Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Full Length Article

Inflammation/coagulopathy/fibrinolysis: Dynamic indicators of COVID-19 progression in patients with moderate COVID-19 in Wenzhou, China

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ABSTRACT

Background: The majority of the coronavirus disease 2019 (COVID-19) non-survivors meet the criteria for disseminated intravascular coagulation (DIC). Although timely monitoring of clotting hemorrhagic development during the natural course of COVID-19 is critical for understanding pathogenesis, diagnosis, and treatment of the disease, however, limited data are available on the dynamic processes of inflammation/coagulopathy/fibrinolysis (ICF).

Methods: We monitored the dynamic progression of ICF in patients with moderate COVID-19. Out of 694 COVID-19 inpatients from 10 hospitals in Wenzhou, China, we selected 293 adult patients without comorbidities. These patients were divided into different daily cohorts according to the COVID-19 onset-time. Furthermore, data of 223 COVID-19 patients with comorbidities and 22 critical cases were analyzed. Retrospective data were extracted from electronic medical records.

Results: The virus-induced damages to pre-hospitalization patients triggered two ICF fluctuations during the 14-day course of the disease. C-reactive protein (CRP), fibrinogen, and D-dimer levels increased and peaked at day 5 (D) 5 and D9 during the 1st and 2nd fluctuations, respectively. The ICF activities were higher during the 2nd fluctuation. Although 12-day medication returned high CRP concentrations to normal and blocked fibrinogen increase, the D-dimer levels remained high on days 17 ± 2 and 23 ± 2 days of the COVID-19 course. Notably, although the oxygenation index, prothrombin time and activated partial thromboplastin time were within the normal range in critical COVID-19 patients at administration, 86% of these patients had a D-dimer level $> 500\,\mu g/L$.

Conclusion: COVID-19 is linked with chronic DIC, which could be responsible for the progression of the disease. Understanding and monitoring ICF progression during COVID-19 can help clinicians in identifying the stage of the disease quickly and accurately and administering suitable treatment.

1. Introduction

The sudden spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, in December 2019, led to the global pandemic—coronavirus disease 2019 (COVID-19). Within two months, the SARS-CoV-2 had spread from Wuhan to all other districts of China [1]. Wenzhou, the second hardest hit area in China, witnessed an exponential increase in COVID-19 cases. Post-mortem analysis revealed that the presence of viral elements within endothelial cells and accumulation of inflammatory cells were COVID-19 non-survival

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contributors [2], which are important initiating factors of disseminated intravascular coagulation (DIC); this is consistent with the findings that 71.4% of non-survivors met the criteria for DIC during their hospital stay [3]. These data suggest that DIC may be the final pathological step leading to the death of COVID-19 patients. Connors and Levy (2020) [4] summarized the current available data related to COVID-19 coagulopathy and revealed that COVID-19 presents with a special feature of coagulopathy: a prominent elevation of D-dimer and fibrin/fibrinogen degradation products, while abnormalities in prothrombin time, partial thromboplastin time, and platelet counts were relatively uncommon, which does not fulfill the usual definition of DIC. Whether the early COVID-19 coagulation changes seen in COVID-19 patients progress linearly with DIC because of virus infection needs to be investigated. The dynamic manifestation of COVID-19 coagulopathy during COVID-19 progression remains unknown. The elucidation of DIC progression during COVID-19 could greatly enhance our understanding of its pathogenesis and eventually lead to better diagnosis and treatments.

The angiotensin-converting enzyme 2 (ACE2) is the critical receptor on cell membranes for mediating SARS-CoV-2 entry into host cells [5]. Endothelial cells and smooth muscle cells are rich in ACE2 receptors [6], indicating that these cells could be the targets of the virus. In addition to lung injuries, vascular damage produced by viral infection could acutely increase the plasma C-reactive protein (CRP) concentration, which is the first acute-phase protein identified during tissue damage or inflammation. CRP induces several prothrombotic activities, including an increase in the expression and activity of the tissue factor (TF), a decrease in the expression of the TF pathway inhibitor [7], an activation of inflammation and blood coagulation, impairment of the endogenous fibrinolytic capacity, and a stimulation or enhancement of platelet adhesiveness and responsiveness [8]. Epidemiological studies have revealed that CRP concentrations are associated with an increased risk of venous thromboembolism (VTE) [9]. Clinically, D-dimers is extensively used for the diagnosis of VTE [10]. Healthy individuals have low serum D-dimer levels, which is a biomarker of fibrin formation and degradation. Although virus-induced overt-DIC has been recognized in COVID-19 patients [11], estimation of overt-DIC (pre-DIC) at an early stage is difficult.

During the COVID-19 pandemic, the patients remembered the exact onset-day of their first COVID-19-related symptom related. Here, in an analytical retrospective study in Wenzhou, Zhejiang province, China, we investigated the activation profiles of CRP and coagulatory systems in COVID-19 patients before hospitalization.

2. Methods

2.1. Study design and participants

2.1.1. Ethics statement

This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Ethics approval has been issued by the Ethics Committee of Wenzhou Medical University (Ref 2,020,002). Given the urgency of the COVID-19 pandemic and global health concerns, the informed consent forms from patients were waived by the Ethics Committee of Wenzhou Medical University.

In this study, 694 patients (consecutive) with confirmed COVID-19 admitted to 10 hospitals in Wenzhou City, Zhejiang Province, China from Jan 17, 2020 to Mar 20, 2020 were enrolled. The diagnosis of COVID-19 was made according to the World Health Organization [12] interim guidelines and confirmed using RNA detection of the SARS-CoV-2 in the clinical laboratory of the hospitals as described previously [13]. Patients with COVID-19 were stratified into mild (mild symptoms and no sign of pneumonia), moderate (pneumonia and oxygenation index >300 mmHg), and severe (pneumonia and oxygenation index <300 mmHg) patients. Exclusion criteria were as follows: all patients with comorbidities, smokers, age <18 or >65 years, and habits/conditions that may alter the clinical parameters during the natural progression of COVID-19. Data related to disease progression were obtained from medical records and from detailed interviews with patients. Patients without record of the onset-time of the first symptoms were classified as failed cases ($n=7$).

