



**Original Article**

**Elevated Serum Pentraxin-3 Levels is Positively Correlated to Disease Severity and Coagulopathy in COVID-19 Patients**

Ming Tong<sup>1,2,3</sup>, Ying Xiong<sup>4</sup>, Chen Zhu<sup>5</sup>, Hong Xu<sup>4</sup>, Qing Zheng<sup>6</sup>, Changping Hu<sup>7</sup>, Yu Jiang<sup>3</sup>, Lianhong Zou<sup>3</sup>, Xiaolin Xiao<sup>4</sup>, Fang Chen<sup>3</sup> and Yimin Zhu<sup>2,3\*</sup>.

<sup>1</sup> Department of Infectious Diseases, The First-affiliated Hospital of Hunan Normal University (Hunan Provincial People's Hospital), Changsha, 410005, Hunan, China.

<sup>2</sup> School of Life Sciences, Hunan Normal University, Changsha, Hunan, China.

<sup>3</sup> Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, The First-affiliated Hospital of Hunan Normal University (Hunan Provincial People's Hospital), Changsha, 410005, Hunan, China.

<sup>4</sup> The Fourth People's Hospital of Yiyang, Yiyang, 413000, Hunan, China.

<sup>5</sup> Department of Pediatrics, Yiyang Central Hospital, Yiyang, Hunan 413099, P.R. China.

<sup>6</sup> Department of Geriatrics, The First-affiliated Hospital of Hunan Normal University (Hunan Provincial People's Hospital), Changsha, 410005, Hunan, China.

<sup>7</sup> Department of Pharmacology, Xiangya School of Pharmaceutical Sciences, Central South University, Changsha 410078, Hunan, China.

**Competing interests:** The authors declare no conflict of Interest.

**Abstract. Background:** Coronavirus disease 2019 (COVID-19) is highly contagious and deadly and is associated with coagulopathy. Pentraxin-3 (PTX3) participates in innate resistance to infections and plays a role in thrombogenesis.

**Purpose:** The present study aimed to investigate the role of PTX3 in coagulopathy in patients with COVID-19.

**Methods:** A retrospective study, including thirty-nine COVID-19 patients, enrolled in Hunan, China, were performed. The patients were classified into the D-dimer\_L (D-dimer < 1mg/L) and D-dimer\_H (D-dimer ≥ 1mg/L) groups basing on the plasma D-dimer levels on admission. Serum PTX3 levels were detected by enzyme-linked immunosorbent assays and compared between those two groups, then receiver operating characteristic (ROC) curve analysis, correlation analysis, and linear regression models were performed to analyze the association between PTX3 and D-dimer.

**Results:** Our results showed that serum PTX3 levels (median values, 10.21 vs. 3.36,  $P < 0.001$ ), computerized chest tomography (C.T.) scores (median values, 10.0 vs. 9.0,  $P < 0.05$ ), and length of stay (LOS) (mean values, 16.0 vs. 10.7,  $P = 0.001$ ) in the D-dimer\_H group were significantly higher than that in D-dimer\_L group. ROC curve analysis revealed that the AUC of white blood corpuscle counts, C-reaction protein, erythrocyte sedimentation rate, and PTX3 for COVID-19 were 0.685, 0.863, 0.846, and 0.985, respectively. Correlation analysis showed that there was a positive relationship between PTX3 and D-dimer ( $r = 0.721$ ,  $P < 0.001$ ), chest CT imaging score ( $r = 0.418$ ,  $P = 0.008$ ), and LOS ( $r = 0.486$ ,  $P = 0.002$ ). Multiple linear regression analysis showed that the coefficient of determination was 0.657 ( $P < 0.001$ ).

**Conclusion:** Serum level of PTX3 was positively correlated with disease severity and coagulopathy. Detection of serum PTX3 level could help identify severer patients on admission and may be a potential therapeutic target for coagulopathy in patients with COVID-19.

