Thrombo-inflammatory features predicting mortality in patients with COVID-19: The FAD-85 score

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Abstract

Background: The roles of inflammation and hypercoagulation in predicting outcomes of coronavirus disease 2019 (COVID-19) are unclear.

Methods: Adult patients diagnosed with COVID-19 from 28 January 2020 to 4 March 2020 in Tongji Hospital, Wuhan were recruited. Data on related parameters were collected. Univariate analysis and multivariable binary logistic regression were used to explore predictors of critical illness and mortality.

Results: In total, 199 and 44 patients were enrolled in the training and testing sets, respectively. Elevated ferritin, tumor necrosis factor-α and D-dimer and decreased albumin concentration were associated with disease severity. Older age, elevated ferritin and elevated interleukin-6 were associated with 28-day mortality. The FAD-85 score, defined as age + 0.01 * ferritin + D-dimer, was used to predict risk of mortality. The sensitivity, specificity and accuracy of FAD-85 were 86.4%, 81.8% and 86.4%, respectively. A nomogram was established using age, ferritin and D-dimer to predict the risk of 28-day mortality.

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Conclusions: Thrombo-inflammatory parameters provide key information on the severity and prognosis of COVID-19 and can be used as references for clinical treatment to correct inflammatory and coagulation abnormalities.

Keywords
COVID-19, inflammation, coagulation, predictor, mortality, model

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Introduction
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread to many countries in recent months, and the number of infected patients has increased dramatically. The resulting coronavirus disease 2019 (COVID-19) is associated with significant mortality. Developing strategies for to predict disease severity and mortality in patients with COVID-19 remains an explored and important direction of research.

Patients diagnosed with mild pneumonia or with evidence of pneumonia\(^1\) accounted for most COVID-19 patients. Although most symptoms of those patients can be alleviated temporarily, their condition can become exacerbated in just a few days. In addition, mortality in patients with severe disease, who are more prone to acute respiratory distress syndrome, is much higher than that in patients with mild disease. Treatment of patients with mild and severe disease varies considerably, and the treatment and monitoring of the latter patients is very challenging.

Easily accessed indicators to evaluate prognosis in the early stages of COVID-19 enable physicians to take timely and effective action to prevent disease exacerbation or mortality. According to the Chinese Guidelines for Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial Version 7), microthrombi were extensively present in multiple organs of patients with complicated COVID-19,\(^2\) which is presumably the consequence of dysregulated inflammation and coagulation processes. Although previous studies have reported that an increased level of D-dimer is a risk factor for COVID-19 mortality,\(^3\)\(^-\)\(^5\) comprehensive and detailed studies on thrombo-inflammatory features are still lacking. Therefore, in this study, we retrospectively analyzed the relationship between thrombo-inflammatory biomarkers and illness severity and outcome in patients with COVID-19. We explored the use of simple tools to determine prognosis, which may have significant value in assisting clinical decision-making to improve outcomes in patients with COVID-19.

Materials and Methods

Study design and population
A retrospective single-center observational study was conducted from 28 January 2020 to 4 March 4 2020 in the Sino-French New City Branch of Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China. Clinical outcomes were monitored up to 23 March 2020. Cases were diagnosed according to the World Health Organization interim guidance.\(^4\) Patients were confirmed to be positive for SARS-CoV-2 nucleic acid by real-time reverse-transcription polymerase chain
reaction of nasal or pharyngeal swab specimens. The presence of SARS-CoV-2-specific immunoglobulin M and immunoglobulin G has been used to diagnose COVID-19 since February 26. Patients with severe liver diseases (e.g. hepatic cirrhosis with an alanine aminotransferase [ALT] or aspartate aminotransferase [AST] level more than five times the upper limit of the normal range), hematological malignancies, advanced carcinoma, rheumatic immune disease, organ transplantation, recent thrombosis or embolism, or end stage renal failure with chronic dialysis were excluded. The study was approved by the ethics committee of Peking University Third Hospital (IRB00006761-M2020060). All data were anonymous and so the requirement for informed consent was waived. Data for enrolled patients were partitioned into two complementary subsets: the training set of patients from four wards was used to establish the predictive model, and the testing set of patients from another ward was used to validate the analysis.

