Complement C5a and Clinical Markers to predict COVID-19 Disease Severity and Mortality in a Multi-ethnic Population

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Abstract

Coronavirus disease-2019 (COVID-19) was declared as a pandemic by WHO in March 2020. SARS-CoV-2 causes a wide range of illness from asymptomatic to life-threatening. There is an essential need to identify biomarkers to predict disease severity and mortality during the earlier stages of the disease, aiding treatment and allocation of resources to improve survival. The aim of this study was to identify at the time of SARS-COV-2 infection patients at high risk of developing severe disease associated with low survival using blood parameters, including inflammation and coagulation mediators, vital signs, and pre-existing comorbidities. This cohort included 89 multi-ethnic COVID-19 patients recruited between July 14th and October 20th 2020 in Doha, Qatar. According to clinical severity, patients were grouped into severe (n = 33), mild (n = 33) and asymptomatic (n = 23). Common routine tests such as complete blood count (CBC), glucose, electrolytes, liver and kidney function parameters and markers of inflammation, thrombosis and endothelial dysfunction including complement component split product C5a, Interleukin-6, ferritin and C-reactive protein were measured at the time COVID-19 infection was confirmed. Correlation tests suggest that C5a is a novel predictive marker of disease severity and mortality, in addition to 40 biological and physiological parameters that were found statistically significant between survivors and non-survivors. Survival analysis showed that. high C5a levels, hypoalbuminemia, lymphopenia, elevated procalcitonin, neutrophilic leukocytosis, acute anemia along with increased acute kidney and hepatocellular injury markers were associated with a higher risk of death in COVID-19 patients. Altogether, we created a prognostic classification model, the CAL model (C5a, Albumin, and Lymphocyte count) to predict severity with significant accuracy. Stratification of patients using the CAL model could help the identification of patients likely to develop severe symptoms in advance so that treatments can be targeted accordingly.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first reported as a novel pneumonia in Wuhan (China), has resulted in above 119 million infections and approximately 2.5 million deaths globally [1]. In particular, SARS-CoV-2 is one of seven coronaviruses that are capable of infecting humans. Three of these viruses can cause severe disease, namely SARS-CoV, MERS-CoV and SARS-CoV-2, the other four HKU1, NL63, OC43 and 229E are linked to mild disease symptoms [2]. Generally, coronaviruses are known to cause respiratory, enteric, hepatic, and neurological symptoms in their host, with a wide spectrum of disease severity [3]. COVID-19 patients have a variable presentation of symptoms including fever, non-productive cough, dyspnea, myalgia, fatigue and pneumonia accompanied with normal or reduced leukocyte counts [4 5]. Thus, COVID-19 disease symptoms range from asymptomatic and mild phenotype, to the severe infection leading to acute respiratory distress syndrome (ARDS) and multiorgan failure with poor survival rates [6 7]. The mortality rate varies among populations and patient demographics and is reported to be higher within the elderly, individuals with pre-existing comorbidities, and immunocompromised patients [8].

In addition, a role for the cytokine storm has been suggested as a crucial player in determining disease severity, including the development of pulmonary intravascular coagulation. Among the mechanisms contributing to multiorgan failure, an interplay between inflammation and coagulation has been shown critical in COVID-19 patients [9]. Exacerbated coagulation, characterized by elevated levels of D-dimer (> 1 µg/ml) were observed in COVID-19 patients, and an association with poor prognosis was reported [10]. Moreover, the presence of a prothrombotic state in multiple organs is supported by autopsies of COVID-19 patients independent of disease time-course [11]. In particular, megakaryocytes and platelet-rich thrombi are found in the lungs, heart and kidneys [12]. Remarkably, COVID-19-associated thrombosis is linked with increased morbidity and mortality in critical cases [13 14 15]. Accumulating evidence suggests a correlation between increased level of inflammatory mediators, including cytokines and chemokines, and COVID-19 disease progression. For instance, SARS-CoV-2 infection severity is linked to high blood levels of C-reactive protein, ferritin, and D-dimers [16 17 18]. The clinical symptoms documented in the severe form of COVID-19 resemble those observed in the cytokine release syndrome (CRS) [19 20 21 22]. CRS is characterized by a robust release of pro-inflammatory cytokines such as IL-6 [23]. A significant correlation between levels of IL-6 and an increased risk of death was observed in COVID-19 patients [20 24]. Hence, tocilizumab and sarilumab were proposed as promising therapeutic strategies to reduce mortality in COVID-19, by blocking IL-6 signalling, and ameliorating the deleterious effects of the inflammatory storm [25]. The RECOVERY trial study recently demonstrated the effectiveness of tocilizumab and dexamethasone combined in improving the survival rate and reducing the risk for ventilatory assistance in COVID-19 patients [26].

Furthermore, the complement system, an important arm of innate immunity, has been proposed as a crucial mediator in lung inflammation in SARS-CoV-2 infection. Generally, the complement system triggers a cascade, upon its activation by specific recognition pathways, leading to the generation of cleavage products that opsonize and eliminate pathogens, regulating inflammatory responses, and coordinating adaptive immunity. Indeed, an exaggerated complement activation induced by excessive stimuli such as viruses, may have a damaging effect by turning the complement system into a pathogenic effector in numerous diseases, especially thrombosis and sepsis [27]. An active cross-talk between the complement system and the coagulation cascade has been established [28 29]. Interestingly, increased levels of complement split product C5a were detected in the plasma and bronchoalveolar lavage fluid (BALF) of COVID-19 patients (25). In addition, a higher expression level of C5aR1 receptors were measured in pulmonary myeloid cells in the blood of SARS-CoV-2 infected patients, supporting a role for the C5a-C5a receptor interaction in the pathophysiology of ARDS [30 28]. In this study, several serological and biological variables were measured in patients with SARS-CoV-2 infection and their association with disease severity was analysed. In particular, we tested the hypothesis that C5a is a novel potential candidate to predict COVID-19 disease severity. Furthermore, we tested the association between C5a and pro-inflammatory and coagulation biomarkers in COVID-19 patients. The results from this study might aid the identification of patients at risk of developing severe COVID-19 disease or mortality, and in discerning pharmacological interventions to improve patient outcomes.

Methods

Study approval

This study was conducted in accordance with the Code of Ethics of the World Medical Association. Ethics committee approval was obtained from the Medical Research Center at Hamad Medical Corporation (MRC-05-084, Immunological and immune-genetic investigations in COVID-19 patients with varying disease severity, 06/21/2020). All the patients gave their informed consent to participate.

Study design and data collection

This prospective cohort study included 89 randomly selected patients, diagnosed with COVID-19 in Doha between July 14th and October 20th 2020. Selection criteria for participants was age (between 35 and 65 years), a positive SARS-CoV-2 PCR test result (a CT value < 30) and residency in Qatar. Upper respiratory tract specimens (throat and nasopharyngeal swabs) were tested for SARS-CoV-2 infection using TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Waltham, Massachusetts), or Cobas SARS-CoV-2 Test (Roche Diagnostics, Rotkreuz, Switzerland). All consented patients were recruited form Hazem Mebairek Hospital, Qatar's centre of communicable disease control (CDC) and Um Gharan quarantine facility. SARS-CoV-2 testing is routinely offered to all individuals presenting with symptoms suggestive of COVID-19, those who had close contact with confirmed cases, and all returning travellers.

Participants were stratified into three categories namely severe, mild and asymptomatic. Patients were categorized in the severe group based on requirement of oxygen support and ICU admission (n = 33). While mild cases were categorized based on clinical symptoms and positive radiographic findings indicating pulmonary involvement (n = 33). COVID-19 patients with mild to severe disease (n = 66) were hospitalized for inpatient management, out of which 33 (50%) were admitted to ICU. In the severe group, 23 patients (70%) needed mechanical ventilation, of which fourteen patients (42%) died with respiratory failure listed as the primary cause of death. Standard of care for hospitalized patients consisted of supportive care and antiviral therapy, with individual regimens selected based on severity of disease, the presence of comorbidities, contra-indications and potential drug-drug interactions. The patients with a positive SARS-CoV-2 PCR and no longitudinal clinical presentation were labelled as asymptomatic cases (n = 23). Blood samples were collected at the time of diagnosis, prior to isolation, or hospitalization. Clinical and laboratory investigations including diastolic blood pressure, body mass index (BMI), viral load, number of comorbidities, routine blood tests including complete blood cell counts were performed. In addition, the blood levels of electrolytes, glucose, albumin, total protein, C-reactive protein, procalcitonin, IL-6, D-dimers, ferritin, urea, and liver enzymes were determined. Complement activation C5a was measured in plasma using Human C5a / Complement C5a ELISA Kit (Sigma, St Lousi, MO, USA). Survival data including whether the patients were still alive or not as the end of survey date was obtained from the CERNER electronic healthcare system. This study was performed in accordance with the Reporting of Observational Studies in Epidemiology (STROBE) recommendations [31].

External validation of existing severity and mortality models in COVID-19 patients

Nowadays, various early prediction models have emerged amid the COVID-19 pandemic, aiming at optimizing patient stratification and reducing morbidity and mortality [32]. In this study, we systematically tested several existing mortality and severity models, based on simplicity and ease of use. The tested mortality models included the CURB-65 (confusion, urea, respiratory rate, BUN, age > 65) [33], the CRB-65 (confusion, respiratory rate, BUN, age > 65) [34], the pneumonia severity index (PSI) [35], and the ANDC score (age, NLR, D-dimer, CRP) [36]. The first three models have been validated and extensively used to predict 30-day mortality in community-acquired pneumonia, whereas the ANDC has only recently been employed to predict COVID-19 mortality. These scoring systems need rigorous external validation, through testing in different populations. Therefore, we further attempted to validate four COVID-19 severity models, including the CALL (comorbidity, age, lymphocyte count, lactate dehydrogenase), CALL-interleukin-6 (IL-6) scores, Haifeng et al. model (lymphocyte count and albumin), and Zhenyu et al. model (age, albumin, comorbidity, CRP) [37].

