

IP-10 and MCP-1 as biomarkers predicting disease severity of COVID-19

Yu Chen

Peking Union Medical College Hospital

Jinglan Wang

Peking Union Medical College Hospital

Chenxi Liu

Peking Union Medical College Hospital <https://orcid.org/0000-0001-7154-1021>

Longxiang Su

Peking Union Medical College Hospital

Dong Zhang

Peking Union Medical College Hospital

Junping Fan

Peking Union Medical College Hospital

Yanli Yang

Peking Union Medical College Hospital

Meng Xiao

Peking Union Medical College Hospital

Jing Xie

Peking Union Medical College Hospital

Yingchun Xu

Peking Union Medical College Hospital

Yongzhe Li (✉ yongzhelipumch@126.com)

Peking Union Medical College Hospital <https://orcid.org/0000-0002-0046-5276>

Shuyang Zhang

Peking Union Medical College Hospital

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Abstract

Background:

COVID-19 is a viral respiratory disease caused by the severe acute respiratory syndrome-Coronavirus type 2 (SARS-CoV-2). Patients with this disease may be more prone to venous or arterial thrombosis because of the activation of many factors involved in it, including inflammation, platelet activation and endothelial dysfunction. Therefore, this study focused on coagulation and thrombosis-related indicators (IP-10, MCP-1 and MIP1a) in COVID-19, with the hope to find biomarkers that can predict patients' outcome.

Methods:

This is a retrospective single-center study involving 74 severe and critically ill COVID-19 patients recruited from the ICU department of the Tongji Hospital in Wuhan, China. The patients were divided into two groups: severe patients and critically ill patients. The serum IP-10, MCP-1 and MIP1a level in both groups was detected using the enzyme-linked immunosorbent assay (ELISA) kit. The clinical symptoms, laboratory test results and the outcome of COVID-19 patients were retrospectively analyzed.

Results:

The serum IP-10 and MCP-1 level in critically ill patients was significantly higher than that in severe patients ($P < 0.001$). However, no statistical difference in MIP1a between the two groups was found. The analysis of dynamic changes showed that these indicators remarkably increased in patients with poor prognosis. Since the selected patients were severe or critically ill, no significant difference was observed between survival and death.

Conclusions:

IP-10 and MCP-1 are biomarkers predicting the severity of COVID-19 disease and could be related to the risk of death in COVID-19 patients. In addition, anti-IP-10 antibody treatment may represent a new approach in COVID-19 patients, especially the ones with thrombotic events.

Introduction

In December 2019, a new coronavirus pneumonia (COVID-19) originated in Wuhan, China, spreading across the country(1). On February 11, 2020, the International Virus Classification Committee announced the official name of this disease caused by a new coronavirus, such as "severe acute respiratory syndrome-Coronavirus type 2" (SARS-CoV-2)(2). The main source of infection is represented by pneumonia patients with new coronavirus infection. As of May 31, the new SARS-CoV-2 has spread to

over 200 countries and regions around the world, with more than 6 million confirmed cases reported abroad and more than 300,000 deaths worldwide, with a mortality rate of approximately 5.44%(3).

Some studies showed that 40% of COVID-19 patients are at risk of venous thromboembolism(4), and among 30 COVID-19 deaths, 46% were affected by a disseminated intravascular coagulation, indicating that the coagulation dysfunction is one of the main cause of death in severe patients with COVID-19(5). SARS-CoV-2 is mainly affecting the alveolar type II epithelial cells, lung macrophages, hilar lymph nodes, spleen and testicular tissue(6). SARS-CoV-2 invades human cells by binding the angiotensin converting enzyme 2 protein distributed on the surface of cells (7) in organs such as heart, lung, kidney, testis and digestive tract (8). As a consequence of SARS-CoV-2 infection, a massive amount of inflammatory factors is released, leading to a systemic inflammatory response syndrome(9). Therefore, the microvascular system is damaged, resulting in an abnormal activation of the coagulation system, causing systemic small vasculitis and extensive microthrombosis(6). Therefore, this study aimed to discover coagulation-related factors that can predict the prognosis of patients.

Several studies showed that IL-1 β , IL-6, FGF-2, MCP-1, CCL3 (MIP1a), and CXCL10 (IP-10) are cytokines related to thrombosis⁹⁻¹¹. Mercler et al. reported that the culture medium of pulmonary endothelial cell from patients with chronic thromboembolic pulmonary hypertension contains a higher level of FGF-2, IL-1 β , IL-6 and MCP-1(10). Mir et al. reported that MIP1a may be used as a potential biomarker to predict the risk of deep vein thrombosis in patients with glioma(11). Several studies reported that MCP-1 may be involved in the recruitment of monocytes into the arterial wall during the formation of atherosclerotic plaques (9). Elevated levels of MCP-1 were detected in patients with venous thrombosis(12). Lupieri et al. reported that improved endothelial healing is a major challenge to prevent arterial thrombosis, and IP-10 can inhibit endothelial healing(13). Since IL-1 β and IL-6 are routinely tested as indicators of inflammation in COVID patients, this study focused on IP-10, MCP-1, and MIP1a level in the blood serum.

IP-10(Interferon gamma inducible protein) is a small 10.8kD protein secreted by many cells in response to interferon-gamma (IFN γ). These cell types include monocytes, endothelial cells and fibroblasts(14). During secretion, IP-10 is cleaved into a 8.7kD bioactive protein, which acts as a chemotactic agent for T cells, NK cells, monocytes / macrophages and dendritic cells. In addition, IP-10 has antitumor activity by inhibiting bone marrow colony formation and angiogenesis. IP-10 works by binding to cell surface chemokine receptor 3 (CXCR3)(14, 15). MCP-1 (monocyte chemoattractant protein-1) is a chemokine that attracts monocytes and basophils, but not neutrophils or eosinophils. MCP-1 plays a role in the pathogenesis of diseases characterized by monocyte infiltration, such as psoriasis, rheumatoid arthritis, or atherosclerosis(16). May be involved in the recruitment of monocytes to the arterial wall in the process of atherosclerosis(17). Macrophage inflammatory protein 1-alpha (MIP1a, also known as CCL3) is a monocyte cytokine with inflammatory and chemotactic properties. MIP1a can be combined with CCR1, CCR4, and CCR5(18). In addition, it is one of the major HIV inhibitory factors produced by CD8 + t cells(19).

These cytokines were measured at different time points in each patient, with the aim to verify whether these coagulation-related factors changed over time or were related to the patient's risk of death.

