Autopsy and Informatics Analysis Evidence Coagulopathy Progress in COVID-19 Patients

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Research

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Abstract

Background

The outbreak of COVID-19 around the world resulted in more than 480 thousand deaths.

Objective

To clarify the thrombotic phenomena with coagulation progress in COVID-19 patients based on epidemiological statistics combining the autopsy and informatics analysis.

Methods

Using 9 autopsy results with COVID-19 pneumonia and the medical records of 407 patients including 39 deceased ones whose discharge status was certain, time-sequential changes of 11 coagulation relevant indices within mild, severe and critical infection throughout hospitalization according to NHC guidelines were evaluated. Informatics tools were applied to calculate the importance and correlation between them and the progression of thrombosis.

Results

At the beginning of the hospitalization, PLT had a significant decrease in critically ill patients. GLU, PT, APTT, and D-dimer in critical patients were higher than those in mild and severe during the whole admission period. The ISTH DIC score also showed the continuous overt DIC in critical patients. At the late stage of non-survivors, the dynamic profiles of PLT, PT, and D-dimer were significantly different from survivors. A random forest model indicated that the most important feature was PT, followed by D-dimer, indicating their crucial roles for the progression of disease.

Conclusions

COVID-19 is constantly spreading wildly around the world, combining autopsy data, time-sequential profiles and informatics methods to explore the dynamic changes of coagulation relevant indices throughout the course of the disease deterioration, which helps guide the therapy and detect the prognosis in different level of COVID-19 infection.

Essentials

The role of coagulation related indices in different severities of COVID-19 remains to be clarified.

Autopsy, coagulation indices after admission or before outcome, and informatics analysis were used to examine coagulopathy process and importance of related parameters.

Continuous abnormalities of coagulation parameters during hospitalization indicated poor prognosis.

Prothrombin time, D-dimer, blood glucose, and age had been demonstrated the most important parameters related to the severities of COVID-19.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China and associated with an outbreak of coronavirus disease 2019 (COVID-19) that affected more than 9 million patients with more than 480 thousand deaths globally.\(^1\)\(^-\)\(^4\) The latest published researches remind us of COVID-19 patients with severe hypercoagulability and venous thrombosis, which cannot be ignored.\(^5\)\(^-\)\(^7\) The coagulation changes in the SARS-CoV-2 infected patients are now described as the COVID-19 associated coagulopathy (CAC).\(^8\) A few clinical reports have been published on patients with COVID-19 who undergo complete autopsy.\(^9\)\(^-\)\(^16\) Notably, several cases find a high incidence of venous thromboses and embolisms as the direct cause of death.\(^17\)\(^-\)\(^19\) CAC correlated thrombotic phenomena in COVID-19 deaths have been suggested its significant poor prognostic features.\(^20\)

Besides, many critical patients with COVID-19 also exhibit abnormal coagulation, most of whom have venous or arterial thromboembolic complications and microvascular thrombosis similar to other systemic coagulopathy associated with severe infections, such as disseminated intravascular coagulation (DIC) or thrombotic microangiopathy.\(^12\)\(^-\)\(^21\)\(^-\)\(^23\) However, these studies are based on relatively small sample sizes and lack time-sequential properties from admission to discharge, and little knowledge is known about the progress of coagulation that leads to death in COVID-19 patients and the combination of the autopsy with the complete coagulation parameters in confirmed discharge status (deceased or discharge without COVID-19) were not well studied.

Here we reported the pathological features of COVID-19 patients with thrombotic phenomena from autopsy and the coagulation disease progress among mild, severe, and critical patients from one hospital in China. Moreover, we also investigated the difference between survivors and non-survivors in patients with critical infection. Last but not least, we evaluated the correlation and contribution of those features regarding the severity of patients by informatic tools.

Methods
Patients

We collected autopsy data from 9 deceased patients and other clinical data from 407 patients in one hospital in China. All patients were confirmed COVID-19 pneumonia. Patients’ medical records contain the essential information and values of detection indices during the whole process from admission to discharge (or decease).