Assessment of accuracy of prediction models

Accuracy of COVID severity and mortality models was assessed using the area under the receiver-operator characteristic curve (AUC). For internal validation of the accuracy estimates and to reduce overfit bias, we used 1000 bootstrap resamples. The area under the curve (AUC) was calculated to detect the model with the best discriminatory capacity. A model with AUROC > 0.8 is known to be of excellent discriminatory ability [36]. In addition, we tested model calibration using the “rms” package.

Statistical analysis

All statistical analyses were conducted with R statistical software (version 4.0.4, R Foundation). Shapiro-Wilk test was used to examine covariates normality and choose an appropriate statistical test. Since all variables had a non-normal distribution, comparisons between different groups of severity and survival were performed using the Mann-Whitney test for continuous variables, and Fisher's Exact Test for categorical. Values were reported as medians and interquartile range [IQR]. Two tailed p-values were calculated and *p*-value < 0.05 was considered statistically significant. Spearman rank correlation tests were used to assess the correlation between different blood parameters. The Kaplan-Meier method was used to plot survival curves.

Results

Clinical characterization of COVID-19 patients upon diagnosis

In order to find a set of early predictors of severity and mortality, we analysed the clinical history, instrumental variables and laboratory tests of 89 patients diagnosed with COVID-19, aged between 35 and 65 years (median, IQR 48 [42–56]). This multi-ethnic study included patients from thirteen nationalities : Qatar, Pakistan, Bangladesh, India, Siri Lanka, Philippines, Nepal, Egypt, Libya, Tunisia, Yemen, Eretria and US. In agreement with previously published studies of COVID-19 cohorts, patients with comorbidities, including diabetes, hypertension, dyslipidemia, cardiovascular disease, renal disease, liver disease and/or asthma experienced more severe symptoms, requiring admission to the intensive care unit (ICU) [38]. In particular, 70% out of 33 severe cases required mechanical ventilation. Within the severe group, 36% had at least 1 comorbidity, 44% and 39% had at least 2 and 3 comorbidities respectively (Table 1). Furthermore, at the time of diagnosis, a low diastolic blood pressure and a high respiratory rate were observed in severe/critical cases that required ICU admission compared to patients with mild and asymptomatic patients (Fig. 1A). Interestingly, out of 69 patients with available BMI data, 71% were overweight (27.9 [24.7, 30.4]) (BMI > 25 kg/m^2) including asymptomatic (26.1 [23.8, 30.1]), mild (28.8 [25.8, 31.7]), and severe (27.3 [24.5, 29.4]) (Fig. 1B and Table 1). The nutritional status of our cohort according to the WHO guidelines was 26% normal weight, 46% pre-obesity, 19% obesity class I, 4% obesity class II, and 3% obesity class III (Table 1) [39 40]. It is noteworthy to mention that hyperglycemia was observed in COVID-19 patients with mild and severe disease, despite insulin treatment (Table 1). While some studies suggest a direct association between viral load and COVID-19 disease severity, the viral load in our cohort did not show a difference between asymptomatic, mild or severe cases at the time of diagnosis (Fig. 1C) [41 42 43].

Changes in complete blood count are associated with disease severity and mortality in SARS-CoV-2 infected patients

The association between hematological and serological biomarkers changes and disease severity in SARS-CoV-2 infection was analysed (Table 1). The analysis of the CBC variables using Wilcoxon rank sum test showed a significant increase in white blood cell count (WBC) (Fig. 2A), absolute neutrophil count (ANC) (Fig. 2B), percentage of neutrophils (Fig. 2C), and neutrophil-to-lymphocyte ratio (Figure-6I) in severe patients compared to mild and asymptomatic cases. In contrast, lymphocyte count (Fig. 2D), percentage of lymphocytes (Fig. 2E), and monocytes (Fig. 2F) showed significantly lower levels in severe cases compared to non-severe counterparts. On the other hand, the total monocyte count, as well as eosinophil and basophil percentages were not different among the three groups with varying disease severity (Table 1). In addition, changes in red blood cell parameters were observed among patients with different COVID-19 disease severity. Decreased red blood cell count (RBC) (Fig. 2G), haematocrit (Hct) (Fig. 2H), haemoglobin (Hgb) levels (Fig. 2I), and mean corpuscular hemoglobin concentration (MCHC) (Fig. 2J) were detected in severe patients compared to mild and asymptomatic cases. On the other hand, mean corpuscular volume (Fig. 2K) and red blood cell distribution width (RDW-CV) (Fig. 2L) were elevated in severe cases compared to patients without symptoms and with mild symptoms. Moreover, compared to patients who survived, non-survivors had significantly higher levels of total WBC (Fig. 3A), ANC (Fig. 3B), neutrophils (%) (Fig. 3C), mean corpuscular volume (MCV) (Fig. 3D), ferritin (Fig. 3E), and RDW-

CV (Fig. 3F). However, patients who did not survive despite medical intervention showed significantly lower levels of lymphocyte count (Fig. 3G), lymphocyte (%) (Fig. 3H), monocyte (%) (Fig. 3I), RBC (Fig. 3J), Hct (%) (Fig. 3K), and Hgb (Fig. 3L).

COVID-19 severity is associated with increased levels of C5a, coagulation and inflammation markers

The plasma concentration of complement split product C5a was measured using ELISA in all 89 COVID-19 patients. Although, the normal plasma level of C5a is less than 120 pg/ml, higher levels were detected in all samples (> 260 pg/ml), including the asymptomatic patients. Interestingly, C5a plasma levels increased proportionally to COVID-19 disease severity ($p < 0.001$) (Fig. 4A). Prothrombin time ($p = 0.002$) was higher in severe patients, whereas fibrinogen was lower in the severe group compared to non-severe patients ($p = 0.021$) (Table-1). Regarding survival outcomes, median circulating C5a levels were further compared between survivors (1,289 [775-1,690]) and non-survivors (1,670 [1,375-2,191]) and were found statistically significant ($p = 0.035$) (Fig. 5A). A procoagulant state characterized by platelet activation, increased mean platelet volume ($p < 0.001$) (Fig. 5B), platelet distribution width ($p = 0.010$) (Fig. 5C), and partial thromboplastin time ($p = 0.006$) (Fig. 5D) was observed in patients with severe disease. Furthermore, within the severe group the higher values were observed in patients that did not survive. Elevated D-dimer levels were also observed in the severe group (Fig. 4B) and particularly in patients who did not survive (4.15 [1.49–5.67]) ($p < 0.001$) (Fig. 5E) as compared to mild and asymptomatic cases.

Recent studies have shown an association between higher levels of inflammatory markers and SARS-CoV-2 infection severity [9]. In this line, increased peripheral leukocytes and neutrophils numbers, and higher levels of C-reactive protein (CRP) were observed in patients with severe disease (27 [6–77]) compared to mild (21 [7–83]) and asymptomatic patients (4 [1–14]) ($p < 0.001$) (Fig. 4C). Procalcitonin, another acute-phase protein usually below the limit of detection in clinical assays in the blood of healthy individuals, was found in high amounts in patients with severe disease (0.44 [0.14–0.83]) compared to patients with no symptoms (0.03 [0.03–0.14]) and to those with mild clinical presentation (0.16 [0.05–0.30]) ($p = 0.007$) (Fig. 4D). Provocatively, higher levels of CRP (Fig. 5F) and procalcitonin (Fig. 5G) were detected in patients that did not survive compared to those who survived, with a prominent increase in CRP levels (74 [21–158] vs 13 [5–40]) ($p = 0.004$). A mildly elevated but statistically non-significant elevation of IL-6 levels was observed in severely ill patients when compared to other groups (Fig. 4E). Another key acute-phase reactant that we investigated was ferritin, whereas an increase in ferritin levels protects the host by limiting the free iron needed for pathogen growth and survival, it can also play a pro-inflammatory role, contributing to the cytokine storm [44]. Within the group of critically ill COVID-19 patients, hyperferritinemia (> 500 µg/L) was observed with a median of 1,131 [536-1,634] vs 396 [181–582] and 404 [220–786] in patients with no or mild symptoms respectively (Fig. 4F). Accumulated evidence is in support of a higher mortality rate of patients with cardiovascular diseases as a result of SARS-CoV-2 infection [45]. In this line higher levels of high-sensitivity cardiac troponin T (HS-TnT) as a

marker of disease progression, was determined in severe cases compared to mild patients (Fig. 4L). Particularly, higher levels were observed in patients that did not survive (Fig. 5M).

SARS-CoV-2 disease severity is associated with changes in kidney and liver function parameters

Acute kidney injury has been reported in adult patients following SARS-CoV-2 infection with multifactorial causality including cytokine storm, hypoxia, increased coagulation and impaired glomerular filtration. Interestingly, we observed significantly higher levels of urea in severe patients compared to asymptomatic and mild patients ($p < 0.001$) (Fig. 4G). High urea levels indicative of impaired renal function were observed in non-survivors (Fig. 5I). To further investigate the association between renal function and COVID-19 disease severity and mortality, electrolytes were measured. Patients with severe complications of COVID-19 presented with hypernatremia (Table 1) and hypocalcemia (Fig. 4K), in which low calcium levels correlated with increased mortality (Fig. 5L). Recently, COVID-19 patients were classified as severe or non-severe based on total protein levels in the serum [46]. In our study, we found that circulating total protein (Fig. 4I) and albumin levels (Fig. 4J) were inversely correlated with disease severity. As a result, calcium values adjusted for the albumin concentration were highest in the severe COVID-19 cases (Fig. 4H). The levels of total protein (Fig. 5J), albumin (Fig. 5K), and calcium (Fig. 5L), were significantly lower in patients who died compared to those who survived.

