Analysis of mortality in patients of COVID-19: clinical and laboratory parameters

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Abstract

Several reports on epidemiological and clinical features of the 2019 coronavirus disease (COVID-19) had been published. However, mortality and morbidity analysis, important for better understanding of the pathogenesis of this disease, are scarce. We examine the clinical and laboratory features of 14 patients who died of COVID-19. The cohort consisted of 11 male and 3 female patients; nine patients aged 70 years or above, and nearly all had underlying diseases. Fever with bilateral pneumonia was the main manifestation. Most patients had consolidations combined with ground glass opacity (GGO) on chest CT scan. Laboratory tests showed lymphocytopenia in 10 patients, high blood glucose in 11, GGT in 5 of the 14 patients, and high LDH in 5 of 6 patients tested. In addition, patients in this cohort had high level of cytokines such as interleukin 6 in all 8 patients tested. The clinical and laboratory parameters in the cohort of fatal cases may be incorporated into future clinical prognosis models, and will be of help in understanding the pathogenesis of this disease.

Key words: coronavirus; SARS-CoV-2; COVID-19; pneumonia; mortality; risk assessment

Introduction

The ongoing outbreak of SARS-CoV-2 infection, which causes COVID-19 (2019 coronavirus disease), also known as the novel coronavirus pneumonia (NCP), has become worldwide pandemic. Although the vast majority of infected patients suffer from a mild form of illness and recover, a portion of patients who fall into the severe case category eventually die of the illness[1, 2] . In a recent study of a cohort of 138 patients from our hospital, the overall mortality is 4.6%[1]. Another study with 402 patients showed higher mortality in male than female, and a mortality of 19% in patients of age 70 or older[3]. To date there have been several studies published about COVID-19 on infection transmission, and clinical characteristics of hospitalized patients[1, 2, 4-6]. However, pathological data are still scarce, and little is known about the pathogenesis and clinical risk factors of COVID-19. The present study was aimed at identifying common clinical and laboratory features from 14 patients who died of COVID in our hospital to gain insight to the underlying pathogenesis.

Methods

Data collection

This study was approved by the Ethics Board of Zhongnan Hospital of Wuhan University. The electronic medical records were retrospectively searched to identify fatal cases of COVID-19 for the month of January of 2020. These patients either had the diagnosis confirmed by a positive nucleic acid test[1], or met the clinical diagnostic criteria as provided in the National Health Commission of China Guidelines(http://www.nhc.gov.cn/yzygj/s7652m/202002/e84bd30142ab4d8982326326e4db22ea.shtml). Epidemiological, clinical, laboratory, and radiological characteristics and

treatment history were recorded according to the data collection forms and then reviewed and double-checked. Data collection forms included demographic data, medical history, history of exposure to infection, symptoms, signs, laboratory findings, chest computed tomographic (CT) scans or chest X-ray results, treatment measures especially history corticosteroid use, and duration of illness.

Statistical Analysis

Statistical analyses were performed using Graphpad Prism version 7.0 software. Wilcox on rank-sum tests was used for nonnormally distributed data. *p* value less than 0.05 was considered statistically significant.

Results

Clinical and radiologic features of the cohort

As shown in table 1, patients ages range from 36 to 92 years (median = 71). The male-to-female ratio is 3.7 to 1. Past medical history included at least one underlying disease such as hypertension, cardiovascular disease, diabetes, lymphoma and carcinoma (cases 6, 13 and 14) in all but 2 patients (case 9, 12). Most patients had high fever with maximal temperature reaching 39.8℃. Two patients were not febrile, one had renal failure suggesting an immunocompromised status and the other had lung cancer (cases 11 and 14). A nuclear acid test for SARS-Cov-2 was positive for 10 patients and unavailable for the 4 patients. However, the latter 4 met the criteria for clinical diagnosis of COVID-19. The patients received routine comprehensive treatments including intravenous antibiotics, assisted oxygenation, specific treatment for the underlying diseases, and supportive treatments. In addition, 11 patients had

glucocorticoid therapy during the course of their hospitalization (not known in 3 patients). The major complications leading to patient demise were acute respiratory distress syndrome (ARDS), heart and respiratory failure, septic shock or multiple organ failures. Case 13 died of gastrointestinal bleeding due to the underlying NK/T cell lymphoma. Duration of the clinical course from onset of illness to death ranged from 5 days to 28 days, with a mean of 15 days.

