Characterization of Confirmed and Suspected COVID-19 Pneumonia Patients in a Retrospective Cohort Study in Wuhan

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Keywords: COVID-19, SARS-CoV-2 negative, suspected patients, retrospective cohort, clinical characteristics

DOI: https://doi.org/10.21203/rs.3.rs-92239/v1

Abstract

Background: A methodical comparison of confirmed and suspected COVID-19 patients has not been previously reported. Therefore, we thoroughly analyzed the demographic and clinical characteristics between these groups to identify mortality risk factors.

Methods: A retrospective cohort of 1,276 hospitalized COVID-19 pneumonia patients at Tongren Hospital (Wuhan, China; January 27 to March 3, 2020) was studied. Cox regression analyses were performed to evaluate multiple mortality risk factors.

Results: Both cohorts of confirmed (n=797) and suspected (n=479) patients exhibited typical demographic, clinical, and radiological characteristics. Treatment methods were consistent and both groups shared similarities in many demographic and clinical characteristics: age (≥65, 45.9% vs 41.8%, *P*=0.378) and lung disease (12.5% vs 14.6%, *P*=0.293). However, confirmed patients exhibited more severe disease manifestations than those in suspected patients: a higher incidence of fever (65.4% vs 58.0%, *P*<0.01), lower lymphocyte count (1.12×10^9/L vs 1.22×10^9/L, *P*=0.022), higher C-reactive protein (CRP) (11.60 mg/L vs 7.61mg/L, *P*=0.021), and more severe radiographic manifestations (lung infection incidence, 3.8% vs 3.0%, *P*=0.014; ground-glass opacity lesion incidence, 2.3% vs 2.0%, *P*=0.033). The dynamic profiles of lymphocytes, monocytes, D-dimer, and CRP, clearly delineated confirmed patients from suspected patients exhibiting critical illness. Cox regression analysis demonstrated that lung disease (adjusted hazard ratio 8.972, 95% CI: 3.782-21.283), cardiovascular disease (3.083, 1.347-7.059), neutrophil count (1.189, 1.081-1.307), age (1.068, 1.027-1.110), and ground-glass opacity lesions (1.039, 95% 1.013-1.065), were the main risk factors for mortality in confirmed patients; lung disease (14.725, 2.187-99.147), age (1.076, 1.004-1.153), and CRP level (1.012, 95% CI 1.004-1.020) were the primary factors in suspected patients.

Conclusions: Suspected patients with serious illness should seek medical attention to reduce mortality. Multiple factors must be assessed to determine the mortality risk and the appropriate treatment.

1. Background

Severe acute respiratory syndrome conoravirus-2 (SARS-CoV-2) was first reported in Wuhan in December 2019 [1] and the World Health Organization (WHO) named it severe coronavirus disease 2019 (COVID-19) on February 11, 2020. As of September 24, 2020, over 30.6 million confirmed cases and 950000 deaths worldwide (WHO Coronavirus Disease (COVID-19) Dashboard) have been reported due to SARS-CoV-2, and the numbers are still rising every day. The experiences in combating COVID-19 and patient management in Wuhan are informative for medical intervention and the formulation of public policy against COVID-19 pneumonia worldwide.

While many reports focusing on confirmed COVID-19 patients have been published, a number of cases of COVID-19-suspected patients (SARS-CoV-2 RNA negative) during the outbreak have been overlooked. The WHO [2–4], Centers for Disease Control and Prevention (CDC) [5, 6] and the National Institute for Health

and Care Excellence (NICE) [7] suggest these patients seek medical attention if they have a fever, cough, and difficulty breathing. Chinese hospital criteria are similar, except mildly ill patients have been asked to isolate in temporary hospitals reconstructed from gymnasiums and exhibition centers [2, 8]. Moreover, many guidelines for the management of suspected COVID-19 patients suggest that for people with a high likelihood of infection, based on exposure history and/or clinical presentation, a single negative test does not completely exclude the possibility COVID-19 infection because false-negatives are possible of PCR test [3, 7, 8].

Some reports [9–11] reveal epidemiological, demographic, and clinical aspects of COVID-19 treatment, and provide valuable information for clinical intervention and the formulation of public policies. However, a significant number of patients, suspected to have COVID-19, who presented with typical symptoms including pneumonia and lung radiographic abnormalities, tested negative for SARS-CoV-2 more than once in the hospital. These suspected patients were treated in a hospital designated for COVID-19 cases in Wuhan to reduce mortality from February 4, 2020 to March 3, 2020. The suspected patients analyzed in this study tested negative for SARS-CoV-2 RNA, even after many tests. The lack of confirmation by a positive RNA test challenges the precision of diagnosis and the rational treatment for these patients, which may undermine the optimal distribution of limited medical resources during the outbreak. Further, the distinguishing clinical characteristics between confirmed and suspected COVID-19 patients are lacking. Currently, there is insufficient evidence to formulate a specific management plan for these suspected patients.