Autopsy and histological examination

We performed full-body autopsies on deceased persons with SARS–CoV-2 positivity as soon as possible after taking proper safety precautions at the biosafety level 3 (BSL-3) following guidelines from the industrial standards of public safety of the People’s Republic of China. Tissue samples for histopathologic examination were fixed in buffered 4% formaldehyde and processed via standard procedure to slides stained with hematoxylin–eosin (H&E stain). All the hematological indices were collected for the last testing before decease.

Data Collection and Procedures

We reviewed the electronic clinical charts, examination records, and laboratory findings for 407 COVID-19 patients (including 39 deceased patients). During the whole process from admission to discharge (or death), time-sequential hematological investigations including 11 indices i.e. platelet (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-dimer, blood glucose (GLU), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were extracted for subsequent analyses. Other clinical characteristics including age, gender, major comorbidities (coronary artery disease [CAD], hypertension [HTN], and diabetes mellitus [DM]), discharge status (survive or die) and the hospitalization time were also analyzed in our study. Following data extraction, those patients were divided into 3 groups (mild, severe or critical) according to the Chinese National Health Commission (NHC) guidelines (7th trial edition) for COVID-19 pneumonia. Furthermore, we focused on the dynamic profiles of these indices between 39 non-survivors and 42 survivors in the critical group to assess the coagulation process of disease deterioration. In addition, correlation using Pearson method and a random forest model was calculated for patient classification for evaluate the importance of 11 indicator for the process of COVID-19. For DIC analysis, the International Society on Thrombosis and Haemostasis (ISTH) diagnostic criteria were applied to all the patients.

Case Definitions

Mild infection, severe infection, and critical infection were characterized throughout the entire hospitalization according to NHC’s guidelines. Briefly, mild infection is characterized with mild symptoms, fever, respiratory symptoms, and imaging findings of pneumonia. Severe infection is with any of the following appears: shortness of breath (RR > 30 times/min), oxygen saturation ≤ 93%, PaO₂/FiO₂ ≤ 300 mmHg. Critical infection is with any of the following appears: respiratory failure requires mechanical ventilation, shock, other organ failure requires ICU monitoring treatment.

Pearson Correlation Coefficient and Random Forest Model Analysis

We labeled the male as 1 and female as 0 in the correlation and random forest model. Due to the limitations of our detection system, the reportable range of D-dimer and TT were 0.22-21 µg/mL and 13–240 s, respectively. Therefore, when it was reported out of this level (e.g. >21 µg/mL for D-dimer), we corrected those values the barrier of the reportable range (e.g. >21 µg/mL for D-dimer as 21 µg/mL). We also labeled the severity of patients as 1 for mild syndrome, 2 for severe syndrome, and 3 for critical syndrome. Then all data were put into one file to calculate the Pearson correlation coefficient (R, 3.6.1, package ‘gpairs’) and random forest model (Python 3.7) according to previous reports.

Statistical Analysis

For categorical variables and baseline indices, median and interquartile range (IQR) were applied in the form of counts and percentages. Mean and standard error were also used to display the line charts of indices changes. Proportions for categorical variables were compared using the χ² test. Continuous variables were compared using Wilcoxon rank sum test. These statistical analyses were performed using R (version 3.6.1) and the graphs were drawn using GraphPad prism (version 8.0.2).

Role of funding source

None of the funders had any role in the study design, data collection, analysis, interpretation or in the writing of the article and the decision to submit it for publication. Independence from funders and sponsors were confirmed by the researchers.

Results

Complete autopsies of 9 deceased COVID-19 patients (5 males and 4 females) with 15 median hospitalization days (IQR, 10–22) before death were performed (Table 1). The median of ages was 67 years old (IQR, 63–78). Except for the missing comorbidity records of 2 cases (cases 6 and 9), the other 7 cases all had comorbidities. To be specific, 7/7 cases had the comorbidity of hypertension, 2/7 (cases 1 and 4) of cerebral infarction, 2/7 (cases 5 and 8) of coronary artery disease, and 1/7 (case 7) of gout. Of note, one case (case 5) had not only hypertension and coronary artery disease but also renal dysfunction, lacunar infarction, and chronic bronchitis with emphysema. 8/9 cases (88.9%) died mainly due to the respiratory failure with multiple organ failure and the other 1/9 (11.1%) died due to sudden cardiac death from acute coronary heart disease.
Table 1
Demographic and clinical characteristics and laboratory indices of 9 autopsy cases