Materials And Methods

Patients

This study is a retrospective single-center study involving 74 ICU patients admitted to the Tongji Hospital, Wuhan City, China, with a diagnosis of severe and critical ill COVID-19 confirmed by polymerase chain reaction (PCR). As of February 7, the ICU of this hospital has been managed by the multidisciplinary medical team of the Peking Union Medical College Hospital, and most COVID-19 patients are severe and critically ill transferred from ICU of hospitals at all levels. The distinction between severe and critically ill COVID-19 patients was realized according to the "New Coronavirus Pneumonia Diagnosis and Treatment Program (Trial Version 7)"(20). Those who meet one of the following conditions are defined as critical ill COVID-19 patients: (1) Respiratory failure occurs and mechanical ventilation is required; (2) Shock occurs; (3) Combined with failure of other organs, and ICU monitoring and treatment is required. Therefore, the patients were divided into severe patients and critically ill patients according to the above instructions. This study was approved by the Ethics Committee of the Peking Union Medical College Hospital, and the informed consent to participate to this study was provided by all the enrolled patients or their families.

Data collection

The laboratory tests, including hematologic parameters (platelet [PLT], plateletcrit [PCT], platelet distribution width [PDW], mean platelet volume [MPV] and platelet larger cell ratio [P-LCR]), routinely tested cytokines (IL-1 β , IL-2R, IL-6, IL-8, IL-10 and TNF α), and coagulation parameters (prothrombin time [PT], prothrombin activity [PTA], international normalized ratio [INR], fibrinogen [FIB], activated partial thromboplastin time [APTT], thrombin time [TT], D-dimer, fibrin degradation products [FDP], and antithrombin [AT]) in COVID-19 patients were retrospectively analyzed. All these parameters were measured according to the standard clinical laboratory methods.

Cytokine determination

Serum was obtained by centrifugation of a 5 ml whole blood sample and was stored at -80 °C until further use. The amount of three inflammatory cytokines, such as IP-10 (ab173194), MCP-1 (ab179886), and MIP1a (ab214569) (all from Abcam Ltd., Cambridge, UK) was measured in the serum using the human enzyme-linked immunosorbent assay (ELISA) kit (Abcam). The assay was performed according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 for Windows (SPSS Inc, Chicago, IL, USA). The figures were generated by GraphPad Prism 7.0 (La Jolla, CA, USA). Categorical variables were expressed as percentages, and frequency was compared using Pearson's χ^2 or Fisher's exact test. Continuous

variables were expressed as median and interquartile range (IQR) values. The comparison of continuous variables between the two groups was performed with Student's *t* test and Mann-Whitney's *U* test, and that correlation analyses were performed using Spearman correlation analysis. A *p* value less than 0.05 was considered statistically significant.

Results

Clinical analysis and laboratory examination of 74 severe and critically ill patients

The clinical analysis and laboratory tests performed on 74 severe and critically ill patients are shown in Table 1. The median age among the 74 enrolled patients was 67 years (IQR, 57-72), and the vast majority were males (45 [60.8%]). The most common symptoms were fever (63 [85.1%]), cough (59 [79.7%]) and dyspnea (46 [62.2%]). Sixty two (83.8%) patients with COVID-19 had one or more complications, and among them, the most common were hypertension (36 [48.6%]), cardiovascular disease (19 [25.7%]) and diabetes (14 [18.9%]). In terms of clinical manifestations, the proportion of males in critically ill patients was higher than that in the severe patients ($P = 0.024$), while the remaining clinical manifestations were not statistically different between the two groups. The content of both IP-10 and MCP-1 in the serum of the critically ill patients was higher than that in severe patients ($P < 0.001$). In addition, critically ill patients had a higher level of IL-6 in the serum compared to that in severe patients ($P < 0.001$). No statistical difference was found in the level of other cytokines. The hematologic indicators PLT and PCT were both lower in critically ill patients compared with severe patients ($P < 0.001$). In addition, the critically ill patients also had a significantly higher level of PDW, MPV and P-LCR compared with the level in the severe patients. Furthermore, most of the coagulation indicators were all significantly increased in critically ill patients, including PT, INR, D-dimer and FDP.

Cytokines and coagulation parameters in 74 patients with COVID-19 stratified according to high (\geq median) versus low ($<$ median) IP-10

As shown in Table 2, the IP-10 results of 74 COVID-19 patients were analyzed, grouped according to severe and critically ill, and the cutoff value was found. The sensitivity of IP-10 in the prediction of critical illness was 69.1%, the specificity was 89.5%, and the AUC was 0.806 when the Youden index was the largest. The decreased IP-10 group included 34 patients while the increased group included 40 patients. The proportion of critically ill patients in the increased group (38/40) was significantly higher than that in the decreased group (17/34) ($P < 0.001$). However, the mortality between the increased group (7/40) and decreased group (3/34) was not significantly different. The increased IP-10 group had higher IL-6, IL-8, IL-10, TNF α , PT, INR, TT, and lower PTA compared with their values in the decreased group ($P < 0.05$).

Cytokines and coagulation parameters in 73 patients with COVID-19 stratified according to high (\geq median) versus low ($<$ median) MCP-1

The MCP-1 results of 73 COVID-19 patients were analyzed, are shown in Table 3, and grouped according to severe and critically ill to find the cutoff value. The data of one patient were missing. The sensitivity of

MCP-1 in the prediction of critical illness was 78.2%, the specificity was 83.3%, and the AUC was 0.852 when the Youden index was the largest. The decreased MCP-1 group included 27 patients, while the increased MCP-1 group included 46 patients. The proportion of critically ill patients in the increased group (43/46) was significantly higher than that in the decreased group (12/27) ($P<0.001$). However, the mortality between the increased group (9/46) and decreased group (1/27) was not significantly different. The level of IL-6 increased, PT, INR, D-dimer and FDP were higher, and PTA decreased in the increased MCP-1 group compared with the decreased MCP-1 group ($P<0.05$).

Coagulation and thrombosis-related ELISA indicators in 71 patients with COVID-19 stratified according to high (\geq median) versus low ($<$ median) D-dimer

The D-dimer results of 71 COVID-19 patients were analyzed (Three patients' D-dimer results were missing). Table 4 shows the grouping according to survival and death, and Table 5 shows the grouping according to severe and critically ill patients. The cutoff value was found after grouping by survival and death, and the sensitivity of D-dimer in the prediction of critical illness was 100%, the specificity was 54.8%, and the AUC was 0.796 when the Youden index was the largest. The increased D-dimer group had a higher IP-10 and MCP-1 level compared with the decreased group ($P<0.05$), while MIP1a was not statistically different between the two groups. When grouped according to severe and critically ill, D-dimer had a sensitivity of 44.4%, a specificity of 94.1%, and an AUC of 0.722. The increased D-dimer group had higher IP-10 and MCP-1 level compared with their level in the decreased group ($P<0.05$), while MIP1a was not significantly different between the two groups.