**Data collection**

Demographic information, comorbidities and laboratory information including complete blood count, ferritin, C-reactive protein (CRP), procalcitonin, inflammatory cytokines and coagulation tests were collected. Demographic information and information on comorbidities were provided by patients or their immediate family members at admission, and personal certificate information was registered. Blood samples were collected within 24 hours of hospitalization. The test instruments were calibrated every day.

**Statistical analysis**

Study data were collected on standard forms, checked for completeness, and double keyed into an Epidata database using Epidata version 3.1 (Odense, Denmark).

The analysis was conducted in two stages. First, data were compared between patients with moderate and severe disease (including severe and critical disease); severity classification was based on the Chinese Guidelines for Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial Version 7). Second, data were compared between non-survivors and survivors at 28 days.

All analyses were conducted using SPSS 25.0 (IBM Corp., Armonk, New York, USA) and R version 3.6.2 (www.r-project.org). Non-normally distributed continuous variables were expressed as medians (interquartile ranges) and normally distributed continuous variables were expressed as means ± standard deviations. Categorical variables were summarized as counts and percentages. The independent t test was used to assess differences between two groups for continuous variables and counts (percentages). A two-sample t-test was used for variables with normal distributions and a nonparametric test (Mann–Whitney U test) was used for continuous variables with non-normal distributions. Pearson’s χ² test or Fisher’s exact test were used to assess differences in proportions for categorical variables.

Multivariable binary logistic regression (backward method) was used to explore factors associated with critical illness and mortality. Factors with P < 0.1 in univariate analyses were included as independent variable. The variables with the highest P values were removed from the model until all P values for the remaining variables were <0.05. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated.

A joint model was established to predict the probability of outcomes according to the results of multivariate analysis and clinical experience. Missing data were handled by complete-case analysis. The predicted
values of binary logistic regression were used as the joint variable. The area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate predictive value. The final linear prediction formula of the joint model was converted using the equal proportion method to facilitate calculation. The cutoff value was determined according to actual clinical needs and Youden’s index. We constructed a nomogram to predict the probability of outcomes more accurately using the rms package in R. The ROC curve was plotted using the ROCR package in R. A two-sided $P$ value $<0.05$ was considered statistically significant. Parameters for patients from the testing set were used to verify the model and the nomogram.

**Results**

**Comparison of patients with moderate and severe disease**

The study flowchart is shown in Figure 1. A total of 234 adult inpatients were enrolled in the study including 199 patients in the training set and 44 patients in the testing set. The training set included 70 (35.2%) patients with moderate disease and 129 (64.8%) patients with severe disease.

The comparison of patients with moderate and severe disease is shown in Table 1. The median age of patients was 62.3 ± 14.0 years old and 100 (50.3%) patients were women. Compared with patients with severe disease, patients with moderate

![Figure 1. Study flowchart.](image-url)
Table 1. Comparison of baseline and laboratory indicators between patients with moderate and severe COVID-19 disease.

