Prediction of severe illness due to COVID-19 based on an analysis of initial Fibrinogen to Albumin Ratio and Platelet count

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Abstract
Concomitant coagulation disorder can occur in severe patients with COVID-19, but in-depth studies are limited. This study aimed to describe the parameters of coagulation function of patients with COVID-19 and reveal the risk factors of developing severe disease. This study retrospectively analyzed 113 patients with SARS-CoV-2 infection in Taizhou Public Health Center. Clinical characteristics and indexes of coagulation function were collected. A multivariate Cox analysis was performed to identify potential biomarkers for predicting disease progression. Based on the results of multivariate Cox analysis, a Nomogram was built and the predictive accuracy was evaluated through the calibration curve, decision curve, clinical impact curve, and Kaplan–Meier analysis. Sensitivity, specificity, predictive values were calculated to assess the clinical value. The data showed that Fibrinogen, FAR, and D-dimer were higher in the severe patients, while PLT count, Alb were much lower. Multivariate Cox analysis revealed that FAR and PLT count were independent risk factors for disease progression. The optimal cutoff values for FAR and PLT count were 0.0883 and 135*10^9/L, respectively. The C-index [0.712 (95% CI = 0.610–0.814)], decision curve, clinical impact curve showed that Nomogram could be used to predict the disease progression. In addition, the Kaplan–Meier analysis revealed that potential risk decreased in patients with FAR<0.0883 and PLT count>135*10^9/L. The model showed a good negative predictive value [(0.9474 (95% CI = 0.845–0.986)]. This study revealed that FAR and PLT count were independent risk factors for severe illness and the severity of COVID-19 might be excluded when FAR<0.0883 and PLT count>135*10^9/L.

Keywords
Coagulation and Fibrinolysis, COVID-19, fibrinogen-to-albumin ratio (FAR), non-Severe Survival (NSS), platelet count (PLT), prediction

METHODS

Patient Selection
One hundred and thirteen patients of COVID-19 were enrolled in Taizhou Public Health Medical Center, Taizhou Hospital, Zhejiang Province, China, from January 23 to February 4, 2020. Clinical diagnosis and classifications were made according to the Chinese management guideline for COVID-19 (version 6.0) [7]. According to the guideline, COVID-19 patients are classified into four categories: 1) Mild, mild symptoms and no pneumonia manifestation; 2) Typical, fever, or respiratory symptoms and imaging manifestation of pneumonia; 3) Severe, having any of the three conditions: respiratory distress, respiratory rate ≥ 30 beats/min; means oxygen saturation ≤93% in a resting state; arterial blood oxygen partial pressure/oxygen concentration ≤300 mm Hg (1 mm Hg = 0.133 kPa); 4) Critical, having one of the three conditions: shock incidence; respiratory failure and requiring mechanical ventilation; admission to ICU with other organ function failure. The endpoint of this study was the occurrence of severe illness. The clinical outcomes were monitored until February 15, 2020. All patients were classified into either the severe or non-severe group, the severe group contained severe and critically severe patients, while the non-severe group included mild and moderate patients. On admission, all patients were classified as non-severe. We collected a total of 28 COVID-19

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patients from February 5 to February 20 as an external validation group. The endpoint of follow-up was March 1, 2020.

Data Collection

The information of epidemiology history, clinical features, radiologic characteristics, and days from on admission were collected from electronic medical records. Two researchers independently reviewed the data collection forms. Laboratory indicators including indexes of coagulation function, hemocyte count, blood chemistry were collected.

Statistical Analysis

Categorical variables were expressed as frequency and percentage, and continuous variables were shown as median, and interquartile range (IQR). χ² test was used to compare categorical variables, while Mann–Whitney U test was conducted for continuous variables. A multivariate analysis was performed to predict the disease progression. Considering the total number of severe cases (n = 22) in our study and to avoid overfitting in the model, four variables were chosen for multivariable analysis on the basis of previous findings and clinical constraints. Cutoff points were identified following Youden’s index of receiver operator characteristic (ROC) curve. Based on the results of multivariate analysis, a Nomogram was established. The C-index, calibration, decision curve, and the clinical impact curve were used to verify the Nomogram. Kaplan–Meier analysis was drawn, and risk stratification was compared by the log-rank test. Sensitivity, specificity, predictive values were calculated. Ninety-five percent confidence intervals (95% CI) of hazard ratio(HR) were used as common measures to assess relative risk. All statistical analysis were performed using SPSS (version 24.0), R program (version 3.6.2). P < .05 were considered to be statistically significance.

