The U-shaped association of serum iron level with COVID-19 severity: Is iron a potential therapeutic target?

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The work was performed at Yokohama City University Hospital and Yokohama Municipal Citizen’s Hospital.

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Abstract

Objective:

To evaluate the association between iron metabolism indicators and disease severity in hospitalized patients with coronavirus disease 2019 (COVID-19).

Design:

Two-center observational study

Setting:

A university hospital and a core hospital in Yokohama, Japan

Patients:

Adults with COVID-19 whose serum iron levels were measured within the first 5 days of hospitalization were included. Patients who refused mechanical ventilation were excluded from the study.

Measurements and Main Results:

One hundred thirty-six patients were included in this study. We analyzed the association between COVID-19 severity and serum iron, total iron binding capacity (TIBC), and transferrin saturation (TSAT) levels. Disease severity was defined as the worst respiratory status during hospitalization. Serum iron levels were significantly lower in patients with mild respiratory failure (RF) (n=55, median serum iron level: 24 [interquartile range: 19–42] mg/dL) than in the non-RF group (n=44, 40 [24–80] mg/dL) and the severe RF group (n=37, 60
[23.5–87] mg/dL); however, there were no significant differences in iron levels between the non-RF and severe RF groups (non-RF vs. mild RF: p=0.019, non-RF vs. severe RF: p>0.999, and mild RF vs. severe RF: p=0.009). That is, there was a U-shaped association between serum iron levels and disease severity. TIBC levels decreased significantly with increasing severity; consequently, TSAT was significantly higher in patients with severe RF than in other patients. Multivariate analysis including only patients with RF adjusted for age and sex demonstrated that higher serum iron or TSAT levels were independently associated with development of severe RF.

**Conclusions:**

A U-shaped association between serum iron level and RF severity in hospitalized COVID-19 patients was observed. Higher serum iron levels in COVID-19 patients with RF are associated with the development of severe RF, indicating that inadequate response to lower serum iron might be an exacerbating factor for COVID-19.
Introduction

Coronavirus disease 2019 (COVID-19) is an emerging infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), provoking worldwide pandemic emergencies. The main organ system affected by SARS-CoV-2 infection is the lungs, and in severe cases, life-threatening respiratory failure occurs. Although the pathophysiology of COVID-19 has not been fully elucidated, complex interactions between the virus and host responses seem to account for disease severity(1).

A host’s exposure to viral infection triggers multiple responses to mitigate the pathogen load and tissue damages, which are termed “resistance” and “tolerance”, respectively(2). Immunological responses exerted by innate and acquired immune systems are among the best-known host resistance responses. Another strategy of host resistance is the deprivation of molecules necessary for the pathogens to survive or replicate. Iron ions are among the nutrients necessary for viral replication(3, 4). When a host is infected with the virus, hepcidin is released from the liver and lowers serum iron levels to limit iron availability(3, 5). Moreover, iron overload induces oxidative stress and subsequent tissue damages(6). Therefore, lowering iron levels also engages host tolerance responses to protect the host itself.

Several previous reports have shown that a decreased serum iron level is observed in patients with severe COVID-19, and serum iron levels can be a prognostic marker (7–10).
However, it is not clear whether the lower iron level is the result of severe infection or the
mediator of disease deterioration. In this study, we evaluated the association between iron
metabolism indicators and disease severity in hospitalized COVID-19 patients with or
without respiratory failure.

Materials and Methods

Study Design

In this two-center retrospective and prospective observational study, we analyzed the
concentrations of total iron, total iron binding capacity (TIBC), transferrin saturation
(TSAT), and ferritin in the serum of patients with COVID-19. Data from patients
admitted to Yokohama City University Hospital (YCUH) from April 1st to August 7th,
2020 and data from those admitted to Yokohama Municipal Citizen’s Hospital (YMCH)
from April 1st to September 11th, 2020 were retrospectively collected. Whereas data from
patients admitted to YCUH from August 8th, 2020 to January 26th, 2021 were
prospectively collected. The study protocol was reviewed and approved by the
institutional review boards of YCUH (Yokohama City University Certified Institutional
Review Board, approval number: B200700099) and YMCH (Yokohama Municipal
Citizen’s Hospital Institutional Review Board, approval number: 20-09-04). The need for
informed consent was waived by the institutional review boards because of the observational design of the study.

Patients

The inclusion criteria were as follows: 1) aged ≥18 years, 2) a diagnosis of COVID-19 by positive results of real-time polymerase chain reaction or SARS-CoV-2 antigen test, and 3) serum iron level measured within first 5 hospital days. Patients who refused mechanical ventilation were excluded from the study.