ROC curves of IP-10, MCP-1, D-dimer and combined indicators in blood tests of COVID-19

Figure 1 showed ROC curves of IP-10, MCP-1, D-dimer and combined indicators

. The AUC for IP-10 was 0.8057, the AUC for MCP-1 was 0.8520, and the AUC for D-dimer was 0.7222. Then, we combined these three indicators to see whether the performance of the model can be improved, and found that the combined AUC of the three could reach 0.8998, proving a good application prospect of the joint detection index of the three.

Dynamic changes of coagulation and thrombosis-related ELISA indicators

Figure 2 lists the dynamic changes of *coagulation and thrombosis-related* ELISA indicators in the two outcomes after the critical illness turned into severe and the critical ill patients eventually died. Patients whose multi-point indicators were greater than three time points were selected for dynamic analysis. The overall index of coagulation and thrombosis-related ELISA indicators in the death group was higher than that in the survival group. Figure A.1 and A.2 show the dynamic changes of IP-10. When the critical illness improved to severe, the IP-10 level increased at first and then decreased after 20-30 days of the disease process in most patients. When the critical illness turned into death, the IP-10 level decreased at first and then increased in approximately 20-30 days of disease progression in most patients. Figure B.1 and B.2 show the dynamic changes of MCP-1. When the critical illness improved to severe, the MCP-1 level

gradually decreased in most patients. When the critical illness turned into death, the MCP-1 level gradually increased in most patients. Figure C.1 and C.2 show the dynamic changes of MIP1a. When the critical illness improved to severe, the MIP1a level gradually decreased in most patients. When the critical illness turned into death, the MIP1a level gradually increased in most patients.

Dynamic changes in blood coagulation indexes

Figure 3 lists the dynamic changes in blood coagulation indexes in the two outcomes when the critical illness improved to severe and the critically ill patients eventually died. Figure D.1 and D.2 show the dynamic changes of PT. When the critical illness improved to severe, the PT level increased at first and then decreased in most patients. When the critical illness turned into death, the PT level decreased at first and then increased in most patients. Figure B.1 and B.2 show the dynamic changes of INR. When the critical illness improved to severe, the INR level increased at first and then decreased of most patients. When the critical illness turned into death, the INR level decreased at first and then increased in most patients.

Correlation analysis among coagulation and thrombosis-related ELISA indicators, cytokines and coagulation-related parameters

Supplementary Table 1 lists the results of the correlation analysis among ELISA detection indexes, cytokines and coagulation parameters. Figure 4 shows the correlation matrix. A significant positive correlation was observed between IP-10 and IL-1 β , IL-6, IL-8, IL-10, TNF α , APTT, and TT ($P \leq 0.001$). MCP-1 was positively correlated only in the presence of a significant INR ($r^2 = 0.235$, $P = 0.048$). Similar to IP-10, a significant positive correlation was found between MIP1a and IL-1 β , IL-6, IL-8, IL-10, TNF α , APTT, and TT ($P \leq 0.001$). However, a significant negative correlation was observed between IP-10, MCP-1, MIP1a and PTA. These results indicated the presence of a correlation between ELISA indicators and various cytokines and coagulation indicators, with IP-10 possessing the highest correlation with IL-1 β ($r^2 = 0.804$, $P < 0.001$).

Discussion

A large amount of pathological evidence from autopsies is revealing that thrombosis is an important consequence of COVID-19 disease(21). The development of thrombosis in patients with COVID-19 is due to the fact that after the infection with the virus, the body reacts with an extreme immune response and a "cytokine storm", leading to the release of "messenger substances" that induce pneumonia. These substances are the ones causing thrombosis and blood vessel blockage(21). This work focused on the relationship between COVID-19 related pneumonia and thrombosis, by the evaluation of several parameters related to the risk of thrombosis in COVID-19 patients, and the dynamic changes of these indicators in patients with different outcomes.

In addition, our study revealed that several indicators were related to the severity of the disease, including platelet associated parameters (PLT, PCT, PDW, MPV, P-LCR), cytokine (IL-6), coagulopathy parameters

(PT, PTA, INR, D-dimer, FDP), and thrombosis-related indicators (IP-10, MCP-1).

Many clinical studies showed that COVID-19 is associated with coagulopathy, but it is different from the disseminated intravascular coagulation with normal platelets, PT and fibrinogen. A report demonstrated that the platelet count is lower in non-survivors than survivors(22), and our study confirmed this result, although we additionally demonstrated that more platelet associated parameters differed between the two groups. Some studies(23) showed that non-survivors have significantly higher levels of D-dimer and FDP, longer PT and live APTT than survivors at admission. In addition, 71.4% most of the non-survivors showed disseminated intravascular coagulation during hospitalization compared to survivors, with abnormal coagulation results in the late stage of the disease(23). Our results are consistent with these results previously published, confirming the abnormal coagulation function in COVID-19 patients. Therefore, we further evaluated the coagulation and thrombosis-related indicators in COVID-19 patients using ELISA.

Huang et al.(22) reported that patients infected with 2019-nCoV show a significant increase in serum proinflammatory cytokine levels, especially IL1 β , IFN γ , IP-10 and MCP-1, which may cause the activation of the T-helper-1 (Th1) cell response. In addition, patients who require ICU admission have higher GCSF, IP-10, MCP-1, MIP1a, and TNF α concentrations than patients who do not require ICU admission, suggesting that the cytokine storm is associated with disease severity(22). Moreover, Qin et.al(24) reported that several inflammatory cytokines such as IL-2R, IL-6, IL-8, IL-10 and TNF- α were increased in severe patients compared with their level in the non-severe patients.

Our study did not find a difference in serum IL-1 β level between severe and critically ill patients, but our results revealed a difference in IL-6 level between these two groups. IL-6 is a potent inducer of the acute phase response. Indeed, it is an endogenous pyrogen mainly produced in the acute and chronic inflammatory sites, causing fever in people with autoimmune diseases or infections. IL-6 is then secreted into the serum to induce transcriptional inflammation through the interleukin 6 receptor alpha. Furthermore, increased IL-6 can cause a cytokine storm(25, 26).