**Keywords:** Coronavirus disease 2019, Pentraxin-3, D-dimer, Coagulopathy, Disease Severity.

Correspondence to: Yimin Zhu. Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, The First-affiliated Hospital of Hunan Normal University (Hunan Provincial People's Hospital), Changsha, 410005, Hunan, China. E-mail: [zhuyimincs@outlook.com](mailto:zhuyimincs@outlook.com)

**Introduction.** In December 2019, a cluster of severe pneumonia cases of unknown cause, lately named Coronavirus disease 2019 (COVID-19), was reported in Wuhan, China. A novel strain of coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified as the pathogen.<sup>1</sup> COVID-19 can be asymptomatic or mild to severe symptomatic. As of September 1, 2020, more than 25 million cases of COVID-19 have been reported worldwide, with over 854 000 deaths, and the number is overgrowing.<sup>2</sup>

Pentraxin-3 (PTX3) is a pentraxin superfamily member and is involved in acute and chronic inflammation and innate immunity.<sup>3</sup> The level of PTX3 fluctuates with the intensity of the immune-inflammatory response.<sup>4</sup> PTX3 is also involved in endothelial dysfunction through various mechanisms and is correlated with acute pulmonary embolism-related deaths,<sup>5</sup> while endothelial dysfunction has been reported in severe COVID-19 and plays a vital role in coagulopathy.<sup>6</sup> As an indirect marker of coagulation activation, the D-dimer level greater than 1µg/mL (i.e. 1mg/L) on admission has been correlated with an increased likelihood of in-hospital death in COVID-19 patients.<sup>7</sup> The present study aimed to detect the serum level of PTX3 in different groups according to the serum level of D-dimer and to analyze the correlation of serum PTX3 levels with disease severity and coagulopathy.

**Materials and Methods.** A retrospective study was conducted. From February 1 to March 10, 2020, thirty-nine adult patients (age ≥18 years) tested positive with SARS-CoV2 of throat-swab samples were admitted into the Infectious Disease Ward in the Fourth People's Hospital of Yiyang in Hunan, China, and they were all recruited into the study. The clinical characteristics and laboratory findings of COVID-19 patients were extracted from the medical records. Upon admission, the patients underwent blood routine tests, biochemical and immunological routine tests, quantifications of plasma C-reaction protein(CRP), erythrocyte sedimentation rate(ESR) and D-dimer, and computerized chest tomography(C.T.) scanning to assess the severity of COVID-19. Diagnostic criteria for COVID-19 severity were based on the guidelines of the National Health Commission of China.<sup>8</sup> The patients were treated with inhaled interferon α-2b and oral lopinavir-ritonavir as antiviral therapies and supportive treatments. In our

setting, no patients died during the observation period.

The patients were discharged at the following conditions: the absence of fever for at least three days; significant improvement in both lungs on chest C.T. scanning; clinical remission of respiratory symptoms; repeated negative in RT-PCR test of throat-swab samples at least 24-hours interval.

The study was approved by the ethics committee of the indicated hospital by the Code of Ethics of the World Medical Association Declaration of Helsinki. Written informed consent was waived due to the nature of our retrospective study.

**Blood sample collection.** PTX3 detections were routinely performed for the patients who had been suspected of systemic infection in our hospital. In the cohort, vein blood samples for PTX3 detection were collected at admission in a fasting state, 6 a.m. the next morning after admission, and immediately centrifuged at 1500×g and stored at -80°C until thawed once and analyzed.

**Chest computerized tomography.** The chest C.T. image analysis and grading were performed by two radiologists with extensive thoracic radiology experience. The final scores and grading were determined using a scoring system described in the previous study;<sup>9</sup> the details are shown in **Table 1**.

**Enzyme-linked immunosorbent assay (ELISA).** Strictly according to the manufacturer's instructions, quantitative determination of the serum PTX3 level of one batch was performed weekly using commercially

**Table 1.** C.T. imaging performance and corresponding score system.

Number	CT Imaging performance	Score
1	unilateral patchy shadows or ground glass opacity	5
2	bilateral patchy shadows or ground glass opacity	7
3	diffuse changes for (1) or (2)	2
4	unilateral solid shadow or strip shadow	2
5	bilateral solid shadow or strip shadow	4
6	unilateral pleural effusion	2
7	bilateral pleural effusion	4
8	increased or enlarged mediastinal lymph nodes	1

available ELISA kits.

**Statistical Analysis.** Categorical variables were reported as the counts and percentages, and significance was detected by the chi-square test. The continuous variables were described using mean and standard deviation if they were normally distributed, or median and interquartile range (IQR) value if they were not normally distributed. Continuous variables were compared using independent group *t*-tests when they were normally distributed; otherwise, the Mann-Whitney *U*-test was used. Correlation analysis was performed by Pearson's correlation coefficient. Statistical analysis was performed by SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). A two-sided *P*-value < 0.05 was considered statistically significant.