| Case | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Age - years | 70  | 66  | 53  | 83  | 80  | 62  | 51  | 78  | 67  |
| (IQR) |     |     |     |     |     |     |     | 67  | (63–78) |
| Gender     | Male | Female | Female | Male | Female | Male | Male | Female | Male |
| Hospital stay - days | 20  | 10  | 22  | 29  | 5   | 15  | 5   | 12  | 22  |
| (IQR) |     |     |     |     |     |     |     | 15  | (10–22) |
| Comorbidities | Cerebral infarction, hypertension | Hypertension | Hypertension | Cerebral infarction, hypertension | Hypertension, coronary heart disease, renal dysfunction, lacunar infarction, slow to emphysema | NA | Hypertension (level 3 with very high risk), gout | Hypertension, coronary artery disease | NA |
| Hyaline thrombus distribution of major organs | Lung | √ | √ | √ | √ | √ | √ | √ | √ |
| Kidney | √ |     |     |     |     |     |     |     |     |
| Heart |     |     | √ |     |     |     |     |     |     |
| Brain | √ | √ | √ |     |     |     |     |     |     |
| Spleen | √ | √ | √ |     |     |     |     |     |     |
| Laboratory findings of coagulation relevant indices | PLT - ×10⁹/L | 63 | 127 | 116 | 134 | 76 | 167 | 161 | 66 | 174 |
| PT - s | 19.2 | 13.5 | 13 | 12 | 14.5 | 13.7 | 126.2 | 19.3 | 12 | 12.5 |
| (IQR) |     |     |     |     |     |     |     | 12 | (12.0–13.1) |
| APTT - s | 45.2 | 27.9 | 23.4 | 35.1 | 46.3 | 41.9 | 83.5 | 41.6 | 35.1 | 31.5 |
| (IQR) |     |     |     |     |     |     |     | 16.5 | (26.8–35.1) |
| TT - s | 65.2 | 14.9 | 16.7 | 16.2 | 18.2 | 16.7 | 27.8 | NA | 16.2 | 16.5 |
| (IQR) |     |     |     |     |     |     |     | 16.5 | (16.2–16.7) |
| FIB - g/L | 12.73 | 45.43 | 18.76 | 3.36 | 1.3 | 1.3 | 15.94 | 77.33 | 3.36 | 1.3 |
| (IQR) |     |     |     |     |     |     |     | 3.6 | (1.3–2.3) |
| D-dimer - µg/mL | 1.5 | 6.2 | 2.2 | 3.6 | 6.1 | 3.6 | 3.6 | NA | NA | 3.6 |
| (IQR) |     |     |     |     |     |     |     | 3.6 | (3.3–3.6) |
| DIC | 6 | 5 | 5 | 5 | 5 | 4 | 5 | 6 | 5 |
| (IQR) |     |     |     |     |     |     |     | 5.0 | (5.0–5.0) |

* blank cell represented no obvious thrombi in those organs. b NA indicated the data is not available

Besides the diffuse alveolar damage in the lung, the predominant histological findings were hyaline thrombi among all the 9 deceased patients (Fig. 1). To be specific, 9/9 cases showed microthrombi in hilar arteriole, alveolar wall capillary and interstitial vascular lumen of the lung, 4/9 (1, 2, 3, and 5) in the subarachnoid arteriole and parenchymal small endovascular lumen of the brain, 4/9 (1, 2, 3, and 5) in the small vascular lumen of the spleen, 2/9 (cases 2 and 9) within the kidney, and 1/9 (case 4) in coronary artery lumen together with hemorrhage. To evaluate the coagulation state before death, we also extracted the last hematological indices relevant to coagulation, i.e. platelet, prothrombin time, activated partial thromboplastin time, thrombin time, fibrinogen, and D-dimer in these cases when they were alive (Table 1). Although all the medians of these indices were within the normal range, the ISTH DIC scores in 8/9 cases matched the grade of overt DIC (≥ 5 points).