Outcome

Patient outcomes were classified into three categories according to their worst respiratory status during hospitalization as follows: non-respiratory failure (non-RF), not requiring oxygen therapy or mechanical ventilation (MV) throughout their hospitalizations; mild RF, requiring oxygen therapy or MV but with the worst arterial oxygen partial pressure / fractional inspired oxygen (P/F) ratio measured with MV or high-flow nasal oxygen therapy (HFNO) maintained above 200; severe RF, the worst P/F ratio measured with MV or HFNO was ≤ 200.

Statistical Analysis
All data are represented as median ± interquartile range (IQR). We compared iron metabolism indicators among the three groups using the Kruskal-Wallis test followed by Dunn’s multiple comparison test. Clinical characteristics and laboratory values were analyzed with the Kruskal-Wallis test (continuous values) or the chi-square test (categorical values). Moreover, we analyzed the effect of serum iron level or TSAT on disease severity among only patients with RF using a multivariate logistic regression model adjusting for patients’ age and sex. The statistical significance level was set at p < 0.05. All statistical analyses were performed using Prism software (version 9.0; GraphPad Software, San Diego, CA, USA).

Results

Patient Characteristics

One hundred thirty-six patients were included in the study among 204 patients with COVID-19 admitted to YCUH (59 patients) or YMCH (77 patients) (Fig.1). Patient characteristics are shown in Table 1. Fifty-five patients were in the non-RF group, and 44 and 37 patients had mild and severe RF, respectively. The patients with mild or severe RF had higher age and more comorbidities than those without RF. Moreover, severe RF patients showed high neutrophil counts and CRP levels and low lymphocyte counts.
Iron metabolism in the hospitalized COVID-19 patients

Serum iron and ferritin concentrations were measured in all patients within the first 5th days of hospitalization. However, TIBC and TSAT data were missing for 16 patients (4 with mild-moderate RF and 12 with severe RF).

Serum iron levels were significantly lower in the mild RF group (median serum iron level: 24 [interquartile range: 19–42] mg/dL) than in the non-RF (40 [24–80] mg/dL, p=0.019) or severe RF groups (60 [23.5–87] mg/dL, p=0.009) (Fig.1A). No significant difference in iron levels was observed between the severe RF and non-RF groups (p>0.999) (Fig.2A). The TIBC levels significantly decreased with increasing severity (Fig.2B). Consequently, TSAT was significantly higher in patients with severe RF (32.7 [13.9–47.6] %) than in those with non-RF (14.0 [8.4–24.9] %, p=0.012) and mild RF (11.8 [7.8–22.2] %, p<0.001) (Fig.2C). Ferritin levels were significantly increased in patients with RF than in those without RF (Fig.2D), probably due to inflammation, irrespective of iron metabolism.

Single logistic regression analysis including only patients with RF also demonstrated that higher serum iron (odds ratio 1.93 [95% CI: 1.24–3.15] per 2 folds increase in serum iron level, p=0.005) or TSAT (odds ratio 1.97 [95% CI: 1.38–3.04] per 10 % increase in TSAT, p<0.001) levels were associated with the development of severe RF. Finally, we performed multivariate analysis including only patients with RF, adjusting for age and
sex, because it is known the serum iron level is affected these parameters. Either higher serum iron (odds ratio 2.02 [95% CI: 1.26–3.36] per 2 folds increase in serum iron level, p=0.005) or TSAT (odds ratio 2.00 [95% CI: 1.38–3.16] per 10 % increase in TSAT, p=0.001) levels were independently associated with severe RF among patients with RF.

Discussion

In the present study, we evaluated the association between serum iron metabolism and COVID-19 disease severity. Serum iron levels were significantly lower in patients with mild RF than in those without RF. However, there were no significant differences in iron levels between the non-RF and severe RF groups; that is, we observed a U-shaped association between serum iron levels and disease severity. Additionally, TSAT was significantly higher in patients with severe RF than in other patients. Finally, logistic regression analysis including only patients with RF revealed that a higher serum iron level or TSAT was independently associated with the development of severe RF. Thus, severe RF induced by COVID-19 is characterized by paradoxically higher serum iron levels and TSAT, suggesting that the derangement of iron metabolic responses is associated with the deterioration of COVID-19.