## Results.

**Patient Characteristics.** According to the plasma D-dimer level on admission, the patients were divided into the D-dimer\_L group (<1mg/L) and the D-dimer\_H group (≥1mg/L). 48.7% (19 cases) were male, and there was no difference in sex ratio between the D-dimer\_L

group and the D-dimer\_H group. The patients' average age was 49.0 years old, and the median age was 49.0 and 54.0 years old in D-dimer\_L and D-dimer\_H groups, respectively (*P*=0.03). There were no significant differences in smoking, cardiovascular disease, diabetes between both groups, while significant differences in length of stay were observed (mean values, 10.7 vs. 16.0, *P*=0.001, **Table 2**).

For laboratory findings, there were no significant differences in white blood corpuscle (WBC) counts, lymphocyte counts, monocyte counts, plasma levels of low-density lipoprotein-cholesterol (LDL-C), triglycerides, and total cholesterol between both groups. In contrast, significant differences in neutrophil counts (*P*=0.002), neutrophil-lymphocyte ratio(NLR) (*P*=0.014), plasma levels of CRP, and ESR (*P*<0.01) were observed. D-dimer's median values in D-dimer\_L and D-dimer\_H groups were 0.35 vs. 4.49mg/L (*P*< 0.001, **Table 2**).

On admission, abnormalities in chest C.T. images were observed for all patients. The representative chest C.T. findings were bilateral ground-glass opacity and sub-segmental areas of consolidation. The CT imaging

**Table 2.** Clinical and laboratory findings in patients with COVID-19.

	All patients(n=39)	D-dimer_L(n=30)	D-dimer_H(n=9)	<i>p</i> -value
<b>Characteristics</b>				
Age(years)	49.0(31.0-56.0)	49.0(24.5-55.3)	54.0(47.0-74.5)	0.030
Gender				
Male(n[%])	19[48.7]	15[50.0]	4[44.4]	1.000
Female(n[%])	20[51.3]	15[50.0]	5[55.6]	1.000
<b>Chronic medical illness</b>				
CVD(n[%])	3[7.7]	1[3.3]	2[22.2]	0.127
Current Smokers(n[%])	4[10.3]	3[10.0]	1[11.1]	1.000
Diabetes(n[%])	4[10.3]	2[6.7]	2[22.2]	0.223
length of stay(days)	12.0±4.3	10.7±3.6	16.0±4.2	0.001
CT scores on admission	9(5-11)	9(5-11)	10(9-15)	0.022
<b>Laboratory Findings</b>				
Leucocytes(×10 <sup>9</sup> /L)	6.27(4.64-7.82)	5.70(4.34-7.42)	7.55(5.47-8.80)	0.096
Lymphocytes(×10 <sup>9</sup> /L)	1.16(0.84-1.68)	1.28(1.02-1.74)	1.04(0.59-1.42)	0.194
Neutrophils(×10 <sup>9</sup> /L)	3.52(2.91-4.44)	3.23(1.92-4.17)	5.20(3.66-7.19)	0.002
NLR	2.59 (1.86-4.28)	2.35(1.72-3.71)	6.19(2.79-9.79)	0.014
Monocytes(×10 <sup>9</sup> /L)	0.46(0.33-0.52)	0.46(0.35-0.51)	0.51(0.27-0.58)	0.920
LDL-C(mmol/L)	1.88(1.55-2.34)	1.85(1.55-2.35)	1.95(1.46-2.33)	0.764
Triglycerides (mmol/L)	1.09(0.93-1.58)	1.24(0.95-1.60)	0.95(0.83-1.07)	0.072
Total-cholesterol(mmol/L)	3.87±0.95	3.82±0.90	4.04±1.00	0.546
C-reaction protein(mg/L)	3.50(0.50-16.50)	1.59(0.50-5.66)	38.88(13.60-66.60)	0.001
ESR(mm/h)	22.0(12.0-39.5)	19.5(10.6-29.4)	41.7(27.0-76.8)	0.002
D-dimer(mg/L)	0.43(0.19-0.88)	0.35(0.15-0.52)	4.49(1.55-7.00)	0.000

Data are expressed as median (IQR), mean ± standard deviation or number[%]. Between group comparisons of continuous variables were analyzed by independent sample *t*-tests or Mann-Whitney *U*-tests, and comparisons of categorical variables by chi-square tests.

