




Article

# Evaluation of Biomarkers of Severity in Patients with COVID-19 Infection

Akitaka Yamamoto <sup>1</sup>, Hideo Wada <sup>2,\*</sup> , Yuhuko Ichikawa <sup>3</sup>, Hikaru Mizuno <sup>3</sup>, Masaki Tomida <sup>1</sup>, Jun Masuda <sup>4</sup>, Katsutoshi Makino <sup>4</sup>, Shuji Kodama <sup>5</sup>, Masamichi Yoshida <sup>5</sup>, Shunsuke Fukui <sup>6</sup>, Isao Moritani <sup>6</sup>, Hidekazu Inoue <sup>6</sup>, Katsuya Shiraki <sup>6</sup> and Hideto Shimpo <sup>7</sup>

<sup>1</sup> Department of Emergency and Critical Care Center, Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan; akitaka-yamamoto@mie-gmc.jp (A.Y.); st25053@yahoo.co.jp (M.T.)

<sup>2</sup> Department of General and Laboratory Medicine, Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan

<sup>3</sup> Department of Central Laboratory, Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan; ichi911239@yahoo.co.jp (Y.I.); solid.works.143@gmail.com (H.M.)

<sup>4</sup> Department of Cardiovascular Medicine, Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan; jun-masuda@mie-gmc.jp (J.M.); katsutoshi-makino@mie-gmc.jp (K.M.)

<sup>5</sup> Department of Respiratory Medicine, Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan; shuuj-kodama@mie-gmc.jp (S.K.); masamichi-yoshida@mie-gmc.jp (M.Y.)

<sup>6</sup> Department of Gastroenterology, Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan; m13092sf@jichi.ac.jp (S.F.); isao-moritani@mie-gmc.jp (I.M.); hidekazu-inoue@mie-gmc.jp (H.I.); katsuya-shiraki@mie-gmc.jp (K.S.)

<sup>7</sup> Mie Prefectural General Medical Center, Yokkaichi 510-0885, Japan; hideto-shimpo@mie-gmc.jp

\* Correspondence: wadahide@clin.medic.mie-u.ac.jp; Tel.: +81-59-345-2321



**Citation:** Yamamoto, A.; Wada, H.; Ichikawa, Y.; Mizuno, H.; Tomida, M.; Masuda, J.; Makino, K.; Kodama, S.; Yoshida, M.; Fukui, S.; et al. Evaluation of Biomarkers of Severity in Patients with COVID-19 Infection. *J. Clin. Med.* **2021**, *10*, 3775. <https://doi.org/10.3390/jcm10173775>

Academic Editor: Tadeusz Robak

Received: 29 July 2021

Accepted: 19 August 2021

Published: 24 August 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** Object: Although many Japanese patients infected with coronavirus disease 2019 (COVID-19) only experience mild symptoms, in some cases a patient's condition deteriorates, resulting in a poor outcome. This study examines the behavior of biomarkers in patients with mild to severe COVID-19. Methods: The disease severity of 152 COVID-19 patients was classified into mild, moderate I, moderate II, and severe, and the behavior of laboratory biomarkers was examined across these four disease stages. Results: The median age and male/female ratio increased with severity. The mortality rate was 12.5% in both moderate II and severe stages. Underlying diseases, which were not observed in 45% of mild stage patients, increased with severity. An ROC analysis showed that C-reactive protein (CRP), ferritin, procalcitonin (PCT), hemoglobin (Hb) A1c, albumin, and lactate dehydrogenase (LDH) levels were significantly useful for the differential diagnosis of mild/moderate I stage and moderate II/severe stage. In the severe stage, Hb levels, coagulation time, total protein, and albumin were significantly different on the day of worsening from those observed on the day of admission. The frequency of hemostatic biomarker abnormalities was high in the severe disease stage. Conclusion: The evaluation of severity is valuable, as the mortality rate was high in the moderate II and severe stages. The levels of CRP, ferritin, PCT, albumin, and LDH were useful markers of severity, and hemostatic abnormalities were frequently observed in patients in the severe disease stage.

**Keywords:** COVID-19; biomarkers; mild; moderate; severe

## 1. Introduction

Since the first outbreak of coronavirus disease 2019 (COVID-19) in China [1,2], COVID-19 infections have spread worldwide, generating a pandemic [3]. The mortality rate of COVID-19 is approximately 2%, with 5–10% of patients developing severe and life-threatening disease [3]. COVID-19 infection predominantly displays hyperinflammation and immune dysregulation, which induce multiorgan damage. The primary cause of mortality due to COVID-19 is severe acute respiratory distress caused by epithelial infection and alveolar macrophage activation in the lungs [4]. Therefore, immune modulation and suppression

therapy may prevent the deterioration of the condition of COVID-19 patients [5]. Some COVID-19 patients develop severe disease or die [6], while many more develop mild or moderate disease [7]. The risk factors for severe disease are reported to include an age  $\geq 65$  years [8], malignant tumor [9], chronic obstructive pulmonary disease [10], chronic renal disease [11], diabetes mellitus [12], hypertension [13], hyperlipidemia [8], obesity [14], smoking [13], and immunodeficiency after transplantation [15]. Biomarkers including white blood cell (WBC) count, lymphocyte count, platelet count; albumin, ALT, lactate dehydrogenase (LDH), D-dimer, ferritin, interleukin-6, and procalcitonin (PCT) levels; and prothrombin time (PT) have been reported as specific biomarkers of severity [16]. In this study, specific biomarkers of severity and hemostatic abnormalities were examined in 152 patients with four disease stages of COVID-19 in Japan.

