C . The ARDS (acute respiratory distress syndrome) and the AKI (acute kidney injury) were defined according to the Berlin Definition [12] and the KDIGO clinical practice guidelines [13], respectively. The sepsis was defined according to 2016 Third International Consensus Definition [14]. The AHF (acute heart failure) and the ALF (acute liver failure) were defined based on Recommendations on pre-hospital and early hospital management of acute heart failure [15] and American Gastroenterological Association Institute Guidelines for the Diagnosis and Management of Acute Liver Failure [16], respectively. The hypoalbuminemia was defined as the blood albumin less than 35 g/L. The shock was defined as systolic pressure lower than 90 mmHg or mean arterial pressure lower than 65 mmHg.

Statistical analysis

Categorical data such as sex, symptoms, comorbidity, vital signs, disease severity status, abnormal blood test, imaging features, treatment and outcomes were compared using the χ^2 test. Non-normal distributed continuous data were compared using the Mann-Whitney-Wilcoxon test, including age, time from illness onset to hospital admission, blood test, time from illness onset to discharge or death. Categorical variables were represented by frequency and percentage, and abnormally distributed continuous variables were described by the median and interquartile range (IQR). Survival data were analyzed by the Kaplan-Meier plot. All statistical data were calculated using SPSS (v23) software and plotted figures by GRAPHPAD PRISM (v8.0). P-value less than 0.05 was considered as statistically significant.

Results

Demographics and clinical characteristics on admission

A total of 896 patients with a confirmed diagnosed with the COVID-19 were enrolled. The median age was 60 years (IQR, 47–69 years), 431 (48.1%) were male; the most common symptoms were fever (80.4%) and cough (50.4%). Some people showed symptoms related to digestive tract, including diarrhea (17.7%), nausea or vomiting (5.8%), abdominal pain and distention (2.3%) and inappetence (13.1%). Few patients had symptoms such as itchiness in the eyes (0.8%) (Table 1).

Table 1
Demographic and clinical findings of patients in different time period

	Total (n = 896)	Jan. (n = 685)	Feb. (n = 211)	p value
Age, years	60 (47–69)	60 (47–69)	59 (47–69)	0.945
Sex (n, %)				0.388
Male	431 (48.1%)	324 (47.3%)	107 (50.7%)	
Female	465 (51.9%)	361 (52.7%)	104 (49.3%)	
Symptoms (n, %)				
Fever	720 (80.4%)	581 (84.8%)	139 (65.9%)	< 0.001
Cough	452 (50.4%)	352 (51.4%)	100 (47.4%)	0.345
Sputum	187 (20.9%)	150 (21.9%)	37 (17.5%)	0.208
Chills	26 (2.9%)	25 (3.6%)	1 (0.5%)	0.016
Myalgia	68 (7.6%)	59 (8.6%)	9 (4.3%)	0.037
Shortness of breath	272 (30.4%)	199 (29.1%)	73 (34.6%)	0.145
Chest congestion	231 (25.8%)	163 (23.8%)	68 (32.2%)	0.019
Chest pain	35 (3.9%)	20 (2.9%)	15 (7.9%)	0.013
Nasal obstruction	8 (0.9%)	7 (1%)	1 (0.5%)	0.689
Rhinorrhea	17 (1.9%)	14 (2%)	3 (1.4%)	0.775
Inappetence	117 (13.1%)	103 (15%)	14 (6.6%)	0.001
Nausea or vomiting	52 (5.8%)	44 (6.4%)	8 (3.8%)	0.179
Abdominal pain and distention	21 (2.3%)	14 (2%)	7 (3.3%)	0.300
Diarrhea	159 (17.7%)	117 (17.1%)	42 (19.9%)	0.355
Sore throat	49 (5.5%)	41 (6%)	8 (3.8%)	0.298
Fatigue	295 (32.9%)	242 (35.3%)	53 (25.1%)	0.006
Headache	41 (4.6%)	28 (4.1%)	13 (6.2%)	0.256
Dyspnea	120 (13.4%)	102 (14.9%)	18 (8.5%)	0.020
Palpitation	18 (2%)	12 (1.8%)	6 (2.8%)	0.397
Itching of eyes	7 (0.8%)	5 (0.7%)	2 (0.9%)	0.670

Data are median (IQR) or n (%). p values were calculated by Mann-Whitney U test, or χ^2 test, as appropriate.