Previous studies have demonstrated that serum iron levels are decreased in patients with severe COVID-19 (7–10). In fact, the COVID-19 patients with RF (including both
mild and severe RF) in this study tended to have lower serum iron levels compared to patients without RF. However, in evaluating only patients with RF, higher serum iron levels and TSAT were associated with the development of severe RF. Our study has some strengths compared to other previous studies. First, we analyzed the association between the worst respiratory status and serum iron levels, whereas some previous studies evaluated disease severity on hospital admission. The severity on admission might not necessarily reflect the true disease severity throughout the disease duration. Second, we defined the patients’ outcomes based on a reliable objective criterion, the P/F ratio measured with MV or HFNO. Therefore, we believe that our analysis reflects the true association between serum iron metabolism and COVID-19 severity.

Iron is required for several viruses to replicate in the host cells(3). Iron overload is known to be associated with a poor prognosis of hepatitis virus B/C(11–13) and human immunodeficiency virus infection(14). Moreover, iron overload induces oxidative stress and tissue damages(6) accompanied with viral infection. Although the requirement of iron for SARS-CoV-2 replication is not known, it is expected that iron deprivation can inhibit SARS-CoV-2 replication(4). Our observation of the U-shaped association of serum iron and severity of COVID-19 indicates that the inadequate decrease in serum iron level relative to disease severity may lead to deterioration of COVID-19.
Serum iron levels are also associated with the hypoxic pulmonary vasoconstriction (HPV)(15). It has been reported that iron overload impairs HPV, while chelating serum iron augments HPV(16, 17). Several reports have shown that acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 infection is characterized by severe arterial oxygenation impairment with relatively high lung compliance(18–20). Therefore, it is assumed that inappropriate pulmonary vascular responses to hypoxia might be involved in the pathophysiology of COVID-19 ARDS(18). Our observation of higher iron levels in severe COVID-19 cases might be associated with the impairment of HPV and resultant hypoxemia. This suggests that lowering serum iron levels might potentially improve arterial oxygenation in severe RF with COVID-19.

Serum iron levels can be lowered by the administration of iron chelators such as deferoxamine(21). These iron chelators are already used for the treatment of iron overload, and drug safety has been established. It is noteworthy that we found three trials to evaluate the efficacy of deferoxamine for COVID-19 registered in the clinical trial registration database as of January 2021. In particular, one study was planned to evaluate the preventive effect of deferoxamine on ARDS development. Our data support the rationale of these trials.

This study has some limitations. First, although we performed multivariate analysis adjusting for age and sex, there might be other confounders in the association between
serum iron levels and disease severity. Second, we could not obtain the patients’ serum iron levels before SARS-COV-2 infection, although it is possible that the iron status before the infection might affect the serum iron levels on admission and the disease severity.

Conclusions

We observed a U-shaped association between serum iron levels and RF severity in hospitalized patients with COVID-19. The higher serum iron levels in COVID-19 patients with RF are associated with the development of severe RF, indicating that inadequate response to lower serum iron might be an exacerbating factor for COVID-19.

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**Table. 1**

Patient clinical characteristics. *p<0.05.