## 2. Materials and Methods

One hundred and fifty-two patients with COVID-19 infection (median age, 53.5 years; 25–75th percentile, 38.0–70.0 years; female,  $n = 78$ ; male,  $n = 74$ ) were admitted to Mie Prefectural General Medical Center from 1 April 2020 to 21 March 2021. The severity of COVID-19 was evaluated based on the Japanese Medical Care Guidelines [15]. There were 63 patients with mild disease (without pneumonia), 48 with moderate I disease (pneumonia without oxygen therapy), 32 with moderate II disease (pneumonia with oxygen therapy without mechanical ventilation), and 9 with severe disease (pneumonia with mechanical ventilation) (Table 1).

**Table 1.** Relationship between age, sex, mortality, and comorbidity and severity of COVID-19.

| Stage                           | Mild   | Moderate I         | Moderate II               | Severe                  |
|---------------------------------|--|--------------------|---------------------------|-------------------------|
| Number                          | 63   | 48                 | 32                        | 9                       |
| Age, years (25–75th percentile) | 41.0 (29.3–57.8)                               | 50.5 * (38.0–64.0) | 66.5 ***, ### (58.5–80.5) | 70.0 **, ## (63.8–74.0) |
| Male (percent in stage)         | 25 (39.7%)                                     | 22 (45.8%)         | 19 (59.4%)                | 8 * (88.8%)             |
| Death (mortality)               | 0  | 0                  | 4 (12.5%)                 | 1 (11.1%)               |
| Died of old age                 | 0  | 0                  | 2 (6.3%)                  | 0                       |
| Pregnant patient, $n$ (%)       | 3 (4.8%)                                       | 0                  | 0                         | 0                       |
| Comorbidity                     | Patient number (percent) in each disease stage |                    |                           |                         |
| No comorbidity                  | 28 (45.0%)                                     | 15 (31.2%)         | 3 **, # (9.4%)            | 0 *                     |
| Hypertension                    | 12 (19.4%)                                     | 10 (20.8%)         | 9 (28.1%)                 | 5 * (55.6%)             |
| Hyperlipidemia                  | 6 (9.7%)                                       | 11 (22.9%)         | 6 (18.8%)                 | 2 (22.2%)               |
| Diabetes mellitus               | 6 (9.5%)                                       | 4 (8.3%)           | 11 **, ## (34.4%)         | 5 **, ## (55.6%)        |
| Heart failure                   | 2 (3.2%)                                       | 1 (2.1%)           | 3 (9.4%)                  | 1 (11.1%)               |
| Renal failure                   | 2 (3.3%)                                       | 3 (6.3%)           | 5 (15.6%)                 | 2 (22.2%)               |
| Other pneumonia *               | 1 (1.6%)                                       | 1 (2.1%)           | 5 * (15.6%)               | 0 (0.0%)                |
| Cerebrovascular accident        | 0  | 1 (2.1%)           | 5 ** (15.6%)              | 1 (11.1%)               |
| Asthma                          | 4 (6.3%)                                       | 5 (10.4%)          | 2 (6.3%)                  | 0                       |
| Neurodegenerative diseases      | 5 (7.9%)                                       | 2 (4.2%)           | 4 (12.5%)                 | 0                       |
| Digestive diseases              | 3 (4.8%)                                       | 2 (4.2%)           | 4 (12.5%)                 | 1 (11.1%)               |
| HIV infection                   | 2 (3.2%)                                       | 1 (2.1%)           | 0                         | 0                       |
| Autoimmune diseases             | 3 (4.8%)                                       | 2 (4.2%)           | 2 (6.2%)                  | 0                       |
| Solid cancer                    | 3 (4.8%)                                       | 0                  | 0                         | 0                       |
| Endocrine diseases              | 2 (3.2%)                                       | 2 (4.2%)           | 1 (3.1%)                  | 0                       |
| Other diseases                  | 11 (17.5%)                                     | 11 (22.9%)         | 13 (40.6%)                | 3 (33.3%)               |

Many comorbidities overlapped. HIV, human immunodeficiency virus; Mild, without pneumonia; Moderate I, pneumonia without oxygen therapy; Moderate II, pneumonia with oxygen therapy; Severe, pneumonia with mechanical ventilation. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  in comparison with mild stage. #,  $p < 0.05$ ; ##,  $p < 0.01$ ; ###,  $p < 0.001$  in comparison with moderate I stage. \* Although PCR was positive for COVID-19 in these patients, CT indicated other pneumonia, or their culture showed a bacterial or fungal infection.