The objective of this cohort study was to compare and contrast the clinical characteristics and outcomes between confirmed and suspected COVID-19 pneumonia patients. Here, we reveal that suspected COVID-19 pneumonia patients display technical differences from confirmed patients. More importantly, we confirm multiple risk factors for mortality in confirmed and suspected patients. Further, our study provides instructive information for combating and managing the COVID-19 pandemic.

2. Methods

2.1 Setting and sample

This cohort study was conducted in the endemic area of COVID-19 at the Tongren Hospital of Wuhan University (Wuhan, Hubei province, China). The hospital originally provided broad service to the local community but was reassigned by the government to exclusively hospitalize COVID-19 patients. All the suspected and confirmed COVID-19 pneumonia patients from Jan 27 to Mar 3, 2020 were included in our study cohort.

2.2 Study design

The diagnosis of confirmed and suspected COVID-19 pneumonia patients was performed in accordance with the tentative fifth revised edition of “The diagnosis and treatment plan of Coronavirus Pneumonia

2019," issued by the National Health Commission of the People's Republic of China [8]. The patients were consecutively admitted to the Tongren Hospital of Wuhan University from Jan 27 to Mar 3, 2020.

2.3 Data collection

Electronic medical records were collected and reviewed by practicing physicians and a research team from Tongren Hospital of Wuhan University. Of 1,369 patients, 93 patients were excluded: 16 had normal computerized tomography (CT) scan results and were not administered an RNA SARS-CoV-2 test, 37 had no CT scan results and no SARS-CoV-2 RNA test results, 40 had no CT scan results and tested negative for SARS-CoV-2 RNA. In total, 1,276 cases were included in this study. 797 patients were confirmed as SARS-CoV-2 positive by quantitative reverse transcription polymerase chain reaction (qRT-PCR). 479 suspected cases tested negative, at least once, for SARS-CoV-2 RNA during their hospital stay. The demographic, clinical, radiographic, laboratory, and patient outcome information was obtained with data collection forms from electronic medical records. The radiographic features of chest CT scans were measured by artificial intelligence technology [12, 13]. The time of disease onset was defined as the day when the first symptoms were noticed. Patients were categorized as critical or non-critical based on their medical records.

2.4 Laboratory procedures

The diagnostic criteria and primer sets recommended by the National Institute for Viral Disease Control and Prevention, China (http://www.chinaivdc.cn/kyjz/202001/t20200121_211337.html) were followed. Briefly, SARS-CoV-2 RNA was detected by a qRT-PCR assay following the manufacturer's instructions (BGI Biotech Ltd, Wuhan, China). A probe specific for the highly conserved region of SARS-CoV-2 was designed and conjugated with a fluorescein (FAM) tag. Briefly, throat swab samples were collected, and RNA was extracted from the samples within 2 hours. A one-step qRT-PCR assay was carried out using the following cycle parameters: 50 °C for 20 min and then 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec, 60 °C for 30 sec. A cycle threshold (Ct) value of less than 37 cycles was defined as positive test result, and a Ct value of 40 or more was defined as a negative test result. A retest was required for confirmation of results with an intermediate Ct value (between 37 and 40). If the retest Ct value was less than 40 cycles and the amplification curve displayed an increasing slope,the sample was verified as positive; otherwise it was determined as negative.

2.5 Definitions

Fever was defined as axillary temperature of at least 37.3 ℃. Moderate cases were defined as patients displaying signs and symptoms which might include fever and respiratory or pneumonia manifestations. Patients were categorized as severe cases if they met one or more of three criteria: respiratory rate ≥ 30 breaths/min, oxygen saturation ≤ 93% on room air, or PaO2/FiO2 ≤ 300 mmHg (1 mmHg = 0.133 kPa). Critical cases were determined based on whether the patient met any three criteria: respiratory failure occurred and mechanical ventilation was required, shock occurred, or the case was complicated with other organ failure that required monitoring and treatment in the ICU.

The chest imaging features of COVID-19 patients was as follows: (1) In the early stage, the radiographs show multiple, small, patched shadows and interstitial changes, especially in the lung periphery. (2) As the disease progresses, the CT scans reveal further development of multiple ground glass shadows and infiltration shadows in both lungs. (3) In severe cases, lung consolidation may occur. Pleural effusion is seldom found in patients with COVID-19 [1].

2.6 Statistical analysis

Statistical analysis was performed with SPSS 22.0 (version 22.0, IBM, Armonk, NY, USA), unless otherwise stated. The normal distribution assumption was tested using the Kolmogorov-Smirnov test ($P > 0.05$). The continuous variables were compared using the Mann-Whitney U test and are presented as the median value (25th percentile – 75th percentile). Categorical variables were compared using the Chi-square test or Fisher's exact probability test and are presented as numbers and percentages. A Cox regression were performed to analyze the effect of several risk factors on survival. P values < 0.05 was considered statistically significant for two-tailed tests. Abundance levels of the clinical index at each individual time point were plotted using GraphPad Prism 8.0.

