Therapeutic Effect of Tocilizumab on Inhibiting Cytokine Release Syndrome in Severe Coronavirus Disease 2019 Patients

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Abstract

**Background and Objectives:** Older adults are more susceptible to coronavirus disease 2019 (COVID-19). Interleukin-6 (IL-6) is an important cytokine in the cytokine release syndrome (CRS), and tocilizumab blocks the IL-6 receptor. The objective is to analyze the effect of tocilizumab on CRS in older patients with severe COVID-19.

**Materials and Methods:** Between February 10 and March 21, 2020, a total of 19 patients aged ≥60 years with severe or critical COVID-19 met the study inclusion criteria at the Tongji Hospital in Wuhan City, Hubei Province, China. The patients were divided into two groups: the tocilizumab group, with IL-6 levels, which exceeded the upper limit of normal by >10-fold and non-tocilizumab group. **Results:** Patients in the tocilizumab group were older (73.20 ± 4.44 vs. 66.21 ± 5.06 years, \(P = 0.014\)), had lower lymphocyte counts (0.71 ± 0.18 vs. 1.18 ± 0.59 × 10⁹/L, \(P = 0.016\)), and higher high-sensitivity C-reactive protein (hsCRP) levels (94.04 ± 57.24 vs. 51.65 ± 45.37 mg/L, \(P = 0.035\)). Increases in ferritin (FER) and hsCRP levels in patients in the tocilizumab group were marked. Except for one patient who died, IL-6, FER, hsCRP levels, and the neutrophil/lymphocyte ratio in the remaining four patients decreased following treatment with tocilizumab. Tocilizumab did not cause any serious adverse reactions. There were no differences in mortality, days until lung computerized tomography improvement, or renal function between the two groups. The total mortality rate was 10.53%. **Conclusions:** Our results support the therapeutic efficacy and safety of tocilizumab in older patients with severe COVID-19.

**Key words:** Cytokine release syndrome, interleukin-6, older, severe coronavirus disease 2019, tocilizumab

INTRODUCTION

Older adults are more susceptible to the novel coronavirus disease 2019 (COVID-19). Patients aged ≥60 years account for 15.1%–37% of patients with COVID-19, among whom 27.0% are diagnosed with severe disease. Compared with younger patients, older adults are more likely to develop severe illness and have an increased risk of intensive care unit admission and death.[1,2] Inflammation is a major pathological process that occurs in patients with severe COVID-19. The...
detection of elevated inflammatory cytokines suggests that a cytokine storm, also known as cytokine release syndrome (CRS), may be an important factor in the transition of patients with COVID-19 to severe disease status.[3] Blocking the signaling of key cytokines in the inflammatory cytokine storm induced by COVID-19 may reduce the damage to the target organs.[4] Therefore, CRS treatment has become an important part of rescuing patients with severe diseases. Interleukin-6 (IL-6) is an important cytokine,[1] and its levels are significantly increased in patients with severe COVID-19 relative to those with nonsevere symptoms.[5] Tocilizumab is a blocker of the IL-6 receptor (IL-6R) and has the potential to be an effective drug for the treatment of patients with severe COVID-19.

In this study, we enrolled 19 older adult patients with severe COVID-19 whose serum IL-6 levels were significantly elevated. Patients with high IL-6 levels were treated with tocilizumab. We analyzed the therapeutic effects of tocilizumab on CRS and its safety in older patients with severe COVID-19.

MATERIALS AND METHODS

Patients
A total of 19 patients tested positive for COVID-19 by qualitative polymerase chain reaction assay met the study inclusion criteria between February 10 and March 21, 2020, in Tongji Hospital in Wuhan City, Hubei Province, China. The inclusion criteria were age ≥60 years, met the severe or critical criteria, as defined in the “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (7th Interim Edition),”[6] IL-6 ≥7.0 pg/mL, and rapidly progressing pneumonia was confirmed by imaging. Exclusion criteria were cancer (solid or hematological tumor), autoimmune diseases, such as rheumatoid arthritis, infections other than pneumonia, such as urinary tract infection, cellulitis, herpes zoster, gastroenteritis, and bacterial arthritis, or other symptoms that researchers have considered inappropriate.

The patients were divided into two groups according to their IL-6 levels: the tocilizumab group, with IL-6 levels exceeding the upper limit of normal by >10-fold, and the nontocilizumab group, with IL-6 levels that did not reach 10-fold the upper limit of normal.

