The iatrogenic elevation of neutrophils possibly aggravates lung injury after COVID-19 infection: A case report

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Case Report

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Abstract

**Background:** Coronavirus disease-2019 (COVID-19) caused by SARS-CoV-2 is a rapidly escalating epidemic in most of countries. Symptom of COVID-19 usually present as the normal or decrease of leucocytes and the decrease of lymphocytes, which may be the body's response for SARS-CoV-2 infection. However, it is unknown that whether rising leukocytes, especially neutrophils, will aggravate lung injury in COVID-19. Here we report a case of aggravated lung injury induced by rising neutrophils with the usage of recombinant human granulocyte stimulating factor (GSF) for the first time.

**Case presentation:** A patient aged 46 years old was infected with SARS-CoV-2 without hypoxemia on admission, but his leucocytes decreased gradually after admission. After following injected with recombinant human granulocyte stimulating factor(GSF) 150 μg , the absolute value of leucocytes reached to 32.81×10⁹ /L, and neutrophils were 31.57×10⁹/L. Then, the patient's condition deteriorated rapidly and he appeared a series of symptoms, such as short breath, hemoptysis, hypoxemia, increased range of lung lesions and secondary acute respiratory distress syndrome (ARDS). However, those symptoms were alleviated and leucocytes recover to normal level gradually after stopping recombinant human GSF treatment. Eventually, Re-examination of CT showed that lung lesions were absorbed significantly and he was cured and discharged from hospital.

**Conclusion:** This case report showed that iatrogenic increase of leucocytes (especially neutrophils) may worsen lung injury and leucocyte increasing agents were used with caution in the early stage of COVID-19 patients. At the same time, the phenomenon remains to be further confirmed in the future study.

Background

Since late December 2019, an outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has occurred in Wuhan, and subsequently spread rapidly to all provinces of China. Currently, COVID-19 has been epidemic in most countries of the world. Clinical data indicate that COVID-19 infection may lead to multiple organ failure (MOF) such as acute respiratory distress syndrome (ARDS), cardiac failure, shock and acute kidney injury (AKI), but the mechanism is still unclear [1-3]. Recent studies indicate that the decrease of leucocyte counts (especially for lymphocytes) and the increase of C-reactive protein (CRP) are positively correlated with the severity of COVID-19[4]. However, so far it has not been reported that whether the increase of leucocytes will worsen the lung injury in the early stage of SARS-CoV-2 infection. Here we reported a case that iatrogenic elevation of leucocytes (especially neutrophils) potentially aggravated lung injury of COVID-19 patient by using recombinant human granulocyte stimulating factor (GSF).

Case Presentation

On January 26th 2020, a 46-year-old man was admitted to a local hospital with symptoms of fever for 3 days and cough for 1 day and he had no any history including hypertension, coronary heart disease, hematopathy, diabetes and genetic disease. The patient was fever up to 39 °C without chills in the early stage. Then, the patient developed symptoms of moderate cough with white sticky sputum, head discomfort, cough, aching pain in right gastrocnemius, without shortness of breath, poor appetite, fatigue, diarrhea and other discomfort. Thoracic computed tomography (CT) scan showed patchy ground glass opacities in bilateral subpleural areas infectious lesions in both lungs (Figure 1a). He was hospitalized for suspected viral pneumonia.

On admission, His body temperature was 38.1°C, and his blood pressure was 124/70mmHg, with heart rate of 96 bpm per minute, respiratory rate of 20 breaths per minute. Clinical laboratory results were showed in Table 1 during hospitalization. On the day of admission (January 26), blood tests revealed leukocytes (5.61×10⁹/L), lymphocytes (0.78×10⁹/L), and neutrophils (4.48×10⁹/L). On January 27, IgG tests for serum chlamydia pneumoniae and mycobacterium tuberculosis were weak positive. IgM tests for chlamydia pneumoniae, mycoplasma pneumoniae, respiratory syncytial virus, parainfluenza virus, influenza A and B virus and legionella pneumophilia were negative. On January 28, the Centers for Disease Control (CDC) confirmed that the patient's oropharyngeal swab tested of SARS-CoV-2 by real-time reverse-transcriptase–polymerase-chain-reaction (rRT-PCR) assay was positive. According to the diagnostic criteria in China (General Office of National Health Commission, 2020), he was diagnosed as a common type of COVID-19.