|                          | Moderate disease (n = 70) | Severe disease (n = 129) | All patients (n = 199) | P   |
|--------------------------|--------------------------|--------------------------|------------------------|-----|
| Age (years)              | 58.1 ± 13.6              | 64.6 ± 13.7              | 62.3 ± 14.0            | 0.001|
| Women                    | 41 (58.6%)               | 59 (45.7%)               | 100 (50.3%)            | 0.08 |
| Men                      | 29 (41.4%)               | 70 (54.3%)               | 99 (49.7%)             |     |
| Comorbidity              |                          |                          |                        |     |
| Hypertension             | 22 (31.4%)               | 56 (43.8%)               | 78 (39.4%)             | 0.09 |
| Diabetes                 | 13 (18.6%)               | 28 (21.7%)               | 41 (20.6%)             | 0.60 |
| Coronary heart disease   | 8 (11.4%)                | 16 (12.4%)               | 24 (12.1%)             | 0.84 |
| COPD                     | 2 (2.9%)                 | 7 (5.4%)                 | 9 (4.5%)               | 0.63 |
| Stroke                   | 2 (2.9%)                 | 3 (2.3%)                 | 5 (2.5%)               | 1.00 |
| Other comorbidities      | 5 (7.1%)                 | 6 (4.7%)                 | 11 (5.5%)              | 0.68 |
| Glucocorticoids prior to admission | 6 (9.0%) | 13 (10.3%) | 19 (9.8%) | 0.76 |
| Duration from illness onset to admission | 12 (9, 16) | 13 (10, 15) | 12 (10, 16) | 0.48 |
| 28-day mortality (%)     | 1 (1.42)                 | 23 (17.8)                | 24 (12.1)              | <0.001|
| Laboratory tests         |                          |                          |                        |     |
| WBCs (×10⁹/L)            | 4.8 (4.2, 5.8)           | 6 (4.8, 8.4)             | 5.5 (4.4, 7.3)         | <0.001|
| Neutrophils (×10⁹/L)     | 2.9 (2.3, 4.1)           | 4.5 (3.3, 7.2)           | 4 (2.7, 5.6)           | <0.001|
| Lymphocytes (×10⁹/L)     | 1.3 (0.9, 1.6)           | 0.8 (0.6, 1.3)           | 1 (0.7, 1.4)           | <0.001|
| Hemoglobin (g/L)         | 127 (116, 138)           | 125 (116.5, 132)         | 125 (116, 137)         | 0.28 |
| Platelets (×10⁹/L)       | 228.0 ± 84.5             | 229.9 ± 92.9             | 229.2 ± 89.8           | 0.89 |
| Platelet distribution width (fL) | 11.6 (10.8, 13.2) | 12.4 (11.3, 14.0) | 12.2 (11.0, 13.7) | 0.050|
| ALT (U/L)                | 17.5 (12.0, 27.0)        | 26.0 (18.0, 42.5)        | 22 (15, 38)            | <0.001|
| AST (U/L)                | 21.0 (16.8, 29.0)        | 33.0 (22.0, 48.5)        | 27 (19, 42)            | <0.001|
| Albumin (g/L)            | 37.7 ± 4.1               | 32.7 ± 4.2               | 34.5 ± 4.8             | <0.001|
| TBil (μmol/L)            | 8.5 (6.8, 11.4)          | 9.8 (7.0, 13.9)          | 9.5 (7.0, 13.4)        | 0.10 |
| LDH (U/L)                | 234.5 (209.8, 272.8)     | 334.0 (268.5, 469.0)     | 287 (232, 389)         | <0.001|
| BUN (mmol/L)             | 3.7 (3.1, 4.9)           | 4.8 (3.7, 6.8)           | 4.4 (3.4, 6.0)         | 0.07 |
| Scr (μmol/L)             | 65.5 (52.0, 80.3)        | 71.0 (59.5, 87.0)        | 70 (57, 85)            |     |
| Ferritin(μg/L)           | 381.9 (248.6, 519.4)     | 955.3 (624.7, 1576.4)    | 703.4 (387.2, 1315.9)  | <0.001|
| CRP (mg/L)               | 6.2 (1.6, 29.8)          | 59.3 (15.4, 121.4)       | 28.1 (6.0, 87.1)       | <0.001|
| PCT (ng/mL)              | 0.0 (0.0, 0.0)           | 0.1 (0.0, 0.2)           | 0.0 (0.0, 0.1)         | <0.001|
| IL-1/β (pg/ml)           | 2.5 (2.5, 2.5)           | 2.5 (2.5, 2.5)           | 2.5 (2.5, 2.5)         | 0.36 |
| IL-2R (U/mL)             | 501.5 (372.0, 657.2)     | 888.0 (578.0, 1331.5)    | 701.0 (461.0, 1145.0)  | <0.001|
| IL-6 (pg/mL)             | 8.0 (2.1, 19.6)          | 28.3 (8.9, 66.7)         | 18.8 (4.8, 47.3)       | <0.001|
| IL-8 (pg/mL)             | 8.8 (5.5, 14.9)          | 16.5 (8.1, 29.1)         | 12.4 (6.9, 24.3)       | <0.001|
| IL-10 (pg/mL)            | 2.5 (2.5, 6.0)           | 2.5 (2.5, 9.8)           | 2.5 (2.5, 8.1)         | 0.011|
| TNF-α (pg/mL)            | 6.4 (4.5, 9.8)           | 9.2 (6.8, 13.3)          | 8.5 (6, 12)            | <0.001|
| Prothrombin time (s)     | 13.7 (13.2, 14.2)        | 14.2 (13.6, 15.0)        | 14.0 (13.5, 14.6)      | <0.001|
| Prothrombin activity (%) | 94.5 ± 10.5              | 86.8 ± 14.1              | 89.5 ± 13.4            | <0.001|
| Fibrinogen (g/L)         | 4.5 ± 1.3                | 5.5 ± 1.6                | 5.1 ± 1.6              | <0.001|