Involvement of different organs is another hallmark of COVID-19 severity. In the current cohort, significantly elevated levels of liver enzymes, alkaline phosphatase (ALP) (Fig. 5N), alanine aminotransferase (ALT) (Figure-5O), aspartate aminotransferase (AST) (Fig. 5P), and lactate dehydrogenase (LDH) (Table 2) were measured in non-survivors compared to survivors.

C5a is correlated with several blood indices in SARS-CoV2 infection

Elevated levels of pro-inflammatory anaphylatoxin C5a has been reported in cases of sepsis involving renal impairment. Neutrophil-to-lymphocyte ratio (NLR) is also recognized as a predictive factor for disease severity in sepsis, a variety of malignancies, and recently for critical illness in patients with SARS-CoV-2 infection. In accordance with these studies, a higher NLR was observed in the severe group patients compared to mild and asymptomatic cases (Fig. 6I). However, it has not yet been established whether there is an association between C5a and other haematological and serological parameters, and the resulting long-term outcomes in COVID-19 patients.

A significantly lower ratio of C5a to NLR (CNLR) was calculated in severe cases of COVID-19 compared to less severe patients. In addition, we report a statistically significant lower median of CNLR in the non-survivor group (Fig. 6R). In order to further investigate the role of C5a in COVID-19 pathogenesis, we calculated the ratios of C5a to several blood indices and found that ratios of C5a to lymphocyte (Fig. 6K), RBC (Fig. 6L), Hgb (Fig. 6M), Hct (Fig. 6N), albumin (Fig. 6O), and calcium (Fig. 6P) were significantly higher in severe patients in comparison with patients with no or mild symptoms (Fig. 6 and Table 3). A

correlation matrix was plotted for all investigated covariates to analyse the relationship between each pair of variables in this dataset (Fig. 9). Moreover, Spearman's Correlation analysis revealed that neutrophil-to-lymphocyte ratio (Fig. 6A), red blood cell distribution width (RDW) (Fig. 6B), urea (Fig. 6C), and glucose (Fig. 6D) were positively correlated with C5a levels. A mild correlation (R = 0.44) observed between C5a and NLR. On the other hand C5a levels had an inverse correlation with albumin (Fig. 6E), Hgb (Fig. 6F), Hct (Fig. 6G) and calcium (Fig. 6H), with a mild inverse correlation (R = 0.52) with albumin. Altogether, C5a might contribute to COVID-19 disease severity by exacerbating innate immune responses and renal and hepatic injury while playing a dual role in inflammation and thrombosis.

C5a is a novel predictive marker for mortality in COVID-19 patients

To investigate mortality risk factors in COVID-19 patients, available clinical and laboratory parameters were stratified based on clinically relevant cut-offs using normal reference intervals. The difference between the time of admission or quarantine and the time of death or end of the survey was used to calculate Kaplan-Meier survival estimates. Increased levels of C5a in the plasma of severe cases, prompted an inclusion of C5a in survival analysis, along with already described risk factors. The survival function graph demonstrated that levels of C5a higher than 1200 pg/ml adversely affect short-term survival in COVID-19 patients (p = 0.033) (Fig. 7A). Notably, the two functions are closer together in the first 30–40 days of follow-up, but thereafter have a widening gap, suggesting that high levels of C5a is more detrimental later during follow-up than it is early on. To further evaluate the relationship between C5a and known prognostic markers with mortality status, ratios of C5a to other covariates were independently stratified into tertiles and assessed as predictors of survival (Fig. 9). We found that ratios of C5a-to-NLR (Fig. 8A), C5a-to-lymphocyte (Fig. 8B), C5a-to-urea (Fig. 8C), C5a-to-glucose (Fig. 8D), and C5a-to-Hgb (Fig. 8E) are good predictors of mortality in SARS-CoV-2 infection. Additionally, analysing CBC variables demonstrated that patients with high cut-off for WBC (> $10x10^3$/μL) (Fig. 7B), ANC (> 7x103/μL) (Fig. 7C), neutrophil percentage (> 80%) (Fig. 7D), neutrophil-to-lymphocyte ratio (> 5) (Fig. 7E), RDW-CV (> 14.5%) (Fig. 7F) exhibited a higher risk for mortality. Furthermore, patients with a low probability of survival showed a higher level of inflammatory marker CRP > 100 mg/L (Fig. 7G), as well as, urea > 8.1mmol/L (Fig. 7H), and creatinine > 124 μmol/L (Fig. 7I). Patients with high sodium levels (> 145 mmol/L) tended to have a higher risk of death (Fig. 7J). Additionally, the study of the survival time using other potential prognostic blood indices revealed several independent risk factors that are associated with fatal outcome including low levels of lymphocyte (= < 20%) (Fig. 7K), albumin (= < 25 g/L) (Fig. 7O), and red blood cell parameters including RBC (= < $4.8x10^6$/μL) (Fig. 7L), Hct (= < 40%) (Fig. 7M), and Hgb (= < 10 g/dL) (Fig. 7N) in SARS-CoV-2 infected patients.

Validation of severity models: CAL is a new predictive model of COVID-19 severity

Based on the original formulas, we were able to calculate severity scores for the CALL, CALL-IL6 and the Haifeng et al. and Zhenyu et al. models in 89 (99%) patients. Comparing the AUROCs for severe COVID-19, only the model by Haifeng et al. was statistically significant with AUC 0.88 (95% CI 0.80–0.95) [37]. Performance of severity models can be found in Fig. 10A.

Multivariable logistic regression considering the Haifeng et al. model variables (lymphocyte count and albumin levels) along with C5a levels as predictors of severe COVID-19, showed that only the latter two variables were significant (OR 0.707, 95% CI 0.5817–0.815) and (OR 1.001, 95% CI 1.0002–1.003), respectively. Thus, we tested the hypothesis that adding C5a to albumin, and lymphocyte and referring to it as the CAL would result in a predictive model with improved discriminative ability. Using the Delong approach to compare AUCs [47], the CAL model performed better than the original version with (AUC 0.94 vs. 0.88, with difference between areas 0.06, P = 0.04) (Figure-10A), but had lower overall calibration (Figure-S1).

Validation of mortality models

Using the original formulas or points, we were able to calculate mortality scores for the CURB-65, CRB-65, PSI and ANDC models in 53 (60%) patients. Only the ANDC and PSI models were significant, with AUCs 0.81 (95% CI 0.69–0.94) and 0.71 (95% CI 0.56–0.86), respectively. Therefore, we tested the performance of both PSI and ANDC combined, which showed better accuracy, AUC 0.85 (95% CI 0.73–0.98) (Figure-10A). The latter model showed better calibration than the ANDC alone (Figure-S).

Models predicting ICU admission

Using the original formulas or points, we tested predicting ICU admission using the CURB-65, CRB-65, and PSI scores in 53 (60%) patients. Only the PSI excellent performance in predicting ICU admission AUC 0.88 (95% CI 0.79–0.97) (Figure-10A) with acceptable calibration (Figure-S).

Discussion

Severe SARS-CoV-2 infection confers a hypercoagulable state along with a robust inflammatory response [10 9]. Evidence gathered during post-mortem examination of COVID-19 patients implicates thrombosis as a major cause of death. Several studies demonstrated that an exaggerated anti-viral inflammatory response can lead to endothelial dysfunction/activation and a procoagulant state, known as thrombotic microangiopathy (TMA) [48]. TMA results in diminished blood flow leading to tissue ischemia and oxidative injury, culminating in multiorgan failure reported in COVID-19 patients. In agreement, high incidence of venous thromboembolism (VTE) has been shown to correlate with disease severity and mortality in hospitalized COVID-19 patients [49]. Accordingly, pulmonary intravascular coagulation observed in the lungs of COVID-19 patients can compromise other organs including the heart and kidneys, leading to multiorgan failure and death [50 11 51 52]. Recent studies demonstrated the role of SARS-CoV-2 in complement activation [53]. The SARS-CoV-2 N protein was reported to bind mannan-binding lectin-associated protease (MASP-2) and activate the lectin pathway, initiating the complement cascade

[54]. Complement activation products were also detected on circulating erythrocytes in hospitalized COVID-19 patients [55]. Particularly, serum levels of anaphylatoxin C5a are significantly increased in COVID-19 patients [56 57]. Furthermore, antibodies against SARS-CoV-2 may also contribute to the activation of the classical and alternative pathways of complement, sustaining high levels of C5a in severe COVID-19 cases [58]. In another study using immunohistochemistry staining and single-cell RNA-Seq, high expression level of C5aR1 across inflammatory cells was detected in the lungs of patients infected with SARS-CoV-2 [43 59 60]. Increased plasma levels of complement terminal complex soluble C5b-9 (sC5b-9) and activated C5a correlated with disease severity. Even more, elevated levels of C5a were found in patients requiring continuous positive airway pressure or mechanical ventilation [57]. In addition, histological studies in COVID-19 patients have shown deposition of viral spike glycoprotein, complement split product C4d, and sC5b-9 in the interalveolar septa and microvessels [61]. Similarly, Jiang et al detected high concentrations of C5a and sC5b-9 in the sera and lung tissue in a mouse model of MERS [56]. Our study is in alignment with previous research demonstrating a crucial role of C5a in the crosstalk between inflammation and thrombosis [43 53 54 55 56 57 59 61]. We demonstrated increased levels of C5a in the plasma of our multi-ethnic cohort of COVID-19 patients, which was proportional to disease severity. Increases in C5a levels were also proportional to the levels of fibrin degradation product, D-dimer, known to be elevated in VTE and disseminated intravascular coagulation. Additionally, we found poor survival outcomes in COVID-19 patients with C5a levels higher than 1200 pg/ml. In our data, both C5a levels and neutrophil count increased proportionally with disease severity. The complement component C5a is a potent chemoattractant, which activates neutrophils and recruits them to the site of inflammation [62]. The activation of C5a results in neutrophil degranulation and tissue factor expression, resulting in a prothrombotic state by triggering the extrinsic coagulation pathway [28 43]. In this line, we found a positive correlation between C5a levels and the number of circulating neutrophils in the blood, in addition to a lower ratio of C5a to NLR (CNLR) in the severe cases of COVID-19. Here we propose “CAL” as a novel prognostic model of COVID-19 severity with an enhanced predictive capacity.