Radiologically, this cohort showed severe radiologic abnormalities. The majority had bilateral pneumonia and 13 had consolidations combined with ground glass opacity (GGO), interstitial thickening, air bronchogram, pleural puckering and parenchymal band, which were different from pure GGO that were seen in mild cases (**Figure 1**). Seven patients had pleural effusion at the same time. For case 1, it was unusual that both CT scan and X-ray showed improvement in his lungs during the third week compared to the second week, but this middle-aged patient's condition deteriorated towards the end (Figure 2).

Laboratory findings

Hematological findings are summarized in table 2. Abnormalities in CBC included marked lymphocytopenia (10/14, 71%), leukocytosis (6/14, 43 %) or occasional leukopenia. Neutrophil count had the same tendency as white blood cell count (WBC). Compared with the WBC and neutrophil count from the group of patients reported in the Wang et al study[1], the present group showed higher counts than the non-ICU group and similar to the ICU group (table 3). As for subsets of peripheral lymphocyte, both $CD4^+$ and $CD8^+$ T lymphocyte subsets decreased significantly and the ratio of $CD4^+$ to $CD8^+$ T cells decreased as well in

some patients (data not shown). Eosinophil count of most patients (12/14, 86%) were zero. While the counts of red blood cell ranged from normal to mild decrease, hematocrit decreased obviously.

As for biochemical parameters or organ functional parameters, a common finding was elevated LDH in most patients tested (5/6). 11(79%) showed elevated AST, 2 (14%) showed elevated ALT and 5 (36%) showed elevated GGT level which reflects function of biliary epithelial cells. 11 (79%) showed elevated fasting blood glucose.

Of interest, analyses of cytokines and complement showed increased release in tested patients. Specifically, interleukin (IL)-6, IL-10 were elevated in all the patients tested and C1q was elevated in 3 patients tested. Other chemokines or cytokines such as Interleukin 4 and tumor necrosis factor decreased (not shown).

Culture results

Pharyngeal swab test for mycoplasma infection was positive in some patients. It was common to detect bacterial colonies in urine samples. These may suggest secondary infections due to impaired immunity and the use of antibiotics. (not shown)

Discussion

SARS-CoV-2 is the seventh member of the family *coronaviridae* that infect human[6]. It shares similarity with MERS-CoV and SARS-CoV in gene sequences, but is different from the latter in many regards[5]. It is believed that SARS-CoV-2 is less pathogenic than SARS-CoV but stronger in transmission competence[7]. The pathogenic mechanism remains poorly understood. We attempted to gain insight to the pathogenesis through an analysis of available clinical data on fatal cases and literature review, partly relying on the homology of SARS-CoV-2 with SARS.

From this cohort of fatal cases, it is evident that male gender and advanced age predominate (12/14, 86%). This is consistent with previous reports[2]. Most of the patients had underlying diseases prior to the infection, which likely contribute to risk for death. Radiographically, these patients presented with bilateral pneumonia and severe abnormalities on chest CT images, indicating another hint for a worse prognosis. Of note, some patients showed improvements radiographically during the clinical course, but deteriorated abruptly later on as case 1.

Elevation of WBC indicates bacterial infection, and viral infection causes lymphocytopenia. Some patients in this cohort exhibit both elevated WBC and lowered lymphocyte count. A number of potential mechanisms may be involved. First, SARS-CoV-2 infection may affect hematopoietic stem/progenitor cells via CD13 or CD66a directly or through development of auto-antibodies or immune complexes as seen in SARS[8]. Atrophy of bone marrow hematopoietic tissue has been seen in SARS and COVID-19[9, 10]. Second,

glucocorticoid treatment can induce lymphocytopenia in some patients[8]. Interestingly, case 14 had no fever during her stay in the hospital. The CBC profile from the first day post-op showed high WBC counts and lymphocytopenia prior to the positive CT and nuclear acid test, suggesting that this CBC profile may serve as a good clue for early diagnosis in the future. Third, as seen in another study of ours[11], superimposed bacterial infection can be seen a portion of patients, which can lead to elevation of WBC.