Fibrinogen (g/L) < 2.4 1 (1.4%) 5 (3.9%) 6 (3.0%) <0.001

Fibrinogen (g/L) > 2.4 to 4.8 42 (60.0%) 35 (27.3%) 77 (38.9%) <0.001

(continued)
Hypertension was the most common comorbidity (39.4%). Higher white blood cell (WBC) counts ($P < 0.001$), neutrophils counts ($P < 0.001$), platelet distribution widths ($P = 0.050$) and rates of lymphopenia ($P < 0.001$) were noted in the severe disease group. Elevated ALT ($P < 0.001$), AST ($P < 0.001$), lactate dehydrogenase ($P < 0.001$), blood urea nitrogen ($P < 0.001$) and hypoalbuminemia ($P < 0.001$) were observed in the severe disease group. Moreover, patients with moderate disease had lower levels of ferritin ($P < 0.001$), CRP ($P < 0.001$), procalcitonin ($P < 0.001$), interleukin-2 receptor (IL-2R) ($P < 0.001$), IL-6 ($P < 0.001$), IL-8 ($P < 0.001$), IL-10 ($P = 0.11$) and tumor necrosis factor (TNF)-$\text{z}$ ($P < 0.001$). Elevated fibrinogen ($P < 0.001$), D-dimer ($P < 0.001$) and fibrinogen degradation products ($P < 0.001$) were found in patients with severe disease. Longer prothrombin times ($P < 0.001$) and thrombin times ($P = 0.001$) were found in the severe disease group.

Factors with $P < 0.1$ in Table 1 were included as independent variables to explore risk factors associated with the severity of COVID-19. Increased concentrations of ferritin (OR = 1.002, 95%CI 1.000, 1.003), TNF-$\text{z}$ (OR = 1.155, 95%CI 1.030, 1.296, $P = 0.013$) and D-dimer (OR = 2.166, 95%CI 1.062, 4.416, $P = 0.033$) and decreased concentration of albumin (OR = 0.848, 95%CI 0.746, 0.965, $P = 0.012$) were associated with severe disease.

**Comparison between survivors and non-survivors**

As shown in Figure 1, there were 170 (87.9%) survivors and 24 (12.1%) non-survivors included in the training set.

Comparisons between survivors and non-survivors are shown in Table 2. Survivors were younger than non-survivors ($P = 0.001$), and fewer survivors had chronic obstructive pulmonary disease ($P = 0.01$) or stroke ($P = 0.013$) than non-survivors. Higher counts of WBCs ($P < 0.001$) and neutrophils ($P < 0.001$) and higher rates of lymphopenia ($P < 0.001$) were noted in non-survivors. Elevated AST ($P < 0.001$), lactate dehydrogenase ($P < 0.001$), blood urea nitrogen ($P < 0.001$) serum creatinine ($P = 0.002$) and hypoalbuminemia ($P = 0.002$) were observed in non-survivors. Moreover, survivors were found to have lower levels of
### Table 2. Comparison of baseline and blood test indicators between surviving and non-surviving COVID-19 patients.