To study the clinical profiles of CRP and coagulatory systems during the natural course of COVID-19, we included only moderate cases in the daily cohort. Two main reasons to exclude the mild and severe patients from the daily cohort studies were as follows: 1) following application of the exclusion criteria, the number of cases in these two groups was small, and 2) the clinical manifestations of either too mild or too severe cases could mask the true feature of the disease and may not be appropriate to demonstrate the dynamic profiles of CRP and coagulatory systems during the natural course of COVID-19. As the illustration of the flow chart, 223 COVID-19 patients with comorbidities and 22 critical cases were rolled in analysis.

According to the onset of the first symptoms before admission, the natural course of COVID-19 was studied every day. The daily distribution of cases is shown in Table 1. Depending on the daily profile of CRP changes, we grouped the nearby days without statistically significant differences of CRP levels and categorized them into three groups (Table 2): day 1–4 (D1–4, $n=123$), day 5–7 (D5–7, $n=91$), and day 8–14 (D8–14, $n=69$). We also investigated the CRP and fibrinogen (FIB) parameters measured at the time of admission in mild patients ($n=10$). (See Fig. 1.)

2.2. Data collection and laboratory procedures

From electronic medical records, we collected epidemiological, demographic, clinical, laboratory, treatment, and outcome data by using a standardized data collection form. Three physicians (CC, BY, and TL) and a researcher (SJ) checked all data and adjudicated any difference in interpretation among the three primary reviewers.

Routine clinical blood examinations, including complete blood count (white blood cell, neutrophils, lymphocyte, and platelet counts), serum biochemical tests (renal and liver function, creatine kinase, and lactate dehydrogenase), myocardial enzymes and CRP, were performed. Coagulation tests, prothrombin time (PT), activated partial thromboplastin time (APTT), FIB, and D-dimer analyses were performed on the day of admission. The circulating levels of IL-2, IL-4, IL-5, IL-6, IL-10, tumor necrosis factor (TNF)- α, and interferon (IFN)-γ were measured using the Human Cytokine Standard 27-Plex Assays panel and the Bio-Plex 200 system (Bio-Rad, Hercules, CA, USA) for all patients according to the manufacturer's instructions.

2.3. Statistical analysis

According to data distribution, the results were given as the mean ± standard deviation, median (interquartile range, IQR), or number (percentage). Distributions were compared with the D'Agostino & Pearson omnibus normality, Shapiro-Wilk normality, and Kolmogorov-Smirnov tests. Categorical variables are shown as frequency (%). Mann-Whitney U test, Kruskal-Wallis test, χ^2 test, Chi-square with Yates' correction, or Fisher's exact test were used for nonparametric data. Spearman correlation coefficients were calculated to determine the relationships between variables. A P-value of <0.05 was considered statistically significant. GraphPad Prism 7.0 software (La Jolla, CA, USA) was used for statistical analyses.

3. Results

In this study, 246 (35.5%) out of the 694 patients (323 females and 371 males), patients were diagnosed with comorbidities, including cardiovascular and cerebrovascular diseases, respiratory system diseases, malignant tumors, chronic liver and kidney diseases, hypercholesterolemia, diabetes mellitus, etc. The mean age at COVID-19 onset was 47.2 years (IQR 37.0–56.0). All patients with COVID-19 underwent

Table 1
CRP and Coagulatory Parameters in Pre-hospitalization Patients ($n = 283$) with a Different Onset-day of COVID-19.

