Increased serum thromboxane A2 and prostacyclin but lower complement C3 and C4 levels in COVID-19: associations with chest CT-scan anomalies and lowered peripheral oxygen saturation

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Abstract

Background. COVID-19 patients suffer from hypercoagulation and activated immune-inflammatory pathways. This study was performed to assay serum complement C3 and C4, and thromboxane A2 (TxA2) and prostacyclin (PGI2) in association with chest CT scan anomalies (CCTAs) and peripheral oxygen saturation (SpO2)

Methods. Serum levels of C3, C4, TxA2, and PGI2 were measured by ELISA and albumin, calcium, and magnesium by spectrophotometric method in 60 COVID-19 patients and 30 controls.

Results. C3 and C4 are significantly decreased and TxA2 and PGI2 significantly increased in COVID-19 patients as compared with controls. Neural networks showed that a combination of C3, albumin, and TxA2 yielded a predictive accuracy of 100% in detecting COVID-19 patients. SpO2 was significantly decreased in COVID-19 patients and was inversely associated with TxA2 and PGI2, and positively with C3, C4, albumin, and calcium. CCTAs were accompanied by lower SpO2 and albumin, and increased PGI2 levels. Patients with positive IgG results show significantly higher SpO2, TxA2, PGI2, and C4 levels than IgG negative patients.

Conclusion. Hypoalbuminemia, which is strongly associated with lung lesions and lowered peripheral oxygen saturation, is characterized by increased TxA2, suggesting that interactions between immune-inflammatory pathways and platelet hyperactivity participate in the pathophysiology of COVID-19 and consequently may play a role in

enhanced risk of hypercoagulability and venous thromboembolism. These mechanisms are aggravated by lowered calcium and magnesium levels.

Introduction

The clinical spectrum of SARS-CoV-2 infection ranges from asymptomatic infection to mild upper respiratory tract disease to severe viral pneumonia with respiratory failure and even death [1]. COVID-19 patients frequently suffer from major symptoms, including acute respiratory distress syndrome (ARDS), as well as inflammation with a possible cytokine storm, hypercoagulation, and thrombosis [2-4].

The COVID-19 virus enters the lung cells after binding of viral Spike proteins-S with the ACE2 receptors [5] and, consequently, the virus may cause histopathological lesions, which appear to be similar to those observed in other forms of ARDS [6]. Coronaviruses activate complement pathways which are a major component of innate immunity and act to remove invading pathogens [7]. Complement activation may result in immune-mediated lung damage [8] and is central to the pathophysiology of ARDS [9] and severe COVID-19 disease, which often resembles complementopathies [7]. Activation of the complement system leads to proteolytic cleavage of the key complement molecules C3 and C4 [10] leading to cleavage products including C3a, C3b, C4a, and C4b, which may trigger inflammatory cell recruitment and neutrophil activation [11]. Ghazavi et al. (2020) detected increased C3 and C4 complement levels in non-severe COVID-19, but lowered levels in severe COVID-19 patients which could be explained by increased consumption through formation of immune complexes [12]. Low serum C3 levels are detected in critically ill COVID-19 patients and are associated with a poor prognosis [13].

The severity of COVID-19 symptoms including end-organ damage is caused by an overzealous inflammatory response in part associated with complement activation, endothelial injury, neutrophil activation, thrombophilia, hypercoagulability, and thrombotic microangiopathy [7, 14-16]. About one third of ICU patients with COVID-19 have thrombotic complications, of which venous thromboembolic events are the most common [17]. The association between complement activation and coagulation mechanisms may cause life-threatening complications and as such the complement-coagulation network is an important drug target [18]. Nevertheless, only few studies in COVID-19 focused on C3 and C4 levels in relation to thromboxane A2 (TxA2) and prostacyclin (PGI2).

Endogenous TxA_2, which is synthesized from arachidonic acid via cyclooxygenase (COX)-1, COX-2, and TxA2 synthase (TxAS), is produced by activated platelets and exerts prothrombotic effects [19]. TxA2 binds to the prostanoid thromboxane receptor, which triggers the binding to G-proteins thereby mediating calcium signaling and facilitating platelet aggregation and vasoconstriction [20, 21]. COX-1, constitutively expressed in platelets, is a dominant source of TxA2 biosynthesis in humans [22]. In COVID-19, interleukin-1 (IL-1), a pro-inflammatory cytokine, stimulates TxA2 production [23]. PGI2 is mainly produced by endothelial and vascular smooth muscle cells [24] via COX-2 [25]. While TxA2 production causes platelet aggregation and vasoconstriction, PGI2 inhibits

platelet aggregation and induces vascular smooth muscle relaxation and endothelium-related vasodilation [26-28]. The endothelial dysfunction following SARS-CoV-2 infection may be caused by lowered endothelial nitric oxide synthase activity and nitric oxide production and VEGF release following systemic hypoxia, whilst PGI2 may enhance angiogenesis and tissue repair through increased VEGF [29, 30].

Recently, we published that chest CT abnormalities (CCTAs) (comprising ground-glass opacities (GGOs), pulmonary densification areas consistent with residual lesions, pneumonic consolidation, and crazy-paving patterns), could be observed in 78.3% of RT-PCR test–proven COVID-19 cases and that the presence of CCTAs was characterized by significantly lowered peripheral oxygen saturation (SpO2) and serum levels of albumin [31]. The latter is a negative acute phase protein which is lowered in response to the systemic inflammatory response in COVID-19 [1, 31-33]. Moreover, lowered SpO2 values were significantly associated with signs of immune activation, and positively with albumin, magnesium, and calcium [31]. In addition, the latter study found that lowered serum calcium was the single best biomarker of acute COVID-19 and was more important than inflammatory biomarkers including interleukin-6 (IL-6) and C-reactive protein (CRP) in discriminating COVID-19 patients from healthy controls. We have argued that beta coronavirus-mediated calcium dyshomeostasis is due to a) hypoalbuminemia with around 45% of calcium being bound to albumin [34]; and b) to activation of store-operated calcium entry (SOCE) channels by endoplasmic-reticulum stress [35, 36], which is a consequence of infections with those viruses [37, 38]. Magnesium has antioxidant [39], anti-inflammatory [40] and antithrombotic [41] properties with about one third of total magnesium levels being bound to albumin [42].