|                                      | Survivors (n = 175) | Non-survivors (n = 24) | P    |
|--------------------------------------|---------------------|------------------------|------|
| **Age, years**                       | 64.0 (51.0, 71.0)   | 69.5 (64.5, 82.75)     | <0.001 |
| **Women**                            | 89 (50.9%)          | 8 (33.3%)              | 0.11 |
| **Men**                              | 86 (49.1%)          | 16 (66.7%)             |      |
| **Comorbidity**                      |                     |                        |      |
| Hypertension                         | 66 (37.9%)          | 12 (50.0%)             | 0.27 |
| Diabetes                             | 33 (18.9%)          | 9 (37.5%)              | 0.06 |
| Coronary heart disease               | 21 (12.0%)          | 2 (8.3%)               | 1.00 |
| COPD                                 | 10 (5.7%)           | 5 (20.8%)              | 0.01 |
| Stroke                               | 2 (1.1%)            | 3 (12.5%)              | 0.013|
| Other comorbidities                  | 11 (6.3%)           | 0 (0%)                 | 0.37 |
| **Duration from illness onset to admission (days)** | 12 (10, 16)        | 12 (9, 14)             | 0.38 |
| **Glucocorticoids prior to admission** | 16 (9.5%)          | 3 (13.0%)              | 0.71 |
| **Laboratory tests**                 |                     |                        |      |
| WBCs ($\times 10^9$/L)               | 5.3 (4.4, 6.8)      | 8.2 (5.5, 13.1)        | <0.001|
| Neutrophils ($\times 10^9$/L)        | 3.8 (2.6, 5.1)      | 7.0 (4.3, 12.3)        | <0.001|
| Lymphocytes ($\times 10^9$/L)        | 1 (0.7, 1.4)        | 0.6 (0.5, 1.0)         | <0.001|
| Hemoglobin (g/L)                     | 136 (117, 143)      | 125 (114, 134)         | 0.052 |
| Platelets ($\times 10^9$/L)          | 230.5 ± 86.5        | 221 ± 114.0            | 0.65 |
| Platelet distribution width (fL)     | 12.2 (11, 13.7)     | 12.9 (12.0, 14.6)      | 0.11 |
| ALT (U/L)                            | 22.0 (14.0, 38.0)   | 21.5 (18.3, 39.5)      | 0.36 |
| AST (U/L)                            | 25.0 (19.0, 40.0)   | 41.0 (33.5, 57.5)      | <0.001|
| Albumin (g/L)                        | 34.9 ± 4.7          | 31.6 ± 4.6             | 0.002 |
| TBil (µmol/L)                        | 9.4 (7.0, 12.3)     | 10.5 (7.4, 16.2)       | 0.14 |
| LDH (U/L)                            | 274 (226, 358)      | 478.5 (315, 702.5)     | <0.001|
| BUN (mmol/L)                         | 4.2 (3.4, 5.5)      | 8.2 (4.9, 13.8)        | <0.001|
| Scr (µmol/L)                         | 68 (56, 82)         | 81.5 (71.3, 103.5)     | 0.002 |
| Ferritin (µg/L)                      | 658.7 (378.8, 1268.7) | 1478.4 (670.7, 2217.0) | 0.001|
| CRP (mg/L)                           | 26.1 (5.3, 78.5)    | 139.8 (51.3, 191.0)    | <0.001|
| PCT (ng/mL)                          | 0.05 (0.03, 0.12)   | 0.33 (0.06, 1.19)      | <0.001|
| IL-1β (pg/ml)                        | 2.5 (2.5, 2.5)      | 2.5 (2.5, 2.5)         | 0.94 |
| IL-2R (U/mL)                         | 658 (443, 1018)     | 1189 (720, 1577)       | 0.001 |
| IL-6 (pg/mL)                         | 15.3 (4, 41.3)      | 64.1 (29.6, 137.6)     | <0.001|
| IL-8 (pg/mL)                         | 11.3 (6.5, 22.5)    | 23.3 (12.8, 35.2)      | 0.003 |
| IL-10 (pg/mL)                        | 2.5 (2.5, 6.9)      | 9.2 (2.5, 17.4)        | <0.001|
| TNF-α (pg/mL)                        | 8.1 (5.8, 11.5)     | 12.0 (7.4, 15.2)       | 0.005 |
| Prothrombin time (s)                 | 14 (13.4, 14.6)     | 14.9 (14.1, 15.7)      | <0.001|
| Prothrombin activity (%)             | 90 (83, 98)         | 80.5 (72.3, 89)        | <0.001|
| Fibrinogen (g/L)                     | 5.1 (3.8, 6.2)      | 5.8 (4.7, 6.4)         | 0.12 |
| Fibrinogen (g/L) (≤ 2.4)             | 4 (2.3%)            | 2 (8.3%)               | 0.035 |
| Fibrinogen (g/L) (> 2.4 to 4.8)      | 72 (41.4%)          | 4 (16.7%)              |      |
| Fibrinogen (g/L) (> 4.8 to 7.2)      | 76 (43.7%)          | 15 (66.7%)             |      |