Similarly, previous reports have detected low serum albumin in patients with severe COVID-19, which was linked to thrombotic events [63 64]. In this study, an association of hypoalbuminemia with patient mortality was identified. A sustained stress on the liver leading to the production of APPs and clotting factors diverting the resources might result in a diminished synthesis of albumin, further aggravating the hemodynamic status. While the insult on the renal system led to increased levels of circulating urea and creatinine promoting multi-organ damage. The role of proteinuria as a cause of hypoalbuminemia needs further exploration. We further attempted to validate several COVID-19 severity models created to aid in clinical decision-making but exhibit limited overall performance. These models are associated with a risk of bias and overfitting, and are not well-reported [65]. Public sharing of anonymized raw data that has been published from COVID-19 studies is necessary to develop and validate better models in large multi-centred settings [65]. We acknowledge that the current study has limited sample size and the model needs further validation in diverse ethnic backgrounds with larger cohort studies. Moreover, the CAL model

might not be easy to adopt in low-income countries due to financial constraints associated with lab investigations in the underdeveloped countries.

In summary, high C5a complement protein and APPs, hypoalbuminemia, and renal insufficiency collectively have an adverse outcome on survival of COVID-19 patients. Altogether, we conclude that patients with an abnormally low level of albumin and lymphocytes and a high number of neutrophils, in addition to anaemic state characterized by low RBC, haemoglobin, and haematocrit counts are at a higher mortality risk. Patients who did not survive had elevated levels of inflammatory (CRP, and procalcitonin) and prothrombotic mediators (C5a, D-Dimer, INR, MPV, and PDW, prothrombin time, and partial thromboplastin time). Based on these results, we propose a mechanistic role of C5a in the pathogenesis and severity of COVID-19, highlighting its crosstalk between inflammation and thrombosis (Figure-11). Lastly, this study identifies biomarkers of COVID-19 severity that can potentially assist clinicians in early recognition of patients at risk for critical complications and mortality and in developing new management strategies.

Declarations

Author contributions

FC, GG, MS: Data curation, methodology, formal analysis, writing the original draft. IA, AD: methodology, investigation, data cleansing, formal analysis, second draft. YS, AE, MD, MA, MM, AZ, AA: Sample acquisition, investigation. FAM, AP: resources, investigation, experimental design EE, RS: Resources, supervision, experimental design FC, GG, MS: Experimental design, conceptualization, resources, supervision, funding acquisition, validation, investigation, visualization, project administration, writing-review and editing.

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Tables

Table-1 Demographic, clinical and laboratory measurements of asymptomatic, mild and severe COVID-19.

Characteristic	N	Total N = 89[1]	Asymptomatic N = 23[1]	Mild N = 33[1]	Severe N = 33[1]	*p*-value[2]
Survival [%]	89		23 (31%)	33 (44%)	19 (25%)	**<0.001**
Gender (Male) [%]	89		21 (26%)	29 (36%)	31 (38%)	0.9
At least 1 Comorbidity	89		17 (31%)	18 (33%)	20 (36%)	0.3
At least 2 Comorbidities	89		7 (22%)	11 (34%)	14 (44%)	0.6
At least 3 Comorbidities	89		5 (28%)	6 (33%)	7 (39%)	>0.9
Hospitilization duration	89	7 (0-32)	0 (0-0)	6 (0-10)	46 (29-86)	**<0.001**
Age [years]	89	48 (42-56)	43 (38-48)	47 (43-55)	54 (46-59)	**0.002**
COVID-19 Average CT	78	25.6 (18.7-29.5)	25.4 (19.2-28.2)	26.2 (18.5-31.7)	24.4 (19.3-28.0)	0.5
Diastolic blood pressure [mmHg]	89	75 (64-83)	78 (71-90)	76 (67-82)	68 (57-80)	**0.019**
Body Mass Index (BMI) [kg/m^2]	68	28.1 (24.8-30.4)	27.6 (23.8-30.4)	28.8 (25.8-31.7)	27.3 (24.5-29.4)	0.4
Nutritional status	68					0.4
Normal weight			4 (22%)	5 (28%)	9 (50%)	
Obesity class I			3 (23%)	6 (46%)	4 (31%)	
Obesity class II			0 (0%)	1 (33%)	2 (67%)	
Obesity class III			0 (0%)	2 (100%)	0 (0%)	
Pre-obesity			2 (6.5%)	12 (39%)	17 (55%)	
Underweight			0 (0%)	0 (0%)	1 (100%)	
Glucose [mmol/L]	89	6.3 (5.3-8.8)	5.5 (4.8-6.4)	6.0 (5.3-8.2)	7.3 (5.9-10.3)	**0.005**
White blood cell count (WBC) [x10^3/µL]	89	7.1 (4.9-10.3)	6.3 (5.2-7.5)	5.9 (4.2-7.5)	11.2 (8.0-13.6)	**<0.001**

Characteristic	N	Total N = 89[1]	Asymptomatic N = 23[1]	Mild N = 33[1]	Severe N = 33[1]	*p*-value[2]
Lymphocyte count [x10^3/µL]	89	1.40 (1.00-1.90)	1.70 (1.60-2.35)	1.30 (1.00-1.90)	1.00 (0.60-1.70)	**0.001**
Absolute neutrophil count (ANC) [x10^3/µL]	89	4.6 (3.1-7.8)	3.7 (2.5-4.7)	3.4 (2.4-5.8)	8.3 (6.3-11.5)	**<0.001**
Neutrophil-to-lymphocyte ratio	89	2.8 (2.0-8.7)	2.1 (1.9-2.5)	2.4 (1.5-3.9)	9.3 (3.6-17.7)	**<0.001**
Monocyte count[x10^3/µL]	89	0.60 (0.40-0.80)	0.60 (0.50-0.80)	0.40 (0.30-0.70)	0.70 (0.40-0.80)	0.10
Eosinophil count[x10^3/µL]	89	0.00 (0.00-0.10)	0.10 (0.00-0.20)	0.00 (0.00-0.10)	0.00 (0.00-0.10)	0.066
Basophil count[x10^3/µL]	89	0.030 (0.010-0.040)	0.030 (0.020-0.030)	0.020 (0.010-0.040)	0.040 (0.010-0.050)	0.2
Lymphocyte [%]	89	23 (9-30)	28 (25-30)	27 (18-35)	8 (5-19)	**<0.001**
Neutrophil [%]	89	68 (58-83)	59 (54-64)	66 (53-74)	84 (73-90)	**<0.001**
Monocyte [%]	89	7.7 (5.2-9.3)	8.8 (7.4-11.7)	7.9 (6.0-9.5)	5.4 (3.4-8.7)	**<0.001**
Eosinophil [%]	89	0.70 (0.00-1.90)	1.80 (0.35-3.10)	0.50 (0.00-1.50)	0.30 (0.00-0.90)	**0.019**
Basophil [%]	89	0.30 (0.20-0.50)	0.50 (0.35-0.50)	0.30 (0.20-0.60)	0.30 (0.10-0.50)	**0.019**
Red blood cell count (RBC) [x10^6/µL]	89	4.70 (3.80-5.30)	5.10 (4.80-5.40)	5.10 (4.70-5.40)	3.40 (3.00-4.10)	**<0.001**
Hematocrit (Hct) [%]	89	40 (33-44)	44 (42-46)	41 (39-44)	31 (27-36)	**<0.001**
Hemoglobin (Hgb) [g/dL]	89	13.00 (10.40-14.70)	14.60 (13.80-15.40)	13.70 (12.90-14.70)	9.60 (7.90-11.50)	**<0.001**
Hemoglobin A1C (HbA1C) [%]	47	6.20 (5.60-7.55)	5.65 (5.40-6.00)	6.90 (5.75-8.85)	6.20 (5.70-7.00)	0.2