### 2.1. Measurement of Laboratory Biomarkers

Blood sampling was performed on the day of admission in hospitalized patients and throughout the clinical course in patients with severe disease. Total protein (TP), albumin, AST, lactate dehydrogenase (LDH), creatinine, and C-reactive protein (CRP) were measured

by an Aqua-auto Kainos TP-II test, Aqua-auto Kainos ALB (Kainos, Tokyo, Japan), LaboFit AST, CicaFit LD-IFCC (Kanto Chemical Co., Inc., Tokyo, Japan), Signasu-auto CRE (Shino-test, Tokyo, Japan), and CRP-Latex X2 (Denka, Niigata, Japan), respectively, using a LABOSPECT006 (Hitachi High-Tech Co., Tokyo, Japan). PCT and ferritin were measured by Elecsys® BRAHMS PCT and Elecsys® Ferritin (Roche Diagnostics K.K., Tokyo, Japan), respectively, using a Cobas 8000 e602 (Roche Diagnostics K.K., Tokyo, Japan). The WBC count, hemoglobin (Hb), total neutrophil count, and total lymphocyte count were measured using a fully automatic blood cell counter XN-3000 (Sysmex Co., Kobe, Japan). HbA1c was measured using an Adams Hybrid AH 8280 (Arkray Factory, Shiga, Japan). The prothrombin time (PT), activated partial thromboplastin time (APTT), and D-dimer level were measured by a Thromborel S, Thrombocheck APTT-SLA, and LIASAUTO D-dimer Neo (Sysmex Co., Kobe, Japan), respectively, using an automatic coagulation analyzer CS-5100 (Sysmex Co., Kobe, Japan).

## 2.2. Statistical Analyses

The data are expressed as the median (25–75th percentiles). The difference in the frequency was analyzed by the chi-squared test. The significance of differences between groups was examined using the Mann-Whitney U test. *p*-Values < 0.05 were considered to indicate statistical significance. Cut-off values were determined by a receiver operating characteristic (ROC) analysis. A multivariate analysis with a stepwise regression test was performed. All statistical analyses were performed using Stat-Flex software (version 6; Artec Co. Ltd., Osaka, Japan).

## 3. Results

The median age and male/female ratio increased with severity (Table 1). The mortality rate was 12.5% in both the moderate II and severe stages. Comorbidity, which was not observed in 45% of mild stage patients, increased with severity (Table 1).

Hypertension, hyperlipidemia, or diabetes mellitus were observed  $\geq 40\%$  of patients with moderate II or severe disease. Heart failure, other pneumonia complications, and cerebrovascular accident were frequently observed among the patients who died within 30 days.

Regarding the laboratory data (Table 2), the neutrophil, lymphocyte, and platelet counts as well as the PT-INR and D-dimer levels in the moderate II and severe stages were significantly different from those in the mild and moderate I disease stages. The CRP, PCT, and ferritin levels in the moderate II and severe stages were significantly higher than those in the mild and moderate I disease stages. In the moderate II and severe stages the total protein and albumin levels, A/G ratio, and estimated glomerular filtration rate (eGFR) were significantly lower, and the levels of AST, LDH, and hemoglobin A1C levels were significantly higher in comparison with the mild and moderate I disease stages.

The ROC analysis of laboratory biomarkers in the mild and moderate I stages vs. moderate II and severe stages (Table 3, Figure S1), CRP (Figure 1), albumin, and A/G ratio showed  $\geq 80.0\%$  sensitivity; CRP, ferritin, albumin, A/G ratio, and hemoglobin A1c showed an odds ratio of  $\geq 10.00$ ; and CRP, PCT, ferritin, albumin, A/G ratio, and LDH showed an area under the curve (ARC)  $\geq 0.800$ . In our multivariate analysis, CRP ( $p = 0.006$ ) and AST ( $p = 0.033$ ) were identified as significant factors in mild to moderate II stage vs. severe stage; albumin ( $p = 0.022$ ), CRP ( $p = 0.001$ ), and LDH ( $p = 0.011$ ) were identified as significant factors in mild to moderate I stage vs. moderate II to severe stage; and LDH ( $p = 0.032$ ) and CRP ( $p = 0.036$ ) were identified as significant factors in mild stage vs. moderate I to severe disease stages.