(continued)
CRP \( (P < 0.001)\), procalcitonin \((P < 0.001)\), ferritin \((P < 0.001)\), IL-2R \((P = 0.001)\), IL-6 \((P < 0.001)\), IL-8 \((P = 0.003)\), IL-10 \((P = 0.005)\) and TNF-\(\alpha\) \((P = 0.006)\). Elevated levels of D-dimer \((P < 0.001)\) and fibrinogen degradation products \((P < 0.001)\) were observed in non-survivors. Compared with survivors, longer prothrombin times \((P < 0.001)\) were observed in non-survivors.

Because of the moderate sample size, 11 factors with \(P < 0.1\) in Table 2 were included as independent variables to explore risk factors associated with COVID-19 mortality. Elevated age \((\text{OR} = 1.130, 95\% \text{CI} 1.054, 1.210, P = 0.001)\), ferritin \((\text{OR} = 1.001, 95\% \text{CI} 1.000, 1.001, P = 0.005)\) and IL-6 \((\text{OR} = 1.009, 95\% \text{CI} 1.000, 1.017, P = 0.040)\) were independent risk factors for mortality.

### Models for prediction of mortality

The efficacy of different combinations of variables in predicting mortality is shown in Appendix 1. As shown in Figure 2, the combination of age, ferritin, and IL-6 had the highest AUC. There was little difference in the AUCs for the first three combinations. The combination of age, ferritin and D-dimer was selected as the predictive model combination. The predictive model was constructed as “0.119*age+0.001* ferritin (\(\mu\)g/L) +0.086* D-dimer (\(\mu\)g/mL)” according to the coefficient of the second combination in the binary logistic regression model (Appendix 2). The model coefficient was converted to facilitate calculation, divided by 10 times the coefficient of ferritin and approximated. The resulting model, \(y = \text{age} + 0.01 \times \text{ferritin (mg/L)} + \text{D-dimer (mg/mL)}\) had an AUC of 0.871. The model was named FAD-85 based on its optimal cut-off value (85) (Appendix 3). The sensitivity, specificity, positive predictive value, negative predictive value, false positive rate and false negative rate were 86.4%, 81.8%, 39.6%, 97.7%, 16.0% and 13.6%, respectively. The mortality rates suggested the following risk categories: FAD-85 < 85 (‘low risk’, mortality = 2.3%); FAD-85 \(\geq 85\) (‘high risk’, mortality = 39.6%). The comparison of the training and validation datasets is described in Appendix 4. With the information of 44 patients from the testing set was calculated according to the FAD-85 score, the accuracy of this score was 86.4%.

### Nomogram for prediction of mortality

To quantitatively describe the risk of mortality, we used a binary logistic regression model consisting of age, ferritin, D-dimer
and non-survival to establish a nomogram (Figure 3a). Scores for each case can be determined according to the values of these three indicators, and the risk of death within 28 days can be calculated according to the total score. The calibration curve of the nomogram (Figure 3b) showed that the predictive effect was good, especially for middle and high scores. Because of the low mortality of this disease and the small sample size of this study, prediction at low scores was unsatisfactory.