The present study was conducted to examine the associations between immune-inflammatory (as measured with albumin, C3 and C4) and thrombosis-related (TxA2 and PGI2) biomarkers in relation to SpO2 and CCTAs

Subjects and Methods

Subjects

The present study recruited sixty patients with confirmed acute SARS-CoV-2 infection and 30 normal controls. The patients were recruited at the Al-Amal Specialized Hospital for Communicable Diseases and Al-Sadr Teaching Hospital in Najaf governorate-Iraq between September and November 2020. The diagnosis of SARS-CoV-2 infection was based on positive test results of COVID-19 nucleic acids by reverse transcription real-time polymerase chain reaction (rRT-PCR) and positive IgM to SARS-CoV-2, and symptoms of acute infectious disease including fever, fatigue, dyspnea, dry cough, dysgeusia, and anosmia. Patients were excluded for the presence of premorbid medical disease including diabetes, liver disease, chronic kidney disease, neurodegenerative and neurologic disorders including multiple sclerosis, and Parkinson's and Alzheimer's disease.

Chest computed tomography (CT) scans were used to examine chest CT abnormalities (CCTAs), comprising GGOs, pulmonary densification areas consistent with residual lesions, pneumonic consolidation, and crazy-paving patterns [43]. We divided the patient group into those with (COVID+CCTA) and without (COVID-CCTA) CCTAs. The patients were further divided according to IgG results into negative-IgG and positive-IgG

subgroups to examine the difference in the measured biomarkers between these subgroups.

We also recruited 30 healthy controls, age, and sex-matched to the patient groups. All controls were free from any systemic disease. However, as a public method to enhance their immunity against COVID-19 infection, some healthy controls were taken zinc and vitamins C and D.

The IRB of the University of Kufa has approved the study (617/2020). All controls and patients gave written informed consent before participation in this study. The study was conducted according to Iraq and international ethics and privacy laws and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Furthermore, our IRB follows the International Guideline for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Measurements

Upon admission of the patient into hospital, RT-PCR tests were conducted using the Lyra® Direct SARS-CoV-2 Assay kits (Quidel Corporation, CA, USA) using the Applied Biosystems® QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific; Life Technologies Holdings Pte Ltd., Marsiling Industrial Estate, Singapore). The Lyra® Direct SARS-CoV-2 assay kit is a real-time RT-PCR assay for the qualitative detection of human coronavirus SARS-CoV-2 from viral RNA extracted from nasal, nasopharyngeal or oropharyngeal swab specimens.

Upon inclusion in the study, fasting blood samples were taken in the early morning directly after awakening. Five milliliters of venous blood samples were drawn and transferred into clean plain tubes. After ten minutes, the clotted blood samples were centrifuged for five minutes at 3000 rpm, and then serum was separated and transported into three new Eppendorf tubes until assay. Hemolyzed samples were rejected. Serum C3, C4, PGI2, and TxA2 were measured using ELISA techniques based on sandwich technique using ready-for-use kits supplied by Melsin Medical Co (Jilin, China). The inter-assay CV values of all assays were less than 12%. Serum albumin, magnesium, and total calcium were measured spectrophotometrically by kits supplied by Biolabo® (Maizy, France). The procedures were followed exactly without modifications according to the manufacturer's instructions. A qualitative ACON® COVID-19 IgG/IgM rapid test was used to detect IgG and IgM in all subjects' sera. The kits have a sensitivity ≥ 99.1 % and a specificity ≥ of 98.2 %.

Statistical analysis

The group-differences among continuous variables were examined by analysis of variance (ANOVA), while associations between nominal variables were checked using analysis of contingency tables (χ^2-test). Pearson's product-moment and Spearman's rank-order correlation coefficients were used to determine the correlations between biomarkers and clinical and cognitive scores. To assess the associations between diagnosis and biomarkers, we used multivariate general linear models (GLM) while adjusting for confounding variables such as tobacco use disorder (TUD), age, sex, and BMI. Consequently, we used tests for between-subject effects to determine the relationships between diagnosis and the separate biomarkers. The effect size was estimated using partial

eta-squared values. We also computed estimated marginal mean (SE) values provided by the GLM analysis and performed protected pairwise comparisons among treatment means. Binary logistic regression analysis was employed to determine the best predictors of COVID-19 versus the control group. Odd's ratios with 95% confidence intervals were computed as well as Nagelkerke values which were used as pseudo-R^2 values. We used multiple-regression analysis to delineate the significant biomarkers predicting symptom domains while allowing for the effects of age, gender, and education. All regression analyses were tested for collinearity using tolerance and VIF values. All tests were two-tailed, with a p-value of 0.05 used to determine statistical significance.

Neural network analysis was conducted with diagnosis (COVID-19 *versus* controls) as output variables and biomarkers as input variables as explained previously [44]. In brief: an automated feed-forward architecture, multilayer perceptron neural network model was employed to check the associations between biomarkers (input variables) and the diagnosis of COVID-19 *versus* controls (output variables). We trained the model with two hidden layers with up to 4 nodes in each layer, 20-50 epochs, minibatch training with gradient descent. One consecutive step with no further decrease in the error term was used as stopping rule. We extracted three samples: a) a holdout sample (33.3%) to check the accuracy of the final network; b) a training sample (47.7%) to estimate the network parameters, and c) a testing sample (20.0%) to prevent overtraining. We computed error, relative error, and importance and relative importance of all input variables were computed. IBM SPSS windows version 25, 2017 was used for all statistical analysis.

Results

Socio-demographic data

Table 1 shows the socio-demographic and clinical data in COVID-19 patients and the healthy control (HC) group. There was no significant difference between study groups in age, BMI, education, residency, marital status, and TUD. Sixty patients were recruited to participate, namely admission room: 35 patients, ICU: 16 patients, and RCU: 9 patients. All patients were on O_2 therapy, and were administered paracetamol, bromhexine, vitamin C, vitamin D, and zinc. Thirty-six patients out of 60 were IgG positive.

Differences in biomarkers among groups

The results of the biomarkers in COVID-19 patients compared with the control group are presented in **Table 2**. There was a significant decrease in SpO_2 in COVID+CCLA patients compared to COVID-CCLA patients and controls. There was also a significant decrease in serum albumin levels in COVID-19 patients compared to controls, with the lowest levels in the COVID+CCLA group. Serum magnesium was not significantly different between the three study groups. Serum PGI2 and TxA2 showed a significant increase in COVID-19 patients compared with controls, with the highest levels in the COVID+CCLA group. The significant increases in TxA2 in COVID-19 remained significant after forced entry of albumin (F=6.93, df=2/86, p=0.002), and albumin and prostacyclin (F=3.33, df-2/85, p=0.040). Serum C3, C4, and total calcium were significantly lower in both patient groups when compared with controls. The differences in serum total calcium were no longer significant after covarying for albumin levels (F=0.16, df=1/86, p=0.850).

Table 1. Socio-demographic and clinical data of COVID-19 patients and healthy controls (HC).