**Table 2.** Laboratory data on the day of admission.

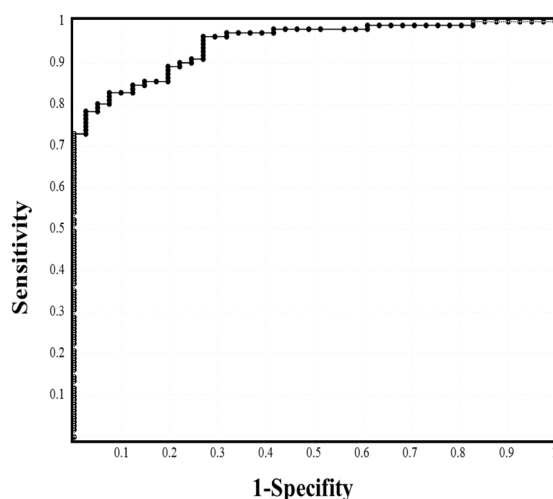
|                                    | Mild                | Moderate I               | Moderate II                  | Severe                       |
|------------------------------------|---------------------|--------------------------|------------------------------|------------------------------|
| WBC<br>( $\times 10^9/L$ )         | 5.2<br>(4.2–6.1)    | 5.5<br>(4.0–6.7)         | 5.7<br>(4.0–7.6)             | 6.0<br>(5.4–8.0)             |
| Neutrophil<br>( $\times 10^9/L$ )  | 2.7<br>(1.7–3.3)    | 3.2<br>(1.9–4.1)         | 4.4 **, ##<br>(2.2–5.9)      | 4.8 ***, ###<br>(4.4–7.1)    |
| Lymphocyte<br>( $\times 10^9/L$ )  | 1.5<br>(1.1–1.7)    | 1.3<br>(1.0–1.7)         | 9.8 **, #<br>(0.6–1.3)       | 0.7 **, ##<br>(0.5–1.1)      |
| Hemoglobin<br>(g/dL)               | 14.4<br>(12.7–15.4) | 14.3<br>(13.5–15.0)      | 13.4<br>(12.4–14.9)          | 14.6<br>(13.4–14.7)          |
| Platelet<br>( $\times 10^{10}/L$ ) | 21.7<br>(18.7–25.2) | 22.0<br>(17.7–26.6)      | 19.5 *<br>(14.5–23.2)        | 13.8 **, ##<br>(10.6–19.0)   |
| PT-INR                             | 0.96<br>(0.90–1.01) | 0.98<br>(0.94–1.02)      | 1.02 **<br>(0.96–1.07)       | 1.02 **, #<br>(0.99–1.10)    |
| APTT<br>(sec)                      | 32.0<br>(29.0–33.0) | 32.0<br>(30.0–35.0)      | 32.0<br>(30.0–35.0)          | 33.0<br>(28.5–34.8)          |
| D-dimer<br>( $\mu\text{g/mL}$ )    | 0.5<br>(0.5–0.7)    | 0.5<br>(0.5–0.9)         | 0.8 ***, #<br>(0.5–2.0)      | 1.2 ***, ##<br>(0.7–1.8)     |
| CRP<br>(mg/mL)                     | 0.20<br>(0.05–0.65) | 0.58 ***,<br>(0.31–2.67) | 5.59 ***, ###<br>(2.69–8.11) | 9.21 ***, ###<br>(7.53–13.0) |
| Procalcitonin (ng/mL)              | 0.05<br>(0.04–0.06) | 0.06 *<br>(0.05–0.08)    | 0.08 ***, ##<br>(0.07–0.10)  | 0.95 **, ##                  |
| Ferritin<br>(ng/mL)                | 150<br>(83–342)     | 258<br>(115–369)         | 541 ***, ###<br>(378–1288)   | 1530 **, ##                  |
| Total protein<br>(g/dL)            | 7.10<br>(6.88–7.50) | 7.10<br>(6.80–7.45)      | 6.65 **, #<br>(6.30–7.25)    | 6.55 **, #<br>(6.30–6.95)    |
| Albumin<br>(g/dL)                  | 4.20<br>(3.90–4.50) | 4.00 *<br>(3.80–4.30)    | 3.20 ***, ###<br>(3.00–3.70) | 3.45 ***, ###<br>(2.80–3.55) |
| A/G ratio                          | 1.40<br>(1.30–1.60) | 1.30 **<br>(1.20–1.40)   | 1.00 ***, ###<br>(0.80–1.10) | 1.05 ***, ##<br>(0.90–1.10)  |
| T-Bil<br>(mg/dL)                   | 0.53<br>(0.37–0.68) | 0.60<br>(0.46–0.77)      | 0.71 ***, ##<br>(0.65–1.09)  | 0.69<br>(0.50–0.98)          |
| AST (U/L)                          | 20.0<br>(16.3–24.0) | 22.0 *<br>(19.0–31.5)    | 35.0 ***, ##<br>(25.5–47.0)  | 43.0 ***, ###<br>(34.0–140)  |
| LDH<br>(U/L)                       | 176<br>(155–195)    | 202 **<br>(175–249)      | 254 ***, ##<br>(216–333)     | 428 ***, ###<br>(318–731)    |
| Creatinine<br>(mg/dL)              | 0.67<br>(0.55–0.82) | 0.69<br>(0.58–0.89)      | 0.76<br>(0.65–1.01)          | 1.24 *<br>(0.68–1.51)        |
| eGFR                               | 84.5<br>(73.5–104)  | 79.5<br>(68.5–92.5)      | 65.0 ***, #<br>(54.3–84.3)   | 45.0 *<br>(37.0–84.0)        |
| Hemoglobin A1c (%)                 | 5.60<br>(5.40–6.08) | 5.80<br>(5.50–6.10)      | 6.45 ***, ###<br>(5.90–7.45) | 6.70 ***, ###<br>(6.55–8.43) |

WBC, white blood cell; PT-INR, prothrombin time—international normalized ratio; APTT, activated partial thromboplastin time; CRP, C reactive protein; A/G ratio, albumin/globulin ratio; T-Bil, total bilirubin; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; Mild, without pneumonia; Moderate I, pneumonia without oxygen therapy; Moderate II, pneumonia with oxygen therapy; Severe, pneumonia with mechanical ventilation. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  in comparison with mild stage. #,  $p < 0.05$ ; ##,  $p < 0.01$ ; ###,  $p < 0.001$  in comparison with moderate I.