Antiviral drug consisting of Lopinavir 200 mg and Ritonavir 50 mg was treated twice a day. On January 29, the patient's condition did not improve, and he was still fever and even as high as 39.5°C. Meanwhile, compared with the CT imaging on January 26, multilobed segments of both lungs were scattered in patchy and flaky high-density foci, and some of them showed more inflated bronchial signs and blurred edges (Figure 1b). Based on the symptoms, the patient was intravenous-drip with methylprednisolone 80 mg daily for anti-inflammatory treatment, because of considering the progression of lung disease. However, the absolute value of leucocytes continued decrease, especially lymphocytes and monocytes. Local doctors took clinic strategic to increase leucocytes through treating with 20mg leucoson, 20mg vitamin B4 and 150 μg recombinant human GSF on January 30.
On January 31, the patient no longer had fever. Meanwhile, re-examination of CT showed that the lung lesions were significantly more severe than before (Figure 1c). On February 1, the patient had obvious shortness of breath, intermittent cough with white sticky sputum and bloodshot sputum. Oxygenation index further decreased to 202 (oxygen concentration 53%). Blood tests indicated that leukocytes reached to 32.81×10^9/L, and neutrophils were 31.57×10^9/L.

Because of the exacerbation of patient's condition including obvious shortness of breath after activity and hypoxemia (moderate ARDS), he was transferred to the First Affiliated Hospital of University of South China on February 1. After ICU admission, the patient received high-flow nasal oxygen (oxygen concentration 30% -45%, flow rate 30L/min) and antiviral therapy including lopinavir 200 mg, ritonavir 50 mg and 5 million units of IFN-α (course 14 days). Meanwhile, 40 mg methylprednisolone and 15g human immunoglobulin were administered intravenously daily (course of treatment for 5 days), and 4.5 g piperacillin-tazobactam continued to be administered intravenously every 8 hours for preventing secondary infection. Considering the abnormal increase of leukocytes with no obvious bacterial infection, we conjectured that it might be associated with the usage of recombinant human GSF. Therefore, we suspended this medicine immediately. Gradually, the patient's leukocytes and neutrophils decreased and recovered to normal level. The patient's respiratory symptoms improved and maintained normal body temperature. Re-examination of CT showed that lung lesions were absorbed significantly (Figure 1d-g). SARS-CoV-2 RNA of oropharyngeal swab remained negative for twice (over 24-hour intervals) by rRT-PCR. The patient stated that there was obvious improvement of his symptoms and was eventually cured discharged from hospital on February 20.

**Discussion And Conclusion**

Lung was the primary organ affected by SARS-CoV-2 infection. Most of patients had mild symptom, but part of them can develop to severe or critical symptom. The risk of death significantly increased with the progress of heart failure, acute kidney injury and ARDS[2, 5, 6]. However, the pathogenesis of SARS-CoV-2 infection was still unclear. It was documented that the C-reaction protein, IL-6 and TNF-α are all significantly upregulated in COVID-19 patients[7]. Among the secondarily symptoms, cytokine storm was identified as the inducer, which could be association with excessive activation of immune cells[8]. Based on these, some researchers presumed that excessive immune response promote cytokine storm and further evoke MOF[9, 10]. However, so far, the role of neutrophils is not clear in lung injury induced by SARS-CoV-2.

We found that, in the early stage of the disease, patient's leucocytes, including neutrophils, lymphocytes and monocytes, decreased with the disease progresses. Although the patient's lung damaged, he did not develop significant hypoxemia. After the treatment with recombinant human GSF, the patient's leucocytes increased rapidly, especially with a significant increase in neutrophils. Afterwards, the patient's condition further deteriorated and developed to moderate ARDS. Considering that leukocyte elevation was not associated with other infections such as secondary bacterial infections, we supposed that the iatrogenic elevation of neutrophils might aggravate lung injury.

In addition, there are several issues that need to be clarified. Firstly, promoting leukocytes, especially neutrophils, may worsen lung injury induced by SARS-CoV-2 infection in early progression stage. In classical pathogenesis of ARDS, the activation of alveolar-macrophages resulted in persistent inflammation and tissue damage, which contributed to produce proinflammatory factor, recruit leucocyte (neutrophils, monocytes/macrophages, effector T cells), and activate alveolar epithelial cells[11-13]. Moreover, many literatures had shown that neutrophils played an important role in the development of ARDS[14-17]. It was shown that chemokines CCL2 and CCL7 synergized with C-X-C Motif Chemokine Ligand 8(CXCL8) to promote neutrophils migration into the pulmonary interstitium and alveoli, aggravating lung injury[18]. Up to now, few studies focused on the role of neutrophils in lung injury induced by viral infection. It was documented that the number of neutrophils in the lower respiratory tract is correlated with disease severity during severe influenza pneumonia and highly pathogenic avian influenza infection[16]. Therefore, improper promotion of neutrophils may aggravate lung injury in SARS-CoV-2 infection early stage.