Variables	HC (n=30)	COVID-19 (n=60)	F/FEPT/χ^2	df	p
Age (years)	40.1 ±8.8	41.0 ±10.2	0.17	1/88	0.681
BMI (kg/m^2)	26.05 ±4.02	27.07 ±3.62	1.50	1/88	0.225
Sex (Female/Male)	6/24	17/43	0.73	1	0.393
Urban/Rural	28/2	52/8	-	-	0.486
Single/married	10/20	17/43	0.24	1	0.626
TUD (No/Yes)	20/10	39/21	0.03	1	0.875
Employment (No/Yes)	9/21	21/39	0.23	1	0.635
Education (years)	10.7 ±3.2	9.8 ±4.0	1.07	1/88	0.303
Admission room / ICU / RCU	-	35 / 16 / 9	-	-	-
Zinc (No/Yes)	23/7	1/59	57.53	1	<0.001
Vitamin D (No/Yes)	27/3	0/60	77.14	1	<0.001
Vitamin C (No/Yes)	23/7	0/60	61.79	1	<0.001
Dexamethasone (No/Yes)		9/51			
Azithromycin (No/Yes)		25/35			
Enoxaparin (No/Yes)		28/32			
O2 therapy (No/Yes)		0/60			
Bromhexine (No/Yes)		21/39			
Paracetamol (No/Yes)	27/3	0/60	77.14	1	<0.001
Omeprazole (No/Yes)		23/37			
Ceftriaxone (No/Yes)		35/25			
IgG (Positive / Negative)		36/24			

Results are shown as mean ±SD, FEPT: Fisher's exact probability test, BMI: body mass index, TUD: tobacco use disorder

Table 2. Biomarkers in COVID-19 patients divided into those with chest CT scan abnormalities (COVID+CCTA) and those without CCTA (COVID-CCTA) and healthy controls (HC).

Biomarkers	HC [A] (n=30)	COVID-CCTA [B] (n=15)	COVID+CCTA [C] (n=45)	F	df	p
SPO$_2$	98.53 ±0.68 [C]	97.13 ±0.74 [C]	92.62 ±3.40 [A, B]	56.37	2/87	<0.001
Albumin g/l	43.10 ±3.11 [B, C]	33.47 ±5.08 [A, C]	29.71 ±3.79 [A, B]	111.12	2/87	<0.001
Magnesium mM	0.933 ±0.102 [C]	1.018±0.223	1.010 ±0.172 [A]	2.42	2/87	0.005
Calcium mM	2.264 ±0.096 [B, C]	2.089 ±0.148 [A]	2.021 ±0.128 [A]	36.02	2/87	<0.001
Thromboxane A2 pg/ml	236.4 ±64.6 [B, C]	337.9 ±64.1 [A]	366.0 ±98.7 [A]	22.17	2/87	<0.001
Prostacyclin pg/ml	104.7 ±36.9 [B, C]	153.6 ±45.4 [A, C]	188.7 ±45.0 [A, B]	35.00	2/87	<0.001
Complement C3 mg/l	872.1 ± 285.5 [B, C]	346.7 ±134.0 [A]	302.9 ±88.2 [A]	92.00	2/87	<0.001
Complement C4 mg/l	381.7 ±138.7 [B, C]	266.3 ±110.0 [A]	273.1±87.3 [A]	9.98	2/87	<0.001

All results are shown as mean (SE). All results of univariate GLM analyses examining the associations between diagnosis (healthy control and COVID-19 patients divided into two groups depending on the presence or absence of CCTA) after adjusting for age, sex, tobacco use disorder, and body mass index.
SPO$_2$ %: oxygen saturation percentage.

Multivariate differences between COVID-19 patients and controls.

Using lowered levels of SpO2, C3 and albumin in a binary logistic regression analysis, we found a significant discrimination between COVID-19 patients and controls with an accuracy of 100%. Nevertheless, the SPSS program does not allow to estimate the regression parameters in this condition. **Table 3** shows the network information of a neural network model which examines the discrimination of COVID-19 versus controls. The network has been trained using one hidden layer with 4 units in layer 1. Hyperbolic tangent was used as an activation function in hidden layer 1 and identity in the output layer. The partitioned confusion matrix showed an AUC ROC=1.000 with an accuracy of 100.0% in the holdout sample and a sensitivity of 100.0% and specificity of 100.0%. **Figure 1** shows the relative importance of the most effective input variables (C3, albumin, TxA2, C4, PGI2, and SPO2) that represent the most important determinants of the model's predictive power.
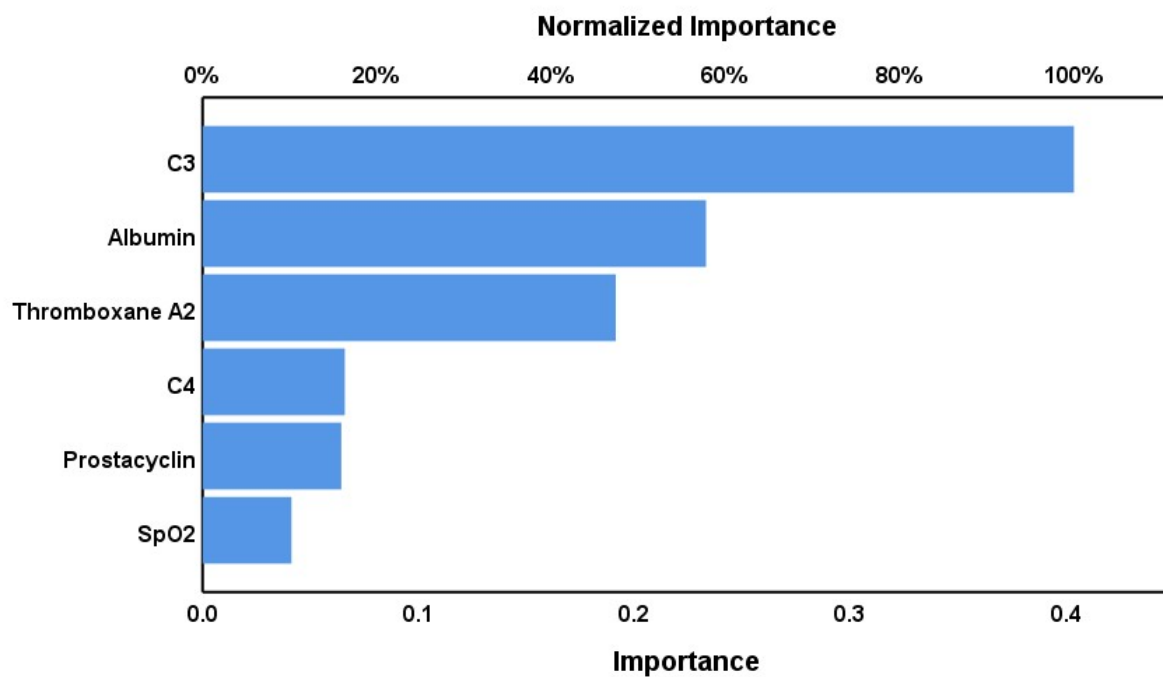

Figure 1. Results of neural network (importance chart) with diagnosis of COVID-19 *versus* controls as output variables and biomarkers as input variables.