Results

Clinical Characteristics of Patients with COVID-19

One hundred and thirteen confirmed patients with SARS-COV-2 infection were included in this study, 91 (80.5%) patients were grouped into non-severe cases and 22 were (19.5%)severe cases, as shown in Table I. The median age was 46 years(IQR, 37–45 years); 64 (56.6%) of them were male; 44 (38.9%) patients had the basic disease; Forty-seven(41.6%) had a fever (with a body temperature> 37.3°C) on admission; X-ray or CT findings showed involvement of chest radiographs in 110 patients (97.3%). Seventy-two (63.7%) had a Wuhan exposure, the others had a close exposure history to those patients withCOVID-19.Four (3.5%) patients developed a secondary infection during hospitalization. The median age of severe patients was older than non-severe patients (54 years vs 44 years, P = .000). Compared with non-severe patients, the Median time from illness onset to admission was much longer in severe patients (3 days vs. 2 days, P = .034).

Comparison of Initial Indexes of Coagulation Function between Severe and Non-severe Patients

It was shown that Fibrinogen, FAR, and D-dimer were significantly higher in the severe group than in the non-severe group (4.23 g/L vs. 3.07 g/L, 0.10 vs. 0.078,0.32 mg/L vs.0.24 mg/L, P = .002,0.046,0.009, respectively), while PLT count and Alb were much lower (166*10⁹/L vs. 199*10⁹/L,38.3 g/L vs.40.6 g/L, P = .034,0.005), as shown in Figure 1.

Dynamic changes of CoagulationIndexes in Severe COVID-19 Patients

To perform the dynamic profile that appeared during COVID-19 progression, we had been following up for 23 days since on admission at 5-day intervals. As of February 4, 2020, 11 severe patients were analyzed (Figure 2). Coagulation time such as PT, aPTT had shortened from the beginning of hospitalization, then

| Table I. Clinical characteristics of patients with 113 COVID-19 between the severe and non-severe group. |
|---------------------------------------------------------------|---------------------------------|-----------------|---------------|
| Age, year                                                    | Non-severe(N = 91) | Severe (N = 22) | ALL (N = 113) | P value  |
| ≤50                                                         | 44 (36–54)          | 54 (47–62)      | 46 (37–55)    | 0.000    |
| >50                                                         | 61 (67.0%)          | 6 (27.3%)       | 67 (59.3%)    | 0.001    |
| Gender                                                      | 30 (33.0%)          | 16 (72.7%)      | 46 (40.7%)    | 0.796    |
| Male                                                        | 51 (56%)            | 13 (59.1%)      | 64 (56.6%)    |          |
| Female                                                      | 40 (44%)            | 9 (40.9%)       | 49 (43.4%)    |          |
| Current smoking                                             | 3 (5.3%)            | 1 (3.9%)        | 4 (3.5%)      |          |
| Comorbidity                                                 | 32 (72.7%)          | 12 (54.5%)      | 44 (38.9%)    | 0.143    |
| Temperature on admission, °C                                | ≤37.3              | 56 (61.5%)      | 10 (45.5%)    | 0.459    |
|                                                               | >37.3               | 35 (38.5%)      | 12 (54.5%)    | 0.416    |
| Symptom                                                     |                    |                 |               |
| Pharyngalgia                                                | 8 (8.0%)            | 2 (9.1%)        | 10 (8.8%)     | 0.743    |
| Cough                                                       | 53 (58.2%)          | 13 (59.1%)      | 66 (58.4%)    | 0.570    |
| Expectoration                                               | 26 (28.6%)          | 7 (31.8%)       | 33 (29.2%)    | 0.797    |
| CT; Involvement of chest radiographs                        | 84 (92.3%)          | 22 (100%)       | 110 (97.3%)   | 0.210    |
| Exposure                                                    |                    |                 |               |
| Outside Wuhan                                               | 33 (36.3%)          | 8 (36.3%)       | 41 (36.3%)    | 0.615    |
| Wuhan                                                       | 58 (63.7%)          | 14 (63.7%)      | 72 (63.7%)    |          |
| Involvement                                                 |                    |                 |               |
| With bacterial infection                                    | 2 (2.2%)            | 2 (9.1%)        | 4 (3.5%)      | 0.504    |
| disturbance of consciousness                                | 0                  | 1 (4.5%)        | 1 (0.8%)      | 0.714    |
| Timeline after onset of illness                             |                    |                 |               |
| Days from illness onset to severe illness                   | 2 (0–5)             | 3 (1–7.0)       | 2 (0–5)       | 0.034    |
| Days from admission time to severe illness                  | 15 (8.3–20)         |                 |               |          |
leveled off after 6-10 days in the hospital. Fibrinogen showed an unstable variation, the minimum level (3.5mg/L) appeared on the 11-15 days after admission, and the maximum level (0.89mg/L) of D-dimer occurred at the same time. The level of FAR elevated with the progression of severe disease, then reached the highest at 6-10 days after admission, but as the patients got better, the FAR decreased gradually. PLT count increased rapidly before the occurrence of severe illness, then decreased from day 15th. All data were displayed by median and inter quartiles range.

