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Letter to the Editor

Cytokine levels in sputum, not serum, may be more helpful for indicating the damage in the lung and the prognosis of severe COVID-19 – A case series

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can cause cytokine storm and acute respiratory distress syndrome (ARDS) or even death. IL-6 and TNF-α levels in serum are independent and important predictors of coronavirus disease 2019 (COVID-19) severity and death. Our research finds that IL-6 and IL-10 are involved in the deterioration of COVID-19. IL-6 levels in bronchoalveolar lavage fluid (BALF) and in the pleural effusion of COVID-19 patients are significantly higher than that in peripheral blood. We aim to explore the relationship between the severity of lung damage and cytokine levels in sputum, BALF and serum. Here we analyze eight severe patients with SARS-CoV-2.

Methods

From January 2021 to March 2021, eight severe COVID-19 patients were admitted and evaluated at COVID-19 Critical Care Center in Heilongjiang Province, China. According to the treatment guidelines of China’s national COVID-19 intensive care expert group, all patients received oxygen therapy, expectoration, daily prone ventilation for 12–20 h, anti-infection, anticoagulant, and symptomatic treatment. Serum samples were collected, and bronchoscopy was performed within 24 h after mechanical ventilation. During the process of bronchoscopy, sputum samples from both lungs were taken, and bronchoscopy alveolar lavage was performed. Cytokine levels were detected by ELISA. Chest computed tomography (CT) examination was performed within 24 h before or after mechanical ventilation.

Results

The median age of the eight patients is 72 years (range 54–79 years) and the median intensive care unit (ICU) hospitalization days is 40.5 days (range 29–66 days). Seven of them have chronic diseases. The most common symptoms before admission are fever (N = 7), fatigue (N = 5), cough (N = 5), sputum (N = 3), and pharyngalgia (N = 3); medium common symptoms are dyspnea (N = 2), dizziness (N = 2). The patients usually take one or two weeks to become severe after the symptom’s onset. Three of them had thrombocytopenia (#1, #2, #5). All of the cases require supplemental oxygen and underwent high-flow oxygen inhalation or non-invasive mechanical ventilation, and finally mechanical ventilation. Except for #4, all patients had a tracheotomy.

Some patients received antibody plasma (#8), glucocorticoid therapy (#1), continuous renal replacement therapy (CRRT) (#7, #8), and extracorporeal membrane oxygenation (ECMO) (#1, #4, #7, #8). So far, four patients have finally recovered, and four patients died (#4, #5, #7, #8), but #8 was died of tumor-related complications after treating COVID-19 successfully and removing the ventilator (Table 1).

The levels of IL-6, IL-10, and TNF-α in sputum and in BALF from both lungs were detected. The sputum sample of patient #3 and the sputum sample from the left bronchus of patient #8 was not obtained during bronchoscopy. Except for #1, IL-6 and TNF-α levels in sputum are slightly higher than that in BALF with small differences but significantly higher than that in serum. The IL-6 levels in BALF and in sputum of three patients who died of COVID-19 are 4.63 to 27.16 times and 13.06 to 37.82 times higher than that in serum, respectively (#4, 4.63 & 13.06; #5, 13.42 & 25.05; #7, 27.16 & 37.82). Levels of IL-6 in BALF and in sputum of COVID-19 recovered patients are 0.57 to 8.84 times and 0.32 to 4.72 times higher than that in serum (#1, 0.57 & 0.32; #2, 1.76 & 2.88; #3, 8.84 & NA; #6, 3.28 & 4.72; #8, 1.33 & 2.43) (Table 2). In addition, levels of IL-6 and TNF-α in sputum and in BALF are related to the severity of lung injury. Except for #7 (CT shows both lungs are extensively affected, but cytokine levels of the left lung are higher than that of the right lung), the cytokine levels in sputum from infected lung (left/right) are consistent with the severity of the corresponding CT imaging. Compared with in sputum, IL-6 and TNF-α levels in BALF from #4 and #5 show some deviation. (Table 3, Fig. 1)