3. Results

This cohort study included 1,276 hospitalized patients: 797 COVID-19 pneumonia patients were SARS-CoV-2 positive, and 479 suspected patients were SARS-CoV-2 negative. The demographic characteristics were comparable between the confirmed and suspected patients regarding age (≥ 65 years, 45.9% vs 41.8%, $P = 0.378$) and gender (female, 50.7% vs 55.1%, $P = 0.125$) (Table 1).

3.1 Clinical characteristics of confirmed and suspected COVID-19 patients

Both confirmed and suspected patients displayed typical clinical symptoms including fever, dry coughing, fatigue, chest tightness, and sputum production. Major comorbidities included hypertension, diabetes, cardiovascular disease, lung disease, liver disease, and chronic kidney disease. Of all the patients, 11.6% were treated under critical conditions and the overall fatality rate was 4.4% (Table 1). Although most laboratory parameters remained within the normal range, we noticed a high alveolar-arterial oxygen gradient ($AaDpO_2$, 90 mmHg), suggesting a defect of lung oxygen exchange due to impaired oxygenation, typical of lung dysfunction in both groups of COVID-19 patients.

Table 1
Basic Characteristics of COVID-19 Patients

Parameter	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
Age, n (%)				0.378
0–14	2 (0.2)	2 (0.3)	0 (0.0)	
15–49	294 (23.0)	179 (22.4)	115 (24.0)	
50–64	414 (32.4)	250 (31.4)	164 (34.2)	
≥65	566 (44.4)	366 (45.9)	200 (41.8)	
Gender, n (%)				0.125
Male	608 (47.7)	393 (49.3)	215 (44.9)	
Female	668 (52.3)	404 (50.7)	264 (55.1)	
Medication, n (%)				
Antibacteria	881 (69.0)	551 (69.1)	330 (68.9)	0.928
Antivirus	1,051 (82.4)	651 (81.7)	400 (83.5)	0.407
Corticosteroids	380 (29.8)	246 (30.9)	134 (28.0)	0.274
Others	1,115 (87.4)	701 (88.0)	414 (86.4)	0.427
Oxygen inhalation, n (%)				
Continuously	687 (53.8)	425 (53.3)	262 (54.7)	0.634
Non-invasive mechanical ventilation **	81 (6.3)	66 (8.3)	15 (3.1)	< 0.001
Invasive mechanical ventilation	28 (2.2)	22 (2.8)	6 (1.3)	0.075
Temperature on admission, n (%), ℃				0.069
≤37.2	1,048 (82.1)	647 (81.2)	401 (83.7)	
37.3–37.9	111 (8.7)	77 (9.6)	34 (7.1)	
38-38.9	92 (7.2)	62 (7.8)	30 (6.3)	
≥39	25 (2.0)	11 (1.4)	14 (2.9)	

a: Data are displayed as the median (IQR), n (%).

Parameter	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
Symptoms on admission, n (%)				
Fever *	799 (62.6)	521 (65.4)	278 (58.0)	0.009
Dry coughing	696 (54.6)	438 (55.0)	258 (53.9)	0.704
Fatigue	213 (16.7)	128 (16.1)	85 (17.8)	0.434
Chest tightness	173 (13.6)	103 (12.9)	70 (14.6)	0.393
Dyspnea	32 (2.5)	17 (2.1)	15 (3.1)	0.269
Sputum	71 (5.6)	46 (5.8)	25 (5.2)	0.677
Diarrhea	55 (4.3)	36 (4.5)	19 (4.0)	0.639
Pharyngalgia	35 (2.7)	20 (2.5)	15 (3.1)	0.510
Headache	17 (1.3)	10 (1.3)	7 (1.5)	0.755
Rhinorrhea	6 (0.5)	2 (0.3)	4 (0.8)	0.205
Asymptomatic	97 (7.6)	59 (7.4)	38 (7.9)	0.729
Comorbidities, n (%)				
Patient No.	761 (59.6)	479 (60.1)	282 (58.9)	0.665
Hypertension	436 (34.2)	280 (35.1)	156 (32.6)	0.350
Cardiovascular disease	210 (16.5)	133 (16.7)	77 (16.1)	0.775
Diabetes	189 (14.8)	111 (13.9)	78 (16.3)	0.251
Lung disease	170 (13.3)	100 (12.5)	70 (14.6)	0.293
Chronic kidney disease	82 (6.4)	46 (5.8)	36 (7.5)	0.219
Liver disease	107 (8.4)	74 (9.3)	33 (6.9)	0.135
Spectrum of disease, n (%)				0.447
Moderate	788 (61.8)	491 (61.6)	297 (62.0)	
Severe	340 (26.6)	207 (26.0)	133 (27.8)	
Critical	148 (11.6)	99 (12.4)	49 (10.2)	
Outcome, n (%) *				0.002

a: Data are displayed as the median (IQR), n (%).

Parameter	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
Non-survivor	56 (4.4)	46 (5.8)	10 (2.1)	
Survivor	1,220 (95.6)	751 (94.2)	469 (97.9)	
Onset of symptoms to hospital admission, median (IQR), a	11 (7–17)	10 (7–17)	11 (7–16)	0.579
Length of stay, median (IQR), a **	16 (12–20)	17 (13–22)	15 (11–18)	< 0.001
a: Data are displayed as the median (IQR), n (%).				