		CRP, mg/L				FIB, g/L				D-dimer, μg/L						Platelets, $\times 10^9$ /L		TBil, mg/dL		IBil, mg/dL	
	Number	median (IQR)	P*	≥10 No./total No. (%)	P†	median (IQR)	P*	>4 No./total No. (%)	P†	median (IQR)	P*	≥300 No./total No. (%)	P†	≥500 No./total No. (%)	P†	median (IQR)	P*	median (IQR)	P*	median (IQR)	P*
D1	35	4.5 (1.3–7.5)	–	7/34(20.6)	–	3.0 (2.8–3.6)	–	3/28(10.7)	–	215 (105–278)	–	4/24(16.7)	–	1/24(4.2)	–	202 (177–265)	–	0.49 (0.37–0.58)	–	0.33 (0.24–0.40)	–
D2	29	5.0 (2.0–7.5)	0.80	5/29(17.2)	0.74	3.1 (2.8–3.7)	0.56	3/25(12.0)	>0.99	220 (122–280)	0.70	4/26(15.4)	> 0.99	2/26(7.7)	0.94	183 (151–219)	0.07	0.53 (0.35–0.81)	0.62	0.34 (0.20–0.53)	0.97
D3	28	6.9 (3.4–15.4)	0.80	9/27(33.3)	0.38	3.5 (3.0–4.0)	0.19	6/25(24.0)	0.28	160 (115–280)	0.82	5/23(21.7)	0.73	0/23	> 0.99	158 (136–203)	0.003	0.55 (0.41–0.71)	0.29	0.34 (0.26–0.46)	0.62
D4	31	7.2 (5.0–13.5)	0.01	10/29(34.5)	0.22	3.9 (3.1–4.5)	0.003	9/23(39.1)	0.02	220 (134–350)	0.37	8/22(36.4)	0.18	2/22(9.1)	0.94	200 (171–256)	0.62	0.54 (0.40–0.78)	0.26	0.37 (0.25–0.54)	0.31
D5	34	10.0 (5.0–28.3)	0.002	16/33(48.5)	0.02	4.1 (3.3–4.8)	<0.001	20/32(62.5)	<0.001	230 (160–380)	0.09	12/31(38.7)	0.13	4/31(12.9)	0.52	186 (148–218)	0.07	0.60 (0.42–0.97)	0.04	0.38 (0.25–0.56)	0.19
D6	22	7.9 (3.4–20.5)	0.12	9/22(40.9)	0.10	3.7 (2.9–4.4)	0.06	8/20(40.0)	0.03	164 (82–383)	0.93	7/20(35.0)	0.19	2/20(10.0)	0.87	168 (141–223)	0.02	0.51 (0.39–0.71)	0.35	0.32 (0.26–0.47)	0.48
D7	35	5.0 (1.7–14.6)	0.29	12/34(35.3)	0.18	3.8 (3.0–4.8)	0.01	15/33(45.5)	0.004	230 (125–410)	0.15	13/33(39.4)	0.08	5/33(15.2)	0.35	192 (155–223)	0.14	0.75 (0.47–0.98)	0.002	0.51 (0.34–0.64)	0.003
D8	11	19.4 (10.5–39.3)	<0.001	9/10(90.0)	<0.001	4.2 (3.4–5.1)	0.003	4/7(57.1)	0.02	300 (130–450)	0.13	4/7(57.1)	0.05	1/7(14.3)	0.41	185 (160–259)	0.42	0.61 (0.53–1.02)	0.04	0.46 (0.36–0.75)	0.03
D9	12	21.6 (12.4–39.8)	<0.001	10/12(83.3)	<0.001	4.6 (4.1–5.1)	<0.001	11/12(91.7)	<0.001	305 (172–425)	0.04	6/12(50.0)	0.05	2/12(16.7)	0.25	169 (152–218)	0.10	0.59 (0.49–0.65)	0.11	0.39 (0.35–0.44)	0.19
D10	26	5.5 (3.2–25.2)	0.07	12/26(46.2)	0.03	4.0 (3.0–5.0)	0.005	10/22(45.5)	0.009	306 (216–410)	0.009	12/22(54.5)	0.01	4/22(18.2)	0.29	240 (189–311)	0.19	0.74 (0.47–0.86)	0.01	0.47 (0.27–0.62)	0.07
D11-12	10	8.7 (6.6–27.6)	0.02	4/9(44.4)	0.20	4.0 (3.8–5.6)	<0.001	4/9(44.4)	0.05	600 (275–845)	0.004	7/9(77.8)	0.002	4/9(44.4)	0.013	231 (195–286)	0.25	0.64 (0.59–1.02)	0.03	0.45 (0.42–0.50)	0.003
D13-14	10	12.4 (3.8–49.0)	0.12	5/9(55.6)	0.09	4.9 (4.6–8.1)	<0.001	6/7(85.7)	<0.001	690 (270–1010)	0.003	5/7(71.4)	0.01	4/7(57.1)	0.0051	261 (221–427)	0.04	0.79 (0.60–0.86)	0.009	0.38 (0.36–0.45)	0.13

SI conversion factors: To convert bilirubin to μmol/L, multiply by 17.104. Abbreviations: CRP, c-reactive protein; FIB, fibrinogen; TBil, total bilirubin; IBil, indirect bilirubin; IQR, interquartile range; D1-14, day 1–14.
P* Mann-Whitney U test. vs. D1. P† χ^2 test, Chi-square with Yates' correction or Fisher's exact test vs. D1.

Table 2
Clinical Characteristics and Laboratory Results of Pre-hospitalization Patients at the Different Stages of COVID-19 Course.

	D1-4 ($n = 123$)	D5-7 ($n = 91$)	D8-14 ($n = 69$)	*P* value*
Age, yr	40 (32–48)	41 (34–50)	47 (40–52)	0.03
Sex				
Male	59 (48.0)	42 (46.2)	42 (60.9)	0.14
Female	64 (52.0)	49 (53.9)	27 (39.1)	–
Laboratory tests				
Oxygenation index, mm Hg	449 (403–486)	457 (404–500)	425 (386–473)	0.07
C-reactive protein, mg/L	5.0 (3.0–11.4)	7.9 (3.7–19.5)	13.7 (5.0–25.2)	<0.001
≥10	31/119 (26.1)	37/89 (41.6)	41/67 (61.2)	<0.001
≥30	8/119 (6.7)	13/89 (14.6)	14/67 (20.9)	0.02
Procalcitonin, ng/mL	0.07 (0.04–0.25)	0.12 (0.03–0.25)	0.07 (0.04–0.10)	0.54
IFN-γ, pg/mL	2.5 (1.8–3.1)	2.5 (1.5–3.3)	2.4 (1.0–3.2)	0.78
TNF-α, pg/mL	1.2 (1.0–2.5)	1.5 (0.9–2.5)	1.2 (0.5–2.3)	0.71
IL-2, pg/mL	1.6 (1.4–2.5)	1.8 (1.4–2.5)	1.6 (0.8–2.5)	0.53
IL-4, pg/mL	2.1 (1.3–2.5)	1.6 (1.1–2.5)	1.9 (1.0–2.7)	0.92
IL-5, pg/mL	1.2 (1.0–1.4)	1.4 (1.0–2.1)	1.5 (1.1–1.9)	0.42
IL-6, pg/mL	4.0 (2.3–5.9)	4.4 (2.1–11.6)	4.0 (2.6–10.0)	0.86
IL-10, pg/mL	2.5 (0.9–3.8)	2.5 (0.9–3.9)	3.3 (1.9–4.7)	0.12
Coagulatory parameters				
Fibrinogen, g/L	3.2 (2.9–3.9)	4.0 (3.0–4.6)	4.2 (3.7–5.2)	<0.001
<2	1/101 (0.99)	0/85	0/57	>0.99
>4	21/101 (20.8)	43/85 (50.6)	35/57 (61.4)	<0.001
D-dimer, μg/L	210 (120–280)	220 (135–388)	320 (220–565)	<0.001
≥300	21/95 (22.1)	32/84 (38.1)	23/62 (37.1)	0.04
≥500	5/95 (5.3)	12/84 (14.3)	9/62 (14.5)	0.09
PT, sec	12.1 (11.2–13.0)	12.5 (11.6–13.0)	12.9 (12.3–13.6)	<0.001
<11.5	34/107 (31.8)	18/87 (20.7)	5/62 (8.1)	0.002
>14.5	4/107 (3.7)	1/87 (1.2)	5/62 (8.1)	0.09
APTT, sec	32.5 (29.5–37.2)	34.1 (29.9–37.6)	34.6 (29.0–38.3)	0.48
Platelet count, $\times 10^9$ /L	192 (159–235)	188 (152–218)	224 (176–277)	0.001
<100	3/122 (2.5)	0/90	0/68	>0.99
>300	11/122 (9.0)	4/90 (4.4)	14/68 (20.6)	0.006