Characteristic	N	Total N = 89[1]	Asymptomatic N = 23[1]	Mild N = 33[1]	Severe N = 33[1]	*p*-value[2]
Ferritin [µg/L]	62	661 (286-1,151)	396 (181-582)	404 (220-786)	1,131 (536-1,634)	**<0.001**
Mean corpuscular volume (MCV) [fL]	89	86 (82-91)	86 (83-89)	83 (78-87)	90 (86-93)	**<0.001**
Mean cell hemoglobin (MCH) [pg]	89	28.60 (26.70-30.00)	28.80 (27.55-30.40)	27.90 (25.80-29.60)	29.20 (27.80-30.00)	0.087
Mean corpuscular hemoglobin concentration (MCHC) [g/dL]	89	33.10 (31.60-33.90)	33.60 (32.25-33.95)	33.60 (32.50-34.20)	32.30 (31.10-33.30)	**0.033**
Red blood cell distribution width (RDW-CV) [%]	89	13.50 (12.50-15.10)	12.30 (12.00-13.15)	13.30 (12.50-13.80)	15.20 (14.10-17.30)	**<0.001**
Platelet [× 10^9/L]	85	246 (194-326)	248 (228-298)	216 (190-306)	269 (190-344)	0.8
Mean platelet volume (MPV) [fl]	86	10.55 (9.70-11.38)	10.60 (9.70-11.20)	10.30 (9.70-10.90)	10.85 (10.10-11.90)	0.2
Platelet distribution width (PDW) [fl]	56	13.5 (11.5-15.7)	15.3 (14.4-15.6)	13.8 (11.0-15.7)	12.6 (11.6-15.8)	0.7
Prothrombin time (PT) [second]	55	12.10 (11.35-13.20)	12.35 (11.80-13.02)	11.40 (11.05-11.70)	12.55 (11.95-13.88)	**0.002**
International Normalized Ratio (INR)	55	1.00 (1.00-1.10)	1.05 (1.00-1.12)	1.00 (1.00-1.00)	1.10 (1.00-1.20)	0.12
D-Dimer [mg/L FEU]	64	0.87 (0.37-2.40)	0.30 (0.26-0.38)	0.43 (0.30-0.57)	2.18 (1.20-4.73)	**<0.001**
Fibrinogen [g/L]	40	4.15 (3.00-5.67)	26.30 (16.55-29.65)	4.60 (3.90-5.90)	3.80 (2.98-4.95)	**0.021**
Partial thromboplastin time (APTT) [second]	55	31 (28-37)	30 (26-33)	31 (28-33)	33 (29-40)	0.2
Complement component 5a (C5a) [pg/ml]	89	1,383 (929-1,786)	1,009 (722-1,265)	1,289 (749-1,687)	1,815 (1,407-2,400)	**<0.001**

Characteristic	N	Total N = 89[1]	Asymptomatic N = 23[1]	Mild N = 33[1]	Severe N = 33[1]	*p*-value[2]
C-reactive protein (CRP) [mg/L]	89	16 (5-58)	4 (1-14)	21 (7-83)	27 (6-77)	**<0.001**
Interleukin-6 (IL-6) [pg/mL]	52	32 (12-57)	14 (8-19)	32 (13-52)	34 (8-82)	0.4
High-sensitivity Troponin-T [ng/mL]	40	26 (8-108)	7 (6-88)	8 (5-10)	36 (24-150)	**<0.001**
Lactic acid [mmol/L]	35	1.70 (1.10-2.20)	1.80 (1.12-2.52)	1.37 (1.10-1.85)	1.80 (1.30-2.30)	0.5
Uric acid [µmol/L]	51	305 (266-384)	326 (277-406)	313 (274-380)	300 (148-376)	0.5
Bicarbonate [mmol/L]	89	25.0 (23.0-28.0)	26.0 (25.0-27.0)	24.9 (23.0-26.0)	26.0 (23.0-32.0)	**0.034**
Sodium [mmol/L]	89	138.0 (136.0-141.0)	138.0 (136.0-140.0)	137.0 (135.0-139.0)	141.0 (137.0-147.0)	**0.006**
Potassium [mmol/L]	85	4.40 (4.00-4.70)	4.60 (4.12-5.10)	4.15 (4.00-4.55)	4.40 (4.00-4.70)	0.3
Chloride [mmol/L]	89	102.0 (99.0-104.0)	102.0 (99.0-103.0)	101.0 (99.0-103.0)	104.0 (98.0-109.0)	0.079
Phosphorus [mmol/L]	46	1.14 (1.00-1.31)	1.30 (1.28-1.32)	1.14 (1.01-1.16)	1.14 (0.98-1.42)	0.4
Magnesium [mmol/L]	60	0.89 (0.83-0.96)	0.87 (0.84-0.90)	0.87 (0.79-0.92)	0.94 (0.87-1.01)	**0.030**
Total Protein [g/L]	80	71 (64-77)	77 (70-80)	72 (68-75)	67 (57-72)	**<0.001**
Albumin [g/L]	89	32 (27-39)	41 (38-44)	35 (32-39)	26 (23-29)	**<0.001**
Bilirubin [mg/dL]	80	8 (5-13)	8 (4-10)	8 (6-12)	8 (6-16)	0.4
Urea [mmol/L]	89	5 (4-10)	4 (3-5)	4 (3-5)	13 (6-21)	**<0.001**
Creatinine [µmol/L]	89	78 (63-92)	81 (72-88)	78 (66-92)	66 (54-98)	0.5

Characteristic	N	Total N = 89[1]	Asymptomatic N = 23[1]	Mild N = 33[1]	Severe N = 33[1]	*p*-value[2]
Creatine kinase (CK) [U/L]	45	103 (58-245)	98 (84-114)	73 (61-133)	133 (57-359)	0.7
Alkaline phosphatase (ALP) [U/L]	80	88 (63-110)	82 (69-92)	86 (62-100)	99 (73-166)	0.14
Alanine aminotransferase (ALT) [U/L]	77	31 (20-64)	25 (18-36)	35 (24-72)	34 (22-76)	0.085
Aspartate aminotransferase (AST) [U/L]	72	28 (23-50)	26 (18-29)	27 (22-49)	36 (25-54)	0.10
Lactate dehydrogenase (LDH) [U/L]	53	347 (249-462)	318 (250-386)	249 (214-339)	429 (336-623)	**<0.001**
Low-density lipoprotein (LDL) [mmol/L]	16	2.85 (2.28-3.70)	2.00 (1.60-2.40)	2.90 (2.41-4.07)	3.19 (2.77-3.43)	0.4
Triglyceride [mmol/L]	52	1.80 (1.35-2.62)	1.10 (0.95-2.15)	1.36 (1.20-1.77)	2.20 (1.70-3.05)	**<0.001**
Vitamin D [ng/mL]	56	18 (13-23)	22 (14-24)	18 (14-21)	17 (12-24)	0.7
Calcium [mmol/L]	89	2.25 (2.14-2.35)	2.34 (2.28-2.42)	2.26 (2.15-2.34)	2.16 (2.06-2.25)	**<0.001**
Adjusted calcium [mmol/L]	89	2.39 (2.30-2.46)	2.31 (2.26-2.37)	2.35 (2.29-2.46)	2.45 (2.39-2.53)	**<0.001**
Procalcitonin [ng/mL]	47	0.27 (0.10-0.55)	0.03 (0.03-0.14)	0.16 (0.05-0.30)	0.44 (0.14-0.83)	**0.007**

[1]Statistics presented: n (%); Median (25%-75%)

[2]Statistical tests performed: Fisher's exact test; chi-square test of independence; Kruskal-Wallis test

Table-2 Demographic and clinical characteristics based on survival and hospital death in COVID-19 patients.

Characteristic	N	Total N = 89[1]	Survivor N = 75[1]	Non-survivor N = 14[1]	*p*-value[2]
Severity	89				**<0.001**
Asymptomatic			23 (100%)	0 (0%)	
Mild			33 (100%)	0 (0%)	
Severe			19 (58%)	14 (42%)	
Gender (Male) [%]	89		67 (83%)	14 (17%)	0.3
At least 1 Comorbidity	89		47 (85%)	8 (15%)	>0.9
At least 2 Comorbidities	89		27 (84%)	5 (16%)	>0.9
At least 3 Comorbidities	89		16 (89%)	2 (11%)	0.7
Hospitilization duration	89	7 (0-32)	6 (0-16)	48 (31-73)	**<0.001**
Age [years]	89	48 (42-56)	46 (41-54)	57 (52-60)	**<0.001**
COVID-19 Average CT	78	25.6 (18.7-29.5)	25.7 (18.7-29.6)	22.1 (19.1-28.1)	0.6
Diastolic blood pressure [mmHg]	89	75 (64-83)	75 (68-84)	60 (51-72)	**0.006**
Body Mass Index (BMI) [kg/m^2]	68	28.1 (24.8-30.4)	28.6 (25.0-30.5)	27.2 (24.3-29.7)	0.6
Nutritional status	68				0.8
Normal weight			13 (72%)	5 (28%)	
Obesity class I			11 (85%)	2 (15%)	
Obesity class II			2 (67%)	1 (33%)	
Obesity class III			2 (100%)	0 (0%)	
Pre-obesity			25 (81%)	6 (19%)	
Underweight			1 (100%)	0 (0%)	
Glucose [mmol/L]	89	6.3 (5.3-8.8)	6.2 (5.2-8.3)	6.8 (6.0-9.3)	0.2
White blood cell count (WBC) [x10^3/µL]	89	7.1 (4.9-10.3)	6.6 (4.8-8.4)	12.9 (11.5-18.6)	**<0.001**
Lymphocyte count [x10^3/µL]	89	1.40 (1.00-1.90)	1.60 (1.15-1.95)	0.95 (0.70-1.08)	**0.007**