The autopsy results of thrombi in the major organs of the body and their overt-DIC before death strongly indicated coagulation abnormalities in COVID-19 patients (Table 1, Fig. 1). Together with previous reports showing the high relevance of blood glucose, total cholesterol, triglyceride, high-density lipoprotein, and low-density lipoprotein with coagulation,27–30 we then included those indices in our clinical data analyses. The clinical time-sequential data included 407
hospitalized patients with confirmed COVID-19. Their demographic and clinical characteristics were shown in Table 2. The median (IQR) age was 62.0 years (51.0–58.0) with the overall range from 6 years to 92 years; 51.8% of the patients were from 40 to 65 years of age. A total of 49.9% were female. Of all the patients, 184 (45.2%) had at least 1 of the following 3 comorbidities: coronary artery disease (40 [9.8%]), hypertension (144 [35.4%]), and diabetes (72 [17.7%]). Since all the patients included in this study had either been discharged from hospital with SARS-CoV-2 negativity (368 [90.4%]) or deceased (39 [9.58%]), the median duration of hospitalization was countable at 15 days (8.0–25.0).

Table 2

| Characteristics | All Patients (n = 407) | Disease Severity |
|-----------------|-----------------------|------------------|
|                 | Mild (n = 253)        | Severe (n = 73)  | Critical (n = 81) |
| Age - Median (IQR) - years | 62.0 (51.0–69.0) | 59.0 (45.0–66.0) | 67.0 (60.0–73.0) | 65.0 (57.0–72.0) |
| Distribution - No. (%) | | | | |
| <40 | 43 (10.6) | 36 (14.2) | 4 (5.5) | 3 (3.7) |
| 40–65 | 211 (51.8) | 150 (59.3) | 22 (30.1) | 39 (48.1) |
| >65 | 153 (37.6) | 67 (26.5) | 47 (64.4) | 39 (48.1) |
| Range | 6–92 | 6–92 | 32–91 | 24–92 |
| Gender – No. (%) | | | | |
| Female | 203 (49.9) | 142 (56.1) | 29 (39.7) | 32 (39.5) |
| Male | 204 (50.1) | 111 (43.9) | 44 (60.3) | 49 (60.5) |
| Major Comorbidities – No. (%) | | | | |
| Coronary artery disease | 40 (9.8) | 17 (6.7) | 11 (15.1) | 12 (14.8) |
| Hypertension | 144 (35.4) | 77 (30.4) | 36 (49.3) | 31 (38.3) |
| Diabetes mellitus | 72 (17.7) | 43 (17.0) | 14 (19.2) | 15 (18.5) |
| Clinical outcome – No. (%) | | | | |
| Discharged | 368 (90.4) | 253 (100) | 73 (100) | 42 (51.9) |
| Died | 39 (9.58) | 0 | 0 | 39 (48.1) |
| Hospital stay - Median (IQR) - days | 15.0 (8.0–25.0) | 13.0 (7.0–22.0) | 21.0 (14.0–26.0) | 19.0 (7.0–35.0) |

Percentages may not total 100 because of rounding.

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range.

Supplementary Materials

Among all the 407 patients with their different symptoms throughout the hospital stay, 253 patients (62.2%) showed mild infection, while 73 patients (17.9%) showed severe infection, and 81 patients (19.9%) showed critical infection. All relevant clinical records were reviewed to classified the patients into critical, severe, or mild groups according to the Chinese National Health Commission (NHC) guidelines (7th trial edition) for COVID-19 pneumonia. The patients with critical or severe infection were significantly older than those with mild infection (median [IQR] age, 65.0 [57.0–72.0] or 67.0 [60.0–73.0] years vs 59.2 [45.0–59.0] years; both P values < 0.0001) and more likely to stay longer at hospital after admission (median 19.0 days [7.0–35.0] or 21.0 [14.0–26.0] vs 13.0 [8.0–22.0]; both P values < 0.001), while there was no difference between severe ones and critical ones (P = 0.5996 for age and P = 0.2806 for hospital stay).