Homeostasis and normal immune system are critical in controlling acute viral infection. By dynamically screening 14 cytokines/chemokines in blood, Jiang et al found that levels of proinflammatory cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 increased in the patients with superinfection, and the mRNAs for these cytokines were also increased in lung tissues[12]. These suggest that elevation of these cytokines/chemokines is a sign of superinfection and may be related to a high risk for death[12]. Complement is also a major component of innate immunity. Mouse model deficient in complement C3 has less weight loss and reduced pathology, improved respiratory function, and lower levels of inflammatory cytokines/chemokines in the lung and periphery blood after SARS-CoV infection, as compared with wild type mice [13] . Therefore it is suggested that inhibiting complement signaling may serve as a strategy for immune therapy. Other theories suggest that lack of antiviral cytokine response such as interferon alpha, IFN-beta, IFN-gamma, and interleukin 12p40 represent a mechanism of immune evasion by SARS-CoV[14]. The elevated IL-6 and a component of complement C1q in our study suggest that innate immunity play an important role in COVID-19 pathogenesis. Dynamic screening the level of these cytokines and component of complement system will be helpful for evaluating the prognosis and clinical treatment.

Another important player in the pathogenesis of SARS-CoV-2 is angiotensin-converting enzyme (ACE2), which has been recognized as the functional receptor for both SARS-CoV and SARS-CoV-2[5, 7, 15]. ACE2 mRNA and protein are known to be present in virtually all organs (respiratory system, gastrointestinal tract, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain)[15]. The surface expression of ACE2 protein in alveolar epithelial cells and gastrointestinal epithelial cells provide the routes of viral entry [16] . These are also related to symptoms such as nonproductive cough, dyspnea and diarrhea. We suspect that there are higher densities of ACE2 in lung alveolar epithelial cells in male than in female patients, which may be the underlying reason for higher morbidity in male. In addition, elevated fasting blood glucose, GGT, and LDH can be closely related to impairment of the microenvironment of the infected tissue and the damage to target cells such as beta cells in the islets, biliary epithelial cells in the liver, and myocardium cell in heart, through ACE2 and the subsequent disruption of different feedback loops[17, 18]. The presence of ACE2 on bone morrow tissue can be one of the causes for hematological changes described above. SARS-CoV-2 may also aggravate the condition of patients with hypertension through the ACE2 in human vascular endothelia and lead to a worse prognosis [16].

Of note, patient 9 was a 36-year old male with no past medical history. He had a rather short course of illness (14 days) before he died of septic shock. Although no underlying disease was identified, he did have high FBG of 18.3, suggesting an unrecognized diabetic status. He also tested 260.6 for IL-6.

In conclusion, we analyzed a cohort of patients who died of severe COVID-19 for clinical and laboratory features. Male gender, advanced age, and underlying diseases appear to be most common associations with fatal outcome of this disease. Most of the patients had

lymphopenia, and elevated level of IL-6 in their blood. Further studies including more cases and ancillary tests, and animal models are necessary to elucidate the mechanism of disease progression and fatal outcome.

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Figure legend

Figure 1. Consolidations combined with ground glass opacity (GGO), interstitial thickening, air bronchogram, pleural puckering and parenchymal band in fatal cases (A, Case 1; B, Case 5; C, Case 7; D, Case12)

Figure 2. Chest X-ray images from Case 1. Compared with the second week (A), there is improvement in the lungs in the third week (B), but worsened changes in the fourth week (C).

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Table 1. Demographic and clinical features in 14 fatal cases of SARS-CoV-2 pneumonia

Patient	Age	sex	Past medical history	CT	Nuclear acid test	Maximal temperature	Glucocorticoid therapy	Complication	Duration of clinical course
1	54	M	hypertension	Bilateral，2nd week confirmed, 3rd week alleviated	positive	39.3	yes	cardiac and respiratory arrest	28
2	78	M	diabetes	Positive (outside hospital)	positive	39.1	yes	cardiac and respiratory arrest	10
3	88	M	encephalotrophy, smoking	Bilateral positive	positive	39	yes	acute respiratory distress syndrome	9

4	84	F	hypertension and diabetes	Bilateral positive	positive	39	yes	heart and respiratory failure, severe pneumonia	6
5	78	M	hypertension and heart failure	Bilateral positive	positive	38.7	yes	cardiac and respiratory arrest	5
6	67	F	chronic bronchitis, hypertension, GI ulceration, liver cirrhosis, esophagus carcinoma	Right side positive	positive	38.3	yes	respiratory failure	28
7	72	M	chronic bronchitis	Bilateral positive	positive	39.4	yes	heart failure	13
8	92	M	brain infarction	Bilateral positive	NA	38.5	yes	acute respiratory distress syndrome	20
9	36	M	unknown	Bilateral positive	NA	39.5	yes	acute respiratory distress syndrome，septic shock	14
10	81	M	cardiac disease	Bilateral positive	NA	38.9	yes	septic shock	7
11	66	M	chronic renal failure, hypertension	Bilateral positive	positive	36.6	NA	cardiac and respiratory arrest	8