Characteristic	N	Total N = 89[1]	Survivor N = 75[1]	Non-survivor N = 14[1]	*p*-value[2]
Absolute neutrophil count (ANC) [x10^3/µL]	89	4.6 (3.1-7.8)	4.3 (2.8-6.3)	10.7 (9.4-12.9)	**<0.001**
Neutrophil-to-lymphocyte ratio	89	2.8 (2.0-8.7)	2.6 (1.9-4.4)	15.9 (6.9-18.6)	**<0.001**
Monocyte count[x10^3/µL]	89	0.60 (0.40-0.80)	0.60 (0.35-0.80)	0.70 (0.40-0.80)	0.3
Eosinophil count[x10^3/µL]	89	0.00 (0.00-0.10)	0.00 (0.00-0.20)	0.00 (0.00-0.10)	0.14
Basophil count[x10^3/µL]	89	0.030 (0.010-0.040)	0.030 (0.010-0.040)	0.035 (0.013-0.057)	0.4
Lymphocyte [%]	89	23 (9-30)	25 (17-31)	5 (4-8)	**<0.001**
Neutrophil [%]	89	68 (58-83)	65 (55-75)	88 (85-91)	**<0.001**
Monocyte [%]	89	7.7 (5.2-9.3)	8.0 (5.8-9.6)	5.0 (3.6-6.9)	**0.004**
Eosinophil [%]	89	0.70 (0.00-1.90)	0.90 (0.10-2.30)	0.20 (0.00-0.50)	**0.008**
Basophil [%]	89	0.30 (0.20-0.50)	0.40 (0.20-0.55)	0.25 (0.10-0.38)	**0.046**
Red blood cell count (RBC) [x10^6/µL]	89	4.70 (3.80-5.30)	4.90 (4.20-5.35)	3.20 (2.68-3.48)	**<0.001**
Hematocrit (Hct) [%]	89	40 (33-44)	41 (37-44)	28 (25-32)	**<0.001**
Hemoglobin (Hgb) [g/dL]	89	13.00 (10.40-14.70)	13.60 (11.90-14.90)	8.95 (7.73-10.25)	**<0.001**
Hemoglobin A1C (HbA1C) [%]	47	6.20 (5.60-7.55)	6.20 (5.57-7.53)	6.20 (6.05-7.15)	0.6
Ferritin [µg/L]	62	661 (286-1,151)	611 (253-1,090)	1,542 (459-4,584)	**0.017**
Mean corpuscular volume (MCV) [fL]	89	86 (82-91)	86 (82-89)	90 (87-95)	**0.012**
Mean cell hemoglobin (MCH) [pg]	89	28.60 (26.70-30.00)	28.60 (26.50-30.00)	28.85 (26.72-29.78)	0.9
Mean corpuscular hemoglobin concentration (MCHC) [g/dL]	89	33.10 (31.60-33.90)	33.30 (31.90-34.05)	32.25 (31.08-33.00)	**0.029**

Characteristic	N	Total N = 89[1]	Survivor N = 75[1]	Non-survivor N = 14[1]	*p*-value[2]
Red blood cell distribution width (RDW-CV) [%]	89	13.50 (12.50-15.10)	13.20 (12.35-14.40)	17.05 (15.27-18.27)	**<0.001**
Platelet [× 10^9/L]	85	246 (194-326)	262 (201-332)	165 (151-231)	**0.013**
Mean platelet volume (MPV) [fl]	86	10.55 (9.70-11.38)	10.50 (9.55-11.10)	12.30 (11.10-12.95)	**<0.001**
Platelet distribution width (PDW) [fl]	56	13.5 (11.5-15.7)	12.9 (10.9-15.5)	16.0 (13.1-20.4)	**0.010**
Prothrombin time (PT) [second]	55	12.10 (11.35-13.20)	12.10 (11.20-12.60)	12.95 (11.80-15.22)	0.12
International Normalized Ratio (INR)	55	1.00 (1.00-1.10)	1.00 (1.00-1.10)	1.05 (1.00-1.28)	0.3
D-Dimer [mg/L FEU]	64	0.87 (0.37-2.40)	0.52 (0.30-1.90)	4.15 (1.49-5.67)	**<0.001**
Fibrinogen [g/L]	40	4.15 (3.00-5.67)	4.65 (3.73-5.83)	3.10 (2.40-4.62)	**0.038**
Partial thromboplastin time (APTT) [second]	55	31 (28-37)	31 (28-34)	40 (32-47)	**0.006**
Complement component 5a (C5a) [pg/ml]	89	1,383 (929-1,786)	1,289 (775-1,690)	1,670 (1,375-2,191)	**0.035**
C-reactive protein (CRP) [mg/L]	89	16 (5-58)	13 (5-40)	74 (21-158)	**0.004**
Interleukin-6 (IL-6) [pg/mL]	52	32 (12-57)	24 (8-53)	43 (32-61)	0.076
High-sensitivity Troponin-T [ng/mL]	40	26 (8-108)	10 (7-33)	84 (32-158)	**0.004**
Lactic acid [mmol/L]	35	1.70 (1.10-2.20)	1.37 (1.00-1.90)	2.30 (1.80-2.70)	**0.010**
Uric acid [µmol/L]	51	305 (266-384)	323 (277-391)	260 (146-292)	**0.028**
Bicarbonate [mmol/L]	89	25.0 (23.0-28.0)	25.0 (23.0-27.0)	27.0 (24.0-31.8)	0.2
Sodium [mmol/L]	89	138.0 (136.0-141.0)	138.0 (136.0-140.0)	144.0 (136.2-149.0)	0.063

Characteristic	N	Total N = 89[1]	Survivor N = 75[1]	Non-survivor N = 14[1]	*p*-value[2]
Potassium [mmol/L]	85	4.40 (4.00-4.70)	4.30 (4.00-4.70)	4.55 (4.32-4.85)	0.2
Chloride [mmol/L]	89	102.0 (99.0-104.0)	101.5 (99.0-104.0)	103.0 (95.8-107.8)	0.8
Phosphorus [mmol/L]	46	1.14 (1.00-1.31)	1.13 (1.02-1.26)	1.27 (0.97-1.47)	0.2
Magnesium [mmol/L]	60	0.89 (0.83-0.96)	0.87 (0.83-0.96)	0.96 (0.87-1.05)	**0.044**
Total Protein [g/L]	80	71 (64-77)	71 (68-77)	56 (54-74)	**0.012**
Albumin [g/L]	89	32 (27-39)	35 (30-40)	22 (19-26)	**<0.001**
Bilirubin [mg/dL]	80	8 (5-13)	8 (5-11)	16 (7-26)	**0.017**
Urea [mmol/L]	89	5 (4-10)	4 (3-6)	21 (15-26)	**<0.001**
Creatinine [µmol/L]	89	78 (63-92)	76 (62-90)	90 (65-226)	0.15
Creatine kinase (CK) [U/L]	45	103 (58-245)	74 (56-182)	204 (67-282)	0.2
Alkaline phosphatase (ALP) [U/L]	80	88 (63-110)	80 (61-99)	161 (129-302)	**<0.001**
Alanine aminotransferase (ALT) [U/L]	77	31 (20-64)	30 (19-52)	73 (32-136)	**0.009**
Aspartate aminotransferase (AST) [U/L]	72	28 (23-50)	26 (20-43)	70 (35-120)	**<0.001**
Lactate dehydrogenase (LDH) [U/L]	53	347 (249-462)	322 (228-408)	576 (382-1,090)	**<0.001**
Low-density lipoprotein (LDL) [mmol/L]	16	2.85 (2.28-3.70)	2.85 (2.28-3.70)	NA (NA-NA)	
Triglyceride [mmol/L]	52	1.80 (1.35-2.62)	1.70 (1.20-2.20)	2.65 (1.88-3.55)	**0.025**
Vitamin D [ng/mL]	56	18 (13-23)	18 (13-23)	16 (14-29)	0.7
Calcium [mmol/L]	89	2.25 (2.14-2.35)	2.29 (2.17-2.36)	2.12 (2.00-2.18)	**<0.001**
Adjusted calcium [mmol/L]	89	2.39 (2.30-2.46)	2.36 (2.30-2.46)	2.46 (2.41-2.56)	**0.019**
Procalcitonin [ng/mL]	47	0.27 (0.10-0.55)	0.15 (0.06-0.31)	0.86 (0.57-5.06)	**<0.001**

Characteristic	N	Total N = 89[1]	Survivor N = 75[1]	Non-survivor N = 14[1]	*p*-value[2]

[1]Statistics presented: n (%); Median (25%-75%)

[2]Statistical tests performed: Fisher's exact test; chi-square test of independence; Kruskal-Wallis test

Table-3

Characteristic	**N**	**Total N = 89[1]**	**Asymptomatic N = 23[1]**	**Mild N = 33[1]**	**Severe N = 33[1]**	**p-value[2]**
C5a : Neutrophil-to-lymphocyte ratio	89	409 (160-611)	464 (353-632)	444 (193-715)	170 (90-527)	**0.002**
C5a : Lymphocyte count	89	1,012 (503-1,687)	528 (398-742)	827 (475-1,604)	1,854 (1,173-2,697)	**<0.001**
C5a : Red blood cell count	89	284 (193-469)	197 (139-247)	278 (139-323)	508 (440-694)	**<0.001**
C5a : Hematocrit	89	35 (21-54)	23 (16-30)	32 (17-41)	58 (48-80)	**<0.001**
C5a : Hemoglobin	89	110 (65-166)	73 (48-90)	100 (50-124)	193 (143-244)	**<0.001**
C5a : Albumin	89	43 (23-64)	25 (19-32)	42 (21-54)	80 (56-95)	**<0.001**
C5a : Calcium	89	603 (394-812)	423 (318-552)	602 (325-751)	821 (664-1,059)	**<0.001**

[1]Statistics presented: n (%); Median (25%-75%)