IP-10 and MIP1a, as well as IL-1 β , IL-6, IL-8, IL-10, and TNF α , are also inflammatory cytokines, and therefore showed a strong positive correlation with each other. IP-10, MCP-1, and MIP1a are parameters related to thrombosis, thus having a significant correlation with the coagulation parameters.

The level of both IP-10 and MCP-1 was higher in critically ill patients than that in serious patients. Therefore, in this study, the 74 enrolled patients were divided according to the level of IP-10 and MCP-1. Our results showed an increased IL-6 level in the IP-10 + MCP-1 increased group compared to the IP-10 + MCP-1 decreased group. PT, INR increased, and PTA decreased in the IP-10 + MCP-1 increased group compared to the IP-10 + MCP-1 decreased group, also confirming the previous statement. Moreover, the proportion of critically ill patients in the IP-10 + MCP-1 increased group was higher than that in the IP-10 + MCP-1 decreased group, further indicating that IP-10 and MCP-1 are biomarkers for predicting the severity of COVID-19 disease. When the IP-10 and MCP1 level was compared between the survival group and the death group, no significant difference was found, which might be due to the fact that the selected

patients were severe or critically ill, resulting in a too high mortality rate, with no difference between survival and death. In addition, several previous studies in Wuhan showed that the D-dimer level in non-survivors are higher than that in survivors(23, 27), suggesting that the increased D-dimer level is an independent risk factors of death in COVID-19 patients (28). Therefore, patients were grouped according to the D-dimer level and the results showed that regardless of the clinical feature, the increased D-dimer group had higher IP-10 and MCP-1 level than the decreased group, while MIP1a was not statistically significant between the two groups. Our further speculation was that IP-10 and MCP-1 could be related to the risk of progress to death in COVID-19 patients.

A report demonstrated that CXCL10 (IP-10) inhibits endothelial recovery independently of any other inflammatory factor, and anti-CXCL10 antibody is under validation in a clinical trial to prevent cardiovascular events (13) because the more severe the COVID-19 patient is, the higher the serum IP-10 level is. Therefore, anti-IP-10 antibody treatment may represent a new approach in COVID-19 patients, especially the ones with thrombotic events.

Patients whose multi-point indicators were greater than three time points were selected for dynamic analysis. The analysis of dynamic changes revealed that the overall index of the death group was higher than that in the survival group. In addition, the indicators remarkably increase in patients with a poor outcome, while some indicators decreased in a later time, suggesting a disease change to a pathophysiological model, although further studies are needed to explain this phenomenon.

This is the first study comparing the coagulation and thrombosis-related ELISA indicators, platelet-related parameters, routinely tested cytokines and coagulation indicators according to the guidelines when serious and critically ill patients are grouped. Furthermore, this study compared the dynamic changes of multiple indicators in the serum of patients with multi-point detection.

This study is a single-center retrospective study, thus these results might not be representative, in addition to the fact that all the included patients were severe and critically ill. Thus, these results could not be compared with the results in mild patients.

However, there are some limitations in the present study. First, the sample size is small due to the limited time and number of patients allocated to PUMCH. Second, only patients with more than three measurements were included in the dynamic changes analysis. Although the more time-points available, the better characterization of dynamics over time is allowed, this approach leaves only 14 patients for the analysis, and it may introduce some bias, as the patients who had blood samples obtained most frequently may also be the most critically ill and may thus not be representative for the entire cohort. More multi-center studies are needed in the future to verify these results and for a comprehensive interpretation of the clinical results.

In conclusion, the level of both IP-10 and MCP-1 in the serum of critically ill patients was higher than that in severe patients, proving that IP-10 and MCP-1 are biomarkers predicting the severity of COVID-19 disease. Moreover, IP-10 and MCP-1 level increased in the D-dimer increased group compared with the

decreased group, suggesting that IP-10 and MCP-1 could be related to the risk of death in COVID-19 patients. Thus, anti-IP-10 antibody treatment may represent a new approach in COVID-19 patients, especially the ones with thrombotic events. However, since the selected patients were severe or critically ill, the results did not show any difference between survival and death, suggesting the need of further research.

Declarations

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Declaration of interests

We declare on conflicts of interest.

Abbreviations

Severe acute respiratory syndrome-Coronavirus type 2	SARS-CoV-2
enzyme-linked immunosorbent assay	ELISA
interferon-gamma	IFN γ
cell surface chemokine receptor 3	CXCR3
monocyte chemoattractant protein-1	MCP-1
Macrophage inflammatory protein 1-alpha	MIP1a
polymerase chain reaction	PCR
platelet	PLT
plateletcrit	PCT
platelet distribution width	PDW
mean platelet volume	MPV
platelet larger cell ratio	P-LCR
prothrombin time	PT
prothrombin activity	PTA
international normalized ratio	INR
fibrinogen	FIB
activated partial thromboplastin time	APTT
thrombin time	TT
fibrin degradation products	FDP
antithrombin	AT
interquartile range	IQR

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Tables

Table 1. Clinical characteristics and laboratory parameters of 74 severe and critically ill patients.