**Table 3.** ROC analysis to determine cutoff values of laboratory data (mild to moderate I vs.  $\geq$ moderate II to severe).

|                | Cutoff Value                 | Sensitivity | Odds Ratio | AUC   |
|----------------|------------------------------|-------------|------------|-------|
| WBC            | $5.5 \times 10^9/L$          | 55.0%       | 1.491      | 0.580 |
| Neutrophil     | $3.5 \times 10^9/L$          | 68.5%       | 5.067      | 0.734 |
| Lymphocyte     | $1.1 \times 10^9/L$          | 65.8%       | 3.990      | 0.695 |
| Hemoglobin     | 14.0 g/dL                    | 57.0%       | 1.898      | 0.580 |
| Platelet       | $20.1 \times 10^{10}/L$      | 59.3%       | 2.269      | 0.662 |
| PT-INR         | 0.997                        | 61.4%       | 2.557      | 0.676 |
| APTT           | 32.8 s                       | 53.2%       | 1.316      | 0.530 |
| D-dimer        | 0.60 $\mu\text{g}/\text{mL}$ | 67.4%       | 4.438      | 0.697 |
| CRP            | 2.36 mg/mL                   | 85.0%       | 33.65      | 0.946 |
| Procalcitonin  | 0.069 ng/mL                  | 74.6%       | 9.387      | 0.853 |
| Ferritin       | 379 ng/mL                    | 78.3%       | 14.12      | 0.872 |
| Total protein  | 6.89 g/dL                    | 62.2%       | 2.558      | 0.703 |
| Albumin        | 3.73 g/dL                    | 81.7%       | 20.44      | 0.882 |
| A/G ratio      | 1.10                         | 84.6%       | 30.67      | 0.897 |
| T-Bil          | 0.67 mg/dL                   | 66.5%       | 4.248      | 0.711 |
| AST            | 27.8 U/L                     | 76.2%       | 11.86      | 0.794 |
| LDH            | 222 U/L                      | 76.9%       | 3.939      | 0.886 |
| Creatinine     | 0.74 mg/dL                   | 62.3%       | 3.163      | 0.630 |
| eGFR           | 75.5                         | 65.3%       | 3.571      | 0.690 |
| Hemoglobin A1c | 6.11%                        | 73.9%       | 10.63      | 0.788 |

WBC, white blood cell; PT-INR, prothrombin time—international normalized ratio; APTT, activated partial thromboplastin time; CRP, C reactive protein; A/G ratio, albumin/globulin ratio; T-Bil, total bilirubin; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; Mild, without pneumonia; Moderate I, pneumonia without oxygen therapy; Moderate II, pneumonia with oxygen therapy; Severe, pneumonia with mechanical ventilation. AUC, area under the curve.

**Figure 1.** ROC analysis to determine the cutoff values of CRP (mild to moderate I vs.  $\geq$ moderate II to severe).

In patients with severe disease, the neutrophil, hemoglobin, and platelet counts; PT-INR, APTT, TP, and albumin levels; and the A/G ratio on the day of each instance of worsening were significantly different from those on the day of admission (Table 4). The frequencies of hemostatic abnormalities were as follows: hemoglobin  $\leq 10$  g/dL, 44.4%; PT-INR  $\geq 1.26$ , 44.4%; D-dimer  $\geq 3.0$   $\mu\text{g}/\text{mL}$ , 44.4%; platelet count  $\leq 10 \times 10^{10}/L$ , 55.5%; and APTT  $\geq 50$  s, 77.8% (Table 5).