	Total (n = 896)	Jan. (n = 685)	Feb. (n = 211)	p value
Asymptomatic	25 (2.8%)	9 (1.3%)	16 (7.6%)	< 0.001
Comorbidity (n, %)				
Hypertension	279 (31.1%)	213 (31.1%)	66 (31.3%)	1.000
Diabetes	138 (15.4%)	110 (16.1%)	28 (13.3%)	0.383
Cardiovascular disease	91 (10.2%)	72 (10.5%)	19 (9%)	0.603
Embolism disease	4 (0.4%)	4 (0.6%)	0 (0.0%)	0.578
Chronic obstructive lung diseases	38 (4.2%)	27 (3.9%)	11 (5.2%)	0.436
Thyroid disease	18 (2%)	14 (2%)	4 (1.9%)	1.000
Chronic kidney disease	20 (2.2%)	15 (2.2%)	5 (2.4%)	0.795
Malignant tumor	39 (4.4%)	31 (4.5%)	8 (3.8%)	0.847
Chronic hepatitis B virus infection	26 (2.9%)	23 (3.4%)	3 (1.4%)	0.166
Cerebrovascular diseases	16 (1.8%)	12 (1.8%)	4 (1.9%)	1.000
Pregnancy	23 (2.6%)	14 (2%)	9 (4.3%)	0.083
Depression	8 (0.9%)	6 (0.9%)	2 (0.9%)	1.000
Vital signs (n, %)				
Respiratory rate(> 24 breaths per min)	125 (14%)	105 (15.3%)	20 (9.5%)	0.031
Pulse (\geq 125 beats per min)	17 (1.9%)	16 (2.3%)	1 (0.5%)	0.143
Systolic blood pressure (< 90 mm Hg)	2 (0.2%)	2 (0.3%)	0 (0.0%)	1.000
Disease severity status (n, %)				
Mild	14 (1.6%)	5 (0.7%)	9 (4.3%)	0.001
Common	280 (31.3%)	210 (30.7%)	70 (33.2%)	0.498
Severe	494 (55.1%)	376 (54.9%)	118 (55.9%)	0.813
Critical	108 (12.1%)	94 (13.7%)	14 (6.6%)	0.005
Time from illness onset to hospital admission, days	10 (7–15)	11 (8–15)	7 (5–13)	0.001
Data are median (IQR) or n (%). p values were calculated by Mann-Whitney U test, or χ^2 test, as appropriate.				

According to time of illness onset, 896 patients were divided into group A (n = 685, 76.5%; patients with onset of illness in January) and group B (n = 211, 23.5%; patients with onset of illness in February). The median age was 60 (IQR 47–69 years) and 59 (IQR 47–69 years) respectively. Approximately 1:1 ratio of male and female were present in both group A and group B. Compared with group B, group A had a higher incidence of fever ($p < 0.001$), chills ($p = 0.016$), myalgia ($p = 0.037$) but a lower rate of chest congestion ($p = 0.019$) and chest pain ($p = 0.013$). The patients with nonspecific symptoms, including inappetence ($p = 0.001$) and fatigue ($p = 0.006$), reduced in group B. Significant decrease in the patients with dyspnea ($p = 0.02$), and increased asymptomatic patients ($p < 0.001$) in group B were observed (Table 1).

Among all 896 COVID-19 patients, 470 (52.5%) had comorbidities. Whereas, hypertension (31.1%), diabetes (15.4%) and cardiovascular disease (10.2%) were the most common comorbidities. There was no significant difference in comorbidity between two groups (Table 1).

Compared with group B, group A had a higher rate of mortality ($p = 0.028$), the higher rate of critical patients ($p = 0.005$), but a lower rate of mild patients ($p = 0.001$). The number of common patients and severe patients had no significant difference between the two groups. There was significant difference in time from the onset of illness to the admission ($p = 0.001$). The median time was eleven days (IQR 8–15 days) in group A and seven days (IQR 5-13days) in group B. The number of patients with a respiratory rate higher than 24 breaths per min during admission were observed to be increased significantly in group A ($p = 0.031$) (Table 1).

Laboratory findings and image feature

Laboratory results were collected during the admission. Among all the patients, the patients with most common abnormal blood test showed increased D-dimer (63.3%), about half of patients demonstrated elevated CRP (60%), fibrinogen (53.6%), LDH (50.1%) and decreased lymphocyte count (48.7%). Whereas, few patients showed kidney injury with increased level of creatinine (7.7%) and urea (13.6%) (Table 2).