P values were calculated using Mann-Whitney U test, χ^2 test, or Fisher's exact test as appropriate. *: P < 0.05; **: P < 0.001

3.2 Vital characteristics demonstrate significant differences between confirmed and suspected patients

We further compared the clinical indices of confirmed and suspected patients. These two groups of patients displayed comparable lengths of time from the onset of symptoms to hospital admission, and both groups presented with a similar spectrum of disease (Table 1). However, these two groups of patients did show significant differences in many aspects. The suspected patients had a lower incidence of fever than confirmed patients (P < 0.01). Additionally, although there was no significant difference (P = 0.447) between the confirmed and suspected patient groups regarding disease classification (moderate, severe, and critical patients), the outcome of disease appeared significantly different (P = 0.002). Consistently, the confirmed patients also required a longer hospital stay (17 days vs 15 days, P < 0.001) (Table 1). The differences in these two group were also reflected by several laboratory parameters, as evidenced by significantly increased C-reactive protein (CRP) (11.60 mg/L vs 7.61 mg/L, P = 0.021) and decreased lymphocytes (1.12 × 109/L vs 1.22 × 109/L, P = 0.022) in confirmed patients compared with that in suspected patients (Table 2).

Table 2
Clinical indices of COVID-19 Patients on Admission to Hospital

Parameter	Normal Range	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
WBC ($\cdot 10^9$/L) *	3.5–9.5	5.1 (4.0-6.5)	5.0 (3.9–6.4)	5.3 (4.2–6.7)	0.008
Lymphocyte ($\cdot 10^9$/L) *	1.1–3.2	1.15 (0.82–1.61)	1.12 (0.81–1.56)	1.22 (0.84–1.65)	0.022
Monocyte ($\cdot 10^9$/L) *	0.1–0.6	0.39 (0.29–0.51)	0.38 (0.28–0.50)	0.40 (0.31–0.52)	0.028
Neutrophil ($\cdot 10^9$/L) *	1.8–6.3	3.24 (2.39–4.49)	3.16 (2.32–4.40)	3.37 (2.52–4.62)	0.033
Platelet ($\cdot 10^9$/L)	125–350	200 (157–263)	200 (152–263)	201 (160–263)	0.330
C-reactive protein (mg/L) *	0–5	9.90 (1.49–44.46)	11.60 (1.83–47.28)	7.61 (1.33-41.00)	0.021
Procalcitonin (ng/mL)	0-0.05	0.05 (0.05–0.05)	0.05 (0.05–0.05)	0.05 (0.05–0.05)	0.736
Alanine aminotransferase (IU/L)	7–40	24 (15–38)	25 (16–39)	23 (15–37)	0.098
Aspartate aminotransferase (IU/L)	0–45	26 (20–37)	26 (20–38)	26 (20–35)	0.500
Albumin (g/L) *	40–55	37.6 (34.0-40.8)	37.3 (33.7–40.5)	38.0 (34.7–41.4)	0.004
Blood Glucose (mmol/l)	3.9–6.1	5.47 (4.73–6.91)	5.42 (4.67–6.95)	5.56 (4.79–6.87)	0.184
Creatinine (μmol/L)	40–105	63.6 (51.7–79.8)	64.0 (51.7–78.4)	63.4 (51.3–82.7)	0.715
Alkaline phosphatase (IU/L)	53–128	60 (49–75)	60 (50–76)	60 (49–74)	0.840
Creatine kinase (IU/L)	30–180	68 (45–120)	67 (45–122)	68 (47–116)	0.548
Lactate dehydrogenase (IU/L)	114–240	201 (161–272)	205 (164–281)	198 (158–262)	0.057
α-HBDH (IU/L) *	72–182	170 (138–226)	172 (139–232)	165 (134–218)	0.034
Creatine kinase isoenzyme (IU/L)	0–25	10 (7–14)	10 (7–14)	10 (7–13)	0.465

Data are displayed as the median (IQR) or n (%). *P* values were calculated using the Mann-Whitney U test. *: $P < 0.05$.