Abbreviations: COVID-19=coronavirus disease 2019; CVD=cardiovascular disease; NLR= neutrophil-lymphocyte ratio; LDL-C=low-density lipoprotein cholesterol; ESR=erythrocyte sedimentation rate; NLR: neutrophil-lymphocyte ratio.

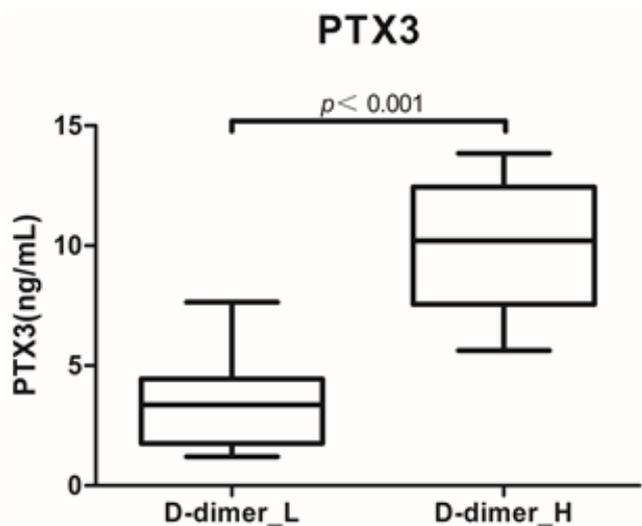
score was higher in the D-dimer\_H group than the D-dimer\_L group (median values, 10.0 vs. 9.0,  $P=0.022$ ; **Table 2**).

The serum level of PTX3 in the D-dimer\_H group was significantly higher than that in the D-dimer\_L group (median values, 10.21 vs. 3.36;  $P<0.001$ ; **Figure 1**). Correlation analysis showed that there was a positive relationship between PTX3 and D-dimer ( $r=0.721$ ,  $P<0.001$ ), chest CT imaging score ( $r=0.418$ ,  $P=0.008$ ), and LOS ( $r=0.486$ ,  $P=0.002$ ) (**Figure 2**). ROC curve analysis revealed that the area under the curve (AUC) of WBC was 0.685 (95% confidence interval (CI) 0.517 - 0.824,  $P=0.010$ ), and the optimum cutoff was  $8.57 \times 10^9/L$  (sensitivity 44.4%, specificity 90.0%). The AUC of CRP was 0.863 (95%CI 0.715 - 0.952,  $P=0.001$ ), and the optimum cutoff was 9.05 (sensitivity 88.9%, specificity 86.7%). The AUC of ESR was 0.846 (95%CI 0.695 - 0.942,  $P=0.002$ ), and the optimum cutoff was 20.17 (sensitivity 100%, specificity 53.3%). The AUC of PTX3 was 0.985 (95%CI 0.883 - 1.000,  $P<0.001$ ), and the optimum cutoff was 5.54 (sensitivity 100%, specificity 90.0%) (**Figure 3**).

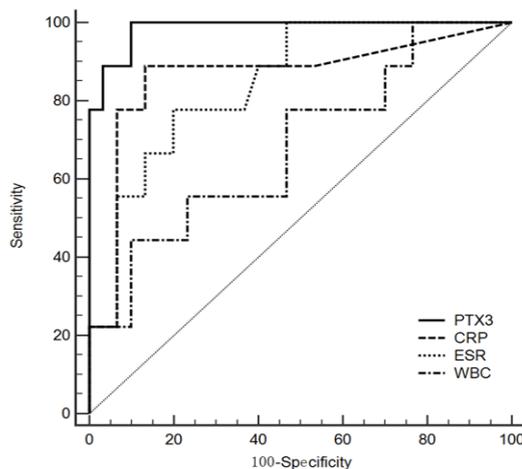
*Univariate analysis of categorical variables.* Univariate analysis of different categorical variables showed no significant group differences in plasma D-dimer levels based on stratification of gender, smokers, diabetes, and cardiovascular diseases (**Table 3**).

*Correlation analysis between continuous variables and plasma D-dimer levels.* Pearson's correlation coefficient analysis showed that there were significant correlations between WBC ( $r=0.339$ ,  $P=0.035$ ), NEU ( $r=0.565$ ,  $P<0.001$ ), NLR ( $r=0.567$ ,  $P<0.001$ ), CRP ( $r=0.625$ ,  $P<0.001$ ), ESR ( $r=0.623$ ,  $P<0.001$ ), PTX3 ( $r=0.721$ ,  $P<0.001$ ) and D-dimer, while no significant correlations between age, lymphocyte counts, monocyte counts, LDL-C, total cholesterol, triglycerides and D-dimer were observed (**Table 4**).