**Table 4.** Laboratory data (data on admission vs. data at time of worsening) in patients with severe stage disease.

|                                 | Admission Data    | Worsening Data     | Worsening Period (Day) | p-Value |
|---------------------------------|-------------------|--------------------|------------------------|---------|
| WBC ( $\times 10^9$ /L)         | 6.0 (5.4–8.5)     | 18.6 (15.4–23.7)   | 10.0; 5.3–12.3         | 0.046   |
| Neutrophile ( $\times 10^9$ /L) | 4.5 (3.8–5.2)     | 16.5 (13.1–21.1)   | 10.0; 6.0–11.5         | 0.044   |
| Lymphocyte ( $\times 10^9$ /L)  | 0.6 (0.2–0.9)     | 0.4 (0.2–0.5)      | 3.0; 1.0–7.3           | 0.229   |
| Hemoglobin (g/dL)               | 14.6 (13.0–14.7)  | 10.2 (9.0–11.4)    | 13.0; 10.5–15.5        | <0.001  |
| Platelet ( $\times 10^{10}$ /L) | 13.8 (10.6–19.0)  | 7.3 (5.3–11.5)     | 1.0; 1.0–10.8          | 0.020   |
| PT-INR                          | 1.02 (0.99–1.11)  | 1.29 (1.17–1.37)   | 12.0; 3.8–12.5         | 0.006   |
| APTT (sec)                      | 33.0 (28.0–33.5)  | 81.5 (58.5–180)    | 2.0; 1.8–9.3           | 0.005   |
| D-dimer ( $\mu\text{g/mL}$ )    | 1.2 (0.7–1.8)     | 3.7 (1.7–8.1)      | 6.0; 1.0–10.3          | 0.155   |
| CRP (mg/mL)                     | 9.21 (7.53–13.02) | 11.67 (9.65–15.27) | 2.0; 1.0–6.5           | 0.728   |
| Total protein (g/dL)            | 6.55 (6.30–6.95)  | 4.20 (4.15–4.55)   | 7.0; 5.5–15.0          | <0.001  |
| Albumin (g/dL)                  | 3.45 (2.80–3.55)  | 1.90 (1.75–2.15)   | 10.0; 6.5–15.0         | <0.001  |
| A/G ratio                       | 1.05 (0.90–1.10)  | 0.80 (0.75–0.90)   | 3.0; 3.0–7.3           | 0.005   |
| T-Bil (mg/dL)                   | 0.69 (0.50–0.98)  | 1.20 (0.61–1.61)   | 8.0; 1.0–12.8          | 0.110   |
| AST (U/L)                       | 43.0 (34.0–139.5) | 65.0 (52.0–119.5)  | 3.0; 1.0–9.0           | 0.900   |
| LDH (U/L)                       | 428 (317–731)     | 476 (395–723)      | 2.0; 1.0–7.8           | 0.803   |
| Creatinine (mg/dL)              | 1.24 (0.68–1.51)  | 1.28 (0.75–1.51)   | 3.0; 1.0–4.3           | 0.879   |
| eGFR                            | 45.0 (36.5–75.0)  | 43.0 (36.0–75.0)   | 1.0; 1.0–3.3           | 0.155   |

WBC, white blood cell; PT-INR, prothrombin time—international normalized ratio; APTT, activated partial thromboplastin time; CRP, C reactive protein; A/G ratio, albumin/globulin ratio; T-Bil, total bilirubin; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; Mild, without pneumonia; Moderate I, pneumonia without oxygen therapy; Moderate II, pneumonia with oxygen therapy; Severe, pneumonia with mechanical ventilation.

**Table 5.** Frequency of hemostatic abnormalities.

|          | Hemoglobin $\leq 10$ g/dL | Platelet Count $\leq 10 \times 10^{10}$ /L | PT-INR $\geq 1.26$ | APTT $\geq 50$ s | D-Dimer $\geq 3.0$ $\mu\text{g/mL}$ |
|----------|---------------------------|--|--------------------|------------------|-------------------------------------|
| Case (%) | 4/9 (44.4%)               | 5/9 (55.5%)                                | 4/9 (44.4%)        | 7/9 (77.8%)      | 5/9 (44.4%)                         |

PT-INR, prothrombin time—international normalized ratio; APTT, activated partial thromboplastin time.

#### 4. Discussion

In the present study, the mortality rate was 0% in the mild and moderate I disease stages of COVID-19 infection, 12.5% in both moderate II and severe disease stages, and 3.3% overall, suggesting a mortality rate similar to other reports [3,16]. These findings suggest that four patients without mechanical ventilation died, and the direct cause of death was not COVID-19 in these patients. In addition to the management of severe COVID-19, the prevention of progression from mild/moderate I to moderate II and severe disease stages is important. Comorbidity, diabetes mellitus, hypertension, and cerebral vascular accident were related to the disease severity of COVID-19 infection. The elevation of Hb A1c and onset of hypertension may have occurred before the onset of COVID-19 infection, suggesting the importance of antihypertensive therapy and glycemic control before the onset of COVID-19 infection.

An increased WBC, especially increased neutrophil counts and decreased lymphocyte counts, was useful for predicting the disease severity of COVID-19. A decrease in lymphocytes due to apoptosis of CD4+ lymphocytes is correlated with the elevation of inflammatory cytokines [17]. CRP and ferritin were also useful biomarkers for predicting the disease severity of COVID-19. Ferritin elevation is believed to be caused by the cytokine storm and secondary hemophagocytic lympho-histiocytosis [18]. Elevation of CRP and ferritin and a reduction in lymphocytes may occur in association with a cytokine storm [19]. Although there was a large difference in the PCT level, this level was within normal range in the mild, moderate I, and moderate II disease stages. PCT is a specific biomarker for bacterial infection and is not markedly elevated in patients with common viral infections [20]. Therefore, PCT elevation may suggest bacterial co-infection or markedly severe inflammation in the severe disease stage of COVID-19 infection.



The change in AST was small and levels fell to within the normal range in patients with moderate II disease. LDH levels have been reported to be associated with Acute Physiology and Chronic Health Evaluation (APACHE) II, Sequential Organ Failure Assessment (SOFA), and computed tomography (CT) semiquantitative rating scores [21], and LDH is suggested to be a useful marker for multiple organ failure. TP and albumin levels, A/G ratio, and eGFR are also related to multiple organ failure. Thus, these biomarkers are associated with organ failure or renal function, indicating a correlation with the disease severity of COVID-19 infection.

