Tocilizumab Treatment Effect on Iron Homeostasis in Severe COVID-19 Patients

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Keywords
COVID-19 · Anemia · Iron · Tocilizumab

Abstract

Background: Tocilizumab has been proposed as an effective treatment for severe COVID-19. We aimed to investigate whether tocilizumab administration is associated with increased availability of serum iron which may possibly be associated with adverse effects on clinical outcomes. Methods: We performed an observational, retrospective cohort study. We included adults, who were hospitalized in ICU with the diagnosis of severe COVID-19 infection eligible for tocilizumab treatment. Laboratory data including serum iron, ferritin, transferrin saturation, hemoglobin, and C-reactive protein levels of all patients were collected shortly before and 24 h, 48 h, and 72 h after tocilizumab administration. Results: During the study period, 15 patients fulfilled the inclusion criteria and were eligible to receive tocilizumab treatment. Tocilizumab therapy was associated with a prominent increase in serum iron and transferrin saturation levels (26 ± 13 μg/dL and 15 ± 8% before treatment and 79 ± 32 μg/dL and 41 ± 15% 72 h after treatment, respectively, \(p < 0.001\)) and decrease in serum ferritin levels (1,921 ± 2,071 ng/mL before and 1,258 ± 1,140 ng/mL 72 h after treatment, \(p = 0.027\)). Conclusion: Treatment of severe COVID-19 patients with tocilizumab is associated with a profound increase in serum iron and ferritin saturation levels along with a decrease in ferritin levels. This may represent an undesirable side effect that may potentiate viral replication.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an enveloped nonsegmented positive-sense RNA virus, is the causal agent of the COVID-19 worldwide pandemic outbreak [1]. Clinical presentation of COVID-19 is heterogeneous and ranges from asymptomatic or mildly affected patients with flu-like symptoms to life-threatening hyperinflammatory syndrome characterized by fulminant hypercytokinemia (also called cytokine storm) with a profile similar to that seen in hemophagocytic lymphohistiocytosis and ensuing multiorgan failure and acute respiratory distress syndrome [2, 3]. Pro-inflammatory cytokines, mainly IL-6, are associated with decreased plasma iron concentration [4]. Since iron
is an essential element for many fundamental physiological processes including viral replication, iron deprivation during infections may serve as a protective mechanism against bacterial and viral infections [5, 6].

Tocilizumab, a monoclonal antibody directed against the interleukin-6 receptor, has been proposed as potential therapy for COVID-19 by mitigating the cytokine storm syndrome associated with severe COVID-19 [7–17]. However, the efficacy of tocilizumab’s therapy is not clear [18–22]. A recently published randomized, double-blind, placebo-controlled trial included 243 patients did not provide support that tocilizumab is an effective treatment for preventing intubation or death in moderately ill hospitalized patients with COVID-19 [23].

The explanation for the failure of tocilizumab to affect the clinical outcome in recent reports, despite the strong pathophysiological basis for its potential beneficial effect is not clear. We hypothesize that the inconclusive efficacy of treatment with tocilizumab may be partially explained by its influence on iron metabolism. Tocilizumab administration has been previously associated with decreased levels of hepcidin and ferritin alongside with increase in total iron-binding capacity (TIBC) and hemoglobin in patients with Castelman’s disease and rheumatic arthritis [24–28].

The clinical significance of the increase in serum iron levels after treatment with tocilizumab is not clear but may represent an increase in labile iron, which may potentially propagate reactive oxygen species [29] generation and potentiate pathogen replication, which is an undesirable side effect of any anti-inflammatory therapy, given during an infectious episode.

We therefore aimed to assess the effect on iron levels in patients treated with tocilizumab. To the best of our knowledge, this is the first study investigating the effect of tocilizumab on iron release and levels in acutely infected COVID-19 patients.

**Methods**

We performed a single-center, observational, retrospective cohort study conducted at Hasharon Campus, Rabin Medical Center, university affiliated tertiary hospital, in Petah-Tikva, Israel. Electronic records of all consecutive adult patients (≥18 years) hospitalized in ICU in our center during the COVID-19 pandemic between April 7, 2020, and July 1, 2020, with the diagnosis of severe COVID-19 infection requiring administration of tocilizumab were identified daily and reviewed by the study coordinators.