Table 2
Laboratory and radiographic findings of patients in different time period

	Total (n = 896)	Jan. (n = 685)	Feb. (n = 211)	p value
Laboratory findings				
White blood cell count, × 10 ⁹ per L	5.6 (4.27–7.48)	5.67 (4.3–7.56)	5.53 (4.27–7.2)	0.344
< 4 (n, %)	182 (20.4%)	140 (20.4%)	42 (20.3%)	0.876
4–10 (n, %)	620 (69.5%)	474 (69.2%)	146 (70.5%)	
> 10 (n, %)	90 (10.1%)	71 (10.4%)	19 (9.2%)	
Neutrophil count, × 10 ⁹ per L	3.645 (2.54–5.5)	3.7 (2.56–5.66)	3.39 (2.47–5.18)	0.126
> 6.3 (n, %)	168 (18.9%)	139 (20.3%)	29 (14%)	0.043
Lymphocyte count, × 10 ⁹ per L	1.11 (0.77–1.57)	1.1 (0.76–1.56)	1.16 (0.84–1.63)	0.122
< 1.1 (n, %)	434 (48.7%)	340 (49.6%)	94 (45.4%)	0.303
Hemoglobin, g/L	124 (114–135)	124 (114–136)	125 (114–135)	0.9
< 110 (n, %)	149 (16.7%)	117 (17.1%)	32 (15.5%)	0.671
Platelet count, × 10 ⁹ per L	216 (166–279)	220 (167–285)	212 (165–259)	0.041
< 100 (n, %)	35 (3.9%)	27 (3.9%)	8 (3.9%)	1
Albumin, g/L	37.3 (33.9–40.2)	37 (33.475–40)	37.9 (35.6–41)	< 0.001
< 35 (n, %)	293 (33.6%)	248 (27.2%)	45 (22%)	< 0.001
ALT, U/L	25 (16–42)	27 (17–43)	20 (14–36)	< 0.001
> 40 (n, %)	228 (26%)	183 (27.3%)	45 (21.7%)	0.123
AST, U/L	25 (19–38)	26 (20–40)	23 (17–34)	0.018
>40 (n, %)	194 (22.1%)	157 (23.4%)	37 (17.9%)	0.103
Creatinine, umol/L	59 (49–73)	59 (49–72)	61 (51–75)	0.067
> 97 (n, %)	69 (7.7%)	50 (7.2%)	19 (9%)	0.46
Urea, mmol/L	4.6 (3.6–6.37)	4.67 (3.6–6.45)	4.56 (3.6–6.1)	0.364

Data are median (IQR) or n (%). p values were calculated by Mann-Whitney U test, or χ^2 test, as appropriate.

	Total (n = 896)	Jan. (n = 685)	Feb. (n = 211)	p value
> 8 (n, %)	122 (13.6%)	98 (14.3%)	24 (11.4%)	0.303
Lactate dehydrogenase, U/L	251 (194–329)	259 (197–342)	232 (185–297)	0.001
> 250 (n, %)	433 (50.1%)	350 (53%)	83 (40.5%)	0.002
Creatine kinase, U/L	56 (35–95)	55 (34–88)	61 (41–114)	0.009
> 310 (n, %)	41 (4.7%)	30 (4.5%)	11 (5.4%)	0.577
CD3 cell count, per ul	742 (456–938)	716 (444–912)	746 (466–1122)	0.015
CD4 cell count, per ul	455 (269–584)	429 (264–569)	446 (266–666)	0.04
CD8 cell count, per ul	261 (149–343)	241 (145–333)	256 (142–395)	0.05
PT, sec	12 (11.5–12.5)	12.1 (11.4–12.5)	11.7 (11.2–12.5)	0.001
> 13 (n, %)	103 (11.5%)	79 (11.5%)	24 (11.4%)	1
APTT, sec	27.7 (25.6–29.6)	27.7 (25.6–29.4)	27.7 (25.9–30.1)	0.206
> 31.3 (n, %)	135 (15.1%)	100 (14.6%)	35 (16.6%)	0.509
Fibrinogen, g/L	4.26 (3.13–4.97)	4.2 (3.3–4.97)	4 (2.7–5.05)	0.119
> 4 (n, %)	480 (53.6%)	378 (55.2%)	102 (48.3%)	0.083
D-dimer, mg/L	0.89 (0.4–3.7)	1.02 (0.44–4.1)	0.59 (0.29–1.4)	< 0.001
> 0.55 (n, %)	563 (63.3%)	456 (66.6%)	107 (52.5%)	< 0.001
PCT > 0.1 ng/ml (n, %)	262 (29.6%)	216 (31.5%)	46 (22.9%)	0.018
CRP > 10 mg/L (n, %)	527 (60%)	423 (61.7%)	104 (53.9%)	0.062
Imaging features				
Ground-glass opacity (n, %)	846 (97.1%)	653 (98.3%)	193 (93.2)	< 0.001
Bilateral pulmonary infiltration (n, %)	762 (87.5%)	594 (89.5%)	168 (81.2%)	0.003
Normal (n, %)	14 (1.6%)	5 (0.7%)	9 (4.3%)	0.001
Data are median (IQR) or n (%). p values were calculated by Mann-Whitney U test, or χ^2 test, as appropriate.				