Discussion

The systemic levels of cytokines caused by COVID-19 may be lower than sepsis, but the local response is more intense. Cytokine levels in circulation may not accurately reflect that in local tissue. The samples from BALF have the highest positive rate of detecting SARS-CoV-2, followed by sputum samples from the lower respiratory tract. Most COVID-19 patients are accompanied by elevated serum IL-6 before their lung lesions becoming worse, and the consistently high IL-6 level may be a risk factor for persistent lung injury. The lung is the core site of SARS-CoV-2 infection that may cause a different mechanism from the other cytokine storm-related diseases. Recently, the intervention methods through the respiratory tract attract more attention. In one animal experiment, intranasal administration of specific dimer lipopeptides can effectively block the host SARS-CoV-2 virus replication. And to inhale

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; CT, computed tomography; ICU, intensive care unit.
**Table 1**
Patient characteristics and treatment.

| Characteristics                  | Patient | #1            | #2            | #3            | #4            | #5            | #6            | #7            | #8            |
|----------------------------------|---------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Sex                              |         | F             | M             | F             | M             | M             | M             | M             | M             |
| Age, y                           |         | 72            | 76            | 66            | 54            | 77            | 72            | 72            | 79            |
| Comorbidity                      |         | HT, DM, AF, CI, CH | AF, HT, CI | HT         | None          | HT, COPD      | CI, Lung carcinoma, Chronic bronchitis | HT, DM, CI | HT, DM, Renal carcinoma, Lung metastases, Emphysema, Fatigue, Pharyngalgia |
| Symptom                          |         | Fatigue, Dyspnea | Fever, Fatigue | Fever, Cough, Fatigue, Pharyngalgia, Dizziness | Fever | Fever, Cough, Sputum, Pharyngalgia, Dizziness | Fever, Cough, Sputum | Fever, Cough, Fatigue |
| Severity on diagnosis of pneumonia |         | Severe      | Severe       | Moderate     | Moderate   | Moderate     | Moderate     | Moderate     | Severe     |
| Disease presentation and course  |         |               |              |              |           |             |              |              |             |
| Interval between symptom onset and ICU admission |   | 5             | 6             | 10            | 7             | 18            | 11            | 15            | 10            |
| ICU hospitalization days         |         | 48            | 29            | 33            | 42            | 62            | 39            | 37            | 66            |
| Steroids                         |         | Methylprednisolone | None          | None         | None         | None         | None         | None         | None         |
| Convalescent Plasma              |         | None          | None          | None         | None         | None         | None         | None         | None         |
| Etezavimab (LY-CoV016, 600 mg, 6 ml) |         | 30ml          | 30ml          | 29ml         | None         | None         | None         | None         | None         |
| Treatment                        |         |               |              |              |             |             |              |              |              |
| Other                            |         | ECMO Recovered | None          | Recovered    | None         | ECMO Death   | None         | ECMO&CRRT    | ECMO&CRRT    |
| Outcome                          |         |               |              |              |             |              |              |              |              |

F, female; M, male; HT = hypertension; DM = diabetes mellitus; AF = atrial fibrillation; CI = cerebral infarction; CH = cerebral hemorrhage; ICU = intensive care unit; CRRT = continuous renal replacement therapy; ECMO = extracorporeal membrane oxygenation.
Table 2
Clinical characteristics and Laboratory findings.