[2]Statistical test performed: Kruskal-Wallis test

Table-4

Nutritional status		N
Underweight	1%	1
Normal weight	26%	18
Pre-obesity	46%	31
Obesity class I	19%	13
Obesity class II	4%	3
Obesity class III	3%	2

Figures

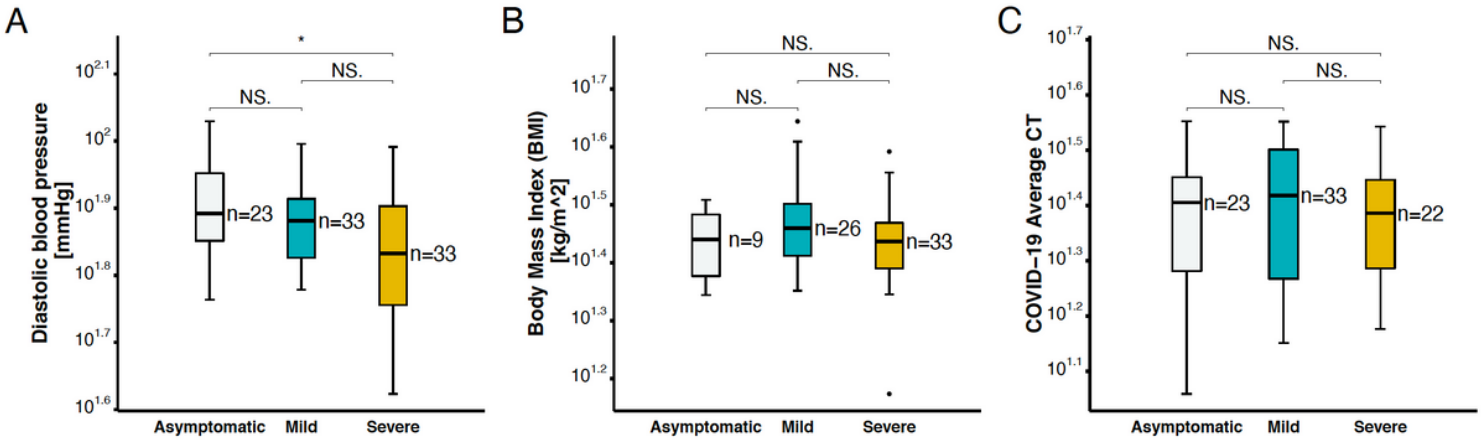

Figure 1

Clinical features in COVID-19 patients across asymptomatic, mild and severe cases (A) diastolic blood pressure, (B) body Mass Index (BMI), and (C) COVID−19 Average CT.

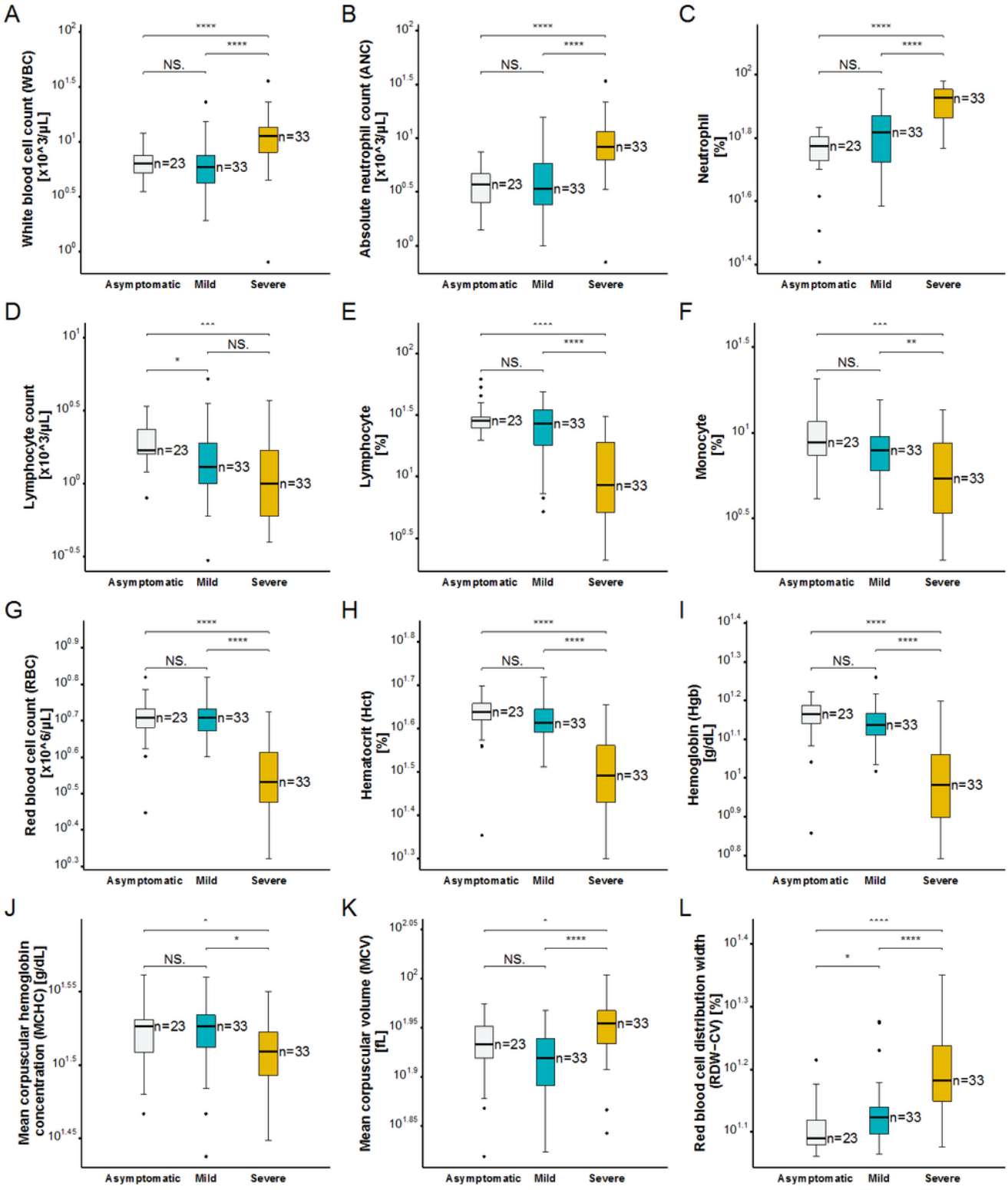

Figure 2

Complete blood count parameters of asymptomatic, mild and severe COVID-19 patients (A) white blood cell count, (B) absolute neutrophil count, (C) neutrophil (%), (D) lymphocyte count, (E) lymphocyte (%), (F) monocyte (%), (G) red blood cell count, (H) hematocrit, (I) hemoglobin, (J)mean corpuscular hemoglobin concentration, (K) mean corpuscular volume, (L) red blood cell distribution width.

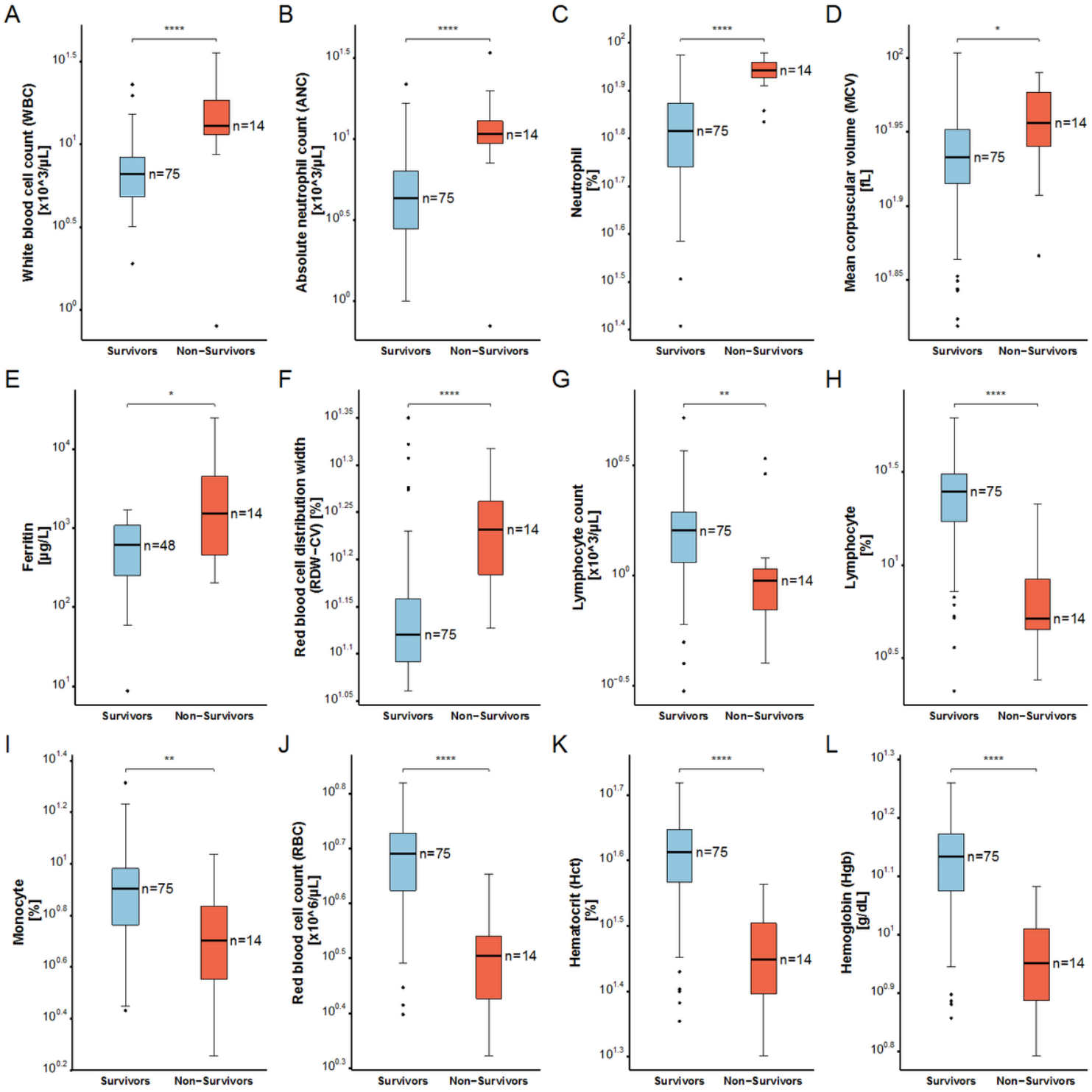

Figure 3

Complete blood count parameters in COVID-19 patients who survived and those who did not survive (A) white blood cell count, (B) absolute neutrophil count, (C) neutrophil (%), (D) mean corpuscular volume, (E) ferritin, (F) red blood cell distribution width, (G) lymphocyte count, (H) lymphocyte (%), (I) monocyte (%), (J) red blood cell count, (K) hematocrit, (L) hemoglobin.