|                      | all (years) | non-RF (years) | mild RF (years) | severe RF (years) | p-value |
|----------------------|-------------|----------------|-----------------|-------------------|---------|
| Age (years)          | 63 (46-73)  | 46 (34-59)     | 68 (56-75)      | 70 (66-78)        | *<0.0001|
| Females/Male         | 46/90       | 23/32          | 14/30           | 9/28              | 0.208   |
| Body Mass Index      | 22.9 (20.7-25.5) | 22.5 (20.2-25.0) | 22.5 (20.3-25.2) | 23.7 (22.6-26.9) | *0.035  |
|                     | 5.9 [8]   | 0.0 [0]   | 0.0 [0]   | 21.6 [8]  | *<0.0001 |
|---------------------|-----------|-----------|-----------|-----------|----------|
| **Death (percent [number])** |           |           |           |           |          |
| **Commorbidities (percent [number])** |           |           |           |           |          |
| Hypertension        | 35.3 [48] | 21.8 [12] | 45.5 [20] | 43.2 [16] | *0.008   |
| Diabetes            | 27.2 [37] | 12.7 [7]  | 36.4 [16] | 37.8 [14] | *0.008   |
| Asthma              | 9.6 [13]  | 9.1 [5]   | 4.5 [2]   | 16.2 [6]  | 0.203    |
| Cardiac Diseases    | 11.0 [15] | 9.1 [5]   | 13.6 [6]  | 10.8 [4]  | 0.772    |
| Hepatic Diseases    | 6.6 [9]   | 7.3 [4]   | 6.8 [3]   | 5.4 [2]   | 0.938    |
| Renal Diseases with Hemodyalysis | 11 [15] | 7.3 [4]   | 13.6 [6]  | 13.5 [5]  | 0.515    |
| **Laboratory data on admission** |           |           |           |           |          |
| WBC count (/μL)     | 6040      | 5360      | 5750      | 7600      | *<0.0001 |
|                     | (4325-8115) | (3720-7080) | (3925-7965) | (6050-11060) |          |
| Neutrophil count (/μL) | 4603  | 3692      | 4070      | 6445      | *<0.0001 |
|                     | (2852-6331) | (2422-5411) | (2604-5789) | (4956-9665) |          |
| Lymphocyte count (/μL) | 834   | 1026      | 788       | 612       | *<0.0001 |
|                     | (579-1109) | (789-1527) | (623-934) | (270-809) |          |
| Platelet count (x1000/μL) | 188   | 193       | 165       | 208       | *0.048   |
|                     | (142-243) | (151-247) | (123-217) | (152-270) |          |
| Hemoglobin (g/dL)   | 13.6     | 14        | 13.6      | 12.9      | *0.026   |
|                     | (12.0-15.0) | (13.2-15.4) | (11.5-17.8) | (11.5-14.5) |          |
| D-dimer (μg/mL)     | 1.2      | 1.06      | 1.27      | 1.24      | *0.014   |
|                     | (1.0-1.6) | (0.86-1.41) | (1.04-2.06) | (1.15-1.47) |          |
| CRP (mg/dL)         | 4.1      | 1.3       | 5.6       | 8.7       | *<0.0001 |
|                     | (0.9-9.3) | (0.3-4.5) | (1.7-10.8) | (4.2-15.4) |          |
| Total bilirubin (mg/dL) | 0.5 | 0.5       | 0.7       | 0.5 (0.4-0.7) | 0.305    |
|                     | (0.4-0.8) | (0.3-0.8) | (0.4-0.8) |          |          |
| Creatinine (mg/dL)  | 0.82     | 0.78      | 0.94      | 0.82      | 0.141    |
|                     | (0.64-1.17) | (0.61-0.91) | (0.63-1.50) | (0.68-1.70) |          |
| **Received Treatment (percent [number])** |           |           |           |           |          |
| Mechanical Ventilation Use | 28.7 [39] | 0.0 [0]   | 6.8 [3]   | 97.3 [36] | *<0.0001 |
| Drug                        | Percentages | p-values |
|-----------------------------|-------------|----------|
| High-Flow Nasal Oxygenation Use | 0.7 [1]     | 0.0 [0]  | 0.0 [0]  | 2.7 [1]     | 0.270 |
| Systemic Steroids           | 58.1 [79]   | 27.3 [15]| 72.7 [32]| 86.5 [32]   | *<0.0001 |
| Ciclesonide                 | 56.6 [77]   | 52.7 [29]| 50.0 [22]| 70.3 [26]   | 0.140 |
| Remdesivir                  | 34.6 [47]   | 3.6 [2]  | 43.2 [19]| 70.3 [26]   | *<0.0001 |
| Favipiravir                 | 27.2 [37]   | 7.3 [4]  | 36.4 [16]| 45.9 [17]   | *<0.0001 |
| Tocilizumab                 | 5.9 [8]     | 0.0 [0]  | 0.0 [0]  | 21.6 [8]    | *<0.0001 |
| Chlorquine                  | 12.5 [17]   | 16.4 [9] | 6.8 [3]  | 13.5 [5]    | 0.353 |
Figure Legend

Figure 1. Flow diagram of the patient inclusion.

Figure 2. Iron metabolism indicators in hospitalized patients with coronavirus disease 2019. (A) serum iron, (B) total iron binding capacity (TIBC), (C) transferrin saturation (TSAT), and (D) ferritin levels. All the values were measured within the first 5 days of hospitalization. Data are presented as median ± interquartile range (IQR). *p<0.05.
204 adult patients (≥18 years old) with COVID-19 who were admitted to Yokohama City University Hospital (YCUH) or Yokohama Municipal Citizen’s Hospital (YMCH) (YCUH n=79, YMCH n=125)

63 patients without serum iron data

141 patients whose serum iron levels were measured within first 5 hospital days

5 patients who refused mechanical ventilation

136 patients included in analysis (YCUH n=59, YMCH n=77)