Moreover, compared to the mild infected patients, patients with severe infection were more likely to have other underlying commodities (47 [64.4%] vs 98 [24.1%]; P = 0.0001) especially hypertension (36 [49.3%] vs 77 [30.4%]; P = 0.0028) (Table S1). However, those 81 critical patients showed slightly close features of the underlying commodities to the mild ones (39 [48.1%] in critical ones, P = 0.1339) and no significant difference of hypertension proportion between them (31 [38.3%] in critical ones, P = 0.1894) (Table S1). Furthermore, though the critical patients showed no statistical difference in duration of hospitalization between the severe ones (P = 0.2806), the median days of critical patients were shorter than that of severe ones. This led us to think about whether the non-survivors displayed any difference in the critical group.

Table S2 demonstrated that when compared with the survivors (42 [51.9%] patients in critical group) in the critical group, fewer non-survivors had underlying commodities (13 [33.3%] vs 26 [61.9%]; P = 0.01), such as hypertension (8 [20.5%] vs 23 [54.8%]; P = 0.002) and diabetes mellitus (3 [7.7%] vs 12 [28.6%]; P = 0.02). 39 non-survivors also had shorter hospital stay after admission than that of 42 survivors (median 10.0 days [6.5–16.5] vs 35.0 [21.3–40.5]; P < 0.0001). In addition, those commodities percentages of commodities and hospital duration in survivors were more similar to the severe group than those in non-survivors from the critical group (Table 2).

To determine the major hematological features that appeared during COVID-19 thrombogenic progression, the dynamic profiles of 11 clinical laboratory indices, including platelet (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-dimer, blood
glucose (GLU), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were tracked on admission until outcome (Table S3). All the 407 patients with definite discharge status were analyzed and displayed using the line chart (Fig. 2). During hospitalization, most patients had increased D-dimer, and those with critical infection stay significantly higher D-dimer after admission until the outcome. Intriguingly, PLT in the critical patients showed a marked down on admission, kept the counts low until day 14 (both P < 0.05 compared to mild and severe patients [Table S3]), and then gradually increased. In addition, the indices, e.g., PT, and GLU in critical patients showed continuous prolonged time, higher score or higher level during the hospitalization than those in severe or mild patients, while others, e.g. TC and HDL in the critical patients were lower at the initial stage and stayed a relatively low level until the outcome. On the other hand, indices e.g. LDL exhibited their changes at the late stage and TT was intermittently prolonged after admission in the critical patients while APTT, FIB, and TG had no discernable difference among patients with different levels of severity during hospitalization. We also found that the continuous overt-DIC in critical patients during the whole hospitalization stay by counting the ISTH DIC scoring system.

To evaluate the severity of the coagulation state in patients with different disease levels, we also ought to evaluate the percentages of 11 indices’ peak value in every individual that ever reached out of normal range during hospitalization. Table S4 and Table S5 summarized the median (IQR) of the level of all the maximum values of 11 indices in every individual patient during the hospitalization, the proportions of out-of-normal-range values of all the patients with different levels of severity and their statistic test results. In line with the previous findings, the median concentrations of D-dimer (µg/mL) in all types were higher than the normal range (all patients, 1.15 [0.39–3.18], normal range, 0.5). Nonetheless, critical patients showed the significantly higher level of D-dimer than those with the other two types (critical median [IQR] vs severe median [IQR] or mild median [IQR]: 16.77 [3.21–12.9] vs 1.60 [0.78–2.94] or 0.53 [0.28–1.39], two P both < 0.0001). Other coagulation parameters i.e. PT (17.9 s [15.6–21.9] vs 14.3 s [13.9–14.8] or 14.0 s [13.4–14.5]; two P both < 0.0001), APTT (52.9 s [44.1–68.7] vs 43.6 s [39.9–47.9] or 40.5 s [37.8–44.0]; two P both < 0.0001) and TT (20.0 s [17.3–27.0] vs 17.4 s [16.8–17.7] or vs 16.8 [16.1–17.8]; two P both < 0.0001) were also prolonged in this peak value evaluation. Consistently, the out-of-normal-range portions of those coagulation parameters in critical patients were significantly larger than those in severe or mild patients, e.g. D-dimer (79/80 [98.8%] vs 64/72 [88.9%], P = 0.0101; or vs 127/247 [51.4%], P < 0.0001), similar in the DIC grades.