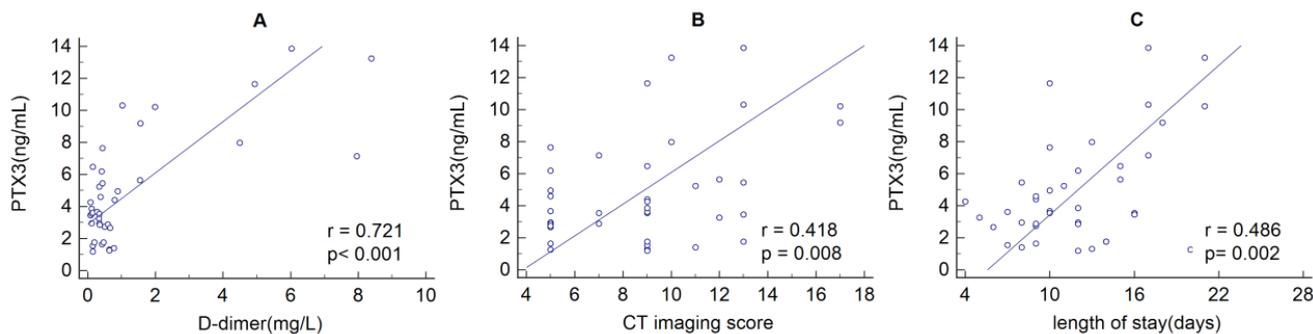
*Linear regression analysis of PTX3 and D-dimer.* D-dimer's influencing factors were analyzed with multiple linear regression models, and the results showed that PTX3 levels were found to be independently positively associated with D-dimer levels ( $\beta = 0.451$ ,  $P=0.006$ ) (**Table 5**).



**Figure 1.** Serum PTX3 levels in D-dimer\_L (D-dimer  $<1\text{mg/L}$ ) and D-dimer\_H (D-dimer  $\geq 1\text{mg/L}$ ) groups. ( $P<0.001$ ).



**Figure 3.** ROC curve for WBC, CRP, ESR, and PTX3 in COVID-19 patients. ROC curve analysis revealed that the AUC of WBC, CRP, ESR, and PTX3 for COVID-19 were 0.685, 0.863, 0.846, and 0.985, respectively.



**Figure 2.** The relationship between serum PTX3 levels and plasma D-dimer(A), chest CT imaging scores(B) and length of stay(C). There was a positive relationship between PTX3 and D-dimer ( $r=0.461$ ,  $P=0.003$ ), chest CT imaging scores( $r=0.418$ ,  $P=0.008$ ) and length of stay( $r=0.486$ ,  $P=0.002$ ).

**Table 3.** Univariate analysis of categorical variables.

Variable	Category	D-dimer	Z	P-value
gender	male	0.35(0.15,0.88)	-0.9	0.368
	female	0.45(0.23,1.37)		
smokers	no	0.42(0.15,0.77)	-1.437	0.151
	yes	0.84(0.46,6.19)		
diabetes mellitus	no	0.43(0.15,0.8)	-1.205	0.228
	yes	2.45(0.36,7.42)		
cardiovascular disease	no	0.43(0.23,0.79)	-1.293	0.196
	yes	7.96(4.06,8.18)		

There were no significant differences in plasma D-dimer levels between different groups based on the stratification of gender, smokers, diabetes mellitus, and cardiovascular disease by nonparametric Mann Whitney U-test.

**Table 4.** Correlation analysis between continuous variables and plasma D-dimer levels

Variables	r	p-value
Age(years)	0.305	0.059
WBC( $\times 10^9/L$ )	0.339	0.035
LYM( $\times 10^9/L$ )	-0.204	0.213
NEU( $\times 10^9/L$ )	0.565	0.000
NLR	0.567	0.000
MON( $\times 10^9/L$ )	-0.118	0.475
CRP(mg/L)	0.625	0.000
ESR(mm/h)	0.623	0.000
LDL-C(mmol/L)	-0.124	0.450
Total cholesterol(mmol/L)	-0.129	0.435
Triglycerides(mmol/L)	-0.254	0.118
PTX3(ng/mL)	0.721	0.000

There were significant correlations between WBC, CRP, ESR, PTX3 and D-dimer by Spearman correlation coefficient analysis.