Values are reported as median (IQR), mean ± SD or number/total number (%) as appropriate. Abbreviations: D1-4, D5-7 and D8-14, day 1–4, 5–7 and 8–14 in COVID-19 course; IL, interleukin; IFN, interferon; PT, prothrombin time; APTT, activated partial thromboplastin time; ALT, IQR, interquartile range. *p values were calculated by Kruskal-Wallis test, one-way ANOVA, χ^2 test, or Fisher's exact test.

antiviral and supportive therapies after the diagnosis was confirmed. After the exclusion of 401 cases, 293 patients (10 mild and 283 moderate) were enrolled in the final analysis.

During the 14-day progression of COVID-19, the oxygenation index was within the normal range in all moderate patients. The CRP level was higher on day 1 (D1) in the moderate patients ($p < 0.05$) when compared to that in the mild patients, but this was not the case for FIB levels (Fig. 2A and B). Daily analysis in the moderate cases revealed that a low fluctuation between day 1–7 (D1–7) was followed by a high fluctuation (D7–14) of serum CRP levels with peaks at D5 and D9 ($p < 0.01$ and $p < 0.001$, respectively, Table 1 and Fig. 2C). The daily changes in serum FIB levels appeared as a tooth-waveform overlapping on the main profile of increasing responses (Fig. 2D). The two peaks of the tooth-waveform of FIB levels were at D5 and D9 (both $p < 0.001$, Table 1). Serum CRP levels were positively correlated with FIB levels ($r = 0.671$, $p < 0.001$, $n = 238$, Fig. 2E). D-dimer levels weakly reflected the dynamic changes in the FIB levels during D1–6. Small fluctuation occurred from D3–6 with a peak at D5 ($p < 0.01$, Fig. 2F). From D6, D-dimer levels increased and reached a significantly high level at D9 ($p < 0.01$ vs. D6), which was then maintained ($p < 0.01$ at D10; both $p < 0.001$ at D11–12 and D13–14 vs. D1, Table 1). Serum D-dimer levels were positively correlated with the FIB levels ($r = 0.496$, $p < 0.001$, $n = 232$, Fig. 2G). The platelet counts were significantly lower at D3 ($p < 0.01$) and D6 ($p < 0.05$) than those at D1 (Table 1).

Notably, the decrease in FIB, D-dimer, and platelet counts, which indicate the increase of fibrin formation from FIB, decrease of fibrinolytic activity, and increase of platelet-thrombi formation, occurred at D6 after the first peak subsided (Fig. 2D, F and Table 1). Further evidence for thrombi-formation was provided by the appearance of homolysis. During the 14-day natural progression of COVID-19, the increase in serum indirect bilirubin (IBil) levels occurred at D7 ($p < 0.05$, D7 vs. D6; $p < 0.01$, D7 vs. D1) and D11–12 (D1 vs. D11–12, $p < 0.01$, Fig. 2H). IBil levels were negatively correlated with D-dimer levels from D6 ($r = -0.354$, $p < 0.001$, $n = 103$, Fig. 2I).

Depending on the number of cases and the characteristics of the peaks of CRP (monitoring infection/inflammatory response) and FIB (monitoring ongoing coagulopathy) responses during the 14-day natural course of COVID-19, we were able to group the first four days (D1–D4, $n = 123$) together as the initiation period for activation of CRP and coagulatory systems, and D5–D7 ($n = 91$) together as the first thrombi-forming period. Due to limited cases and lack of statistically significant CRP levels among D8–14, we grouped D8–14 together as the second peak of the inflammation/coagulatory period (Table 2). Table 2 validates the findings presented in Table 2 that the serum CRP, FIB, D-dimer, and platelet counts increased significantly in the second peak of the inflammatory/coagulatory period when compared to that in the first peak. The CRP, FIB, and D-dimer levels reached the maximum levels of 13.7 (IQR 5.0–25.2) mg/L, 4.2 (IQR 3.7–5.2) g/L, and 320 (IQR 220–565) μg/L, respectively at D8–14 (all $p < 0.001$). Table 2 reveals two new features of the disease: 1) the incidence of diarrhea and fatigue occurred more often during the first thrombi-forming period D5–7 ($p < 0.001$) and 2) the markers of liver damage and serum total and direct bilirubin, showed abnormal levels ($p < 0.001$ and $p < 0.05$ respectively) in the second inflammatory/coagulatory period, indicating that digestive system damage had occurred.

In 69 moderate patients with D8–14 onset-day of COVID-19 progression, although 6- and 12-day (equal to 17 ± 2 and 23 ± 2 days in COVID-19 course) medical interventions (antiviral, corticosteroids, and Chinese herbs treatments) gradually returned CRP levels to normal (Table 3) and blocked a further increase in FIB levels, D-dimer and IBil levels remained significantly high on post-treatment day 6 (P6) and P12 when compared to those on D1–4 (Table 3). No overt-DIC in these patients was observed, as both PT and APTT did not show clinically significant changes (Table 3). The acute respiratory distress syndrome (ARDS) was not observed in these patients.

Considering that severe COVID-19 and DIC mostly occurs in patients with comorbidities, we further analyzed the patients with comorbidities and COVID-19. Compared to patients with moderate COVID-19, the severe and critical patients were four and 11 years older, respectively (Table 4, $p < 0.05$ and < 0.01 respectively). Although the oxygenation index was normal in patients with severe and critical COVID-19 at administration, however, CRP levels in these patients were significantly higher than those in patients with moderate COVID-19 (both $p < 0.01$). Many patients with moderate, severe, and critical COVID-19 had fibronogen levels >4 g/L (Table 4). Notably, although the oxygenation index, PT, and APTT levels were within the normal range in critical COVID-19 patients at administration, 86% of these patients had a D-dimer level > 500 μg/L (Table 4, $p < 0.001$).