Parameter	Normal Range	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
Cystatin C (mg/L)	0-1.26	0.93 (0.77–1.15)	0.94 (0.78–1.15)	0.92 (0.74–1.15)	0.174
hs-C-reactive protein (mg/L) *	0–3	10.00 (2.12-10.00)	10.00 (2.38-10.00)	7.83 (1.90–10.00)	0.031
B-type natriuretic peptide (pg/mL)	2-121	29.75 (12.52–69.69)	28.77 (12.16–68.15)	30.34 (13.27–76.42)	0.171
D-dimer (mg/L)	0-0.5	0.63 (0.35–1.37)	0.62 (0.36–1.30)	0.65 (0.35–1.48)	0.805
Myoglobin (ng/mL)	0-110	44.46 (28.29–76.14)	46.46 (28.67–82.56)	41.83 (27.75–68.56)	0.090
High sensitivity troponin I (ng/mL)	0-0.04	0.002 (0-0.010)	0.002 (0-0.010)	0.002 (0-0.010)	0.928
Serum amyloid protein (mg/L)	0–10	6.32 (5.09–8.70)	6.67 (5.09–11.21)	5.78 (5.06–7.46)	0.094
IgM (g/L)	0.3–2.2	1.2 (0.9–1.9)	1.2 (0.9–1.9)	1.2 (0.9–1.9)	0.873
IgA (g/L)	1.0-4.2	2.52 (1.88–3.27)	2.53 (1.90–3.25)	2.48 (1.86–3.29)	0.704
IgG (g/L)	8.6–17.4	13.0 (11.2–15.4)	13.1 (11.2–15.4)	13.0 (11.3–15.4)	0.718
Lactic acid (mmol/L)	1.65–2.90	2.85 (2.33–3.50)	2.86 (2.34–3.50)	2.84 (2.31–3.46)	0.872
Urea nitrogen (mmol/L)	3.1–7.2	4.3 (3.4–5.6)	4.2 (3.4–5.6)	4.4 (3.3–5.7)	0.718
Total bilirubin (μmol/L) *	2–21	8.6 (6.5–11.9)	8.9 (6.6–12.0)	8.2 (6.4–11.7)	0.040
Prothrombin time (s)	10–13	11.6 (11.1–12.1)	11.6 (11.1–12.2)	11.6 (11.1–12.1)	0.272
Activated partial thromboplastin time (s)	21–35	29.3 (26.2–32.7)	29.3 (26.3–32.9)	29.1 (26.0-32.6)	0.384
Thrombin time (s) *	13–21	19.8 (18.3–21.8)	20 (18.5–22.0)	19.6 (18.1–21.5)	0.014
pH	7.35–7.45	7.41 (7.36–7.47)	7.42 (7.36–7.46)	7.41 (7.37–7.49)	0.604

Data are displayed as the median (IQR) or n (%). *P* values were calculated using the Mann-Whitney U test. *: $P < 0.05$.

Parameter	Normal Range	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
$AaDpO_2$ (mmHg)	-	87.0 (32.0-176.5)	89.0 (38.5-211.5)	67.5 (5.3-156.3)	0.113
Partial pressure of oxygen (mmHg) *	80–100	89 (58–146)	79 (54–125)	132 (65–173)	0.023
Partial pressure of carbon dioxide (mmHg)	35–45	36.3 (31.2–40.0)	36.3 (31.0-39.7)	36.2 (31.3–43.9)	0.371

Data are displayed as the median (IQR) or n (%). *P* values were calculated using the Mann-Whitney U test. *: P < 0.05.

3.3 Artificial intelligence-aided analysis of CT scans in confirmed and suspected patients

The chest CT scans among both groups of patients, quantified by artificial intelligence-aided technology, also exhibited significant differences in the incidence of lung infection. The radiographic data revealed that an overall 3.5% of the total COVID-19 patients suffered lung infection (Table 3). Notably, the confirmed patients displayed lung infection more frequently than suspected patients, as detected by CT scan (3.8% vs 3.0%, *P* = 0.014). Ground-glass opacity lesions in the lungs are a unique abnormality for COVID-19 patients [14].As expected, the confirmed patients more commonly exhibited ground-glass opacity than suspected patients (2.3% vs 2.0%, *P* = 0.033) (Table 3). These observations suggest that confirmed patients tend to be more severe than suspected patients. Of note, the confirmed patients showed a higher incidence of fatality than suspected patients (5.8% vs 2.1%, *P* < 0.01) (Table 1); however, the multiple factors analysis found that clinical type (confirmed vs suspected) was not significantly associated with mortality (Table 4).

Table 3
SARS-CoV-2 RNA Test and Chest CT Scan Results in COVID-19 Patients

Parameter	Total (n = 1,276)	Confirmed (n = 797)	Suspected (n = 479)	*P* value
SARS-CoV-2 RNA test **				
No. of RNA tests, median (IQR)	3 (2–4)	3 (2–4)	2 (2–3)	< 0.001
Cycle threshold value on admission, median (IQR)				
Cycle threshold value: Critical (n = 77) *	-	33.03 (28.79–34.63)	-	0.001[a]
Cycle threshold value: Non-Critical (n = 404)	-	34.10 (31.93–35.88)	-	
Cycle threshold value: Survivor (n = 250) *	-	34.09 (31.74–35.90)	-	0.005[b]
Cycle threshold value: Non-Survivor (n = 37)	-	32.03 (26.82–35.02)	-	
SARS-CoV-2 antibody test				
Patient No., n (%)	289 (22.6)	170 (21.3)	119 (24.8)	0.147
IgM or IgG positive, n (%) **	232 (80.3)	149 (87.6)	83 (69.7)	< 0.001
Incidence of Lung infection, median (IQR), % *	3.5 (0.7–11.3)	3.8 (0.9–12.0)	3.0 (0.4–10.0)	0.014
Incidence of Ground-glass opacity lesion rate, median (IQR), % *	2.1(0.4–6.1)	2.3 (0.5–6.6)	2.0 (0.3–5.9)	0.033
Data are as displayed as the median (IQR), n (%).				