Discussion

This retrospective cohort study found that thrombo-inflammatory abnormalities were implicated in the progression of COVID-19. Simple tools were developed for prognosis evaluation that may be useful for physicians making treatment decisions after admission using only common laboratory results.

Several studies have reported that men had higher COVID-19 mortality than women.6–8 This finding is in general agreement with a report of the Chinese Center for Disease Control and Prevention.9 However, we found no statistical difference in mortality between men and women, although there was a trend toward more men than women having severe disease (70.7% vs 59%) and fatal disease (16.2% vs 8%). Gebhard et al. and Scully et al.7,8 summarized the latest evidence and found that male patients were more likely to be hospitalized and had higher mortality rates than female patients, which may be explained by hormone-regulated expression of genes coding for the SARS-CoV-2 entry receptors angiotensin converting enzyme 2 and transmembrane protease, serine 2. Sex hormone-driven innate and adaptive immune responses as well as gender-specific differences in lifestyle may also play a role. The question of whether male

Figure 2. Characteristic curves for mortality prediction of patients with coronavirus disease 2019 (n = 199).
patients with abnormal inflammatory and coagulation function may require more attention from physicians requires further study.

Lymphopenia was observed more frequently in patients with severe disease and non-survivors. In addition, non-survivors tend to develop severe lymphopenia during hospitalization.\textsuperscript{10} Recent studies noted that the number of T cells significantly decreased in severe COVID-19 cases.\textsuperscript{11} IL-2R and IL-6 complement CD8+ T cell function,\textsuperscript{12} and their levels correlated with disease severity in this study. Recent studies showed that initial IL-8 and TNF-\textit{\alpha} concentrations were higher in COVID-19 patients than in healthy adults. Additionally, concentrations of TNF-\textit{\alpha} were higher in

\textbf{Figure 3.} (a) The nomogram was used to predict the mortality probability of COVID-19 patients. (b) The calibration curve of the nomogram predicting 28-day risk of mortality in patients with COVID-19.
intensive care unit patients than in other patients. Elevated levels of cytokines may be associated with mortality or disease aggravation and are attributable to heightened immuno-inflammation triggered by viral infection.

The pathological features of COVID-19 pneumonia resemble those of severe acute respiratory syndrome. Viral infection can lead to exogenous damage mediated by pathogen-associated molecular patterns. Proteins or molecules expressed within cells are released following cell damage, including damage-associated molecular patterns (DAMPs). Serum ferritin is an acute-phase reactant. Ferritin synthesis is increased in hepatic cells and is often released by injured tissue, and thus its levels mirror the degree of acute inflammation and DAMP release. The concentration of ferritin at the time of hospital admission was associated with COVID-19 mortality; moreover, when patients began to recover, ferritin concentrations began to decrease. Therefore, hyperferritinemia may suggest extensive inflammatory injury and activation of monocytes and macrophages, corresponding with recent reports of alveolar macrophage activation and cytokine storms associated with the pathogenesis of severe COVID-19.

There is a complex relationship and crosstalk between inflammation and thrombosis. Inflammatory reactions usually lead to endothelial damage, then platelet activation and coagulation factor activation, thus forming microvascular thrombi. In turn, coagulation system abnormalities would augment inflammatory responses. IL-6 and IL-8, two key inflammatory cytokines, are typically implicated in pro-inflammatory coagulation. Elevated IL-6 levels by the action of pathogen-associated molecular patterns and DAMPs acting on Toll-like receptors upregulate tissue factor and activates the extrinsic pathway. IL-8 secreted by alveolar epithelial cells typically promotes procoagulant activity by triggering platelet activation. Therefore, elevated levels of IL-6 and IL-8 can cause hypercoagulation and result in a disheveled fibrin clot, in agreement with the findings of this study. These results could explain part of the vascular endothelium exfoliation, intimal inflammation and thrombosis in COVID-19 patients, which was described in the pathological report of the Chinese Guidelines for Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial Version 7). Inevitably, extensive microthrombosis within microvasculature leads to microvascular embolism, resulting in lung, kidney, heart and liver injury. Blood urea nitrogen elevation and renal failure was reported by anti-2019-nCoV volunteers, as well as elevated highly sensitive cardiac troponin I and acute heart injury. As a consequence, imbalances between host coagulation and fibrinolysis pathways mediated by inflammation may be a key risk factor resulting in pneumonia progression, diffuse alveolar damage and acute lung injury.