Abbreviations: WBC=White blood corpuscle counts; LYM=lymphocyte counts; NEU=neutrophil counts; NLR=neutrophil-lymphocyte ratios; MON=monocyte counts; CRP=C-reaction protein; ESR=erythrocyte sedimentation rate; LDL-C=low-density lipoprotein cholesterol; PTX3=pentraxin-3.

**Table 5.** Multiple linear regression analysis of the relationship between laboratory findings and plasma D-dimer levels.

	B	SE	$\beta$	t	P-value
(Constant)	-0.938	0.671		-1.397	0.172
WBC	-0.094	0.166	-0.116	-0.565	0.576
NEU	0.170	0.222	0.192	0.768	0.448
CRP	0.014	0.012	0.197	1.191	0.242
ESR	0.018	0.013	0.223	1.324	0.195
PTX3	0.282	0.095	0.451	2.969	0.006
R <sup>2</sup>	0.657				
F	12.647				
p-value	0.000				

Abbreviations: WBC=White blood corpuscle counts; NEU=neutrophil counts; CRP=C-reaction protein; ESR=erythrocyte sedimentation rate; PTX3=pentraxin-3.

**Discussion.** To our knowledge, this is the first study on the role of PTX3 in coagulopathy in patients with COVID-19. In this study, we divided 39 COVID-19 patients into two groups based on the plasma D-dimer

levels on admission, and ELISA was performed to detect the serum levels of PTX3 in both groups. Our results proved that higher plasma concentrations of D-dimer were positively associated with higher serum levels of

PTX3, which were also positively associated with higher plasma levels of CRP and ESR, higher C.T. imaging scores, and longer durations of in-hospital stay, indicating that the serum levels of PTX3 may contribute to disease severity and coagulopathy in patients with COVID-19.

The spectrum of COVID-19 ranges from asymptomatic, fever and dry cough, gastrointestinal symptoms, coagulation dysfunction, to multiple organ dysfunction, and it has been described as a process of systemic inflammation and immune dysfunction, with a large amount of interleukin (I.L.)-1 $\beta$ , interferon(IFN)- $\gamma$ , tumor necrosis factor(TNF)- $\alpha$ , and other cytokines present in the system.<sup>10</sup> PTX3 is upregulated and released by hematopoietic and stromal cells in response to pro-inflammatory stimuli, such as IL-1 $\beta$  and TNF- $\alpha$ .<sup>11</sup> It is an essential component of innate immunity's humoral arm, participating in innate resistance to infections of fungal, bacterial, and viral pathogens.<sup>12</sup> Besides, PTX3 is an opsonin for pathogens, facilitating recognition and phagocytosis by neutrophils in a Complement- and Fc $\gamma$ R-dependent manner and by neutralizing virus infectivity.<sup>13</sup> Moreover, PTX3 exerts its regulatory function on the inflammatory response by modulating complement activity, recruiting inflammatory cells through interacting with the adhesion molecule P-selectin, or affecting apoptotic cells' engulfment.<sup>12</sup> Thus, PTX3 plays a vital role in innate immunity and inflammation.

Acute-phase proteins, such as CRP, serum amyloid A, and ferritin have been well investigated in patients with COVID-19,<sup>14</sup> while the role of PTX3, which is frequently used to diagnose, predict, and evaluate many inflammatory diseases,<sup>3,15</sup> has not been reported in COVID-19. Elevated PTX3 has been reported to be associated with disease severity and outcome in infectious diseases. Studies proved that PTX3 elevated significantly in hospitalized adult patients with community-acquired pneumonia,<sup>16</sup> and the admission levels of PTX3 were useful for predicting the severity of community-acquired pneumonia, independent of possible pathogens,<sup>17</sup> suggesting it could be used as a biomarker to assess disease severity and the detection of PTX3 on admission might be useful for clinical judgment. Although whether PTX3 plays a compensatory protective role or a detrimental role in COVID-19 patients is unclear, in a mouse model with a severe acute respiratory syndrome (SARS), PTX3 has been demonstrated to play a protective role in coronavirus-induced acute lung injury.<sup>18</sup> Due to the close similarity of viral genome and pathogenicity for SARS-CoV and SARS-CoV2,<sup>19</sup> it is reasonable to envisage that PTX3 may play a similar protective role in the process of immune responses, although further research is needed.