Prediction of Severity Degree of COVID-19 Patients

We then investigated the ability of the indexes of coagulation function to predict the progression of severe disease. Multivariate analysis revealed that FAR (HR=4.058, 95% CI=1.246-13.222, P=0.020) and PLT (HR=4.047, 95% CI=1.313-12.472, P=0.015) were independent factors for disease progression (Table II). The cut-off value of FAR was 0.0883 and PLT count was 135*10^9/L (Figure 3e). Area under the ROC curve (AUC) of FAR, PLT and FAR-PLT combined were 0.730, 0.637, and 0.754 (P=0.001, 0.015, 0.030, respectively).

Establishment and Accuracy Prediction of a Novel Nomogram

We then performed a novel Nomogram that integrated FAR and PLT count for 10-day non-severe survival and 20-day nonsevere survival to predict the disease progression for each COVID-19 (Figure 3a). The Harrell’s C-indexes was 0.712 (95% CI=0.610–0.814), which showed the model had a potential value to predict disease progression. Through internal verification, the calibration curve (Figure 3d) did not deviate from the reference line. The decision curve (Figure 3b) and the clinical impact curve (Figure 3c) also indicated that the Nomogram had good net benefits for the identification of severe of COVID-19 patients. The model showed a good negative predictive value (0.9474, 95% CI=0.845–0.986) through clinical calculation (Table III). Kaplan–Meier analysis showed that FAR<0.0883 and PLT>135*10^9/L were associated with non-severe survival (Figure 3f).

External Validation

Twenty-eight newly confirmed patients were enrolled to perform an external validation, 7(25%) were classified into severe group and 21 (75%) were non-severe. The sensitivity was 0.857 (95% CI = 0.420–0.992) and the negative predictive value was 0.900 (95% CI = 0.541–0.994), as shown in Table III.

Discussion

In this paper, we studied on 113 COVID-19 patients from Taizhou Public Health Medical Center, Taizhou Hospital, Zhejiang Province, as of February 4, 2020. To our knowledge, the sample size was largest outside the Wuhan region and composite indexes of coagulation function were surveyed in our study. The most important finding was that FAR and PLT count were independent risk factors to predict the development of severe
illness in COVID-19. Patients with FAR<0.0883 and PLT count>135*10^9/L were unlikely to develop into severe disease.

The results of this study showed that numerous differences of indexes of coagulation function were detected between severe and non-severe patients. Severe patients suffered a higher level of Fibrinogen and D-dimer at the earliest stage and became more remarkable as the disease progressed. Previous studies had uncovered that markedly elevated D-dimer levels were common in death patients with COVID-19 [8,9]. Similar to their results, we presumed that severe patients with COVID-19 had increased coagulation and fibrinolysis activity, marked by elevated D-dimer concentrations [10,11]. Notably, one severe patients in our study was developed into critical disease 7 day after admission with a D-dimer level of 13.61 mg/L, which further validated our speculation.