Although D-dimer is a well-known biomarker of thrombosis in COVID-19 [22], the increase of D-dimer was small in the present study. These findings suggest that in the present study there were a few patients with severe disease with no resulting thrombotic complications. In the severe disease stage, Hb and platelet counts were significantly decreased, and the PT-INR and APTT were significantly prolonged, suggesting that the hemostatic abnormalities occurred after admission in patients with severe COVID-19. A high frequency of hemostatic abnormalities, including Hb, platelet count, PT-INR, and D-dimer abnormalities, suggests that a coagulopathy similar to thrombotic microangiopathy (TMA) [23] with disseminated intravascular coagulation (DIC) [24] may occur in patients with severe COVID-19. DIC and TMA produce similar pathological states and are associated with poor outcomes due to marked platelet activation [25]. Accordingly, aspirin treatment was reported to improve the mortality of patients with COVID-19 [26].

There are several limitations associated with the present study. Specifically, this study was observational and thus affected by comorbidities and varying therapies. Several patients died of old age or due to comorbidities rather than due to the severity of their COVID-19 infection. For most patients without severe disease, blood sampling was performed on the day of admission. In addition, the study population was relatively small, and there were few cases in the severe stage.

## 5. Conclusions

The mortality rate was high in COVID-19 patients with moderate II/severe disease. CRP, ferritin, PCT, albumin, and LDH levels were useful markers of severity, and hemostatic abnormalities were frequently observed in patients with severe disease.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/jcm10173775/s1>, Figure S1: An ROC analysis to determine the cutoff values of the A/G ratio (a), albumin (b), ferritin (c) and procalcitonin (d) (mild to moderate I vs.  $\geq$  moderate II to severe).

**Author Contributions:** Conceptualization, H.W.; methodology, Y.I. and H.M.; data curation, M.T., J.M., S.K. and S.F.; writing—original draft preparation, H.W. and A.Y.; writing—review and editing, K.S.; investigation, K.M., M.Y., H.I. and I.M.; supervision, H.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by a Grant-in-Aid from the Ministry of Health, Labour and Welfare of Japan.

**Institutional Review Board Statement:** The study protocol (2020-44) was approved by the Human Ethics Review committees of Mie Prefectural General Medical Center, and informed consent was obtained from each patient.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

**Acknowledgments:** We thank the physicians and our coworkers for managing COVID-19 patients and the laboratory technicians (Chief technicians, Motoko Tanaka) for measuring the laboratory biomarkers at Mie Prefectural General Medical Center Hospital.