12	70	M	unknown	Bilateral positive	NA	39	yes	cardiac and respiratory arrest	18
13	38	M	lymphoma	Right lung positive	positive	39.8	NA	GI bleeding	19
14	84	F	lung cancer, hypertension, diabetes	Bilateral positive	positive	normal	NA	multiple organ failure	28

Table 2. Laboratory tests in 14 fata cases of SARS-CoV-2

Patient	WBC ($\times 10^9$/L)	Neutrophil ($\times 10^9$/L)	Lymphocyte ($\times 10^9$/L)	HCT (%)	Eosinophil ($\times 10^9$/L)	AST (U/L)	ALT (U/L)	GGT (U/L)	LDH (U/L)	FBG (mmol/L)	C1q (mg/L)	IL-10 (pg/ml)	IL-6 (pg/ml)
normal range	3.5-9.5	1.8-6.3	1.1-3.2	35-45	0.02-0.52	13-35	7-45	8-57	125-243	3.9-6.1	159-233	0.1-5.0	0.1-2.9
1	16.5	15.3	0.6	34.8	0.0	49.0	33.5	232.5	402.0	7.4	246.1	33.1	101.2
2	4.3	3.9	0.3	35.2	0.0	46.0	30.5	45.0		23.0	129.1		
3	11.5	10.2	0.8	30.2	0.0	680.5	276.5	44.0	1592.5	7.0	130.9		
4	5.4	3.4	1.7	36.0	0.0	58.0	44.0	40.0		9.6			
5	12.7	11.8	0.4	43.7	0.0	54.0	22.5	30.0	683.0	6.8	172.2		
6	7.5	5.9	1.1	32.7	0.0	18.5	12.5	15.0	168.0	6.0	176.9		54.8
7	5.5	4.8	0.6	29.7	0.0	101.0	40.0	133.0		5.8			
8	2.6	2.0	0.4	29.0	0.0	57.0	26.0	36.0	254.0	6.0	441.5		119.7
9	13.7	10.3	2.7	43.8	0.0	81.5	52.0	106.5		18.3	54.5		260.6
10	4.2	3.3	0.6	39.1	0.0	18.0	13.0	12.0		10.8	100.1		128.3
11	4.1	3.6	0.3	40.5	0.0	66.0	38.0	81.0	277.0	11.6	274.5		167.0

12	4.3	3.9	0.3	38.4	0.0	46.0	30.5	45.0		9.9			138.9
13	15.4	11.7	2.7	18.7	0.1	28.0	32.0	43.0		6.3	173.4		23.5
14	23.5	21.7	1.2	39.8	0.1	52.5	38.5	67.5		10.4	140.5		
mean	9.4	8.0	1.0	35.1	0.0	96.9	49.3	66.5	562.8	9.9	185.4	33.1	124.2

WBC: White Blood Cell; HCT: hematocrit; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GGT, Gamma-glutamyl transpeptidase; LDH, Lactate dehydrogenase; FBG: Fasting blood glucose; IL, Interleukin

Table 3. Comparison of laboratory parameters between this cohort and groups in the Wang et al[1] study

	Normal range	ICU (n = 36)	Non-ICU (n = 102)	Alive (n=138)	Fatal (n=14)	*P* value (Overall)	*P* value (ICU)	*P* value (Non-ICU)
White blood cell count ($\times 10^9$/L)	3.5-9.5	6.6 (3.6-9.8)	4.3 (3.3-5.4)	4.5 (3.3-6.2)	9.4（2.6-23.5）	0.0243*	0.3098	0.0125*
Neutrophil count ($\times 10^9$/L)	1.8-6.3	4.6 (2.6-7.9)	2.7 (1.9-3.9)	3.0 (2.0-4.9)	8.0（2.0-21.7）	0.0183*	0.2177	0.0066*
Lymphocyte count ($\times 10^9$/L)	1.1-3.2	0.8 (0.5-0.9)	0.9 (0.6-1.2)	0.8 (0.6-1.1)	1.0（0.3-2.7）	0.5948	0.3489	0.7863
Alanine aminotransferase (U/L)	9-50	24 (16-40)	35 (19-57)	23 (15-36)	49.3（12.5-276.5）	0.2097	0.2549	0.5675