Secondly, it was considered that the mechanism of organ damage caused by virus infection was related to the induced immune response. The overactivated leucocytes (monocytes, T lymphocytes, and macrophages) can lead to ARDS and sequential organ failure[9, 19]. At present, the detection results from clinical lab showed that SARS-CoV-2 infection usually led to the decrease of leucocytes (including neutrophils, monocytes and lymphocytes)[4]. Maybe the cytokine storm was also weakened with the decrease of inflammatory cells, which may conducive to delaying the progress of the disease. Thus, enhancing leucocytes, especially neutrophils may accelerate the deterioration of illness at this moment.

Finally, it was difficult for us to find the direct evidence of the increase of neutrophils in lung tissue and exclude the possibility of progress of the disease itself. This case report showed that there is a certain correlation between the progress of the lung injury and the rise of iatrogenic leucocytes, especially neutrophils. Further studies are warranted to confirm this correlation.

This case report showed that iatrogenic increase of leucocytes (especially neutrophils) may worsen lung injury and leucocyte increasing agents were used with caution in the early stage of COVID-19 patients. At the same time, the phenomenon remains to be further confirmed in the future study.
**Abbreviations**

AKI: Acute kidney injury; ARDS: Acute respiratory distress syndrome; CCL2: Chemokine 2; CCL7: Chemokine 7; CDC: Centers for Disease Control; COVID-19: Coronavirus disease-2019; CRP: C-reaction protein; CT: Computed tomography; CXCL8: C-X-C Motif Chemokine Ligand 8; GSF: Granulocyte stimulating factor; IL-6: Interleukin-6; MOF: Multiple organ failure; rRT-PCR: Real-time reverse-transcriptase–polymerase-chain-reaction; SARS-COV-2: Severe acute respiratory syndrome coronavirus 2; TNF-α: Tumor necrosis factor-α.

**Declarations**

**Ethics approval and consent to participate**

This study is approved by the Ethics Committees from the First Affiliated Hospital of University of South China and the patient is consent to participate.

**Consent for publication**

Written informed consent for publication of the clinical details and/or clinical images was obtained from the patient.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Competing interests**

The authors declare that they have no conflict of interest regarding the publication of this case report.

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**Authors’ contributions**

JT, XX, QW, BH and HF contributed to the clinical patient care and management. JZ, HF and QW contributed to the acquisition and interpretation of data. BS and JZ contributed to the manuscript preparation. JT, JC and XZ carried out the data analysis. XT contributed to the manuscript verification. All authors have read and approved the manuscript.