Study design and procedures
This was a retrospective cohort study. All patients received standard treatment in accordance with the relevant guidelines (2020), including oxygen therapy, nutrition therapy, antibiotics, antiviral drugs (abidom and oselamivir), glucocorticoids, and Chinese medicine. The tocilizumab dose was 8 mg/kg in a single intravenous injection, and a second dose was administered if the patient’s symptoms did not improve after 8 h.

All patients underwent the following examinations upon admission and regular review: routine blood tests (white blood cells [WBC], neutrophils [N], and lymphocytes [L]), ferritin (FER), procalcitonin, and IL-6. A lung computed tomography (CT) scan was performed upon admission and re-checked every 7 days thereafter. Oxygen saturation (SpO₂) levels were measured daily.

Statistical analysis
All statistical data were analyzed using Microsoft Excel 2007 and Statistical Package for the Social Sciences (SPSS) 22.0 (IBM, Armonk, NY, USA). Data conforming to the normal distribution are expressed as the mean ± standard deviation. A t-test was used for comparisons of means between the two groups, whereas the χ² test was used for the comparison of rates. Statistical significance was set at P < 0.05.

RESULTS

Demographic characteristics
The baseline data from the two patient groups are presented in Table 1. Patients in the tocilizumab group were older (73.20 ± 4.44 vs. 66.21 ± 5.06 years, P = 0.014), with lower lymphocyte counts (0.71 ± 0.18 vs. 1.18 ± 0.59 × 10⁹/L, P = 0.014), and higher hsCRP levels (94.04 ± 57.24 vs. 51.65 ± 45.37 mg/L, P = 0.035). Patients in the tocilizumab group also had higher FER levels; however, the difference was not statistically significant.

Table 1: Baseline data from the two patient groups

|                     | No Tocilizumab (n = 14) | Tocilizumab (n = 5) | t/χ² | P    |
|---------------------|-------------------------|---------------------|------|------|
| Age (years)         | 66.21 ± 5.06            | 73.20 ± 4.44        | -2.726 | 0.014 |
| WBC (×10⁹/L)        | 6.47 ± 2.55             | 7.75 ± 1.30         | -1.059 | 0.304 |
| N (×10⁹/L)          | 4.57 ± 2.64             | 6.61 ± 1.13         | -1.650 | 0.117 |
| L (×10⁹/L)          | 1.18 ± 0.59             | 0.71 ± 0.18         | 2.676  | 0.016 |
| N/L                 | 5.21 ± 4.22             | 9.73 ± 2.52         | -2.289 | 0.030 |
| IL-6 (pg/mL)        | 23.51 ± 17.61           | 139.94 ± 141.39    | -3.925 | 0.005 |
| hsCRP (mg/L)        | 47.29 ± 39.43           | 94.04 ± 57.24       | -2.221 | 0.035 |
| FER (μg/L)          | 1186.26 ± 1236.26       | 1803.20 ± 2031.35   | -0.794 | 0.439 |
| ESR (mm/h)          | 51.65 ± 45.37           | 94.04 ± 57.24       | 0.381  | 0.708 |
| PCT (ng/mL)         | 0.12 ± 0.13             | 0.19 ± 0.18         | -0.972 | 0.344 |

Abbreviations: ESR, erythrocyte sedimentation rate; IL-6, Interleukin-6; FER, Ferritin; F, female; hsCRP, high-sensitivity C-reactive protein; LYMHP, lymphocyte; M, male; NEUT, neutrophil; N/L, lymphocyte/neutrophil; PCT, procalcitonin; SpO₂, oxygen concentration.
Details of the tocilizumab group
The demographic characteristics of the patients before tocilizumab administration are presented in Table 2. There were four men and one woman in the tocilizumab group. The levels of inflammatory markers, including IL-6, FER, N/L ratio, hsCRP, and ESR, were elevated in all patients. In particular, the levels of FER and hsCRP were very high; however, procalcitonin levels were almost normal in all participants. In addition, the patients’ SpO₂ levels were <90% despite receiving oxygen therapy.

Tocilizumab use and prognosis
The tocilizumab administration and prognosis for each patient are presented in Figure 1a-e. Patient number 3 died because of COVID-19. IL-6, FER, and other inflammatory factors were not controlled following treatment with tocilizumab [Figure 1c]. Furthermore, when her condition deteriorated, her IL-6 levels exceeded the detection limit. Tocilizumab treatment showed positive effects in the other four patients, whose IL-6, FER, N/L, and hsCRP levels decreased after tocilizumab administration. All four patients had good SpO₂ levels, and their WBC counts were normal and did not change throughout the entire period of hospitalization.