Table 3. Results of neural networks with diagnosis of COVID-19 versus heathy controls (HC) as output variables and biomarkers as input variables.

	Model	COVID-19 *vs.* HC
Input Layer	Number of units	6
	Rescaling method	Normalized
Hidden layers	Number of hidden layers	1
	Number of units in hidden layer 1	4
	Activation Function	Hyperbolic tangent
Output layer	Dependent variables	COVID-19 *vs.* HC
	Number of units	2
	Activation function	Identity

	Error function	Sum of squares
Training	Sum of squares error term	0.420
	% incorrect or relative error	0.0%
	Prediction (sens, spec)	100%, 100%
Testing	Sum of Squares error	0.252
	%incorrect or relative error	0.0%
	Prediction (sens spec)	100%, 100%
	AUC ROC	1.00
Holdout	%incorrect or relative error	0.0%
	Prediction (sens, spec)	100%, 100%

AUC ROC: area under curve of receiver operating curve, sens: sensitivity, spec: specificity.

Associations of biomarkers with anti-SARS-CoV-2 IgG antibodies, CCTAs and SpO2.

Table 4 shows the biomarkers in COVID-19 patients with positive versus negative anti-SARS-CoV-2 IgG antibody titers. The positive IgG group showed a significant increase in SpO2, TxA2, PGI2, and C4 compared with the patients with negative IgG antibodies. These differences remained significant after FDR p-correction (at p=0.016). The same table shows that SpO2 and albumin were significantly lower and prostacyclin significantly higher in COVID+CCTA as compared with COVID-CCTA patients. These differences remained significant after FDR p-correction (at p=0.033).

Table 5 shows the intercorrelation matrix between SpO2 and the biomarkers. SpO2 was significantly and negatively correlated with TxA2 and prostacyclin, and positively with C3, C4, albumin, and calcium. TxA2 was significantly and positively correlated with prostacyclin and negatively with C3, albumin, and calcium. Prostacyclin levels were significantly and positively correlated with magnesium and inversely with C3, albumin, and calcium.

Table 6 shows the regression of TxA2 on immune-inflammatory biomarkers. The first regression shows that 39.3% of the variance in TxA2 was explained by the regression on albumin (inversely) and prostacyclin (positively). The second regression shows that 42.0% of the variance in TxA2 was explained by the regression on C3 (inversely) and C4 and prostacyclin (both positively).

Table 4. Differences in biomarkers between COVID-19 patients with and without anti-SARS-CoV-2 IgG antibodies and with and without chest CT scan anomalies (CCTAs).

Biomarker	Negative IgG N=24	Positive IgG N=36	F	df	P
SpO_2	92.25 ±4.31	94.75 ±2.56	7.97	1/58	0.007
Albumin g/l	29.83 ±4.37	31.19 ±4.43	1.37	1/58	0.246
Magnesium mM	1.017 ±0.201	1.009 ±0.174	0.02	1/58	0.880
Calcium mM	2.012 ±0.132	2.056 ±0.137	1.54	1/58	0.219
Thromboxane A2 pg/ml	306.3 ±57.6	394.1 ±93.8	16.78	1/58	<0.001
Prostacyclin pg/ml	160.2 ±40.9	193.1 ±47.2	7.78	1/58	0.007
Complement C3 mg/l	329.0 ±120.2	303.7 ±88.4	0.88	1/58	0.351
Complement C4 mg/l	227.8 ±81.1	300.4 ±89.2	10.23	1/58	0.002
Biomarkers	**No CCTAs n=15**	**CCTAs n=45**	**F**	**df**	**p**
SpO_2	97.13 ±0.74	92.62 ±3.40	25.94	1/58	<0.001
Albumin g/l	33.47 ±5.08	29.71 ±3.79	9.25	1/58	0.004
Magnesium mM	1.0178 ±0.233	1.010 ±0.172	0.02	1/58	0.895
Calcium mM	2.089 ±0.147	2.038 ±0.135	2.94	1/58	0.092
Thromboxane A2 pg/ml *	337.9 ±64.1	366.0 ±98.7	1.09	1/58	0.301
Prostacyclin pg/ml	153.6 ±45.4	188.7 ±45.0	6.81	1/58	0.011
Complement C3 mg/l **	346.7 ±134.0	302.9 ±88.2	1.92	1/58	0.171
Complement C4 mg/l	266.3 ±110.0	273.4 ±87.3	0.59	1/58	0.809

All results of univariate GLM analysis; results are shown as mean ±SE.
*Processed in Ln and ** square root transformation
SpO_2 %: oxygen saturation percentage.

Table 5. Intercorrelation matrix between oxygen saturation percentage (SpO2) and different biomarkers of COVID-19.

Biomarkers	SpO2	Thromboxane A2	Prostacyclin
Thromboxane A2	-0.362 (0.001)	-	-
Prostacyclin	-0.380 (0.001)	0.539 (0.002)	-
Complement C3	0.598 (0.001)	-0.544 (0.002)	-0.593 (0.001)
Complement C4	0.355 (0.001)	0.028 (0.800)	-0.014 (0.9)
Albumin	0.617 (0.001)	-0.549 (0.002)	-0.572 (0.001)
Magnesium	-0.208 (0.055)	0.178 (0.115)	0.308 (0.005)
Calcium	0.490 (0.001)	-0.379 (0.002)	-0.496 (0.001)

All results of partial correlation analysis after covarying for age, sex, body mass index and tobacco use disorder. Shown are false discovery rate corrected p values.

Table 6. Results of multiple regression analysis with PxA2 as dependent variable and immune-inflammatory mediators and prostacyclin as explanatory variables.

Dependent Variables	Explanatory Variables	β	t	p	F model	df	p	R^2
#1. TxA2	**Model**				28.13	2/87	<0.001	0.393
	Albumin	-0.387	-3.923	<0.001				
	Prostacyclin	0.328	3.319	0.001				
#2. TxA2	**Model**				20.78	3/86	<0.001	0.420
	C3	-0.525	-4.471	<0.001				
	C4	0.241	2.498	0.014				
	Prostacyclin	0.227	2.118	0.037				

Discussion

Changes in complement in COVID-19

The first major finding of the present study is that C3 and C4 are significantly decreased in COVID-19 patients. As reviewed in the introduction, there were some reports that C3 is significantly lowered in severe COVID-19 as compared with controls. This may be explained by increased cleavage during activation and increased consumption following immune complex formation [12]. C3 levels tend to increase gradually in recovered COVID-19 patients, whilst C3 levels were decreased in non-survivors and associated with increased risk of in-hospital death [13]. The levels of complement C4 were decreased from day 0 to day 10 in patients hospitalized for more than two weeks, but not in patients who were discharged earlier [45]. C3 polymorphism may be a significant risk factor of mortality due to COVID-19 [46]. However, in a previous analysis, no major variations in complement C3 or C4 levels were observed between severe and less severe COVID-19 study groups [47], whereas another report found increased C3 and C4 in COVID-19 patients [48].