During the progressive thrombo-inflammatory response to COVID-19, elevation of D-dimer is prominent, further supporting the presumed mechanism of injury via microthrombi and resulting in end organ damage. Anticoagulation therapy is recommended for COVID-19 patients when the D-dimer value is four times higher than the normal upper limit, except for patients with anticoagulant contraindications. Zhou et al. found D-dimer greater than 1 mg/L was associated with fatal outcomes of COVID-19. During SARS-CoV-2 infection, overactive coagulation can lead to uncontrolled thrombosis and coagulopathy while underactive coagulation leads to pulmonary hemorrhage and edema. However, extremely high D-dimer in COVID-19 seemed not to be attributed to disseminated intravascular coagulation (DIC). Previous studies reported that DIC
may occur in critical ill patients at the end of their lives, but it occurred in only one critically ill patient enrolled in this study. In addition, it was very unusual that decreases in platelets, fibrinogen and antithrombin were rarely observed in patients with severe COVID-19 or non-survivors; this finding was very different from patients with bacterial sepsis, H7N9 influenza pneumonia or highly pathogenic H5N1 influenza. These results require further exploration.

All parameters in the FAD-85 score, which could be used as a tool to estimate the outcome of COVID-19 infection, are easy to obtain clinically and all laboratory tests are recommended to be performed on hospital admission. Other tools, such as the 2007 Infectious Diseases Society of America/American Thoracic Society Criteria for Defining Severe Community-acquired Pneumonia, the sepsis-related organ failure assessment, the acute physiology and chronic health evaluation scoring system II, and the simplified acute physiology score II are rather complicated, and may not be practical for a pandemic like COVID-19. The FAD-85 score is simple and has good predictive capacity and accuracy, thus showing promise for risk stratification of patients hospitalized with COVID-19. The results of our study suggest using the test on admission to classify early mortality risk. The FAD-85 scores of six patients were inconsistent with 28-day mortality, including two patients under 65 years old who died and four patients more than 80 years old who survived. These discrepancies may be related to the large role of age in the model. Moreover, mortality probability could be predicted using a nomogram. The results of our study may assist clinicians in making appropriate decisions and optimizing the use of hospital resources. However, these tools are not suitable to predict outcomes for patients receiving anticoagulants or immunosuppressive drugs, or those with other thrombo-inflammatory abnormalities such as thrombi, decompensated cirrhosis, or acute rheumatic immune diseases.

There were several limitations to our study. First, its retrospective single-center design was associated with missing data and unavoidable biases in identifying and recruiting participants. Respiratory tract specimens were used to diagnose COVID-19 through real-time reverse-transcription polymerase chain reaction. The negative predictive value of this assay was 80.61%, resulting in a relatively small missing population for building prediction scores. Second, some D-dimer laboratory results were above the upper limit, which might lead to underestimated associations with severity and outcome. Nonetheless, the present study was designed to respond to clinical demand and showed that thrombo-inflammatory abnormalities are closely related to the severity and outcome of COVID-19.

Conclusions

Thrombo-inflammatory biomarkers were closely associated with the severity and outcome of COVID-19. The FAD-85 score and nomogram, based on three parameters routinely acquired at hospital admission, were strongly predictive of 28-day mortality. This tool not only helps judge the severity and prognosis of the disease, but also provides guidance to make further clinical decisions. Moreover, this study provides a reference for treatment of COVID-19 patients, including inflammatory disorders and coagulation abnormalities, which would be further studied in the near future.

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