4. Discussion

Virus-induced overt-DIC has been observed in patients with COVID-19 [3,4]. As DIC progresses rapidly, COVID-19 develops into a hypercoagulable status, which results in severe thrombi formations, thus,

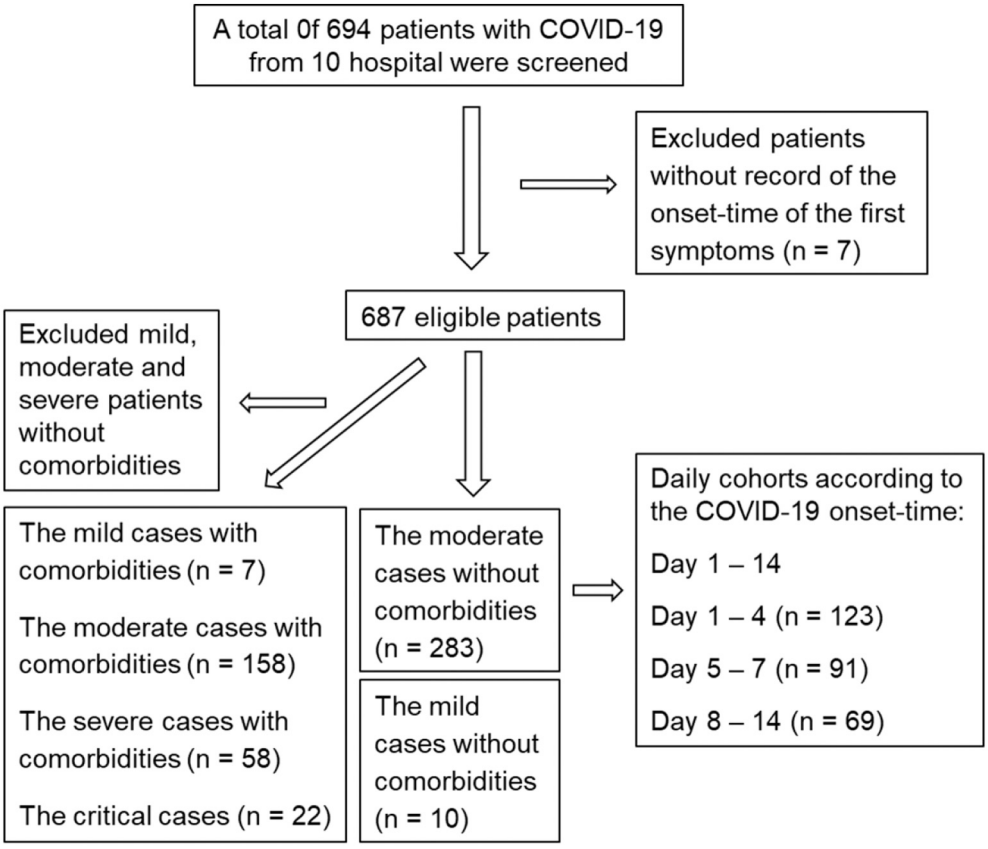

Fig. 1. Flow chart of the study.

leaving no time for therapy [3]. Therefore, quick and accurate identification of the pre-DIC stage during COVID-19 is critical for clinicians to make correct diagnosis and administer treatment. However, the pre-DIC or the chronic DIC (CDIC) stage is difficult to identify. According to the medical records of the patients at the time of admission, we studied the progression of COVID-19 every day without medical intervention. Here, we show that damage to pre-hospitalization patients with COVID-19 triggered two vicious cycles (initiating and amplifying phases) during the 14-day course of the disease. In the initiating phase, CRP and FIB systems were activated at the beginning of the infection. CRP can increase the expression and activity of TF, decrease TF pathway inhibitor levels, activate inflammation and blood coagulation, impair endogenous fibrinolytic capacity, and stimulate or enhance platelet adhesiveness and responsiveness [7]; CRP concentrations are also associated with an increased risk of VTE [8]. FIB can play overlapping roles in blood clotting, fibrinolysis, cellular and matrix interactions, inflammatory response, wound healing, and neoplasia [14]. Thus, elevated circulating CRP and FIB levels could act as significant risk indicators for the development or progression of the pre-DIC stage. We observed that the decrease in FIB levels at D6 and D10 was associated with a reduction in platelet counts, which is an indicator of thrombus formation. This finding was supported by a mild but significant elevation of a homolysis marker, IBil, at D7 and D11–12. Because red blood cells (RBCs) are pushed through the thrombi compromised of tiny vessels in the microvasculature, hemolysis occurs and serum IBil increases [15]. Although COVID-19 in the initiating phase produces limited fibrin thrombi in arterioles and capillaries, it could result in ischemia and multiorgan damage. This would initiate strong inflammation, aggravate tissue damage, and lead to a stronger responsive phase—the amplifying phase.

In the amplifying phase, the CRP peak level at D9 was higher than that at D5; both FIB and D-dimer levels were also higher and positively correlated. D-dimer levels were 3-fold higher at D13–14 than those at D5. D-dimers are produced from intravascular thrombi and clots that have recently been formed [15,16]. Hence, the 3-fold higher D-dimer levels indicate 3-fold higher thrombosis in the amplifying phase.