Compared with group B, group A had a higher rate of increased D-dimer ($p < 0.001$) and PCT ($p = 0.018$), as well as neutrophilia ($p = 0.043$) and elevated levels of LDH ($p = 0.002$). Group B showed significant increase in CD3 cells count ($p = 0.015$) and CD4 cells count ($p = 0.04$). Whereas, in group A, liver injury indices including ALT and AST, were significantly higher than that in group B. More hypoalbuminemia occurred in group A ($p < 0.001$). However, there was no significant difference between two groups in white blood cell count, lymphocyte count and CRP (Table 2).

Compared with group B, more CT scan results in group A showed bilateral pulmonary infiltration ($p = 0.003$) and ground-glass opacity ($p < 0.001$), however, the group B exhibited increased normal CT scan results ($p = 0.001$) (Table 2).

Treatments and outcomes

Among 896 patients, most patients received antibiotics (77%), Lianhua Qingwen capsule (71.8%) and antiviral treatment (93.4%) which including Oseltamivir, Arbidol and Ganciclovir. Compared to group B, group A received more treatment of antibiotics ($p = 0.009$), corticosteroids ($p = 0.036$), intravenous immunoglobulin ($p < 0.001$) and α -interferon ($p = 0.008$). We recorded the most intense level of oxygen support, and 701 (78.24%) patients showed the need of oxygen support. More treatments of high-flow nasal cannula oxygen therapy ($p = 0.028$) and non-invasive mechanical ventilation ($p = 0.027$) were administrated in patients of group A (Table 3).

Table 3
Treatments and outcomes of patients in different time period

	Total (n = 896)	Jan. (n = 685)	Feb. (n = 211)	p value
Treatments*				
Antibiotics (n, %)	689 (77%)	542 (79.1%)	147 (70%)	0.009
Antiviral treatment (n, %)	837 (93.4%)	654 (95.5%)	196 (92.9%)	0.153
Lianhua qingwen capsule (n, %)	643 (71.8%)	493 (72%)	150 (71.1%)	0.794
Corticosteroids (n, %)	353 (39.4%)	283 (41.3%)	70 (33.2%)	0.036
Intravenous immunoglobulin (n, %)	396 (44.2%)	335 (48.9%)	61 (28.9%)	< 0.001
a-interferon (n, %)	151 (16.9%)	128 (18.7%)	23 (10.9%)	0.008
High-flow nasal cannula oxygen therapy (n, %)	82 (9.2%)	71 (10.4%)	11 (5.3%)	0.028
Non-invasive mechanical ventilation (n, %)	30 (3.3%)	28 (4.1%)	2 (0.9%)	0.027
Invasive mechanical ventilation (n, %)	7 (0.8%)	5 (0.7%)	2 (0.9%)	0.67
ECMO (n, %)	2 (0.2%)	1 (0.1%)	1 (0.5%)	0.416
Renal replacement therapy (n, %)	8 (0.9%)	6 (0.9%)	2 (0.9%)	1
Outcome				
ARDS (n, %)	77 (8.6%)	69 (10.1%)	8 (3.8%)	0.003
Shock (n, %)	21 (2.3%)	14 (2%)	7 (3.3%)	0.3
Sepsis (n, %)	38 (4.2%)	30 (4.4%)	8 (3.8%)	0.846
ALF (n, %)	6 (0.7%)	6 (0.9%)	0 (0.0%)	0.345
AKI (n, %)	14 (1.6%)	13 (1.9%)	1 (0.5%)	0.208
AHF (n, %)	20 (2.2%)	15 (2.2%)	5 (2.4%)	0.795
Death (n, %)	81 (9%)	70 (10.2%)	11 (5.2%)	0.028
Hospital length of stay, days	18 (9–32)	18 (9–32)	19 (11–31)	0.379
Illness onset to discharge or death, days	33 (21–44)	33 (20–45)	33 (24–40)	0.343
Data are median (IQR) or n (%). p values were calculated by Mann-Whitney U test, or χ^2 test, as appropriate. *The most intense level of oxygen support during hospitalization was recorded.				