Changes in lung computed tomography scans
Patient number 3 deteriorated and died, which was reflected in the lung CT scans. In other patients, CT scans improved 3–8 days after tocilizumab administration. Lung lesion opacity was reduced in the other four patients and normalized in two patients after 20 and 23 days [Table 3].

Prognosis in the two patient groups
As shown in Table 4, the length of hospitalization was greater in the tocilizumab group. However, after tocilizumab treatment, the number of days until lung CT improvement was the same as that in the group that did not receive tocilizumab, and there was no difference in mortality between the groups.

Following tocilizumab administration, no bacterial infection, other viral infections (such as herpes), impaired liver function, hypertension, leukopenia, neutropenia, peripheral edema, allergies, or other adverse reactions were observed during the entire hospitalization period.

The creatinine level of the tocilizumab group was 98.20 ± 37.52 μmol/L at the time of onset and higher than that of the non-tocilizumab group (72.46 ± 16.40 μmol/L). At the time of discharge after treatment, the creatinine level in the tocilizumab group decreased to 73.00 ± 24.00 μmol/L, and the nontocilizumab group was stable (68.69 ± 14.89 μmol/L).

One patient died in each group, with a total mortality rate of 10.53%.

DISCUSSION
COVID-19 is more serious and has a higher mortality rate in older adults. The global mortality rate of COVID-19 is 4.84%.[7] However, a retrospective study of 3418 black and white patients (mean age, 54 years) reported a mortality rate of 9.36%.[8] In our study of older patients with CRS, the mortality rate was 10.53%, which is higher than that in the reports cited above; however, age ≥65 years is recognized as a risk factor for mortality from COVID-19,[9] which was the rationale for the focus of our study on these patients.

CRS refers to an overactive, uncontrolled immune response that involves an overwhelming release of pro-inflammatory mediators. Increased levels of inflammatory cytokines and chemokines, including IL-6, IL-1β, inducible protein 10, and monocyte chemoattractant protein 1, may also cause fatal complications in patients with COVID-19. In addition to antiviral treatment, IL-6 is the main mediator of CRS toxicity, and its levels are related to the severity of CRS induced by chimeric antigen receptor (CAR) T cell therapy.[5,10] A

Table 2: Clinical manifestations of patients before tocilizumab treatment

| Patient number | Sex | Age (years) | Days from onset to tocilizumab | IL-6 (pg/mL) | FER (μE/L) | WBC (BC) | NEUT (EUT⁺ /L) | LYMPH (LYMP⁺ /L) | N/L | hsCRP (mg/L) | ESR (mm/h) | PCT (ng/mL) | SpO₂ (percentage with oxygen supply) |
|----------------|-----|-------------|-------------------------------|--------------|------------|----------|--------------|-----------------|-----|-------------|-----------|-----------|-------------------------------|
| 1              | Male| 75          | 13                            | 74.24        | 1243.0     | 4.97     | 3.88         | 0.69            | 5.62| 136.3       | 82        | 0.09      | 98.4                          |
| 2              | Male| 78          | 45                            | 64.50        | 670.5      | 4.59     | 4.19         | 0.31            | 13.52| 126.8       | 25        | 0.08      | 98.8                          |
| 3              | Female| 66        | 14                            | 72.77        | 1109.6     | 7.68     | 6.70         | 0.69            | 9.71| 49.8        | 25        | 0.07      | 86.5                          |
| 4              | Male| 74          | 19                            | 392.00       | 5380.4     | 6.3      | 5.67         | 0.42            | 13.50| 100.2       | 72        | 0.50      | 85.1                          |
| 5              | Male| 73          | 14                            | 133.4        | 768.2      | 6.90     | 5.24         | 0.99            | 5.29| 91.01       | 56        | 0.10      | 88.0                          |

WBC: White blood cell, N/L: Lymphocyte/neutrophil, IL-6: Interleukin-6, hsCRP: High-sensitivity C-reactive protein, ESR: Erythrocyte sedimentation rate, PCT: Procalcitonin, FER: Ferritin, LYMPH: Lymphocyte, NEUT: Neutrophil, SpO₂: Oxygen concentration.
systematic review and meta-analysis showed that patients with severe COVID-19 have significantly higher serum IL-6 levels than those with non-severe disease and that increased mean IL-6 levels were associated with higher patient mortality. In five patients treated with tocilizumab in this study, initial levels of IL-6 were increased by >10-fold compared with normal levels and increased at the beginning of the disease. After treatment with tocilizumab, IL-6 levels decreased significantly; however, the IL-6 levels of one patient who died continued to increase significantly, suggesting that IL-6 could be a predictor of prognosis.