P values were calculated using the Mann-Whitney U test or χ^2 test. a: Critical vs Non-critical; b: Survivor vs Non-survivor. *: P < 0.05; **: P < 0.001

Table 4
Cox Regression Analysis Reveals Multiple Risk Factors in Confirmed or Suspected Patients

Clinical index	B	Adjust hazard ratio (95% CI)	*P* value
All patients (n = 1,276)			
Confirmed type	0.256	1.292 (0.505–3.308)	0.593
Confirmed patients (n = 797)			
Age (y)	0.065	1.068 (1.027–1.11)	0.001
Lung disease	2.194	8.972 (3.782–21.283)	< 0.001
Cardiovascular diseases	1.126	3.083 (1.347–7.059)	0.008
Neutrophil count ($\cdot 10^9$/L)	0.173	1.189 (1.081–1.307)	< 0.001
Myoglobin (ng/mL)	0.004	1.004 (1.003–1.005)	< 0.001
Ground-glass opacity lesion rate (%)	0.038	1.039 (1.013–1.065)	0.003
Suspected patients (n = 479)			
Age (y)	0.073	1.076 (1.004–1.153)	0.039
Lung disease	2.69	14.725 (2.187–99.147)	0.006
C-reactive protein (mg/L)	0.012	1.012 (1.004–1.02)	0.003
B-type natriuretic peptide (pg/mL)	0.001	1.001 (1.000-1.001)	0.001

In the Cox model, death is 1 and survival is 0. A patient with lung disease history or cardiovascular disease history is 1 and all others are 0. A patient been confirmed is 1 and else is 0.

3.4 Dynamic profiles of laboratory parameters in critical COVID-19 patients

We extracted the laboratory parameters from critical cases and compared confirmed patients with suspected patients. The dynamic profiles of several laboratory parameters were clearly distinguishable between confirmed and suspected patients (Fig. 1). The confirmed patients maintained low counts of lymphocytes and monocytes overtime, whereas suspected patients tended to recover at late stage (Fig. 1A, B). The confirmed patients also displayed higher D-dimer and CRP levels (with a bell-shaped curve) than the suspected patients (with a flatter curve) (Fig. 1C, D).

3.5 Risk factors associated with mortality in confirmed patients and suspected patients

We further assessed risk factors for the mortality of COVID-19. A Cox proportional hazard model was used to analyze the risk factors for mortality in all confirmed and suspected patients. Eighteen variables of clinical indices upon admission were used for analysis in the Cox regression model: lymphocyte count, neutrophil count, monocyte count, D-dimer, CRP, albumin, myoglobin, Lactate dehydrogenase (LDH), B-type natriuretic peptide, blood glucose, presence of ground-glass opacity lesions, age, gender, diabetes, hypertension, lung disease, cardiovascular disease, and cerebrovascular disease.

Six variables were identified as risk factors for mortality in confirmed patients: lung disease (adjusted hazard ratio 8.972, 95% CI 3.782–21.283), cardiovascular disease (3.083, 1.347–7.059), neutrophil count (1.189, 1.081–1.307), age (1.068, 1.027–1.110), presence of ground-glass opacity lesions (1.039, 1.013–1.065), and myoglobin levels (1.004, 1.003–1.005) (Table 4).

Four variables were identified as risk factors for mortality in suspected patients: lung disease (14.725, 2.187–99.147), age (1.076, 1.004–1.153), CRP levels (1.012, 1.004–1.02), and B-type natriuretic peptide levels (1.001, 1.000-1.001) (Table 4).

4. Discussion

The rapid transmission of SARS-CoV-2 has caused a global outbreak of COVID-19, which often overwhelms the capacities of local hospitals in the affected areas. This situation is complicated by the fact that a large number of patients presenting to these local hospitals were negative for SARS-CoV-2, and repeat testing was required to properly categorize them (the median number of RNA tests per patient is 2, range 1–9 in this study). These suspected patients displayed typical COVID-19 symptoms and lung abnormalities, which challenged the precise diagnosis and administration of appropriate treatment. Due to the exacerbating situation, the suspected patients were clinically diagnosed and treated as COVID-19 in Wuhan from February 4, 2020 to March 3, 2020. Nevertheless, these cases have been insufficiently characterized due to a lack of sufficient clinical evidence at the time. In this retrospective cohort study, we have thoroughly analyzed the clinical data from these patients to improve the diagnosis and treatment of suspected and confirmed COVID-19 patients in the ongoing pandemic.

In fact, the suspected patients were largely comparable to the confirmed patients (Tables 1–2), except that the suspected patients tended toward a less severe presentation, for example, shorter hospital stays and less frequent ventilator usage. This observation is supported by another study [15]. It is worth noting that our serological test for SARS-CoV-2 antibodies revealed that most of the suspected patients were positive for SARS-CoV-2 IgM or IgG (Table 3). A previous study also found that the suspected patients who originally tested negative via qRT-PCR eventually turned out to be positive patients when repeat tests (up to 6) were performed over time [16]. These observations strongly argue that the majority of suspected patients meeting hospital criteria for moderate to critical illness are indeed real COVID-19 patients. They do have typical pneumonia symptoms and may progress to severe illness (10.2%, Table 1), or even death (2.1%, Table 1). Therefore, these patients must be properly treated and managed. Self-isolation and

closely monitoring viral RNA levels and disease progression may be the optimal choice for managing these suspected patients during the COVID-19 outbreak, as practiced in affected areas.