Our autopsy results and 81 critical patients strongly suggested the existence difference between survivors and non-survivors, so the progression analyses of laboratory hematological indices were also taken to evaluate the severity of coagulation. Since the destinations of all patients were confirmed either discharged with SARS-CoV-2 negativity or deceased, we defined the date of discharge or decease as day 1 before outcome and the previous dates increased backward (Fig. 3, Table S6). Interestingly, most indices of survivors and non-survivors shared similar trends, medians, and proportions of abnormal values with the whole critical patients. However, when dividing the critical patients into survivors and non-survivors, several indices exhibited a significant difference between them. Combining analysis with the maximum values of individual patients, non-survivors presented with fewer platelets (× 10^3/L) [216.0 [142.2–273.5] vs 287.5 [199.0–370.0]; P = 0.0035), prolonged prothrombin time (20.2 s [18.1–25.3] vs 16.7 s [15.3–18.0]; P < 0.0001), elevated D-dimer (21.00 µg/mL [13.42–21.00] vs 6.79 µg/mL [2.82–20.61]; P = 0.0013) and DIC score (6 [5–7] vs 5 [5–6]; P = 0.0002) than survivors (Table S7, Table S8), strikingly when it came close to the destination date (P = 0.0027 and P = 0.0051 for PLT and PT at day 11 before outcome, respectively; P = 0.0063 and P = 0.0193 for D-dimer at day 12 before outcome)(Fig. 3, Table S6). Notably, while no obvious change could be found when divided all patients into 3 groups (Fig. 2), the subgroup of non-survivors manifested a significantly higher level of fibrinogen than that of survivors at days 7 and 9 before outcome (Fig. 3, Table S6).

To further explore the underlying correlation between these groups, the heat map was applied to visualize the Pearson correlation coefficient between each clinical feature or laboratory indices (Fig. 4). “Label” in the heat map indicated the severity of COVID-19, i.e. mild, severe, and critical classifications. As indicated by the heat map, the features that positively correlated with patient classifications (label) included coagulation indices e.g. PT (Pearson correlation 0.46), APTT (Pearson correlation 0.31), and D-dimer (Pearson correlation 0.46) and others e.g. age (Pearson correlation 0.22), GLU (Pearson correlation 0.42) whereas indices including TC (Pearson correlation −0.42), HDL (Pearson correlation −0.54), and LDL (Pearson correlation −0.54) showed a significantly negative correlation with severity. We further applied those data to the normal distribution curve to estimate those features’ relationship with the severity (Fig. 4). Unlike age-severity distribution with the critical group’s mean between severe group and mild group (Fig. 4), coagulation indices-severity distributions including PT, APTT, and D-dimer all complied with the mild-severe-critical distribution positively and other indices such as TC, HDL, and LDL negatively (Figure S1). To explore which indices played an indispensable role, a random forest model was constructed according to patient classifications. The best accuracy of the model is 83.8%, the maximum depth of the tree is 9, the number of classifiers is 50 (Figure S1). Then the model showed us the importance of each feature (Fig. 4, Figure S1). The most important feature was PT, followed by D-dimer. These two features contributed to the 40% importance of total. The red dotted line together with the black one separated the features that totally 90% importance. Taken together, those data suggested the important role of coagulation and hematological indices during the deterioration of COVID-19 progress.