Among all the patients, the median time of hospital stay was 18 days (IQR 9–32 days). Further 81 patients (9%) died. Whereas, 77 (8.6%) patients developed the ARDS and sepsis occurred in 38 (4.2%)

patients. The Shock was observed in 21(2.3%) patients. Among the patients with shock, 9 (42.9%) patients were caused by sepsis, 10 (47.6%) patients were contributed to heart failure and 3 (14.3%) patients were caused by acute gastrointestinal bleeding. None of the patients complicated with shock were survived. Compared with group B, group A had a higher rate of mortality ($p = 0.028$) (Fig. 2). The incidence of developing to ARDS ($p = 0.003$) was higher in group A. Among all the patients, the median time from illness onset to death or discharge was 33 days (IQR 21–44 days) and there was no significant difference observed between the two groups (Table 3).

Discussion

In our study, we found distinction of severity of COVID-19 in different time period significantly. Compared with patients whose onset of illness began in January, the illness condition was improved in patients with onset of illness in February, which represented with lower mortality, lower rate of critical patients, lower level of neutrophil, D-miner, LDH, PCT, ALT, AST and a higher level of CD3 and CD4 cells count. Also, we observed significant increase in mild types of patients, asymptomatic patients and patients with normal CT scan whose illness onset began in February.

Increased neutrophil was positively related to poor outcome in coronavirus diseases. The study carried out by Wu et al demonstrated that neutrophilia is a risk factor related to the progression of ARDS and development from ARDS to death in COVID-19 patients [17]. The neutrophilia was also a risk factor for intubation in SARS patients [18]. The severity of lung damage positively correlated with neutrophils counts in the peripheral blood in patients with MERS [19, 20]. The neutrophil was able to produce chemokines and cytokines which can resist against the virus but at the same time it led to an excess inflammatory response that resulted in multiple organ injury. Whereas, D-miner was an independent risk factor for mortality in COVID-19 patients [17, 21]. Moreover, coagulation disorders was a displayed in the COVID-19 patients [22] and non-survivor had a higher level of D-miner, FDP and prolonged PT, APTT in COVID-19 patients [23]. Anticoagulant therapy might play the key role in relieving hypoxemia due to existence of micro-thrombosis in the lung of COVID-19 patients [24]. The possible mechanism might be that the inflammatory cascade triggered by a virus and secondary infection led to injury of microvascular wall and release of procoagulant factors which resulted in ischemia and thrombosis. Evidence revealed that higher CD3 and CD4 T-cell counts protected patients from developing ARDS [17]. A recent study showed using electron microscopy that SARS-CoV-2 were in CD4 cells of COVID-19 patients, however, SARS-CoV-2 showed no productive replication [25]. This indicate that CD4 might control the replication of SARS-CoV-2. The incidence of secondary liver injury was positively related to poor outcome in COVID-19 patients [26]. A recent study showed that the liver injury was caused by activated cytotoxic T lymphocyte triggered by SARS-CoV-2 [27], other than drug-induced liver injury. Increased LDH was also associated with poor outcome in COVID-19 patients [17, 21]. Mild patients and asymptomatic patients increased with onset of illness in February, a recent study showed that COVID-19 patients who had mild or no symptoms, were dominant with virus in the upper respiratory tract [28, 29].

The mortality was reduced, symptoms and results of laboratory test were improved in patients with onset of illness in February compared with patients with onset of illness in January, however, there was no evident difference in age, sex, underlying diseases and environmental factors (district and seasonality) between them. Therefore, we speculate that the improved outcome might contribute to the diminishing virulence.

Recently, variants [30], mutations [31] and genetic diversity [32, 33] is found in SARS-CoV-2, which might be an explanations for diminishing virulence. A 382-nt deletion appeared during SARS-CoV-2 evolution [34], and a 29-nt deletion appeared during SARS-CoV evolution. Both of the deletions covered open reading frame 8 (ORF8). Evidence showed that the 29-nt deletion resulted in the decreased replicative ability of SARS-CoV in-vitro independent of the cell system [6]. The 382-nt deletion might also reduce virulence of SARS-CoV-2. An 81-nucleotide deletion had been found in ORF7 of SARS-CoV-2, leading to a 27 amino-acid in-frame deletion [35], which might also change pathogenicity of SARS-CoV-2. In the United States, researchers found discrepancy in mortality of COVID-19 patients between the East Coast and West Coast of America. They speculated that the discrepancy probably existed due to the change in amino-acid of viral spike protein in SARS-CoV-2 [36]. The study carried out by Li et al similarly found distinct pathogenicity of SARS-CoV-2 in vitro. The Vero-E6 cells were used to be infected with 11 SARS-CoV-2 viral isolates from COVID-19 patients. It showed huge discrepancy in pathogenicity on the Vero-E6 cells [37]. In addition, reduced virulence probably contributed to generation of antibody. A report showed that the improvement of clinical outcome in five COVID-19 patients who received an infusion of plasma from five patients who recovered from the COVID-19 [38].