Clinical characteristics	Total (N=74)		Severe patients, N (%)		Critically ill patients, N (%)		P
Age [median(IQR),years]	67(57-72)		60(55-73)		66(59-71)		0.176
Gender							
Male	45(60.8%)		8(42.1%)		37(67.3%)		0.024
Female	29(39.2%)		11(57.9%)		18(32.7%)		0.053
Common symptoms							
Fever	63(85.1%)		16(84.2%)		47(85.5%)		0.895
Cough	59(79.7%)		14(73.7%)		38(69.1%)		0.706
Dyspnoea	46(62.2%)		11(57.9%)		36(65.5%)		0.555
Comorbidity	62(83.8%)						
Hypertension	36(48.6%)		8(42.1%)		28(50.9%)		0.508
Diabetes	14(18.9%)		5(26.3%)		9(16.4%)		0.34
Cardiovascular disease	19(25.7%)		5(26.3%)		14(25.5%)		0.941
	Total (N=74)		Severe patients (N=19)		Critically ill patients (N=55)		P
Laboratory findings	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	
ELISA							
IP-10, pg/mL	74	364.8(203.7-939.4)	19	193.1(123.0-300.5)	55	531.3(278.0-1217.3)	<0.001
MCP-1,pg/mL	73	642.2(293.2-1207.5)	18	230.7(137.9-338.7)	55	837.0(425.6-1374.0)	<0.001
MIP1a, pg/mL	74	28.6(15.2-79.7)	19	25.5(13.8-86.1)	55	30.6(16.4-77.6)	0.338
Cytokines							
IL-1 β , pg/mL	29	5.0(5.0-5.0)	4	5.0(5.0-5.0)	25	5.0(5.0-5.9)	0.284
IL-2R, U/mL	30	1055.5(464.5-1609.5)	5	740.0(325.0-1314.5)	25	1258.0(518.5-1820.0)	0.331
IL-6, pg/mL	48	74.2(17.0-157.5)	13	10.4(5.4-31.1)	35	103.0(43.0-323.2)	<0.001
IL-8, pg/mL	29	51.7(12.5-114.7)	4	11.1(8.1-60.8)	25	62.3(19.9-142.5)	0.077
IL-10, pg/mL	29	11.4(5.0-20.1)	4	5.0(5.0-8.0)	25	11.9(5.0-25.1)	0.049
TNF α , pg/mL	27	12.7(7.5-28.7)	4	7.8(6.4-9.1)	23	13.3(7.5-30.3)	0.414
Hematologic parameters							
Platelets, $\times 10^9$ /mL	72	132.5(70.8-232.0)	17	254.0(158.5-370.5)	55	88.0(56.0-191.0)	<0.001
Platelet distribution width (PDW), fL	64	14.4(12.9-16.6)	17	13.4(11.5-14.4)	47	14.5(13.1-18.6)	<0.001
Mean platelet volume (MPV), fL	64	11.6(11.0-12.6)	17	11.2(10.3-11.7)	47	11.9(11.2-13.1)	0.005
Platelet larger cell ratio (P-LCR), %	64	37.9(33.1-45.2)	17	33.2(26.9-38.2)	47	40.3(34.2-47.6)	0.003
Plateletcrit (PCT), %	64	0.19(0.10-0.26)	17	0.28(0.19-0.37)	47	0.13(0.08-0.23)	<0.001
Coagulation function							
Prothrombin time (PT), s	71	15.9(14.9-18.2)	17	14.9(14.0-15.6)	54	16.9(15.5-18.5)	<0.001
Prothrombin activity (PTA), %	71	69.0(55.0-80.0)	17	80.0(72.5-89.0)	54	63.0(53.3-72.5)	<0.001
International normalized ratio (INR)	71	1.26(1.15-1.49)	17	1.15(1.07-1.23)	54	1.36(1.22-1.54)	<0.001
Fibrinogen (FIB), g/L	71	4.2(3.1-5.2)	17	4.2(3.5-4.9)	54	4.2(3.0-5.3)	0.845
Activated partial thromboplastin time (APTT), s	71	44.5(39.3-52.6)	17	41.5(37.0-46.6)	54	46.4(40.7-53.9)	0.089
Thrombin time (TT), s	71	15.3(14.5-16.5)	17	15.0(14.3-15.7)	54	15.5(14.7-16.9)	0.083
D-dimer, μ g/mL FEU	71	3.9(1.7-13.5)	17	2.4(1.3-3.6)	54	6.2(2.3-15.2)	0.006
Fibrin degradation products (FDP), μ g/mL	30	17.2(6.2-68.6)	6	4.9(4.0-7.8)	24	33.6(13.6-102.6)	0.003
Antithrombin (AT), %	34	80.5(65.5-88.5)	8	85.0(74.3-93.0)	26	78.5(63.0-86.5)	0.219

Table 2. Cytokines and coagulation parameters in 74 COVID-19 patients stratified according to high (\geq median) versus low ($<$ median) IP-10.

	Total (N=74)		IP-10				P
			low (N=34)		high (N=40)		
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	
IL-1 β , pg/mL	29	5.0(5.0-5.0)	11	5.0(5.0-5.0)	18	5.0(5.0-7.3)	0.253
IL-2R, U/mL	30	1055.5(464.5-1609.5)	12	784.0(362.8-1256.3)	18	1311.5(721.0-1957.0)	0.099
IL-6, pg/mL	48	74.2(17.0-157.5)	23	26.3(9.6-95.9)	25	114.8(54.6-343.1)	0.001
IL-8, pg/mL	29	51.7(12.5-114.7)	11	19.5(10.1-51.7)	18	78.8(22.3-156.0)	0.018
IL-10, pg/mL	29	11.4(5.0-20.1)	11	5.0(5.0-9.0)	18	16.8(8.5-36.4)	0.001
TNF α , pg/mL	27	12.7(7.5-28.7)	10	8.6(5.5-10.7)	17	16.2(8.9-37.1)	0.021
Prothrombin time (PT), s	71	15.9(14.9-18.2)	32	15.6(14.7-17.3)	39	16.9(15.3-19.0)	0.029
Prothrombin activity (PTA), %	71	69.0(55.0-80.0)	32	71.5(60.0-80.8)	39	63.0(51.0-74.0)	0.025
International normalized ratio (INR)	71	1.26(1.15-1.49)	32	1.24(1.14-1.41)	39	1.36(1.20-1.58)	0.026
Fibrinogen (FIB), g/L	71	4.2(3.1-5.2)	32	4.0(3.5-4.7)	39	4.6(2.5-5.7)	0.675
Activated partial thromboplastin time (APTT), s	71	44.5(39.3-52.6)	32	43.0(37.5-50.2)	39	46.8(40.7-57.4)	0.069
Thrombin time (TT), s	71	15.3(14.5-16.5)	32	15.1(14.4-15.8)	39	15.8(14.9-17.7)	0.016
D-dimer, μ g/mL FEU	71	3.85(1.68-13.46)	32	2.52(1.32-8.56)	39	5.73(2.33-18.00)	0.056
Fibrin degradation products (FDP), μ g/mL	30	17.2(6.2-68.6)	13	9.4(4.0-52.3)	17	17.7(13.6-130.5)	0.089
Antithrombin (AT), %	34	80.5(65.5-88.5)	16	83.0(66.8-92.3)	18	78.5(62.5-85.8)	0.48

Table 3. Cytokines and coagulation parameters in 73 COVID-19 patients stratified according to high (\geq median) versus low ($<$ median) MCP-1.