We also found that lowered SpO2 is associated with lowered C3 and C4 levels. In this respect, it is interesting to note that, in COVID-19 patients, systemic complement activation is associated with respiratory failure [49]. Complement activation mediates in part the systemic immune-inflammatory response in SARS-CoV infection [8] and activation of complement C3 can worsen SARS-COV-related ARDS [50].

Increased TxA2 and PGI2 in COVID-19

The second major finding of this study is that TxA2 is significantly increased in COVID-19 patients when compared with controls. Platelets produce significant amounts of TxA2 and prostaglandins dependent upon the activity of COX-1, COX-2, and TxA2. On platelets, TxA2 binds to the prostanoid thromboxane receptor thereby initiating an amplification loop leading to further platelet activation, aggregation and TxA2 formation [51], which may, consequently, lead to a prothrombotic state with increased mortality risk [17, 52, 53]. Increased platelet activity and aggregability has been reported in patients with COVID-19 [54] and is associated with an increased mortality risk [55]. In addition, coagulopathies are often observed in COVID-19 with up to one-third of patients having thrombotic problems [56].

In our study, we observed a significant intertwined upregulation in TxA2 and PGI2 levels. Prostaglandins, including PGI2, are usually elevated in response to inflammatory or toxic stimuli [57]. Endothelial PGI2 binds to the Gs-coupled PGI2 receptor on platelets thereby reducing platelet reactivity, which can be critical to minimizing the risk for atherothrombotic events [58]. PGI2 signaling induces cytosolic cAMP thereby preventing platelet activation [59] and may reduce viral-induced illness by suppressing the induction of type-I interferons [60]. Moreover, PGI2 protects against cytokine toxicity by attenuating nuclear factor-κB activity and possesses strong anti-inflammatory and immune-regulatory properties [61]. As such, increased levels of prostacyclin may attenuate the thrombotic and immune effects of increased TxA2. Nevertheless, in our study, we found that the increases in TxA2 in COVID-19 patients remained significant after adjusting for albumin and prostacyclin. In this regard it is important to note that PGI2 signaling may lead to an increased production of IL-6 from stromal cells [62] and may promote T helper-1 differentiation possibly through cAMP-PKA signaling [63].

The massive platelet activation in COVID-19 is probably not a direct consequence of the virus itself because SARS-CoV-2 has rarely been found in the serum of infected patients [64]. One of the mechanisms causing severe COVID-19 is believed to stem from an exaggerated immune-inflammatory response with complement-induced-coagulation, massive endothelial damage and systemic microangiopathy [65]. In severe COVID-19, widespread endothelial dysfunction and coagulopathies and complement-induced thrombosis may cause systemic microangiopathy and thromboembolism, which may lead to multi-organ failure thereby increasing the mortality rate [65]. Moreover, in COVID-19, activation of the alternative complement pathway is associated with microvascular injury and thrombosis [66]. Consequently, a pro-coagulative endothelium may induce endothelins thereby mediating infiltration of inflammatory cells in the lungs leading to ARDS in COVID-19 [67-69]. On the other hand, the endothelium mediates antithrombotic and anti-inflammatory functions by releasing active endothelium-derived factors such as nitric oxide PGI$_2$ [70].

Lowered albumin, calcium, and magnesium in COVID-19

In agreement with Al-Hakeim et al. (2020) and other studies reviewed in the latter paper [31], this study found that serum albumin, and total calcium and magnesium were significantly lowered in COVID-19. Hypoalbuminemia may be explained by the acute phase responses in COVID-19 with increased breakdown of albumin and increased production of positive acute phase proteins [71], and by enhanced capillary permeability leading to the leaking of albumin to the interstitial space [72]. Interestingly, in the current study we found a significant and inverse associations between TxA2, C3 and albumin levels, suggesting platelet hyperactivity – immune-inflammatory interactions in COVID-19. A previous study showed that hypoalbuminemia, especially when serum albumin is < 35 g/L, is associated with elevated D-dimers and enhanced risk of artery and venous thrombosis [73, 74]. The association between hypoalbuminemia and hypercoagulability and venous thromboembolism may be explained by the anticoagulant and antiplatelet activities of albumin [75]. Not only platelet-platelet but also platelet-leukocyte interactions play a key role in COVID-19 [76]. Activation of the prostanoid TxA2 receptor mediates not only thrombosis and angiogenesis, but also vascular inflammation [23]. In ARDS,

platelets may function as effectors in immunity and inflammation [77, 78] and virus-platelet interactions increase thrombotic risk by fostering procoagulant and inflammatory states during viral infection [79]. The current study found that a combination of C3, albumin and TxA2 could be used as an extern validating criterion for the diagnosis of COVID-19. These data further underscore that the combined intertwined activities of immune-inflammatory and hemostasis pathways underpin the pathophysiology of COVID-19.

Moreover, in agreement with Al-Hakeim et al. (2021), this study found that CCTAs are accompanied by lowered SpO2 and hypoalbuminemia, and additionally that lowered SpO2 is associated with low serum albumin [31]. Such findings indicate that the lesions caused by inflammatory lung damage may result in diminished lung oxygenation and systemic inflammation with hypoalbuminemia [31]. Interestingly, CCTAs are associated with the severity of COVID-19 [80] and are an important risk factor for myocarditis in COVID-19 patients [80].

As reviewed in our Introduction both calcium and magnesium are partially bound to albumin and therefore hypoalbuminemia may explain at least part of the lowered magnesium and calcium levels in COVID-19 [31]. Importantly, hypocalcemia is more common in severely ill COVID-19 patients than in mild cases, and may detect with high specificity the more critically ill patients or those with a poorer outcome [81, 82] and may predict hospitalization of COVID-19 patients [83].