In addition to diminishing virulence, some other factors which might have caused a difference in severity of COVID-19 in a different time period. At the early stage of the outbreak, the time from illness onset to hospital admission was longer, probably because of a shortage of medical resources and lack of awareness for COVID-19. It might aggravate illness after a few days of delay. Moreover, in the later time of outbreaks, more strict managements were employed [39], more number of Fangfang shelter hospital were established and the healthcare environment was improved. Furthermore, experiences and researches accumulated in early time played a crucial role in the improvement of clinical outcomes. (Fig. 3).

However, there were few limitations in our study. First, in the early stage of outbreak, many patients with clinical improvement were transferred to other isolated sites for further observation and waited for viral clearance, thus we did not obtain the time of viral clearance. Second, due to the retrospective study, some data in the analysis is missing. Third, some patients with onset of illness in February were not included as they were not discharged from the hospital yet. Moreover, our hospital was designated for to enroll the patients with severe and critical type patients with COVID-19, therefore the disease severity status and mortality in our research could not reflect the actual situation in Wuhan city. Nonetheless, our study was conducted at a single-center hospital with limited sample size.

Conclusion

We analyzed clinical characteristics and outcomes of COVID-19 during the different time period in Wuhan. Our research suggested that the patients with onset of illness in February showed improved clinical outcome compared to the patients with onset of illness in January. This probably indicate reduced virulence from the aspect of clinical perspective, but more factors should be taken into consideration. Further, virus sequence and population immunity surveillance are required to confirm our hypothesis.

Abbreviations

COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2; SARS, severe acute respiratory syndromes; MERS, middle east respiratory syndrome; RNA, ribonucleic acid; CRP, C-reaction protein; PCT, Procalcitonin; LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; ALT, alanine aminotransferase; AST, aspartate transaminase; CT, computerized tomography; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; AHF acute heart failure; ALF, acute liver failure; ECMO, extracorporeal membrane oxygenation.

Declarations

Ethics approval and consent to participate

Oral informed consent was obtained from all the patients. This retrospective study was approved by the Medical Ethics Committee of Renmin Hospital of Wuhan University, Wuhan, China. (No.WDRY2020-K019).

Consent for publication

Not applicable.

Competing interests

No conflicts of interest to be declared.

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Authors' contributions

Ke Hu and Ruhao Yang contribute to study design, data analysis, and protocol writing. Weihua Hu contribute to protocol writing, collection of data, and data analysis. Jie Wei contributes to study design, protocol writing and is responsible for the statistical analyses. Jun Xiong and Menglin Liu contribute to data collection. The authors read and approved the final manuscript.

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Availability of data and materials

Data and Materials are available on justified request. Please contact the corresponding author Dr. Ke Hu if data are requested.

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 40. Legends.