Since the outbreak of COVID-19, it was characterized as highly contagious and deadly.

Coagulopathy has been reported repeatedly in recent studies, and the high incidence of massive pulmonary embolism and deep venous thrombosis suggests a pivotal role of coagulopathy in the deaths of COVID-19.<sup>20</sup> As the smallest fibrinolysis-specific degradation product found in the circulation, D-dimer is a very sensitive biomarker for intravascular thrombus and is markedly elevated in disseminated intravascular coagulation and pulmonary embolus. It has been reported that higher concentrations of D-dimer are independently associated with in-hospital mortality in COVID-19 patients,<sup>21</sup> and the value greater than 1 mg/L helps to identify patients with poor prognosis at an early stage.<sup>7</sup> In our study cohort, serum PTX3 levels were associated with higher plasma D-dimer levels, independent of diabetes mellitus, current smoking, cardiovascular diseases, aging, and dyslipidemia, which implies that PTX3 may contribute to coagulopathy in patients with COVID-19.

Endothelial cells express angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2, and the interaction of SARS-CoV-2 and ACE2 possibly mediates endothelial activation, which has also been confirmed pathologically in patients with COVID-19.<sup>22</sup> At present, it is believed that PTX3 is dramatically related to endothelial dysfunction, and several pathogenic pathways, such as inhibition of nitric oxide (NO) and P-selectin, have been identified. NO signaling pathway plays a central role in maintaining endothelial cell functions, regulating platelet aggregation, adhesion, and clot formation.<sup>23</sup> The inhibition of PTX3 on NO synthesis leads to endothelial dysfunction, resulting in an imbalance in vascular homeostasis and a prothrombotic state.<sup>24</sup> P-selectin is a pivotal factor in the initiation of leukocyte and endothelial cell adhesion,<sup>25</sup> while the dysregulated expression of P-selectin contributes to pathological inflammation and deep vein thrombosis.<sup>26</sup> Through interacting with P-selectin, PTX3 promotes vascular inflammatory response and endothelial dysfunction.<sup>27</sup> Besides, PTX3 may influence coagulation through activating tissue factors (T.F.). T.F. is the high-affinity receptor and cofactor for FVII/VIIa. The TF-FVIIa complex is the primary initiator of blood coagulation and plays a crucial role in hemostasis. By increasing T.F. expression in endothelial cells, activated monocytes, and monocyte-derived dendritic cells, PTX3 potentially has a thrombophilic activity and plays a role in thrombogenesis.<sup>28-30</sup> In our cohort study, although apparent thrombosis formation was excluded by Doppler ultrasound in deep veins in the lower extremities and repeated chest C.T. scans, plasma D-dimer levels were elevated in nine patients with COVID-19, we speculate that the relationship between pre-thrombosis levels of D-dimer and thrombotic disease is likely attributable to microvascular thrombosis formation.<sup>31</sup>

Considering that PTX3 is produced and released

rapidly by damaged tissue cells and inflammatory cells, it could rapidly respond to the systemic inflammation at an early stage. Meanwhile, PTX3 was found to be significantly elevated in severe COVID-19 patients and independently predicted coagulation abnormalities in our study. Therefore, it is of great urgency to evaluate the potential therapeutic effects of inducers or inhibitors of PTX3, anti-PTX3 antibodies,<sup>32</sup> simvastatin,<sup>33</sup> and small interfering RNA (siRNA)<sup>34</sup> in patients with COVID-19.

Limitations should be noted when interpreting the results of this study. First, the number of patients was too small (only 39 cases), leading to statistical deviation. Second, since we did not measure the coagulation system's direct biomarkers and endothelial dysfunction, the specific disturbed pathways and mechanisms are still

unknown, which deserves further study. Third, the lack of data on longitudinal samples limited the evaluation of the prognostic value of PTX3. Fourth, the relationships between PTX3 and other inflammatory markers (such as IL-6 and fibrinogen) are unclear since those markers were untested in the study.

**Conclusions.** In conclusion, although PTX3 is likely to participate in coagulation and may serve as a therapeutic target in this direction, the role of PTX3 in COVID-19 is still unclear, and further studies are needed to determine its reliability.

**Acknowledgments.** On behalf of the authors, we sincerely thank medical staffs in The Fourth People's Hospital of Yiyang, Hunan, China.

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