Diagnosis of COVID-19 was made according to CDC diagnostic criteria using authorized nucleic acid or antigen detection assays [30]. Tocilizumab was administered to all patients considered with hyperinflammatory state according to the following criteria: C-reactive protein (CRP) >90 mg/L, ferritin >500 ng/dL, and interferon gamma-induced protein 10 >1,000 pg/mL. All ICU patients considered as hyperinflammatory were administrated a total dose of 800 mg tocilizumab, given in 2 separate doses of 400 mg, 12 h apart.

All patients given tocilizumab were treated with methylprednisolone (1.5 mg/kg divided into 3 doses) or equivalent dosing of another corticosteroid. Additionally, patients also received antiviral therapy that was based on combinations of hydroxychloroquine, azithromycin, zinc, and lopinavir/ritonavir at the discretion of the infectious disease consultant and was discussed daily.

Laboratory data including serum iron, serum ferritin, transferrin saturation, hemoglobin, and CRP of all patients were collected at hospital admission, shortly before tocilizumab administration and 24 h, 48 h, and 72 h after tocilizumab administration.

**Statistical Analysis**

The analysis was performed using the IBM SPSS statistics 26; IBM Corporation, Armonk, NY, USA. In order to assess the change in measured laboratory levels before and 24 h, 48 h, and 72 h after tocilizumab administration, we used repeated measures ANOVA test with Bonferroni correction method. To test the assumption of sphericity, Mauchly’s Test was implemented (null hypothesis is rejected at $p < 0.05$). In case of violation of sphericity assumption Greenhouse-Geisser test was used. We reported $F$-statistic from a repeated measures ANOVA.

**Results**

During the study period from April 7, 2020, and July 1, 2020, 132 consecutive patients were hospitalized with the diagnosis of COVID-19 in our hospital. Of them, 52 were hospitalized in ICU and 15 fulfilled the inclusion criteria and were eligible to receive tocilizumab treatment.

Median age was 76 years (IQR 47–90). Twenty percent of patients were female (3/15) and 80% were ventilated (12/15).

The median duration of hospitalization was 18 days (IQR 17–23). The median duration of mechanical ventilation was 16 days (IQR 11–17). Three (20%) patients died.

Before treatment, 80% (12/15) of our patients had low transferrin saturation (<20%) with mean of 15 ± 8% and 93% (14/15) had low levels of serum iron (<50 μg/dL) with mean of 26 ± 13 μg/dL.

All 15 patients had increased CRP levels prior tocilizumab treatment with mean levels of 25 ± 11 mg/dL. Tocilizumab therapy was associated with a significant gradual increase in mean serum iron levels from 26 μg/dL (SD ± 13) shortly before therapy up to 79 μg/dL (SD ± 32) 72 h after therapy ($F(3,42) = 28, p ≤ 0.001$) (Fig. 1).
A gradual and significant increase was also observed in mean serum transferrin saturation levels shortly before therapy (15%, SD ± 8) and 24 h (33%, SD ± 17), 48 h (39%, SD ± 15), and 72 h (41%, SD ± 15) after drug administration ($F(3,42) = 21$, $p ≥ 0.001$) (Fig. 2).

Additionally, we observed a significant decrease in measured mean ferritin levels after tocilizumab administration, from 1,921 ng/mL (SD ± 2,071) shortly before treatment down to 1,258 ng/mL (SD ± 1,140) 72 h after treatment ($F(1,16) = 5.4$, $p = 0.027$).

A significant decrease was also measured in mean CRP levels after administration of first tocilizumab dose (25 ± 11 mg/dL shortly before treatment, 21 ± 10 mg/dL after 24 h, 11 ± 5 mg/dL after 48 h and 5 ± 2 mg/dL after 72 h, $F(1.4,20.6) = 54.4$, $p ≤ 0.001$). No significant change in measured mean hemoglobin levels were observed before and after tocilizumab therapy (11.4 ± 1.6 shortly before treatment, 10.9 ± 1.3 24 h after treatment, 11.4 ± 1 48 h after treatment and 11.2 ± 1.45 72 h after treatment, $F(3,42) = 2.2$, $p = 0.098$) (Table 1).