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Table

Table 1 The detection results from clinical laboratory
| Measure                                | Reference Range | 1 Jan 26 | 2 Jan 27 | 3 Jan 28 | 4 Jan 29 | 5* Jan 30 | 6* Jan 31 | 7 Feb 1 | 8 Feb 2 |
|----------------------------------------|-----------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Leukocyte Count (x10^9/L)              | 4.00-10.00      | 5.61    | 3.94$^5$| 3.45$^5$| -       | 2.22$^5$| 8.43    | 32.81$^k$| 26.18$^k$|
| Lymphocyte Count (x10^9/L)             | 0.8-4.0         | 0.78    | 1.04    | 0.52$^5$| -       | 0.46$^5$| 0.37$^5$| 0.60$^5$| 0.63$^5$|
| Neutrophil Count (x10^9/L)             | 2.0-7.0         | 4.48    | 2.68    | 2.85    | -       | 1.67$^5$| 7.84$^k$| 31.57$^k$| 25.05   |
| Monocyte Count (x10^9/L)               | 0.12-0.8        | -       | 0.22    | 0.08$^5$| -       | 0.09$^5$| 0.22    | 0.61    | 0.50    |
| Eosinophil Count (x10^9/L)             | 0.05-0.5        | -       | 0.00$^5$| 0.00$^5$| -       | 0.00$^5$| 0.00$^5$| -       | -       |
| Basophil Count (x10^9/L)               | 0.0-0.1         | -       | 0.00    | 0.00    | -       | 0.00    | 0.00    | 0.03    | -       |
| Total protein (g/L)                    | 60-83           | -       | 67.00   | 62.00   | -       | 60.00   | 55.00$^4$| 59.00$^4$| 60.30   |
| Albumin (g/L)                          | 33-55           | -       | 41.30   | 37.20   | -       | 35.50   | 31.80$^4$| 32.50$^4$| 36.00   |
| Glutamic oxaloacetic transaminase (U/L)| 0-41            | -       | 27.0    | 30.0    | -       | 54.0$^6$| 46.0$^6$| 40.0    | 33.5    |
| Dehydrogenase (U/L)                   | 125-300         | -       | 267.0   | 287.0   | -       | 324$^6$| 297     | 321$^6$| 457$^6$|
| Glutamic oxaloacetic transaminase isoenzyme (U/L) | 20-220         | -       | 198.0   | 147.0   | -       | 105.0  | 75.0    | 51.0    | 45.0    |
| Troponin (ng/L)                        | 0-14            | -       | Negative| Negative| -       | Negative| -       | -       | -       |
| Pro-BNP (pg/mL)                        | 1-450           | -       | -       | -       | -       | -       | -       | 506.80$^k$| -       |
| Procalcitonin (ng/mL)                  | 0.02-0.05       | -       | 0.02    | 0.01    | -       | -       | 0.02    | 0.02    | <0.02   |
| C-reactive protein (mg/L)              | 0-8.2           | -       | 61.54$^6$| 34.17$^6$| -       | 41.71$^6$| 14.9$^6$| 24.39$^6$| 31.62$^6$|
| PH                                     | 7.35-7.45       | -       | -       | -       | 7.49$^k$| -       | 7.465$^k$| -       | 7.487$^k$|
| PaCO₂ (mmHg)                           | 32-45           | -       | -       | -       | 32.1$^5$| -       | 30.1$^5$| 28.5$^5$| 27.1    |
| PaO₂ (mmHg)                            | 83-108          | -       | -       | -       | 101.00  | -       | 121.30  | 107.10  | 61.00   |
| FiO₂ (%)                               | 21-100          | 25      | 25      | 25      | 29      | 40      | 50      | 50      | 30      |
| Finger pulse oxygen (%)                | 0-100           | 97      | 95      | 98      | 98      | 95      | 94      | 93      | 93      |
| Blood Lactate (mmol/L)                 | ≥300            | -       | -       | -       | 348     | -       | 242.6   | 214.2   | 203     |

Note: $^5$ indicates values within the normal range.

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| Measure                                      | Reference Range | Hospital Day 9 Feb 3 | Hospital Day 10 Feb 4 | Hospital Day 11 Feb 5 | Hospital Day 12 Feb 6 | Hospital Day 13 Feb 7 | Hospital Day 14 Feb 8 | Hospital Day 16 Feb 10 | Hospital Day 18 Feb 12 |
|---------------------------------------------|-----------------|----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Leukocyte Count (x10^9/L)                   | 4.00-10.00      | 17.66^k              | 11.98^k               | 9.94                  | 11.94^k               | 7.71                  | 7.06                  | 5.16                  | 7.15                  |
| Lymphocyte Count (x10^9/L)                  | 0.8-4.0         | 0.72^k               | 0.58^k                | 0.78^k                | 0.76^k                | 0.57^k                | 0.39^k                | 0.94                  | 2.44                  |
| Neutrophil Count (x10^9/L)                  | 2.0-7.0         | 16.39^k              | 10.90^k               | 8.47^k                | 10.39^k               | 6.62                  | 6.23                  | 4.09                  | 3.98                  |
| Monocyte Count (x10^9/L)                    | 0.12-0.8        | 0.55                 | 0.48                  | 0.63                  | 0.74                  | 0.42                  | 4.70                  | 0.12                  | 0.65                  |
| Eosinophil Count (x10^9/L)                  | 0.05-0.5        | 0.00^k               | 0.00^k                | 0.02^k                | 0.05                  | 0.06                  | 0.08                  | 0.00                  | 0.08                  |
| Basophil Count (x10^9/L)                    | 0.0-0.1         | 0.00                 | 0.02                  | 0.05                  | 0.00                  | 0.05                  | 0.03                  | 0.01                  | 0.00                  |
| Total protein (g/L)                         | 60-83           | 64.8                 | 67.2                  | 60.9                  | 61.80                 | -                    | 58.40                 | 59.70                 | -                     |
| Albumin (g/L)                               | 2.44            |                      |                       |                       |                       |                       |                       |                       |                       |

Continued

# The patient was treated with taking 20mg leucoson and 20mg vitamin B4. * The patient was treated with 150 μg recombinant human granulocyte stimulating factor administered subcutaneously. & The value in the patient was above normal. $ The value in the patient was below normal.

**Figures**
Figure 1

The CT imaging change of patient's lung at different time point