In both phases, both PT and APTT were only slightly prolonged (or normal) in the moderate patients (Tables 1–3). This usually occurs in CDIC that develops when patients are exposed to small amounts of thrombin [13]; when the coagulation materials are consumed, the patient is partially able to compensate through their increased production. In this study, the platelet counts were only mildly altered, which is a typical manifestation of CDIC. Both endothelial cells and smooth muscle cells are rich in ACE2 receptors [5], which could be SARS-CoV-2 targets. Therefore, the vasculitis caused by SARS-CoV-2 may be one of the factors leading to CDIC. SARS-CoV-2 spreads through the bloodstream and mainly lodges in the lungs, gastrointestinal tract, and heart. The median time for dyspnea at D7 (4–9) in severe patients calculated in a previous study [17] was consistent with the mild homolysis time (D7) in the moderate patients in our study. Consistenting with the thrombi-forming time during the initial strike of the SARS-CoV-2, the incidence of diarrhea and fatigue increased in patients with moderate COVID-19 during D5–7. These data indicate that some local tissues (lungs, digestive system, and muscles) may have thrombi formation during the first strike, which leads to tissue damage and a more severe *sec*ond strike. During the second strike, the SARS-CoV-2 combined with the endogenous damages of the pre-DIC stage, initiates a double increase in CRP response, and both FIB and D-dimer responses were accumulative. The second thrombi-forming period was during D9–12 and obvious homolysis was observed around D11–12, which is consistent with the time from illness onset to ARDS and the time from illness onset to ICU admission in severe patients, respectively [17]. The reasons for these two peaks of blood CRP and FIB levels were at D5 and D9 remain unclear, but one possible explanation is that these 4–5 days period may result from the incubation time of SARS-CoV-2 virus. It is known that the

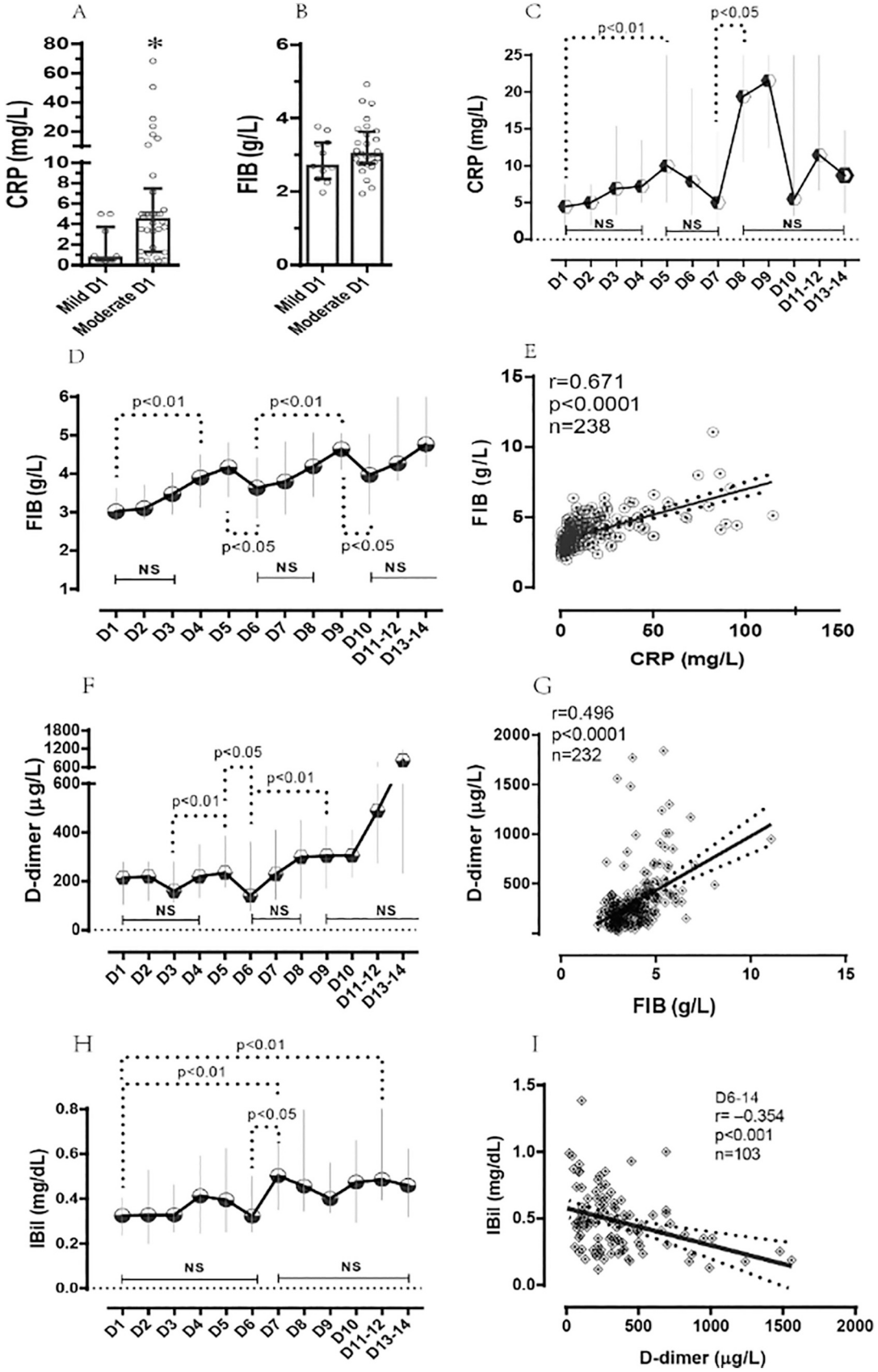

Fig. 2. (A–B) C-reactive protein (CRP) and fibrinogen (FIB) levels in mild and moderate cases on day 1. (C–D) CRP and FIB daily levels in moderate cases. (E) Correlation between CRP and FIB levels. (F) Daily D-dimer levels in moderate cases. (G) Correlation between FIB and D-dimer. (H) Indirect bilirubin (IBil) daily levels in moderate cases. (I) Correlation between IBil and D-dimer. Refer to Table 1 for the number of patients. D1, day 1; D13-14, day 13–14; D6-14, day 6 to day 14.

Table 3
Laboratory Findings in COVID-19 Patients with Medications.