	Total (N=73)		MCP-1				P
			low (N=27)		high (N=46)		
	n	Median (IQR)	n	Median (IQR)	n	Median (IQR)	
IL-1 β , pg/mL	29	5.0(5.0-5.0)	8	5.0(5.0-5.0)	21	5.0(5.0-5.9)	0.491
IL-2R, U/mL	29	1059.0(460.0-1642.0)	8	1075.0(382.0-1674.5)	21	1059.0(518.5-1754.0)	0.391
IL-6, pg/mL	48	74.2(17.0-157.5)	20	19.1(8.7-93.1)	28	114.3(38.2-353.1)	0.001
IL-8, pg/mL	29	51.7(12.5-114.7)	8	20.3(10.6-30.6)	21	77.0(16.2-163.0)	0.064
IL-10, pg/mL	29	11.4(5.0-20.1)	8	5.0(5.0-16.4)	21	11.7(5.4-31.6)	0.095
TNF α , pg/mL	27	12.7(7.5-28.7)	8	8.6(6.4-13.8)	19	13.3(7.9-30.5)	0.27
Prothrombin time (PT), s	71	15.9(14.9-18.2)	26	15.0(14.2-16.1)	45	17.0(15.6-19.0)	0.001
Prothrombin activity (PTA), %	71	69.0(55.0-80.0)	26	77.5(68.3-88.0)	45	62.0(51.0-71.5)	0.001
International normalized ratio (INR)	71	1.26(1.15-1.49)	26	1.17(1.09-1.28)	45	1.37(1.23-1.58)	0.001
Fibrinogen (FIB), g/L	71	4.2(3.1-5.2)	26	4.1(3.4-5.1)	45	4.4(3.0-5.3)	0.878
Activated partial thromboplastin time (APTT), s	71	44.5(39.3-52.6)	26	43.3(37.9-50.6)	45	45.4(40.0-56.1)	0.228
Thrombin time (TT), s	71	15.3(14.5-16.5)	26	15.2(14.5-16.1)	45	15.4(14.6-17.6)	0.316
D-dimer, μ g/mL FEU	71	3.85(1.68-13.46)	26	2.03(1.25-5.48)	45	6.30(2.52-15.76)	0.005
Fibrin degradation products (FDP), μ g/mL	30	17.2(6.2-68.6)	9	5.7(4.0-38.7)	21	32.6(13.6-130.5)	0.019
Antithrombin (AT), %	34	80.5(65.5-88.5)	11	84.0(78.0-93.0)	23	75.0(60.0-86.0)	0.12

Table 4. Coagulation and thrombosis-related ELISA indicators in 71 COVID-19 patients stratified according to high (\geq median) versus low ($<$ median) D-dimer, grouped according to survival and death.

	Total (N=71) Median (IQR)	D-dimer		<i>P</i>
		Low (N=34) Median (IQR)	High (N=37) Median (IQR)	
IP-10	367.0(207.2-906.8)	267.0(167.2-433.0)	643.4(291.1-1217.5)	0.009
MCP	646.6(291.6-1219.8)	355.0(199.7-879.8)	837.0(463.2-1836.7)	0.001
MIP1a	28.7(15.2-77.6)	29.0(15.1-75.9)	28.4(16.0-81.8)	0.822

Table 5. Coagulation and thrombosis-related ELISA indicators in 71 COVID-19 patients stratified according to high (\geq median) versus low ($<$ median) D-dimer, grouped according to severe and critically ill.

	Total (N=71) Median (IQR)	D-dimer		<i>P</i>
		Low (N=40) Median (IQR)	High (N=31) Median (IQR)	
IP-10	367.0(207.2-906.8)	294.1(179.6-530.5)	650.1(300.5-1217.8)	0.014
MCP-1	646.6(291.6-1219.8)	363.3(249.3-979.0)	739.5(500.7-1753.8)	0.008
MIP1a	28.7(15.2-77.6)	29.9(15.2-93.6)	24.4(15.2-63.1)	0.527

