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.
Keywords: COVID-19, C3, C4, inflammation, cytokines, biomarkers, thromboxane A2, prostacyclin.
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 TxA2, 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\textsuperscript{\textsubscript{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\textsuperscript{\textsubscript{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 ($\chi^2$-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 O₂ 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 SpO₂ 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).

*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.

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).

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 nonsurvivors 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 PGI2 [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.

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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/χ² | df  | p    |
|----------------------------|-----------|-----------------|-----------|-----|------|
| Age (years)                | 40.1 ±8.8 | 41.0 ±10.2      | 0.17      | 1/88| 0.681|
| BMI (kg/m²)                | 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            | -         | -   | -    |
| 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.
Table 3. Results of neural networks with diagnosis of COVID-19 versus healthy 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.
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     |
|----------------------------------|-------------------|-------------------|-----|-----|-------|
| SpO₂                             | 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        |     |     |       |
| SpO₂                             | 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
SpO₂ %: 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  | R²  |
|---------------------|-----------------------|-------|-------|--------|---------|----|----|-----|
| #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  |         |    |    |     |
Figure 1. Results of neural network (importance chart) with diagnosis of COVID-19 \textit{versus} controls as output variables and biomarkers as input variables.