	D1-4 (n = 123)	D8-14 (*n* = 69)	D17 ± 2 (n = 69)	D23 ± 2 (n = 69)	P value*				
	No medication	No medication	Medication P6	Medication P12	D1-4 vs. P6	D1-4 vs. P12	D8-14 vs. P6	D8-14 vs. P12	P6 vs. P12
Oxygenation index, mm Hg	449 (403–486)	425 (386–473)	390 (327–483)	432 (375–491)	0.008	0.46	0.08	>0.99	0.21
C-reactive protein, mg/L	5.0 (3.0–11.4)	13.7 (5.0–25.2)	6.4 (2.4–21.4)	2.6 (0.8–5.3)	0.46	<0.001	0.02	<0.001	0.002
≥10	31/119 (26.1)	41/67 (61.2)	14/38 (36.8)	7/49 (14.3)	0.20	0.10	0.02	<0.001	0.01
D-dimer, μg/L	210 (120–280)	320 (220–565)	730 (390–1740)	490 (270–740)	<0.001	<0.001	0.003	0.10	0.23
≥300	21/95(22.11)	34/57(59.65)	11/13(84.6)	9/15(60.0)	<0.001	0.002	0.17	0.98	0.22
≥500	5/95(5.3)	16/57(28.1)	8/13(61.5)	7/15(46.7)	<0.001	<0.001	0.02	0.17	0.48
Fibrinogen, g/L	3.2 (2.9–3.9)	4.2 (3.7–5.2)	4.4 (3.2–5.2)	3.6 (3.3–4.6)	0.02	0.02	0.51	0.07	0.75
<2	1/101 (0.99)	0/57	0/13	0/17	>0.99	>0.99	NA	NA	NA
>4	21/101 (20.8)	35/57 (61.4)	7/13 (53.9)	6/17 (35.3)	0.009	0.19	0.62	0.06	0.31
PT, sec	12.1 (11.2–13.0)	12.9 (12.3–13.6)	13.0 (12.2–14.0)	12.9 (12.7–13.7)	0.01	0.02	0.55	0.72	0.72
APTT, sec	32.5 (29.5–37.2)	34.6 (29.0–38.3)	37.9 (29.5–40.2)	35.8 (31.3–42.3)	0.16	0.06	0.36	0.28	0.86
Platelet count, $\times 10^9$ /L	192 (159–235)	224 (176–277)	275 (208–326)	293 (234–348)	<0.001	<0.001	0.005	<0.001	0.43
<100	3/122 (2.5)	0/68	0/43	0/50	0.57	0.56	NA	NA	NA
>300	11/122 (9.0)	14/68 (20.6)	18/43 (41.9)	24/50 (48.0)	<0.001	<0.001	0.02	0.002	0.55
Total bilirubin, mg/dL	0.51 (0.37–0.73)	0.69 (0.48–0.95)	0.70 (0.54–0.93)	0.56 (0.44–0.85)	<0.001	0.18	0.66	0.10	0.06
Direct bilirubin, mg/dL	0.18 (0.15–0.27)	0.27 (0.19–0.39)	0.26 (0.19–0.43)	0.23 (0.17–0.27)	<0.001	0.16	0.99	0.02	0.05
Indirect bilirubin, mg/dL	0.34 (0.24–0.46)	0.43 (0.33–0.65)	0.47 (0.35–0.65)	0.46 (0.32–0.58)	<0.001	0.003	0.56	0.85	0.80

Values are reported as median (IQR) or number/total number (%), as appropriate. SI conversion factors: bilirubin to μmol/L, multiply by 17.104. Abbreviations: D1-4 and D8-14, day 1–4 and 8–14 in COVID-19 course; D17 ± 2 and D23 ± 2, day 17 ± 2 and 23 ± 2 in COVID-19 course; P6 and P12, post-treatment day 6 and 12; NA, not applicable. Other abbreviations are explained in Table 1. *p values were calculated by Mann-Whitney U test. χ^2 test, Chi-square with Yates' correction, or Fisher's exact test.

Table 4
Clinical Characteristics and Laboratory Results of Pre-hospitalization Patients with comorbidities and mild, moderate, severe or critical COVID-19.

	Mild (n = 7)	Moderate (*n* = 158)	Severe (*n* = 58)	Critical (*n* = 22)	P value*
Age, yr	42 (38–55)	52 (44–61)	56 (48-68)$^{a/b}$	63 (53-75)$^{aa/bb}$	<0.001
Sex					
Male	6 (85.7)	87 (55.1)	37 (63.8)	14 (63.6)	0.25
Female	1 (14.3)	71 (44.9)	21 (39.2)	8 (36.4)	–
Laboratory tests					
Oxygenation index, mm Hg	481 (373–490)	443 (376–500)	336 (257-411)^{bbb}	481 (405-543)^{ccc}	<0.001
C-reactive protein, mg/L	0.5 (0.3–1.9)	10.5 (3.1–30.1)	27.3 (12.6–58.9)^{bb}	45.6 (13.3–84.1)$^{aa/bbb}$	<0.001
≥10	1/7 (14.3)	80/155 (51.6)	47/58 (81.0)$^{aaa/bbbb}$	20/21 (95.2)$^{aaa/bbb}$	<0.001
≥30	1/7 (14.3)	39/155 (25.2)	28/58 (48.3)^{bb}	13/21 (61.9)^{bbb}	<0.001
Fibrinogen, g/L	2.9 (2.1–3.5)	4.0 (3.1–5.3)	4.2 (3.5–5.4)	5.2 (3.7–6.6)a	0.08
<2	0/7	2/147 (1.4)	0/51	2/22 (9.1)	0.08
>4	0/7	73/147 (49.7)^{a}	29/51 (56.9)^{a}	15/22 (68.2)^{aa}	0.01
D-dimer, μg/L	260 (96–340)	344 (211–528)	335 (173–680)	1170 (660–1745)	0.35
≥300	2/7 (28.6)	83/140 (59.3)	27/50 (54.0)	20/22 (90.9)$^{aa/bb/cc}$	0.003
≥500	0/7	37/140 (26.4)	19/50 (38)	19/22 (86.4)$^{aaa/bbbb/ccc}$	<0.001
PT, *sec*	12.0 (11.6–13.1)	12.5 (11.7–13.4)	12.8 (11.6–13.3)	13.0 (12.5–13.6)	0.30
APTT, sec	32.7 (29.5–38.8)	33.0 (29.2–39.2)	32.6 (29.3–35.9)	38.3 (34.8–43.8)^{c}	0.04
Platelet count, $\times 10^9$ /L	190 (150–205)	189 (161–238)	177 (138–215)	176 (158–221)	0.37
<100	0/7	3/156 (1.9)	3/58 (5.2)	0/22 (0)	0.70