Figures

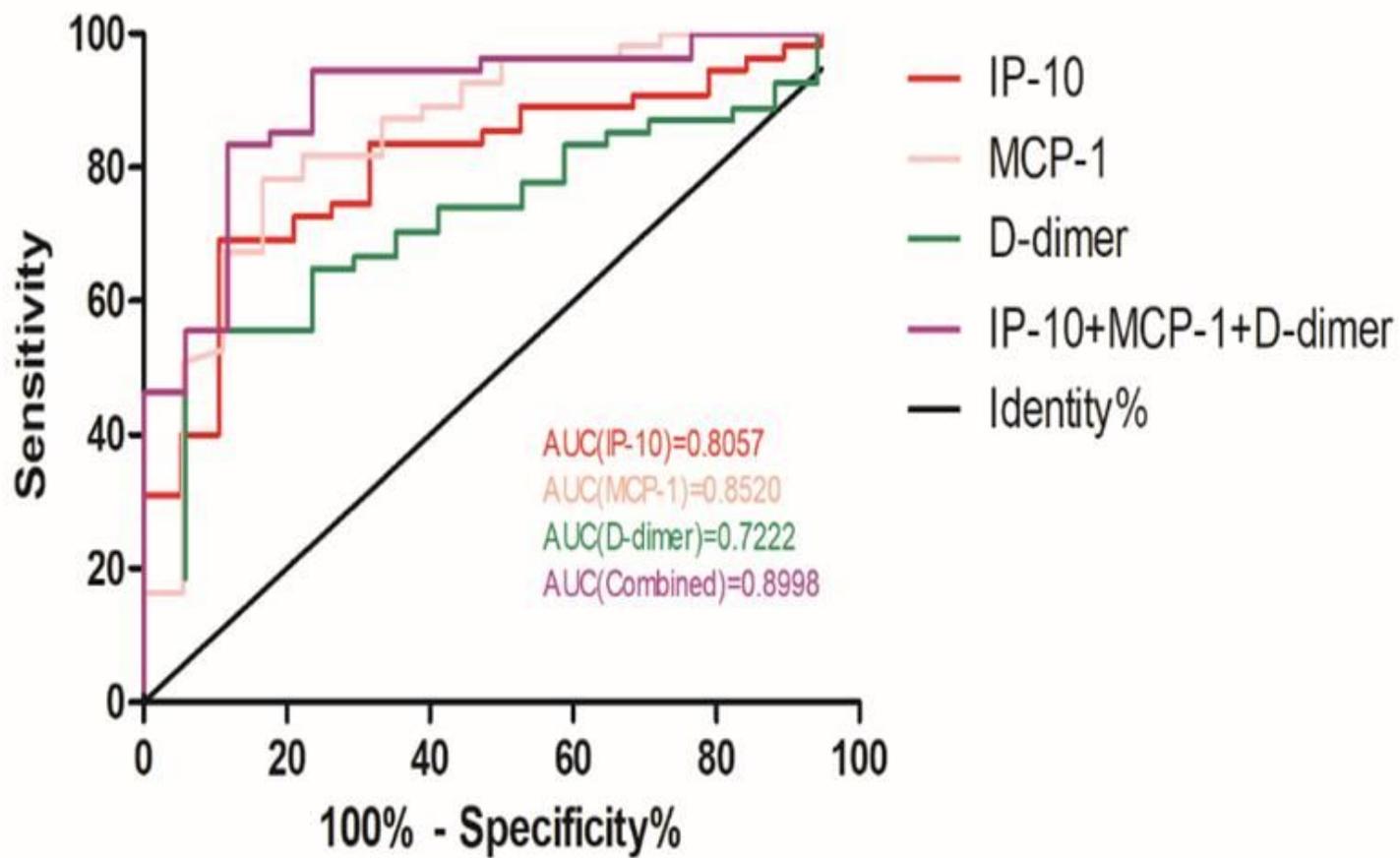


Figure 1

ROC curves of IP-10, MCP-1, D-dimer and combined indicators in blood tests of COVID-19.

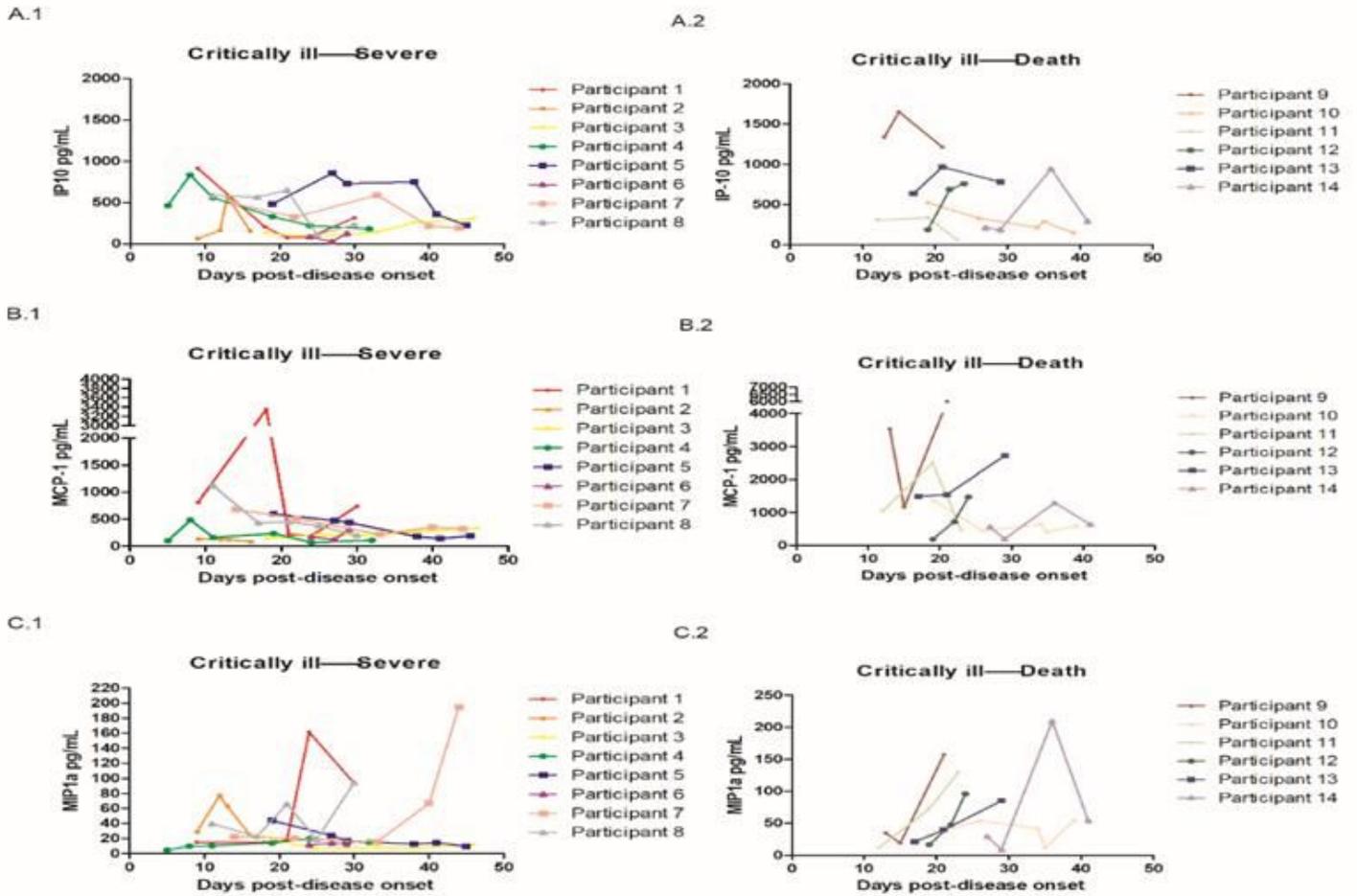
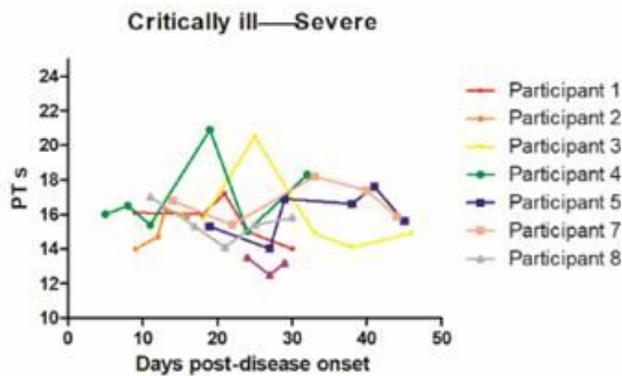