| Variables                        | Patient | #1  | #2  | #3  | #4  | #5  | #6  | #7  | #8  |
|----------------------------------|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| **Clinical characteristics**     |         |     |     |     |     |     |     |     |     |
| Body temperature, °C             |         | 37.2| 37.3| 37.3| 37.6| 38.1| 37.8| 36.9| 37.4|
| PaO2/FiO2                        |         | 100 | 167.2| 168 | 117.2| 216.3| 152 | 123.6| 82.7|
| Mechanical ventilation, d        |         | 26  | 16  | 15  | 36  | 59  | 27  | 33  | 63  |
| ECMO, d                         |         | 16  | NA  | NA  | 24  | NA  | NA  | 20  | 21  |
| **Laboratory findings**          |         |     |     |     |     |     |     |     |     |
| Serum                            |         |     |     |     |     |     |     |     |     |
| IL-6 (1.18–5.3 pg/ml)            |         | 92.65| 2512.21| 85.06| 311.76| 300.11| 323.98| 197.09| 765.86|
| IL-10 (0.19–4.91 pg/ml)          |         | 3.78 | 27.19 | 6.63 | 16.34 | 9.38 | 7.35 | 3.23 | 9.63 |
| TNF-α (0.1–2.31 pg/ml)           |         | 1.43 | 1.04 | 1.5  | 1.18 | 1.95 | 1.37 | 0.84 | 0.8  |
| C-reactive protein (0–10 mg/L)   |         | 96.85| 234.64| 52.85| 242.68| 227.04| 228.96| 122.38| 180.73|
| **Sputum**                       |         |     |     |     |     |     |     |     |     |
| IL-6 (1.18–5.3 pg/ml)            |         | 5.69 | 7227.85| NA  | 889.08| 7517.66| 777.46| 7454.47| NA  |
| Right                            |         | 29.90| 1907.96| NA  | 4072.92| 3113.14| 1529.61| 5111.11| 1859.87|
| TNF-α (0.1–2.31 pg/ml)           |         | 12.63| 244.02| NA  | 73.78 | 21.71 | 6.58 | 712.30| NA  |
| Left                             |         | 213.55| 40.94 | NA  | 286.76| 17.88 | 37.7 | 421.76| 127.26|
| **BALF**                         |         |     |     |     |     |     |     |     |     |
| IL-6 (1.18–5.3 pg/ml)            |         | 44.13| 4434.00| 373.30| 1444.92| 1473.43| 367.08| 5352.55| 363.26|
| Right                            |         | 52.76| 2518.69| 751.52| 411.39| 4026.66| 1062.52| 4750.29| 1018.25|
| TNF-α (0.1–2.31 pg/ml)           |         | 17.10| 123.05| 55.39| 87.38 | 10.22 | 16.55| 92.55 | 4.19 |
| Left                             |         | 17.96| 66.56 | 136.86| 26.93 | 15.82 | 28.38| 29.38 | 33.53|
| **CT Left/Right serious**        |         |     |     |     |     |     |     |     |     |
| Right                            |         |     |     |     |     |     |     |     |     |
| Left                             |         |     |     |     |     |     |     |     |     |
| **BALF = bronchoalveolar lavage fluid; ECMO = extracorporeal membrane oxygenation.**

Table 3
The cytokines level in Sputum and BALF associated with CT images.

| Variables                        | Patient | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 |
|----------------------------------|---------|----|----|----|----|----|----|----|----|
| IL-6 and TNF-α in Sputum         |         | L | H  | NA | L  | H  | L  | H  | NA |
| Left                             |         | H | L  | NA | H  | L  | H  | L  | H  |
| Right                            |         | H | L  | H  | L  | H  | L  | H  | H  |
| IL-6 and TNF-α in BALF           |         | L | H  | L  | H  | L  | H  | L  | H  |
| Left                             |         | H | L  | H  | L  | H  | L  | H  | H  |
| Right                            |         | L | H  | NA | L  | H  | NA | L  | H  |
| CT Left/Right serious            |         | Left Right | Right | Right Left | Right Both | Right |
| Right                            |         | Left Right | Right Right | Left Right Both Right |

**BALF = bronchoalveolar lavage fluid; CT = computed tomography; L = lower, H = higher, compare with the contralateral lung.**

![Fig. 1. CT imaging of eight COVID-19 patients.](image-url)
CD24 protein by exosomes into the lungs may inhibit the cytokine storm.

Conclusion

The inflammatory response plays a key role in COVID-19 and the cytokine storm aggravates the severity of patients. Compared with in serum, IL-6 and TNF-α in sputum and in BALF may be more directly reflect the severity of COVID-19 critical patients. Cytokine levels in the sputum may be helpful for indicating the extent of damage in the lung and IL-6 may predict the prognosis of patients with severe COVID-19. Local intervention through the respiratory tract may be a reasonable treatment for high-risk patients with severe COVID-19.

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Availability of data and materials

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Human research ethics committee approval for the study was provided by local institution. Patients informed consent. Not applicable.

Consent for publication

Not applicable.

Declaration of Competing Interest

None of the authors reports any competing interests.

CRediT authorship contribution statement

Changsong Wang: Methodology. Kai Kang: Methodology. Xiwen Lan: Writing – original draft. Dongsheng Fei: Writing – original draft. Qian Wang: Writing – original draft. Xianyong Li: Writing – review & editing. Yang Chong: Writing – review & editing. Yan Gao: Writing – review & editing. Huaqian Wang: Writing – review & editing. Xueling Li: Writing – review & editing. Mingyan Zhao: Writing – review & editing. Kaijiang Yu: Writing – review & editing.

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