**Conflicts of Interest:** The authors declare no conflict of interest in association with the present study.

## References

1. Zhou, F.; Yu, T.; Du, R.; Fan, G.; Liu, Y.; Liu, Z.; Xiang, J.; Wang, Y.; Song, B.; Gu, X.; et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet* **2020**, *395*, 1054–1062. [CrossRef]
2. Jiang, F.; Deng, L.; Zhang, L.; Cai, Y.; Cheung, C.W.; Xia, Z.J. Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). *Gen Intern Med.* **2020**, *35*, 1545–1549. [CrossRef] [PubMed]
3. Gavriatopoulou, M.; Ntanasis-Stathopoulos, I.; Korompoki, E.; Fotiou, D.; Migkou, M.; Tzanninis, I.-G.; Psaltopoulou, T.; Kastiris, E.; Terpos, E.; Dimopoulos, M.A. Emerging treatment strategies for COVID-19 infection. *Clin. Exp. Med.* **2020**, *21*, 167–179. [CrossRef] [PubMed]
4. Machhi, J.; Herskovitz, J.; Senan, A.M.; Dutta, D.; Nath, B.; Oleynikov, M.D.; Blomberg, W.R.; Meigs, D.D.; Hasan, M.; Patel, M.; et al. The Natural History, Pathobiology, and Clinical Manifestations of SARS-CoV-2 Infections. *J. Neuroimmune Pharmacol.* **2020**, *21*, 1–28.
5. Hertanto, D.M.; Wiratama, B.S.; Sutanto, H.; Wungu, C.D.K. Immunomodulation as a Potent COVID-19 Pharmacotherapy: Past, Present and Future. *J. Inflamm. Res.* **2021**, *14*, 3419–3428. [CrossRef]
6. Berlin, D.A.; Gulick, R.M.; Martinez, F.J. Severe Covid-19. *N. Engl. J. Med.* **2020**, *383*, 2451–2460. [CrossRef]
7. Gandhi, R.T.; Lynch, J.B.; Del Rio, C. Mild or Moderate Covid-19. *N. Engl. J. Med.* **2020**, *383*, 1757–1766. [CrossRef] [PubMed]
8. Matsunaga, N.; Hayakawa, K.; Terada, M.; Ohtsu, H.; Asai, Y.; Tsuzuki, S.; Suzuki, S.; Toyoda, A.; Suzuki, K.; Endo, M.; et al. Clinical epidemiology of hospitalized patients with COVID-19 in Japan: Report of the COVID-19 Registry Japan. *Clin. Infect. Dis.* **2020**, *28*, eiaa1470. [CrossRef] [PubMed]
9. Liang, W.; Guan, W.; Chen, R.; Wang, W.; Li, J.; Xu, K.; Li, C.; Ai, Q.; Lu, W.; Liang, H.; et al. Cancer patients in SARS-CoV-2 infection: A nationwide analysis in China. *Lancet Oncol.* **2020**, *21*, 335–337. [CrossRef]
10. Lippi, G.; Henry, B.M. Chronic obstructive pulmonary disease is associated with severe coronavirus disease 2019 (COVID-19). *Respir. Med.* **2020**, *167*, 105941. [CrossRef]
11. Myers, L.C.; Parodi, S.M.; Escobar, G.J.; Liu, V.X. Characteristics of Hospitalized Adults With COVID-19 in an Integrated Health Care System in California. *JAMA* **2020**, *323*, 2195–2198. [CrossRef] [PubMed]
12. Fadini, G.P.; Morieri, M.L.; Longato, E.; Avogaro, A. Prevalence and impact of diabetes among people infected with SARS-CoV-2. *J. Endocrinol. Investig.* **2020**, *43*, 867–869. [CrossRef] [PubMed]
13. Zheng, Z.; Peng, F.; Xu, B.; Zhao, J.; Liu, H.; Peng, J.; Li, Q.; Jiang, C.; Zhou, Y.; Liu, S.; et al. Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *J. Infect.* **2020**, *81*, e16–e25. [CrossRef]
14. Popkin, B.M.; Du, S.; Green, W.D.; Beck, M.A.; Algaith, T.; Herbst, C.H.; Alsukait, R.; Alluhidan, M.; Alazemi, N.; Shekar, M. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. *Obes. Rev.* **2020**, *21*, e13128. [CrossRef] [PubMed]
15. Latif, F.; Farr, M.A.; Clerkin, K.J.; Habal, M.V.; Takeda, K.; Naka, Y.; Restaino, S.; Sayer, G.; Uriel, N. Characteristics and outcomes of recipients of heart transplant with coronavirus disease 2019. *JAMA Cardiol.* **2020**, *5*, 1165–1169. [CrossRef] [PubMed]
16. Available online: <https://www.mhlw.go.jp/content/000785119.pdf> (accessed on 25 July 2021).
17. Sun, H.-B.; Zhang, Y.-M.; Huang, L.-G.; Lai, Q.-N.; Mo, Q.; Ye, X.-Z.; Wang, T.; Zhu, Z.-Z.; Lv, X.-L.; Luo, Y.-J.; et al. The changes of the peripheral CD4+ lymphocytes and inflammatory cytokines in Patients with COVID-19. *PLoS ONE* **2020**, *15*, e0239532. [CrossRef] [PubMed]
18. Cheng, L.; Li, H.; Li, L.; Liu, C.; Yan, S.; Chen, H.; Li, Y. Ferritin in the coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *J. Clin. Lab. Anal.* **2020**, *34*, e23618. [CrossRef]
19. Soy, M.; Keser, G.; Atagündüz, P.; Tabak, F.; Atagündüz, I.; Kayhan, S. Cytokine storm in COVID-19: Pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin. Rheumatol.* **2020**, *39*, 2085–2094. [CrossRef]
20. Hamade, B.; Huang, D.T. Procalcitonin: Where Are We Now? *Crit. Care Clin.* **2020**, *36*, 23–40. [CrossRef]
21. Han, Y.; Zhang, H.; Mu, S.; Wei, W.; Jin, C.; Tong, C.; Song, Z.; Zha, Y.; Xue, Y.; Gu, G. Lactate dehydrogenase, an independent risk factor of severe COVID-19 patients: A retrospective and observational study. *Aging* **2020**, *12*, 11245–11258. [CrossRef]
22. Cui, S.; Chen, S.; Li, X.; Liu, S.; Wang, F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J. Thromb. Haemost.* **2020**, *18*, 1421–1424. [CrossRef]
23. Wada, H.; Matsumoto, T.; Yamashita, Y. Natural History of Thrombotic Thrombocytopenic Purpura and Hemolytic Uremic Syndrome. *Semin. Thromb. Hemost.* **2014**, *40*, 866–873. [CrossRef] [PubMed]
24. Taylor, F.B., Jr.; Toh, C.H.; Hoots, K.; Wada, H.; Levi, M. Towards a definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb. Haemost.* **2001**, *86*, 1327–1330. [CrossRef] [PubMed]
25. Wada, H.; Matsumoto, T.; Suzuki, K.; Imai, H.; Katayama, N.; Iba, T.; Matsumoto, M. Differences and similarities between disseminated intravascular coagulation and thrombotic microangiopathy. *Thromb. J.* **2018**, *16*, 14. [CrossRef] [PubMed]
26. Salah, H.M.; Mehta, J.L. Meta-Analysis of the Effect of Aspirin on Mortality in COVID-19. *Am. J. Cardiol.* **2021**, *142*, 158–159. [CrossRef]