Tocilizumab is a humanized recombinant monoclonal anti-IL-6R antibody. It binds to soluble IL-6R and membrane-bound IL-6R to inhibit IL-6 mediated cis- and trans-signaling. Tocilizumab has been approved by the United States Food and Drug Administration for the treatment of severe CAR-T cell-induced CRS. After tocilizumab was administered once or twice, 69% of patients with CAR-T-cell therapy-induced CRS responded within 14 days, and their fever and hypotension subsided within a few hours, allowing vasopressors to be stopped within a few days. However, clinical experience with tocilizumab in the treatment of viral disease is very limited, and the increased risk of opportunistic infections (including tuberculosis, fungal, or other viral infections) must be taken seriously.

A retrospective study of 21 patients with COVID-19 by Xu et al. reported a 75.0% reduction in oxygen uptake.
CT scans showed 90.5% clearance of lung lesion opacity and a significant 84.2% reduction in CRP within 5 days of tocilizumab treatment. No adverse reactions were observed. These data provided evidence that treatment with tocilizumab could immediately improve symptoms and effectively reduce mortality in patients with severe and critical COVID-19.\[16\] Another study involving 15 patients showed that tocilizumab treatment could quickly reduce CRP levels in all patients, while IL-6 levels tended to increase first and then decrease. In contrast, untreated patients exhibited a continuous and significant increase in IL-6 levels. Hence, repeated tocilizumab treatment is recommended for critically ill patients with elevated IL-6.\[17\] In our study, we observed that clinical indicators were quickly relieved, and lung CT improved significantly shortly after the administration of tocilizumab in patients with severe COVID-19. These results are similar to those of previous studies and support the positive therapeutic effects of tocilizumab in patients with severe COVID-19. Further, a systematic review indicated that preliminary investigations of the use of tocilizumab to treat COVID-19 demonstrated benefits, while further studies, such as randomized controlled trials, are needed.\[18\]

The most interesting aspect of this study was the comparison between the two patient groups. We analyzed disease severity and prognosis in all older patients with elevated IL-6 levels and found that those in the tocilizumab group had serious disease; however, after the timely application of tocilizumab treatment, the rate of CT improvement and mortality was consistent with those in the group of patients with milder disease. These findings demonstrate that tocilizumab plays a positive role in the treatment of CRS and is effective against severe COVID-19. In addition, the serum creatinine level in the tocilizumab group was higher than the normal level at the time of onset, but after tocilizumab treatment, the serum creatinine level had returned to the normal level. This may also suggest a positive therapeutic significance of tocilizumab.

Given the efficacy of tocilizumab in CRS and the key role of IL-6 in COVID-19, Liu et al. suggested that tocilizumab should be considered under the following circumstances.

| Table 4: Comparison of prognosis in the two patient groups |
|----------------------------------------------------------|
|                  | No tocilizumab (n = 14) | Tocilizumab (n = 5) | t/χ² | P |
| Days in hospital | 23.92±9.98               | 38.75±5.19         | −3.121 | 0.005 |
| Days until CT improved | 8.17±2.72 | 5.75±2.06         | −1.612 | 0.129 |
| Deaths (n)      | 1                        | 1                  | 0.647  | 0.468  |

CT: Computerized tomography

(1) Diagnostic criteria: early diagnosis of CRS in patients with COVID-19 and rapid initiation of immunomodulatory therapy may be beneficial. (2) Disease severity grading system: tocilizumab is only suitable for critically ill patients, whereas the risk-benefit assessment is focused on the symptomatic treatment of patients with mild disease. (3) Combined antiviral treatment: based on the experience with corticosteroids, immunosuppressants may delay viral clearance. Therefore, the combination of immunomodulators with antiviral drugs may be beneficial.\[19\] In our study, tocilizumab did not cause any serious adverse reactions.

CONCLUSIONS

This was a retrospective cohort study with a small number of patients. These results support the therapeutic efficacy and safety of tocilizumab in the treatment of older adults with severe COVID-19. However, randomized controlled trials are required to generate higher-level evidence.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Beijing Hospital, Beijing, China (2020BJYYEC-097-01). The Ethics Committee waived the requirement for informed consent.

Consent for publication
All participants had consent for publication.

Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflicts of interest
There are no conflicts of interest.

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