Biomarkers and IgG positivity
Another major finding is that patients with positive IgG titers showed higher SpO2, TxA2, PGI2 and C4 levels. Previously, we reported that patients with IgG positivity showed higher IL-6, sRAGE and ACE2 levels as compared to patients with negative IgG titers [31]. We found 60.0% (the current study) and 66.7% [31] of COVID-19 patients showed positive IgG levels, whereas a previous study reported that 77.9% of COVID-19 patients were IgG positive [84]. The antibody dynamics observed in COVID-19 patients are quite similar to the Ig responses in other viral infections, with an initial increase in IgM and increasing IgG levels when IgM starts to decrease [85]. Not only increased IL-6 may drive B-cell mediated IgG formation [86], but also complement activation promotes humoral immunity [87]. As we discussed previously, increased IgG titers are more pronounced in severe than in mild cases [88], and, therefore, the association between positive IgG titers and TxA2 and PGI2 found in the current study could reflect severity of illness.

Conclusions
COVID-19 is characterized by significantly decreased SpO2, C3 and C4 and significantly increased TxA2 and PGI2. A combination of C3, albumin, and TxA2 yielded a predictive accuracy of 100% in discriminating COVID-19 patients from healthy controls. SpO2 was significantly and positively associated with C3, C4, albumin, and calcium, and negatively with TxA2 and PGI2. Furthermore, CCTAs were accompanied by lower SpO2 and albumin, but higher prostacyclin. The strong association between CCPA-associated hypoalbuminemia and increased TxA2 suggests that intertwined interactions between

immune-inflammatory pathways and platelet hyperactivity participate in the pathophysiology of COVID-19. These mechanisms may be aggravated by lowered calcium and magnesium, which may increase risk of hypercoagulability and venous thromboembolism.

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Author's contributions
HA-H and SA-H measured serum biochemical biomarkers. MM performed the statistical analyses. All authors collaborated in the design of the analysis, the discussion of the findings, the drafting and editing of the manuscript, and the final version of the manuscript.

Data Access Statement.

The dataset generated during and/or analyzed during the current study will be available from the corresponding author upon reasonable request and once the dataset has been fully exploited by the authors.

References

1.   Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
2.   Tan CW, Tan JY, Wong WH, Cheong MA, Ng IM, Conceicao EP, *et al.* Clinical and laboratory features of hypercoagulability in COVID-19 and other respiratory viral infections amongst predominantly younger adults with few comorbidities. Scientific Reports. 2021;11(1):1793.
3.   Song J-W, Zhang C, Fan X, Meng F-P, Xu Z, Xia P, *et al.* Immunological and inflammatory profiles in mild and severe cases of COVID-19. Nature communications. 2020;11(1):1-10.

4.   Hasan SS, Capstick T, Ahmed R, Kow CS, Mazhar F, Merchant HA*, et al.*
Mortality in COVID-19 patients with acute respiratory distress syndrome and
corticosteroids use: a systematic review and meta-analysis. Expert Rev Respir Med.
2020;14(11):1149-63.

5.   Amini Pouya M, Afshani SM, Maghsoudi AS, Hassani S, Mirnia K.
Classification of the present pharmaceutical agents based on the possible effective
mechanism on the COVID-19 infection. Daru : journal of Faculty of Pharmacy, Tehran
University of Medical Sciences. 2020:1-20.

6.   Merdji H, Mayeur S, Schenck M, Oulehri W, Clere-Jehl R, Cunat S*, et al.*
Histopathological features in fatal COVID-19 acute respiratory distress syndrome. Med
Intensiva. 2021.

7.   Java A, Apicelli AJ, Liszewski MK, Coler-Reilly A, Atkinson JP, Kim AH*, et al.*
The complement system in COVID-19: friend and foe? JCI insight. 2020;5(15).

8.   Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR*, et
al.* Complement activation contributes to severe acute respiratory syndrome coronavirus
pathogenesis. MBio. 2018;9(5).

9.   Sarma VJ, Huber-Lang M, Ward PA. Complement in lung disease.
Autoimmunity. 2006;39(5):387-94.

10.   Dunkelberger JR, Song W-C. Complement and its role in innate and adaptive
immune responses. Cell Research. 2010;20(1):34-50.

11.   Bosmann M, Ward PA. Role of C3, C5 and anaphylatoxin receptors in acute lung
injury and in sepsis. Adv Exp Med Biol. 2012;946:147-59.

12.   Ghazavi A, Mosayebi G, Keshavarzian N, Rabiemajd S, Ganji A. Reduction of
Inflammatory C3 and C4 Complement Proteins in Severe COVID-19 Patients. 2020.

13.   Fang S, Wang H, Lu L, Jia Y, Xia Z. Decreased complement C3 levels are
associated with poor prognosis in patients with COVID-19: A retrospective cohort study.
International Immunopharmacology. 2020;89:107070.

14.   Gavriilaki E, Brodsky RA. Severe COVID-19 infection and thrombotic
microangiopathy: success does not come easily. British journal of haematology.
2020;189(6):e227-e30.

15.   Satyam A, Tsokos MG, Brook OR, Hecht JL, Moulton VR, Tsokos GC.
Activation of classical and alternative complement pathways in the pathogenesis of lung
injury in COVID-19. Clinical Immunology. 2021;226:108716.

16.   Campbell CM, Kahwash R. Will complement inhibition be the new target in
treating COVID-19–related systemic thrombosis? Circulation. 2020;141(22):1739-41.

17.   Klok F, Kruip M, Van der Meer N, Arbous M, Gommers D, Kant K*, et al.*
Incidence of thrombotic complications in critically ill ICU patients with COVID-19.
Thrombosis research. 2020;191:145-7.

18.   Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B*, et al.* Molecular
Intercommunication between the Complement and Coagulation Systems. The Journal of
Immunology. 2010;185(9):5628.

19.   Offermanns S. Activation of platelet function through G protein-coupled
receptors. Circ Res. 2006;99(12):1293-304.

20.   Ruan K-H. Advance in understanding the biosynthesis of prostacyclin and
thromboxane A2 in the endoplasmic reticulum membrane via the cyclooxygenase
pathway. Mini reviews in medicinal chemistry. 2004;4(6):639-47.

21.   Ruan K-H. Thrombogenesis and anti-thrombogenesis in atherosclerosis mediated by prostanoids through the cyclooxygenase pathway: Nova Science Publishers Inc New York; 2005.

22.   FitzGerald GA, Oates JA, Hawiger J, Maas RL, Roberts LJ, Lawson J, *et al.* Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. The Journal of clinical investigation. 1983;71(3):676-88.

23.   Conti P, Caraffa A, Gallenga C, Ross R, Kritas S, Frydas I, *et al.* IL-1 induces throboxane-A2 (TxA2) in COVID-19 causing inflammation and micro-thrombi: inhibitory effect of the IL-1 receptor antagonist (IL-1Ra). Journal of biological regulators and homeostatic agents. 2020;34(4).

24.   Chu L-y, Liou J-Y, Wu KK. Prostacyclin protects vascular integrity via PPAR/14-3-3 pathway. Prostaglandins & other lipid mediators. 2015;118:19-27.