**Discussion**

This study combined 9 autopsy results with the epidemiological and clinical characteristics of 407 COVID-19 patients to explore the dynamic changes in coagulation function profiles during the entire hospitalization. Based on the evaluation of 11 hematological indices on admission to discharge, we found several interesting phenomena that were not reported before. These hematological indices such as PT, APTT, PLT, and D-dimer showed significant changes among different types of patients. Notably, in our study, deceased patients were categorized in critical patients. Mortality among critically ill patients was as high as 48.1%. Moreover, a high level of FIB in the non-survivors at days 5–10 before the outcome was found in our study, which was different from previous reports. Considering the same critical patients as the control group and the intact period of hospitalization, our data were more likely to elucidate the underlying coagulation process. In the same period when FIB was higher in the non-survivors, the other coagulation related indices such as PLT, PT, and D-dimer were all deviated from the normal range, indicating hypercoagulation state in the non-survivors. Of note, PLT was significantly lower in the critical group, and then gradually went up at the late stage of hospitalization (Fig. 2). However, when separating the critical group into subgroups, we found that there were not so many critical changes in the survivors and we could reason that the decline of PLT was the result of non-survivors’ thrombocytopenia (Fig. 2, Fig. 3). In concert with the previous study levels of D-dimer showed a marked elevation twice after admission and before death in non-survivors (Fig. 3), suggesting the

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coagulation activation and secondary hyperfibrinolysis condition during thrombosis. Considering so many coagulation related abnormalities, we also calculated the ISTH DIC score to evaluate the DIC state in all patients along the time axis. Despite the increased level of FIB in non-survivors, the DIC score showed the critical patient reaching the limit nearly all the time with no significant difference in its survivor or non-survivor subgroup (Fig. 3). This phenomenon of overt DIC together with the observation of thrombotic from autopsy histological results showed different coagulopathy among different levels of severity in COVID-19 patients.

Other coagulopathy relevant indices are LDH and HDL. Surprisingly, our observation along the hospitalization showed a significant decrease in these 2 indices in critical patients instead of an increase in the previous report. Since the protective effect of HDL through inhibiting blood vessel aggregation, inflammation, oxidation, endothelial damage and thrombosis in several hematological diseases, the low-level HDL and LDL in our observation in critical patients indicated the disturb hematological system, which might contribute to the disease deterioration. Although GLU exhibited much higher in critical group and diabetes mellitus has been found the risk factor of COVID-19 progression especially for deaths in previous and our studies, we should still be careful giving suggestions between diabetes and COVID-19 unless more definite conclusions are made through detail researches. Previous studies have shown that several COVID-19 patients have increased concentrations of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL), especially induces a cytokine storm that might lead to the activation of the coagulation cascade in severe cases. Besides, diabetes can also affect vascular abnormalities and promote the increased synthesis of glycosylation end products (AGEs) and pro-inflammatory cytokines, oxidative stress to mediate inflammation. Taken together, all these showed us the complex CAC progresses in COVID-19 patients with thrombotic complications.

Limitations

Our study has notable limitations. First, the number of patients included in our study is still not large sample, especially for deceased patients. This may bias the proportion of commodities and other observations. It would be better to include more patients over the world and among different countries. Second, indices are still not enough to evaluate the comprehensive aspects of thrombogenesis since thrombogenesis is a complex complication especially when a patient is infected with a virus of high infectivity. Third, we start the records from the admission instead of the onset of illness, which might lose part of the coagulation information.

Conclusions

In summary, our study provides the full spectrum of coagulation progress with the definite discharge status and also shows the existence and dynamic changes of DIC along with this progress. Importantly, we combined the autopsy histology and informatics analysis to reveal the significance of coagulopathy relevant indices during thrombosis. Those results might help guide the therapy and detect the prognosis in different levels of COVID-19 infections.

Declarations

Contributors

Z.G. Xue, L. Liu, and W.J. Wang designed the study, had full access to all data, and take responsibility for the integrity and accuracy of the data and data analysis. B. Lv, C.Y. Li, W.Q. Li, Y.Q. He, S.W. He and T. Zhang contributed to data analysis. B. Lv, C.Y. Li, and T.B. Jiang contributed to interpreting the analysis. Y.Y. Wang and L. Liu contributed to the autopsy and histological examination. B. Lv and C.Y. Li contributed to the writing of the article. H.X. Liu and G.G. Zhang contributed to the critical revision of the data. All authors contributed to data acquisition, data analysis, or data interpretation, and reviewed and approved the final version.