**Discussion**

Results of our retrospective analysis suggest that treatment of severe COVID-19 with tocilizumab is associated with rapid and persistent increase in serum iron and transferrin saturation levels. In all of our patients, serum iron levels increased by up to 6 folds, and transferrin saturation levels were increased up to 7.5 folds during 72 h following treatment. Moreover, in 87% of our patients, tocilizumab had an immediate and profound effect on decreasing ferritin levels. We believe that this observation reflects the rise of free serum iron levels secondary to tocilizumab therapy.
The direct upregulation of pro-inflammatory cytokines observed in patients with severe COVID-19, particularly IL-6 is well associated with increased levels of hepcidin, ferritin, and haptoglobin [4, 5, 29, 31]. Hepcidin leads to a decrease in intestinal iron absorption and decreased iron mobilization from macrophage reticuloendothelial stores. Ferritin in turn scavenges free iron and enables its sequestration in the reticuloendothelial stores and in this way prevents iron availability for invading pathogens [32]. This leads to functional iron deficiency, defined as normal or increased total body iron stores which are unavailable for incorporation into erythroid precursors for erythropoiesis [33, 34]. Indeed, results of our study are in line with this pathophysiologic basis and the vast majority of patients in our cohort had low serum iron levels and decreased transferrin saturation levels at presentation.

On the one hand, the altogether effect of hemoglobinopathy and iron dysmetabolism may seriously harm the host by compromising the capacity of erythrocytes to perform oxygen transport leading to subsequent hypoxia and inducing hyperferritinemia-related tissue alterations [29]. On the other hand, iron plays a key role in many fundamental biological processes including DNA/RNA synthesis and ATP generation necessary for viral replication [35]. Iron overload in patients with infection of hepatitis B/C viruses has been associated with poor prognosis [36, 37]. Thus, iron depletion may possibly have a marked beneficial antiviral effect.

Tocilizumab, an anti-interleukin 6 receptor antibody is approved for treatment of various rheumatic diseases, such as rheumatoid arthritis as well as other conditions including Castleman’s disease and cytokine release syndrome [38]. Based on its anticytokine effect and subsequent attenuation of the hyperactivated immune response tocilizumab has been proposed as a potential treatment for COVID-19 by several open-label trials and nonrandomized case series [7–14, 16, 39]. A recently published meta-analysis by the WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group evaluated the efficacy of IL6 inhibitors for the treatment of COVID-19 in reducing mortality at 28 days. As a result of the positive outcomes of this study, different expert and governmental guideline groups (WHO, NIH, IDSA) recommend tocilizumab in the treatment of COVID-19 [40].

However, there are growing evidence that the role of tocilizumab in COVID-19 therapy is inconclusive [18–22]. A recently published randomized, double-blind, placebo-controlled trial failed to demonstrate beneficial effect of tocilizumab treatment for preventing intubation or death in moderately ill hospitalized patients with COVID-19 [23]. This study included 243 patients. The hazard ratio for intubation or death in the tocilizumab group as compared with the placebo group was 0.83 (95% confidence interval [CI], 0.38–1.81; p = 0.64). The reason for the lack of tocilizumab beneficial effect on clinical outcome despite the strong theoretical pathophysiologic basis is unclear. We propose that a potential explanation lays in the effect of tocilizumab on iron hemostasis.

The beneficial effects of tocilizumab on anemia have been previously described in several rheumatologic and inflammatory diseases [24–28, 41–43]. These reports are in line with our results suggesting increased availability of free serum iron after tocilizumab administration. The effect of tocilizumab on iron metabolism is thought to be mediated by two possible mechanisms: hepcidin suppression by blocking IL-6 effect [43] and by increased erythropoietin signaling. The erythropoietin receptor and IL-6R complex share the JAK-STAT signaling pathway and excessive IL-6 signaling induces expression of intracellular factors that inhibit the JAK-STAT pathway [44]. Hence, tocilizumab treatment may result in increased erythropoietin signaling and hemapoiesis.