Figures

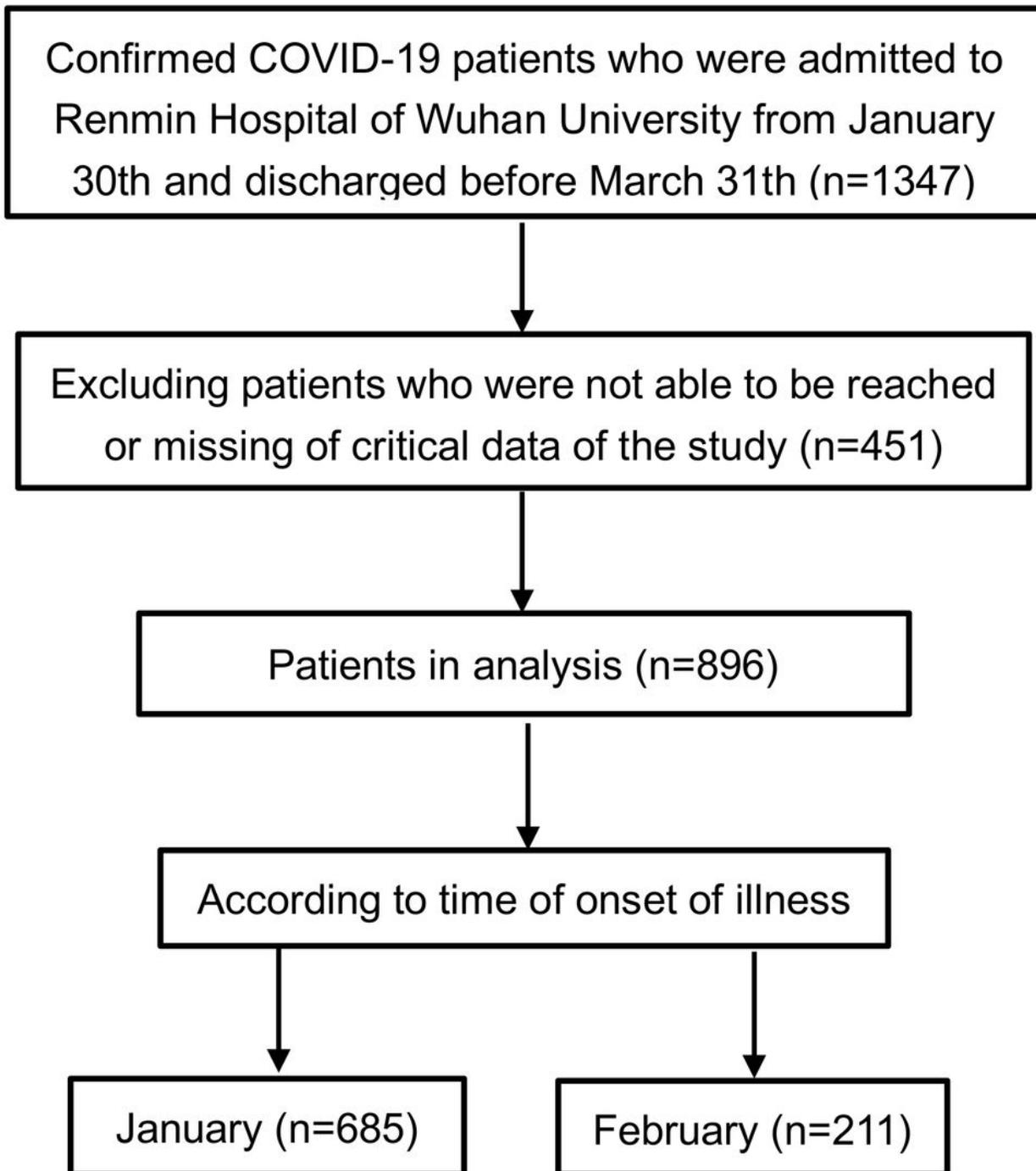


Figure 1

Flow chart of design

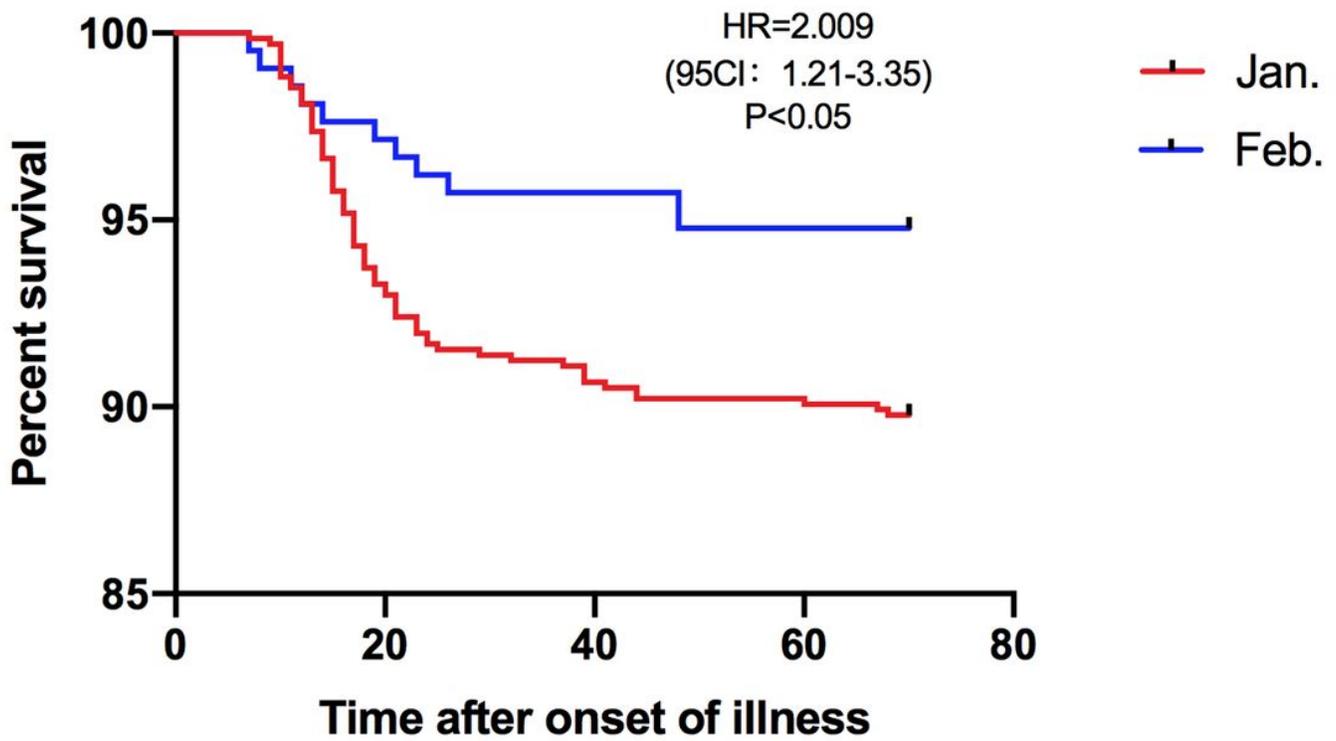


Figure 2

Survival curve in COVID-19 patients with illness onset in January or in February