25.   McAdam B, Catella-Lawson F, Mardini I, Kapoor S, Lawson J, FitzGerald G. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proceedings of the National Academy of Sciences. 1999;96(1):272-7.

26.   Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, *et al.* Role of prostacyclin in the cardiovascular response to thromboxane A2. Science. 2002;296(5567):539-41.

27.   Fleming I. The factor in EDHF: Cytochrome P450 derived lipid mediators and vascular signaling. Vascular pharmacology. 2016;86:31-40.

28.   Vila L. Cyclooxygenase and 5-lipoxygenase pathways in the vessel wall: Role in atherosclerosis. Medicinal research reviews. 2004;24(4):399-424.

29.   Manieri NA, Mack MR, Himmelrich MD, Worthley DL, Hanson EM, Eckmann L, *et al.* Mucosally transplanted mesenchymal stem cells stimulate intestinal healing by promoting angiogenesis. The Journal of clinical investigation. 2015;125(9):3606-18.

30.   Martini R. The compelling arguments for the need for microvascular investigation in COVID-19 critical patients. Clinical hemorheology and microcirculation. 2020(Preprint):1-8.

31.   Al-Hakeim HK, Al-Jassas HK, Morris G, Maes M. Increased angiotensin-converting enzyme 2, sRAGE and immune activation, but lowered calcium and magnesium in COVID-19: association with chest CT abnormalities and lowered peripheral oxygen saturation. medRxiv. 2021:2021.03.26.21254383.

32.   Huang J, Cheng A, Kumar R, Fang Y, Chen G, Zhu Y, *et al.* Hypoalbuminemia predicts the outcome of COVID-19 independent of age and co-morbidity. J Med Virol. 2020;92(10):2152-8.

33.   Zhang Y, Zheng L, Liu L, Zhao M, Xiao J, Zhao Q. Liver impairment in COVID-19 patients: A retrospective analysis of 115 cases from a single centre in Wuhan city, China. Liver Int. 2020;40(9):2095-103.

34.   James MT, Zhang J, Lyon AW, Hemmelgarn BR. Derivation and internal validation of an equation for albumin-adjusted calcium. BMC clinical pathology. 2008;8(1):1-6.

35.   Chen S, Zhang Z, Wu Y, Shi Q, Yan H, Mei N, *et al.* Endoplasmic Reticulum Stress and Store-Operated Calcium Entry Contribute to Usnic Acid-Induced Toxicity in Hepatic Cells. Toxicological sciences : an official journal of the Society of Toxicology. 2015;146(1):116-26.

36.   Zhang IX, Ren J, Vadrevu S, Raghavan M, Satin LS. ER stress increases store-operated Ca(2+) entry (SOCE) and augments basal insulin secretion in pancreatic beta cells. The Journal of biological chemistry. 2020;295(17):5685-700.

37.   Fung TS, Liu DX. Coronavirus infection, ER stress, apoptosis and innate immunity. Frontiers in microbiology. 2014;5(296).

38.   Morris G, Athan E, Walder K, Bortolasci CC, O'Neil A, Marx W*, et al.* Can endolysosomal deacidification and inhibition of autophagy prevent severe COVID-19? Life sciences. 2020;262:118541.

39.   Güzel A, Doğan E, Türkçü G, Kuyumcu M, Kaplan İ, Çelik F*, et al.* Dexmedetomidine and Magnesium Sulfate: A Good Combination Treatment for Acute Lung Injury? J Invest Surg. 2019;32(4):331-42.

40.   Abiri B, Vafa M. Effects of vitamin D and/or magnesium supplementation on mood, serum levels of BDNF, inflammatory biomarkers, and SIRT1 in obese women: a study protocol for a double-blind, randomized, placebo-controlled trial. Trials. 2020;21(1):225.

41.   Sheu JR, Hsiao G, Shen MY, Lee YM, Yen MH. Antithrombotic effects of magnesium sulfate in in vivo experiments. Int J Hematol. 2003;77(4):414-9.

42.   Djagbletey R, Phillips B, Boni F, Owoo C, Owusu-Darkwa E, deGraft-Johnson PKG*, et al.* Relationship between serum total magnesium and serum potassium in emergency surgical patients in a tertiary hospital in Ghana. Ghana medical journal. 2016;50(2):78-83.

43.   Kwee TC, Kwee RM. Chest CT in COVID-19: what the radiologist needs to know. RadioGraphics. 2020;40(7):1848-65.

44.   Moustafa HE-DS, El-Seddek M. Accurate diagnosis of covid-19 based on deep neural networks and chest x-ray images.(dept. e (electronics)). Bulletin of the Faculty of Engineering Mansoura University. 2020;45(3):11-5.

45.   Marcos-Jiménez A, Sánchez-Alonso S, Alcaraz-Serna A, Esparcia L, López-Sanz C, Sampedro-Núñez M*, et al.* Deregulated cellular circuits driving immunoglobulins and complement consumption associate with the severity of COVID-19. medRxiv. 2020:2020.06.15.20131706.

46.   Delanghe JR, De Buyzere ML, Speeckaert MM. C3 and ACE1 polymorphisms are more important confounders in the spread and outcome of COVID-19 in comparison with ABO polymorphism. European journal of preventive cardiology. 2020;27(12):1331-2.

47.   Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y*, et al.* Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis. 2020;71(15):762-8.

48.   Peffault de Latour R, Bergeron A, Lengline E, Dupont T, Marchal A, Galicier L*, et al.* Complement C5 inhibition in patients with COVID-19 - a promising target? Haematologica. 2020;105(12):2847-50.

49.   Holter JC, Pischke SE, de Boer E, Lind A, Jenum S, Holten AR*, et al.* Systemic complement activation is associated with respiratory failure in COVID-19 hospitalized patients. Proc Natl Acad Sci U S A. 2020;117(40):25018-25.

50.   Ciceri F, Beretta L, Scandroglio AM, Colombo S, Landoni G, Ruggeri A*, et al.* Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome

(MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. Critical care and resuscitation. 2020;22(2):95.

51.   Reilly M, Fitzgerald G. Cellular activation by thromboxane A2 and other eicosanoids. European heart journal. 1993;14:88-93.

52.   Gupta A, Kalantar-Zadeh K, Reddy ST. Ramatroban as a Novel Immunotherapy for COVID-19. J Mol Genet Med. 2020;14(3).

53.   Wise J. Covid-19 and thrombosis: what do we know about the risks and treatment? : British Medical Journal Publishing Group; 2020.

54.   Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben C, et al. Platelet gene expression and function in patients with COVID-19. Blood. 2020;136(11):1317-29.