Data sharing

After the publication of the study findings, the data that support the findings of this study will be available for others from the corresponding author based on reasonable request. We will provide an email address for communication once the data are approved to be shared with others under the supervision of the corresponding author and the NHC’s guidelines.

Declaration of interests

These authors declare no competing interests.

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Supplementary Materials

Table S1. Statistical test results related to Table 2.

Table S2. Demographic and clinical features between survivors and non-survivors of 81 critically ill patients with COVID-19.

Table S3. Median, interquartile and statistical test results across different hospitalization days among mild, severe, critical patients related to Figure 2.

Table S4. Laboratory indices of maximum values in individual patients with mild, severe, critical syndromes during the hospital stay.

Table S5. Statistical test results related to Table S4.

Table S6. Median, interquartile and statistical test results across different hospitalization days between survivors and non-survivors at different days before outcome related to Figure 3.

Table S7. Laboratory indices among survivors and nonsurvivors of 81 critically ill patients with COVID-19.

Table S8. χ² test of proportion between survivors and non-survivors related to Table S7.

Figure S1. Informatic analysis of mild, severe and critical patients with COVID-19. Histograms showing the distribution among features, i.e. age (A), APTT (B), GLU (C), TC (D), and HDL (E) within three patient categories. The abscissa represents the current feature value, and the ordinate represents the total number of samples with the feature current value. The blue bar is the histogram of all samples, indicating the distribution of data. The three fitting curves are black, purple, and red, corresponding to the three patient categories: mild, severe, and critical. (F) The parameters of random forest model.

Figures

![Histopathologic findings of deaths with COVID-19 (H&E stain, original magnification). A-C, thrombi in the lung, small intravascular microthrombus (A)(case 1; 100×), alveolar capillary and interstitial vascular microthrombi (B)(case 4; 40×), microthrombus of right hilar arteriole (C)(case 4; 25×); D-E, thrombi in the brain, thrombosis of the subarachnoid arteriole in the right parietal lobe (D)(case 1; 40×), microthrombus was observed in the lumen of small vessels in parenchyma (E)(case 3; 40×); F-G, small intravascular microthrombus (F: case 2; 200×; G: case 9; 200×); H, thrombi in the kidney (case 2; 100×); I, coronary artery lumen together with hemorrhage (case 4; 25×).](image-url)
Figure 2

Temporal changes in laboratory indices after admission to discharge/death in patients with COVID-19. Timeline charts illustrate the daily changes of laboratory indices in 407 patients with COVID-19 (253 mild patients, 73 severe patients and 81 critical patients) during hospitalization. The error bars display mean and standard error. The horizontal dotted lines display the upper or the lower normal limits of platelet (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-dimer, blood glucose (GLU), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and disseminated intravascular coagulation (DIC) score, respectively. Orange triangles, blue squares and green dots indicates critical patients, severe patients and mild patients, respectively. Sample size less than 3 were excluded due to the statistically meaningless in the analysis.
Dynamic profile of laboratory indices including coagulation relevant parameters in critical patients with COVID-19 including platelet (PLT), prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB), D-dimer, blood glucose (GLU), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and disseminated intravascular coagulation (DIC) score. Timeline charts illustrate the daily changes of parameters in 81 critical patients (orange line with triangles, 39 non-survivors and 42 survivors) before discharge (light green line with squares) or death (dark green lines with dots). Sample size less than 3 were excluded due to the statistically meaningless in the analysis.

Informatic analysis of mild, severe and critical patients with COVID-19. A. Pearson correlation showing the correlation coefficient between different feature with three patient categories. Feature 'Label' indicated three patient categories. B-C. Histograms showing the distribution of PT (B) and D-dimer (C) within three patient categories. The abscissa represents the current feature value, and the ordinate represents the total number of samples with the feature current value. The blue bar is the histogram of all samples, indicating the distribution of data, where the three fitting curves are black, purple, and red, corresponding to the three patient categories: mild, severe, and critical. D. Random forest model results with dotted lines indicating the 90% importance of the model. DM, diabetes mellitus; HTN, hypertension; CAD, coronary artery disease. *P < 0.05 for the correlation significance.

Supplementary Files

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