Aspartate aminotransferase (U/L)	15-40	31 (24-51)	52 (30-70)	29 (21-38)	96.9（18-680.5）	0.1616	0.2028	0.3394
Lactate dehydrogenase (U/L)	125-243	261 (182-403)	435 (302-596)	212 (171-291)	562.8（168-1592.5）	0.1845	0.2657	0.6309

* *p* value less than 0.05

Figure 1

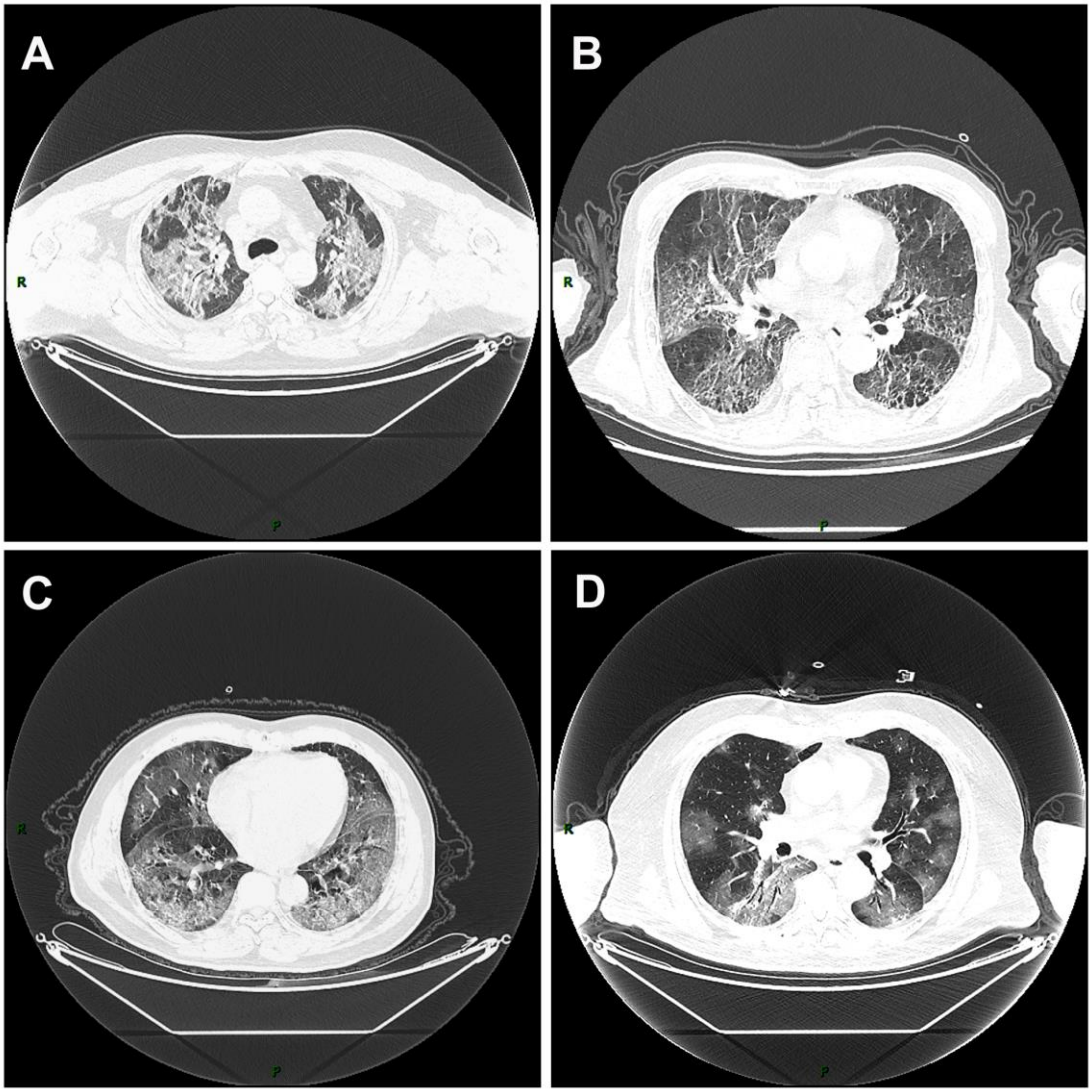

Figure 2