The result of this study showed that FAR and PLT count were closely associated with the disease progression. FAR levels in patients with severe disease were much higher than those with non-severe. As the illness recovered, FAR returned to normal levels gradually. FAR has been widely used as an effective marker of inflammation and tend to elevated extremely among various conditions such as severe infection and malignant disorders [12]. We speculated that the increased level of FAR may be related to cytokine storms induced by virus invasion [4]. In this study, compared with non-severe cases, thrombocytopenia was detected in severe patients. As severe disease occurred, a rapid decrease of PLT count appeared simultaneously. Lippi, G [13] had revealed that low platelet count was associated with an increased risk of severe disease and mortality in patients with COVID-19. Hyper activity of Fibrinolysis often lead to the increase of platelets consumption. Corticosteroids may further cause thrombocytopenia when they had been widely used in the treatment of severe COVID-19 patients [14]. This two reasons mentioned above may explain the similar phenomenon we had observed.

**Limitation**

There are limitations in our study, for we just displayed the changes of laboratory index and explored possible explanations, mechanism researches such as proteomics and metabonomics are needed to confirm our hypothesis.

**Conclusion**

According to our results, we believed the dynamic changes of FAR and PLT count were related to the disease progression. Furthermore, the model integrated FAR and PLT count could be used to predict the development of severe illness. Patients on admission with FAR<0.0883 and PLT count>135*10^9/L had a low probability of severe illness development.
Author Contributions
X.B and Z.S analyzed the data, composed the manuscript. L.C collected data. M.P and S.C revised the paper and approved the final version. H.Y and J.D role the lab investigation, J.W and GE collected the data and revised the paper. B.S and J.L designed the study.

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The researchers identified no potential conflict of interest with any entity regarding the content discussed.

Figure 3. Prediction of severity degree of COVID-19 patients. Nomograms were conveyed using PLT and FAR to predict 10-day non-severe survival and 20 day non-severe survival of COVID-19 patients (A). Decision curve (B) and Clinical impact curve (C) of the Nomogram for non-severe survival in the 2019-nCoV cohort, in which the predicted probability of survival was compared well with the actual survival and had superior standardized net benefit. Calibration plot for 10-day non-severe survival using Nomograms with FAR and PLT count are shown (D), the c-index = 0.712 (95% CI = 0.610–0.814). (E) Area under the ROC curve (AUC) of FAR, PLT and FAR-PLT combination was 0.730, 0.637 and 0.754, P = .001, 0.015, 0.030, respectively. The optimal cutoff values for FAR and PLT count were 0.0883 and 135*10/L. (F) Kaplan–Meier plots were determined by using the cutoff values, Group A vs. Group B long-rank $\chi^2 = 7.511$ P = .006, Group A vs. Group C long-rank $\chi^2 = 20.944$ P = .000, while Group B vs. Group C long-rank $\chi^2 = 3.615$ P = .056; Group A: PLT $\geq 135*10/L$ and FAR $\leq 0.0883$, Group B: PLT < $135*10/L$ or FAR $> 0.0883$, Group C: PLT > $135*10/L$ and FAR > 0.0883.

Table III. Sensitivity, specificity, predictive value of 113 and newly confirmed 28 patients with COVID-19.

|                  | Severe illness | Total | Sensitivity      | Specificity      | Negative Predictive Value | Positive Predictive Value |
|------------------|----------------|-------|------------------|------------------|---------------------------|---------------------------|
| 113 patients     | +              | 37    | 56               | 0.863 (0.640–0.964) | 0.593 (0.485–0.694) | 0.9474 (0.845–0.986) | 0.339 (0.222–0.479) |
| Prediction model | –              | 54    | 3                |                  |                           |                           |                           |
| Total            | 101            | 22    | 113              |                  |                           |                           |                           |
| Newly confirmed 28 patients | + | 6 | 12 | 0.857 (0.420–0.992) | 0.429 (0.226–0.556) | 0.9 (0.541–0.994) | 0.333 (0.143–0.588) |
| Prediction model | –              | 2     | 8                |                  |                           |                           |                           |
| Total            | 8              | 20    | 28               |                  |                           |                           |                           |

http://vassarstats.net/clin1.html

Author Contributions
X.B and Z.S analyzed the data, composed the manuscript. L.C collected data. M.P and S.C revised the paper and approved the final version. H.Y and J.D role the lab investigation, J.W and GE collected the data and revised the paper. B.S and J.L designed the study.

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Informed Consent
Informed consent was obtained from all individual participants included in the study. Research involving human participants. The Faculty of Medicine’s Research Ethics Medical Review Board at Taizhou hospital of Zhejiang province has approved the protocol for the study under number K20200211.

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