Our observations on suspected patients may reflect special feature of coronavirus infection. A previous study reported that MERS-CoV-negative patients showed typical MERS symptoms, display less severe disease manifestation, and survived much better than MERS-CoV-positive patients [17]. Given that viral load is a very important factor in disease severity and outcome, suspected patients may simply have a viral load that is lower than the limit of detection. To support this, we found that the qRT-PCR Ct value was correlated with disease severity and death (Table 3). This study provides data for improving the current management guidelines for suspected patients in various countries; a negative RNA test alone should not exclude patients from the diagnosis of COVID-19. RNA test results should be considered in combination with radiologic and serological features. Negative RNA test results may also be due to errors in throat swab collection, or simply due to the fact that throat swabs may not be the best specimen for SARS-CoV-2 detection [14, 18]. Further, we could not exclude that variant SARS-CoV-2 strains may exist in these patients that could not be recognized by the present primer sets. Neither could we exclude the possibility that other unidentified pathogens may have caused the pneumonia in these patients.

Risk factors for mortality have been implicated in several studies. Patients in these studies are from the very early stages of the COVID-19 outbreak in Wuhan, and the reported fatality is relatively high (54 deaths out of 191 patients in one study) [19]. These patients are likely highly selected and may not be representative of the global patient population. In this study, our patients were admitted from Jan 27 to Mar 3, 2020, a time period that spanned the major course of the epidemic in Wuhan.

Furthermore, we used multiple-variable Cox regression, instead of logistic regression, so that the potential effect of the length of the hospital stay on the clinical outcome was considered. It is worth pointing out that the effect of clinical type (confirmed vs suspected) on mortality was not significant in the Cox model (Table 4). This further supports the need to treat both types of patients if they present with moderate to serious illness. In addition to age (a well-known risk factor), lung disease was found to be a significant factor in our model. It is conceivable that underlying lung disease may worsen lung dysfunction and failure, representing a major cause of severe COVID-19 [20]. The cardiovascular system is known to be an important target for SARS-CoV-2, due to the expression of ACE2, and myoglobin and LDH levels reflect impairment in the heart [21]. Our results are consistent with the idea that COVID-19 mortality is most likely due to multiple organ failure. Additionally, we observed that CRP and B-type natriuretic peptide levels were associated with life-threatening infections and impaired heart function, leading to death in suspected patients. Ground-glass opacity lesions represent a unique feature of COVID-19, and the increased incidence of ground-glass opacity lesions was identified in our model as a significant risk factor. Our findings demonstrate the valuable application of using AI-aided quantification of chest CT scan in precise diagnosis of COVID-19. Interestingly, the decreased adjusted hazard ratio of albumin was also identified, which supports the use of albumin in combating SARS-CoV-2 infection. Monitoring these factors on admission may help predict the prognosis of patients and aid in the formulation of rational patient management.

4.1 Limitations of this study

This study has several limitations. First, all of the data in this study were collected from a single hospital; a multiple-center study may help reveal more aspects of this disease. Second, only throat swabs were used, and the serum of patients was not obtained to evaluate viremia. Third, many patients still remained in hospital, or had been transmitted to other hospitals, by the last follow-up day. Therefore, the information of these patients is missing for the assessment of risk factors. However, the above limitations do not negate our main findings.

5. Conclusion

Suspected patients with moderate to severe illness, who may have a fever and difficulty breathing, should seek medical attention to reduce fatality. Multiple factors on admission need to be considered to assess the mortality risk of confirmed and suspected patients for the administration of appropriate clinical management.

Abbreviations

C. reactive protein (CRP); Severe acute respiratory syndrome conoravirus-2 (SARS-CoV-2); Severe coronavirus disease 2019 (COVID-19); World Health Organization (WHO); Centers for Disease Control and Prevention (CDC); National Institute for Health and Care Excellence (NICE); Quantitative reverse transcription polymerase chain reaction (qRT-PCR); Computerized Tomography (CT); 6-Carboxyfluorescein (FAM); Cycle threshold value (Ct value); Interquartile range (IQR); Lactate dehydrogenase (LDH)

Declarations

Acknowledgements

We extend special thanks for the contribution of consultant experts and healthcare practitioners from Wuhan Third Hospital for their participation.

Author Contributions: X. H., Z. H. and P.C. L. designed study and supervised the study. Q.M.L., D.X., Y.L.Y., Y.W.W., J.B.S., Y.Q.T and J.W. collected and reviewed clinical, laboratory, and radiological data. M.M.X., D. C., and L.L.L. performed statistical data analysis. F.D., H.R.G., M.B., Z.H.L., M.L., A.L.P., Q. M.W, W.G.X and J.H.W. provided valuable suggestions for study design and data analysis. X. H., Z. H., P.C. L., D.C. and M.M.X. wrote the manuscript and all authors have approved the final version of this paper.