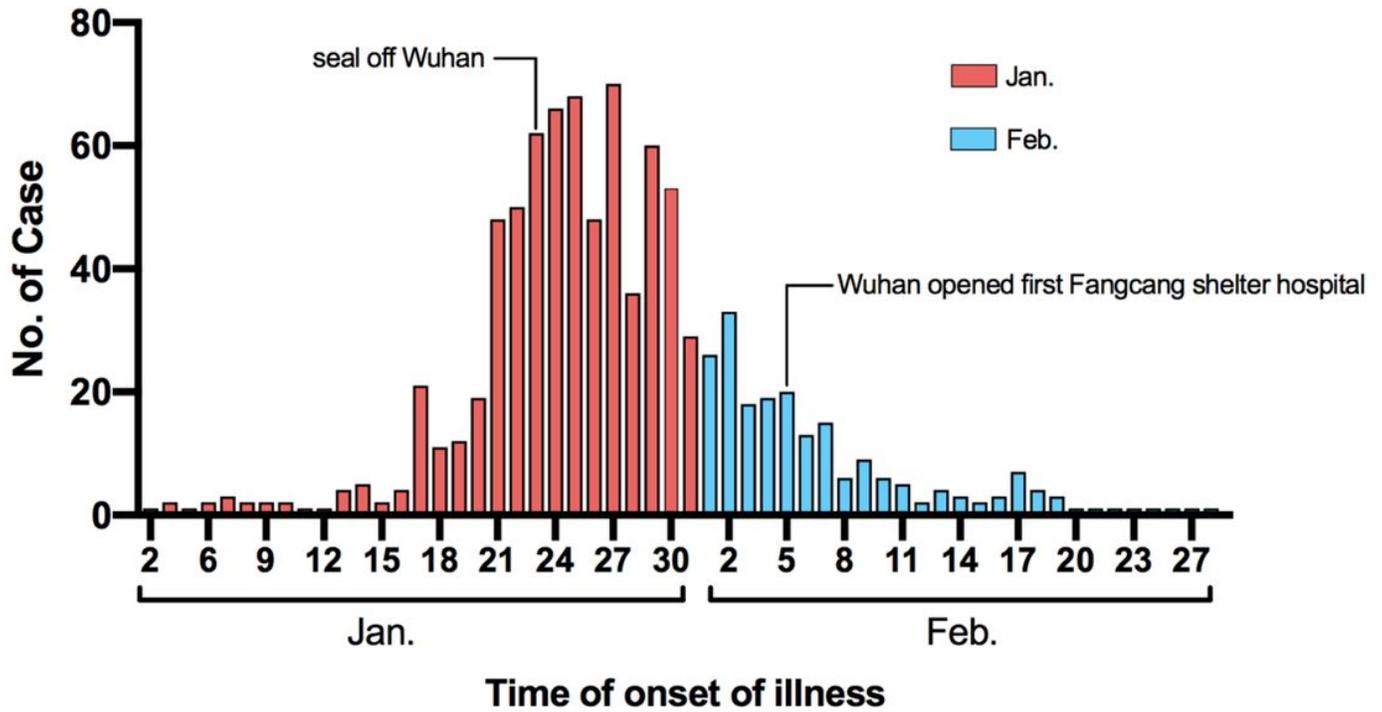


Figure 3

Onset of illness among 896 confirmed cases of COVID-19 in Wuhan, China The decline in incidence after February 1st due to the measures taken to seal Wuhan and the time after an incubation period