The relevance of the rapid increment in iron levels following tocilizumab treatment observed in our patients

### Table 1. Mean values and repeated measures ANOVA of laboratory measurements shortly before treatment, 24 h, 48 h, and 72 h after tocilizumab administration

|                      | Shortly before therapy | After treatment 24 h | After treatment 48 h | After treatment 72 h | F test, p value |
|----------------------|------------------------|----------------------|----------------------|----------------------|----------------|
| Serum iron levels, μg/dL | 26±13                  | 51±26                | 66±31                | 79±32                | F(3,42) = 28, p < 0.001 |
| Serum ferritin levels, ng/mL | 1,921±2,071           | 1,876±1,944          | 1,742±1,779          | 1,258±1,140          | F(1,16) = 5.4, p = 0.027 |
| Transferrin saturation, % | 15±8                   | 33±17                | 39±15                | 41±15                | F(3,42) = 21, p < 0.001 |
| Serum transferrin, mg/dL | 131±36                 | 119±31               | 127±31               | 142±31               | F(3,36) = 10.5, p < 0.001 |
| Hemoglobin, g/dL | 11.4±1.6               | 10.9±1.3             | 11.4±1.4             | 11.2±1.45            | F(3,42) = 2.2, p = 0.098 |
| CRP, mg/dL | 25±11                  | 21±10                | 11±5                 | 5±2                  | F(1,420,6) = 54.4, p < 0.001 |

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might be associated with reactive oxygen species generation and tissue damage. Moreover, in many pathogens including viruses, replication can be influenced by iron [45], and iron chelation has been shown in vitro to inhibit viral proliferation [46, 47]. Dalamaga et al. [48] hypothesized that iron chelator, particularly deferoxamine, may possess beneficial immunomodulatory and antiviral actions against SARS-CoV-2. The authors suggested that higher iron levels may promote the course of viral infections and development of acute respiratory distress syndrome and pulmonary fibrosis. They also speculated that iron chelators might decrease SARS-CoV-2 replication via decreasing iron availability which plays an important role in viral replication. In a recent letter to the editor, Abobaker [49] suggested that deferoxamine could be beneficial in adjunction with antiviral drugs to treat COVID-19. In addition, deferoxamine has been shown to decrease the level of IL-6 and endothelial inflammation in vitro, which could reduce the severity of COVID-19 [50]. If that is the case, then anti-inflammatory medication as tocilizumab given too early in the disease course, may potentiate possibly viral replication through release of iron from activated macrophages and may potentially have adverse effects on clinical outcomes.

However, Garrick and Ghio [51] suggested that iron chelation treatment might actually exacerbate cytokine storm and may harm patients with COVID-19. The coronavirus is an RNA virus, with its replication relying on a RNA duplex intermediate. Such viruses do not need iron to replicate their genome unlike DNA or retroviruses, so iron withholding could be counterproductive and part of the "cytokine storm."

To the best to our knowledge, this is the first report evaluating the effect of tocilizumab therapy on iron metabolism in severe COVID-19 patients. The major limitation of our study is the small sample size used for the analyses and the retrospective nature.

Additional limitation is the lack of a control group and the possibility of cofounding results due to other concomitant therapy and to the disease itself. Moreover, patients included in this study received drugs that are no longer used for the treatment of COVID-19. However, these drugs are not known to effect iron hemoestasis.

Conclusions

Tocilizumab had an immediate and profound effect on iron and ferritin levels in severe COVID-19 patients. This observation reflects the increase in availability of serum iron level following tocilizumab treatment. This response was sustained for at least 72 h after drug administration and may reflect a possible relationship between tocilizumab and iron levels in COVID-19 patients.

Statement of Ethics

The study was a retrospective analysis and data were derived from patient charts. Therefore, informed consent was not required. The study was approved by the institutional research Ethics Committee of the Rabin Medical Center (Approval No. 0257-20-RMC).

Conflict of Interest Statement

There are no conflicts of interest.

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This study has not been sponsored.

Author Contributions

All authors contributed to the final version of the manuscript, provided critical feedback, and processed the experimental data.

Data Availability Statement

All data generated or analyzed during this study are included in this article online supplementary material files (for all online suppl. material, see www.karger.com/doi/10.1159/000522307) (online suppl. Table 1).

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