55.   Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. Journal of thrombosis and haemostasis. 2020;18(4):844-7.

56.   Bilaloglu S, Aphinyanaphongs Y, Jones S, Iturrate E, Hochman J, Berger JS. Thrombosis in Hospitalized Patients With COVID-19 in a New York City Health System. Jama. 2020;324(8):799-801.

57.   Robb CT, Goepp M, Rossi AG, Yao C. Non-steroidal anti-inflammatory drugs, prostaglandins, and COVID-19. British Journal of pharmacology. 2020;177(21):4899-920.

58.   Midgett C, Stitham J, Martin KA, Hwa J. Prostacyclin receptor regulation-from transcription to trafficking. Current molecular medicine. 2011;11(7):517-27.

59.   Kim S, Kunapuli SP. P2Y12 receptor in platelet activation. Platelets. 2011;22(1):54-8.

60.   Toki S, Reiss S, Goleniewska K, Moore ML, FitzGerald G, Peebles RS, Jr. Prostaglandin I$_2$ Receptor (IP) Signaling Inhibits Lung Type I Interferon Expression by RSV Infection. Journal of Allergy and Clinical Immunology. 2013;131(2):AB191.

61.   Dorris SL, Peebles RS. PGI2 as a regulator of inflammatory diseases. Mediators of inflammation. 2012;2012.

62.   Honda T, Segi-Nishida E, Miyachi Y, Narumiya S. Prostacyclin-IP signaling and prostaglandin E2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. The Journal of experimental medicine. 2006;203(2):325-35.

63.   Nakajima S, Honda T, Sakata D, Egawa G, Tanizaki H, Otsuka A, et al. Prostaglandin I2–IP signaling promotes Th1 differentiation in a mouse model of contact hypersensitivity. The Journal of Immunology. 2010;184(10):5595-603.

64.   Canzano P, Brambilla M, Porro B, Cosentino N, Tortorici E, Vicini S, et al. Platelet and Endothelial Activation as Potential Mechanisms Behind the Thrombotic Complications of COVID-19 Patients. JACC Basic Transl Sci. 2021;6(3):202-18.

65.   Perico L, Benigni A, Casiraghi F, Ng LFP, Renia L, Remuzzi G. Immunity, endothelial injury and complement-induced coagulopathy in COVID-19. Nature Reviews Nephrology. 2021;17(1):46-64.

66.   Magro C, Mulvey JJ, Berlin D, Nuovo G, Salvatore S, Harp J, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Translational Research. 2020;220:1-13.

67.   Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F*, et al.* Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. New England Journal of Medicine. 2020;383(2):120-8.

68.   Teuwen L-A, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. Nature Reviews Immunology. 2020;20(7):389-91.

69.   Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS*, et al.* Endothelial cell infection and endotheliitis in COVID-19. The Lancet. 2020;395(10234):1417-8.

70.   Golshiri K, Ataei Ataabadi E, Portilla Fernandez EC, Jan Danser AH, Roks AJM. The importance of the nitric oxide-cGMP pathway in age-related cardiovascular disease: Focus on phosphodiesterase-1 and soluble guanylate cyclase. Basic Clin Pharmacol Toxicol. 2020;127(2):67-80.

71.   Maes M. A review on the acute phase response in major depression. Reviews in the neurosciences. 1993;4(4):407-16.

72.   Soeters PB, Wolfe RR, Shenkin A. Hypoalbuminemia: pathogenesis and clinical significance. Journal of Parenteral and Enteral Nutrition. 2019;43(2):181-93.

73.   Ronit A, Kirkegaard-Klitbo DM, Dohlmann TL, Lundgren J, Sabin CA, Phillips AN*, et al.* Plasma albumin and incident cardiovascular disease: results from the CGPS and an updated meta-analysis. Arteriosclerosis, thrombosis, and vascular biology. 2020;40(2):473-82.

74.   Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M*, et al.* ISTH interim guidance on recognition and management of coagulopathy in COVID-19. Journal of Thrombosis and Haemostasis. 2020;18(5):1023-6.

75.   Paar M, Rossmann C, Nusshold C, Wagner T, Schlagenhauf A, Leschnik B*, et al.* Anticoagulant action of low, physiologic, and high albumin levels in whole blood. PLoS One. 2017;12(8):e0182997.

76.   Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben C*, et al.* Platelet gene expression and function in patients with COVID-19. Blood, The Journal of the American Society of Hematology. 2020;136(11):1317-29.

77.   Yadav H, Kor DJ. Platelets in the pathogenesis of acute respiratory distress syndrome. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015;309(9):L915-L23.

78.   Middleton EA, Weyrich AS, Zimmerman GA. Platelets in pulmonary immune responses and inflammatory lung diseases. Physiological reviews. 2016;96(4):1211-59.

79.   Koupenova M, Clancy L, Corkrey HA, Freedman JE. Circulating Platelets as Mediators of Immunity, Inflammation, and Thrombosis. Circ Res. 2018;122(2):337-51.

80.   Ma K-L, Liu Z-H, Cao C-F, Liu M-K, Liao J, Zou J-B*, et al.* COVID-19 Myocarditis and Severity Factors: An Adult Cohort Study. medRxiv. 2020:2020.03.19.20034124.

81.   Yang C, Ma X, Wu J, Han J, Zheng Z, Duan H*, et al.* Low serum calcium and phosphorus and their clinical performance in detecting COVID-19 patients. J Med Virol. 2021;93(3):1639-51.

82.   Torres B, Alcubilla P, González-Cordón A, Inciarte A, Chumbita M, Cardozo C*, et al.* Impact of low serum calcium at hospital admission on SARS-CoV-2 infection outcome. International Journal of Infectious Diseases. 2021;104:164-8.

83.   Di Filippo L, Formenti AM, Rovere-Querini P, Carlucci M, Conte C, Ciceri F*, et al.* Hypocalcemia is highly prevalent and predicts hospitalization in patients with COVID-19. Endocrine. 2020;68(3):475-8.

84.   Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F*, et al.* Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis. 2020;71(15):778-85.

85.   Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W*, et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. nature. 2020;579(7798):270-3.

86.   Maeda K, Mehta H, Drevets DA, Coggeshall KM. IL-6 increases B-cell IgG production in a feed-forward proinflammatory mechanism to skew hematopoiesis and elevate myeloid production. Blood, The Journal of the American Society of Hematology. 2010;115(23):4699-706.

87.   Nielsen C, Fischer E, Leslie R. The role of complement in the acquired immune response. Immunology. 2000;100(1):4.

88.   Zeng F, Dai C, Cai P, Wang J, Xu L. Jianyu Li1, Guoyun Hu1, LW (2020). A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: a possible reason underlying different outcome between gender. BMJ, February 2019.1-13.