Values are reported as median (IQR), mean ± SD or number/total number (%) as appropriate. Abbreviations: PT, prothrombin time; APTT, activated partial thromboplastin time. *p values were calculated by Kruskal-Wallis test, one-way ANOVA, χ^2 test, or Fisher's exact test. a, other groups vs mild group; b, other groups vs moderate group; c, other groups vs severe group. a,b,c, $p < 0.05$; aa,bb,cc, $p < 0.01$; aaa,bbb,ccc, $p < 0.001$.

median incubation period of SARS-CoV-2 virus in adults usually was 3.0–6.4 days [13,18–23]. There was a possibility that each incubation with SARS-CoV-2 virus over the course of the disease would result in cell death, and sequentially inflammatory / coagulopathy / fibrinolysis responses.

The third strike during COVID-19 progression was interrupted by medical intervention in the present study. Medications gradually returned thee CRP concentration to normal levels on post-treatment D12 (day 23 ± 2 in the COVID-19 course) and blocked the increase of FIB levels. Although COVID-19 progression was interrupted by medications, coagulation/fibrinolysis remained high. However, although medication stopped further damages induced during COVID-19 progression, the anterior thrombus led to an increase in fibrinolytic activities to dissolve the anterior thrombi and obtain coagulation/fibrinolysis homeostasis. The median time was calculated for illness onset to death or discharge was calculated at D21 (D17–25) in severe patients [17], suggesting that the three strikes by SARS-CoV-2 in 21 days may be the complete natural course of COVID-19.

Due to the absence of a gold standard for the diagnosis of DIC, the diagnostic rates of the DIC criteria cannot be properly assessed [24,25]. Here, we show that the dynamic changes and significantly positive relationships of the triple-parameters (CRP/FIB, FIB/D-dimer) can improve the detection of the pre-DIC stage or the CDIC process during COVID-19 progression. CRP induces several prothrombotic activities [6,7]. FIB can play overlapping roles in coagulatory and inflammatory responses [14]. D-dimer is a valuable marker of the activation of coagulation and fibrinolysis [10]. Serum D-dimer has been used for the prognosis Novel Influenza A (H1N1) in 2009 [26]. An interesting finding in the present study is that although the oxygenation index, PT, and APTT levels were within the normal range in critical COVID-19 patients at administration, 86% of these patients had a D-dimer level > 500 μg/L. Thus, dynamic monitoring of serum CRP, FIB, and D-dimer levels in patients with COVID-19 would assist in the timely diagnosis and treatment of the patients.

In the present study, we did not find significant changes of cytokines during the natural course of COVID-19 in the moderate patients. Clinical studies have demonstrated that COVID-19 infection leads to a cytokine storm (severe systemic elevation of several pro-infammatory cytokines) in some elderly critical adults, which links the pathophysiology mechanisms of "infamm-aging"and cytokine storms in some elderly adults with severe COVID-19 infection [27,28]. Both the age and the severity of COVID-19 infection are correlated to an increased risk of a cytokine storm [27,28] and/or abnormal immunological features [29,30]. In our previous study, we found that age and $CD8^+$ T cell counts were negatively correlated ($r = -0.435$, $p < 0.0001$) in patients with COVID-19. Moreover, SARS-CoV-2 infection age-dependently increased the plasma CRP concentrations (2.0, 5.0, 9.0, 11.6, and 36.1 mg/L in the five [2–25, 26–38, 39–57, 58–68, and 69–79-year-old] age-groups, respectively). However, the IL-6 concentration in the 39–57-year-old age group was <7 pg/ml, i.e., within the normal range [30]. In the present study, the mean age of moderate patients with COVID-19 was 40 years (interquartile range [IQR] 32–48), which is the compatible with the 39–57-year-old age group. In contrast to retarded responses of cytokine in moderate patients with COVID-19, the CRP levels were altered during the natural course of the disease, indicating that CRP is a sensitive marker of the inflammatory responses to COVID-19.

This study has several limitations. First, due to the retrospective study design, all laboratory tests were not done for all patients, including coagulation tests and IL-6 measurements. Therefore, their role might have been underestimated. Second, by excluding patients with any comorbidities, some patients might have had unrecognized abnormalities, and this might have had certain influences on the data analysis. Third, as a small number of mild patients with mild COVID-19 were enrolled, the interpretation of our findings might be limited by the sample size. Fourth, although people were very cautious about their health during the COVID-19 pandemic, we could not rule out the possibility that few patients did not remember the onset-time accurately, and this might have influenced the data analysis.

To the best of our knowledge, this is the first retrospective cohort study to reveal the pre-DIC stage or the CDIC progress in the natural course of COVID-19. We found that systemic inflammation and pre-DIC progression, a constant dysregulation of the coagulation cascade and the subsequent formation of intra-alveolar or systemic fibrin clots, is a prominent feature in COVID-19 patients associated with pneumonia and vasculitis. These pathogenetic pathways during COVID-19 can be attributed to the prothrombotic response, which attempts to stop the diffuse alveolar and systemic vascular hemorrhage, but can instead lead to overt-DIC with detrimental effects in patient recovery and survival.

Authorship contributions

ML, XL and SJ contributed equally to this paper and are joint corresponding authors. HA JZ and TZ are joint first authors. The corresponding and first authors conceived and designed the study. ML, XL and SJ had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. ML, XL, SJ, HA, JZ and TZ drafted the paper. ML, SJ, HA, JZ, ZX, and TZ performed data analysis, and all authors critically revised the manuscript for important intellectual content and gave final approval for the version to be published. TL, CC, SL, CH and BY collected the data. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of Competing Interest

None declared.

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