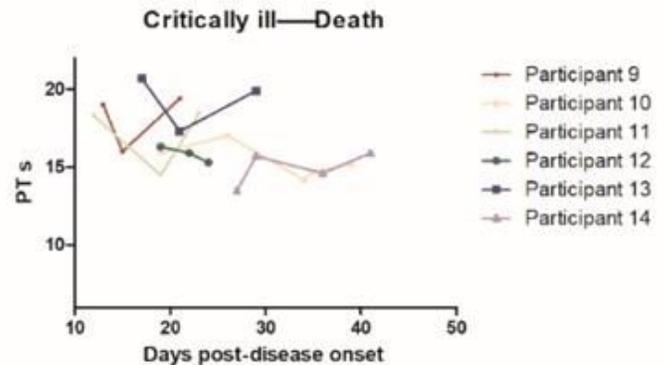
Figure 2

Dynamic changes of coagulation and thrombosis-related indicators. Dynamic changes of coagulation and thrombosis-related indicators in the two outcomes after the critical illness turned into severe and the critically ill patients eventually died. A.1 and A.2. Dynamic changes of IP-10: when the critical illness improved to severe, the IP-10 level in most patients increased at first and then decreased along the 20-30 days of the disease. When the critical illness turned into death, the IP-10 level in most patients decreased at first and then increased in approximately 20-30 days of disease progression. B.1 and B.2. Dynamic changes of MCP-1: when the critical illness improved to severe, the MCP-1 level gradually decreased in most patients. When the critical illness turned to death, the MCP-1 level gradually increased in most patients. C.1 and C.2. Dynamic changes of MIP1a: when the critical illness improved to severe, the MIP1a level gradually decreased in most patients. When the critical illness turned into death, the MIP1a level gradually increased in most patients.

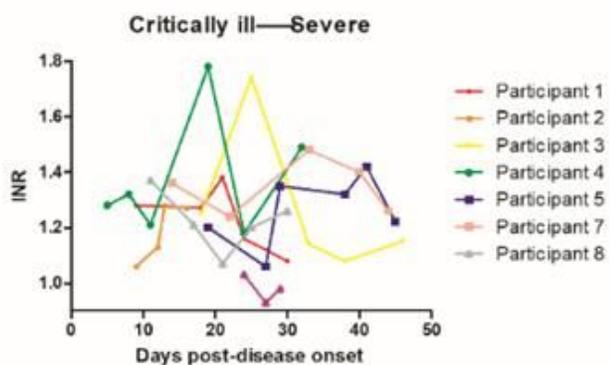
D.1



D.2



E.1



E.2

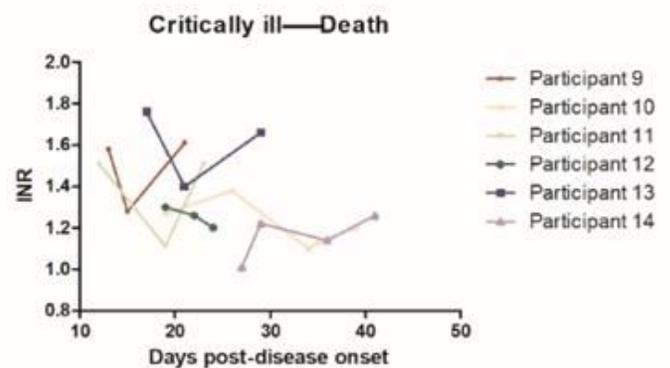


Figure 3

Dynamic changes of coagulation indexes. Dynamic changes in blood coagulation indexes in the two outcomes when the critical illness improved to severe and the critical ill patients eventually died. D.1 and D.2. Dynamic changes of PT: when the critical illness improved to severe, the PT level increased at first and then decreased in most patients. When the critical illness turned into death, the PT level decreased at first and then increased in most patients. E.1 and E.2. Dynamic changes of INR: when the critical illness improved to severe, the INR level increased at first and then decreased in most patients. When the critical illness turned into death, the INR level decreased at first and then increased in most patients.

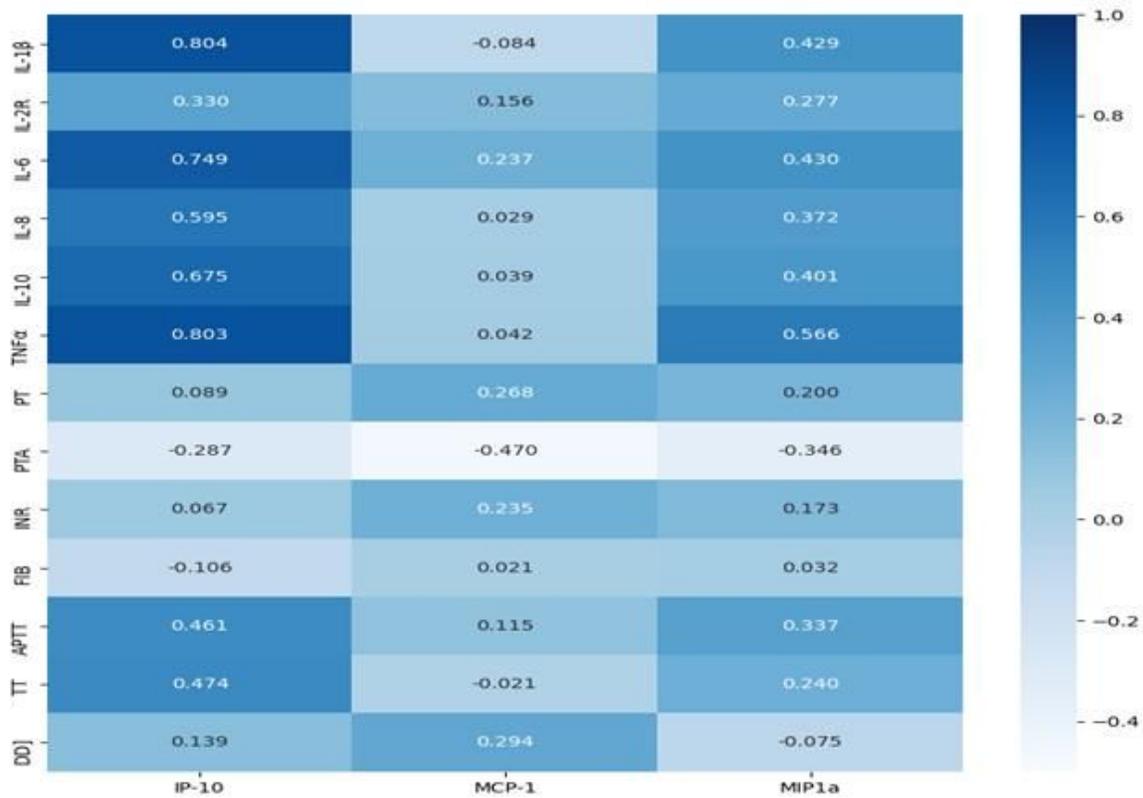


Figure 4

Correlation analysis among coagulation and thrombosis-related ELISA indicators, cytokines and coagulation-related parameters.

Supplementary Files

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- [Supplementarymaterials.docx](#)