Funding: This work was supported by the National Natural Science Foundation of China (Grant Nos. 91957109 and 81870427 to Dr. Z. Huang; Grant No. 81770688 to Dr. P. Luo) and Leading Medical Talents in Hubei Province to Dr. P. Luo.

Availability of data and materials

The data-sets used and/or analyzed during the current study are available from the corresponding author on request.

Ethics approval and consent to participate

This study was conducted with the approval of the Ethics Committee of Tongren Hospital of Wuhan University (KY2020-020).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

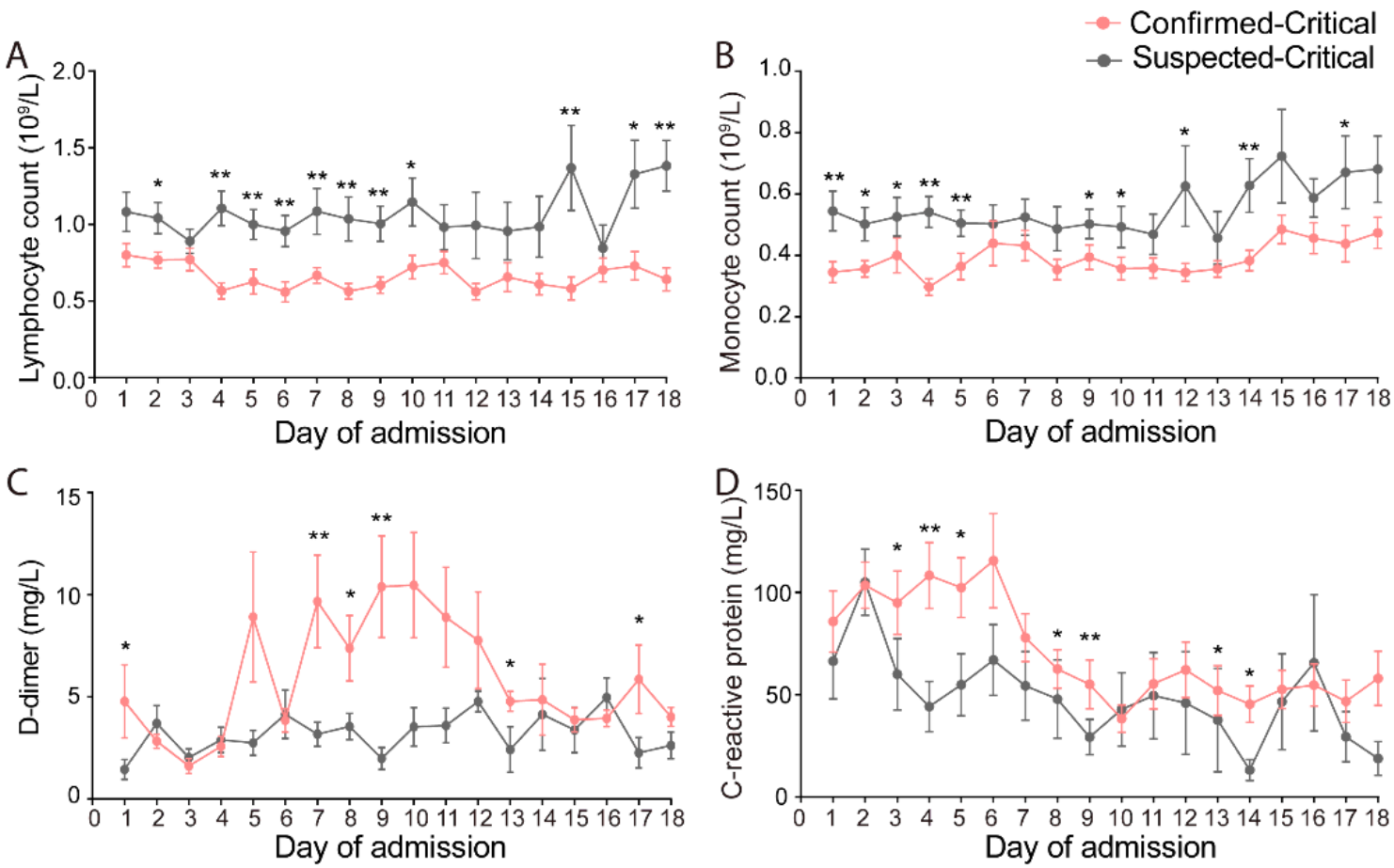

Figure 1

Dynamic Profiles of Laboratory Parameters in COVID-19 Patients in Critical Illness. A-D Timeline illustration of the daily laboratory parameters during hospital stay. A. Lymphocyte; B. Monocyte; C. D-dimer; D. C-reactive protein (CRP). The data were represented as mean ± SEM. The confirmed COVID-19 patients were separated from suspected patients in critical illness. *: P<0.05; **: P<0.01