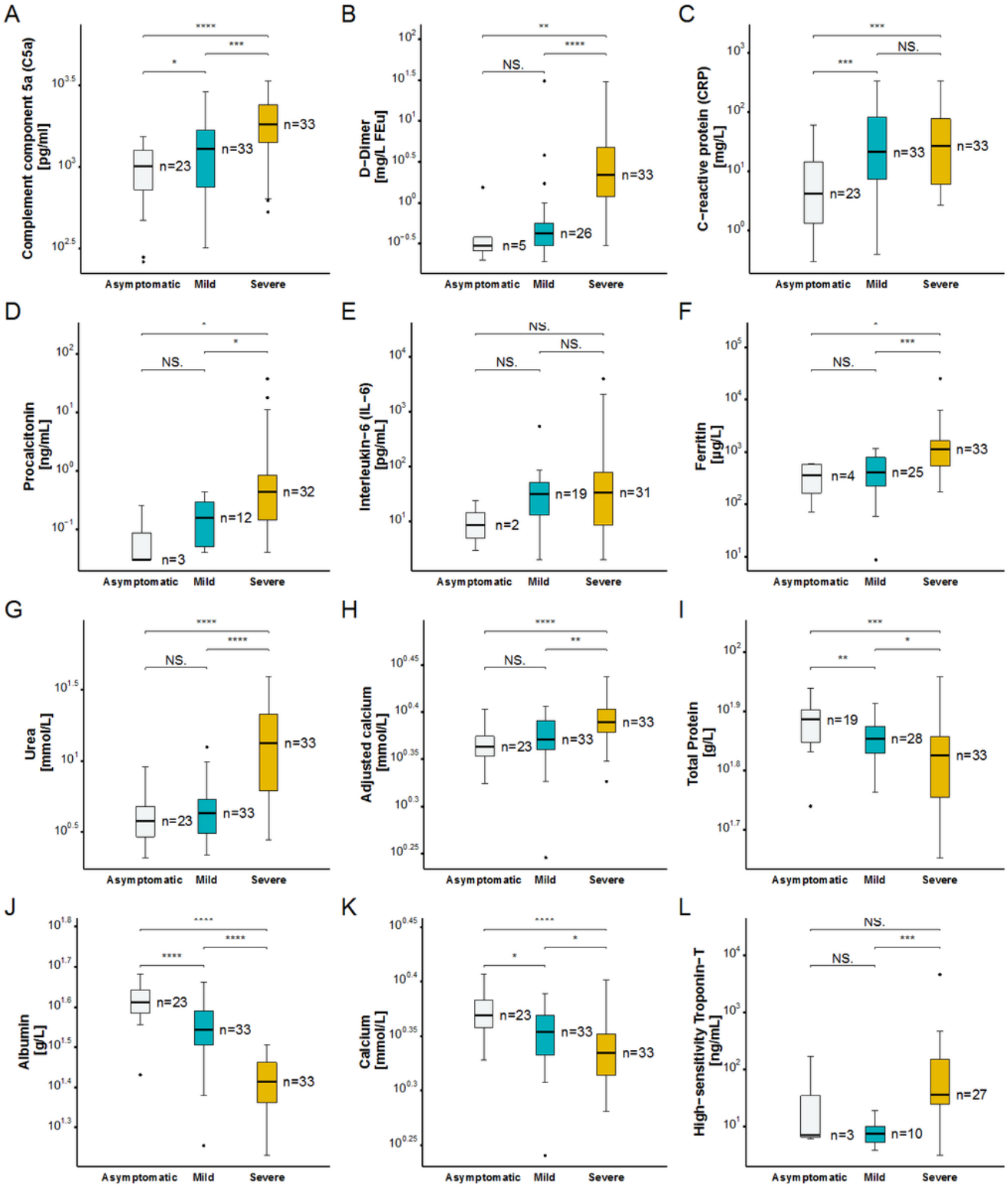

Figure 4

Coagulation and inflammatory markers in asymptomatic, mild and severe COVID-19 patients (A) complement component 5a, (B) D–Dimer, (C) C–reactive protein, (D) procalcitonin, (E) Interleukin–6, (F) ferritin, (G) urea, (H) adjusted calcium, (I) total protein, (J) albumin, (K) calcium, (L) high–sensitivity Troponin–T.

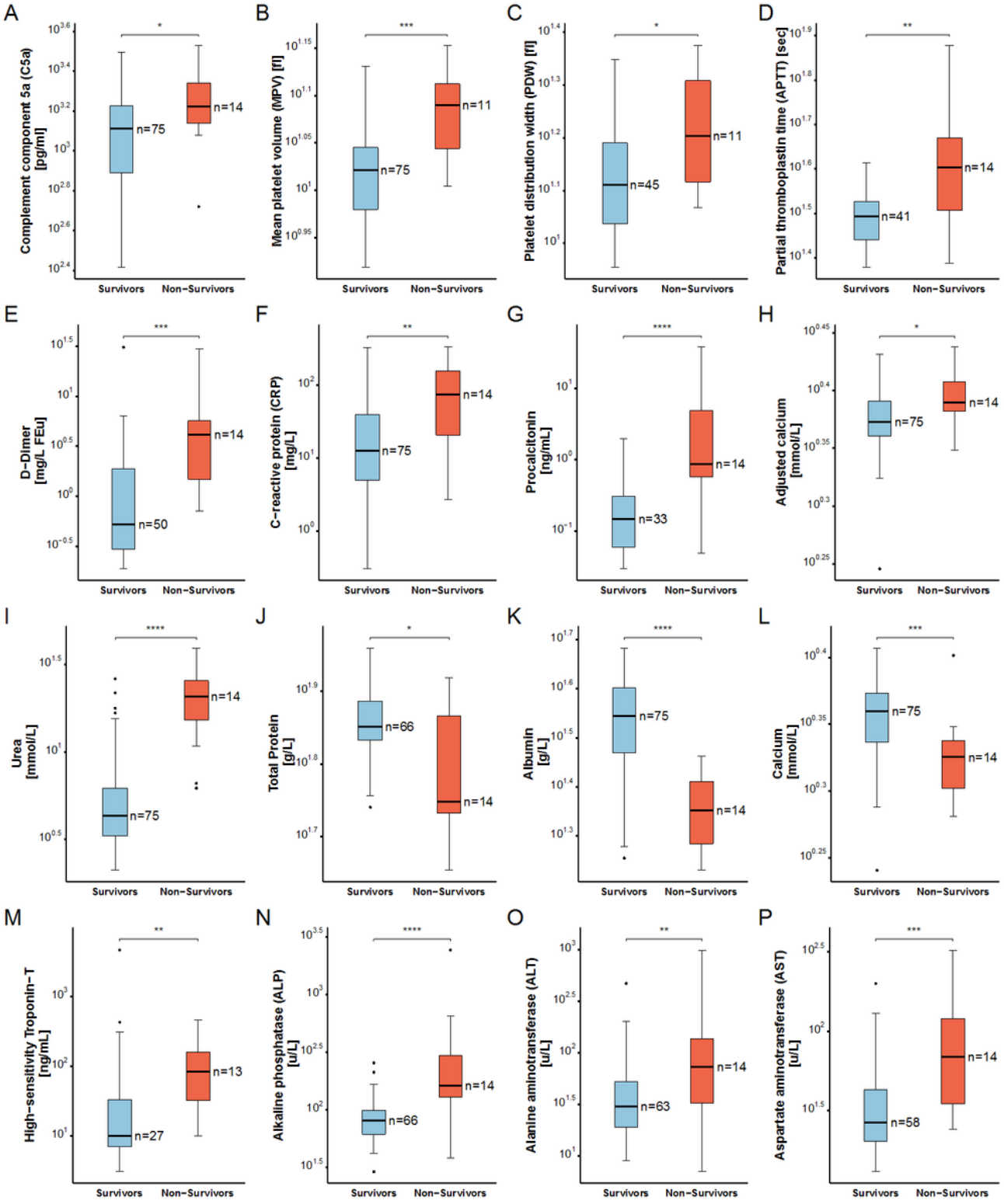

Figure 5

Coagulation and inflammatory markers in COVID-19 patients who survived and those who did not survive (A) complement component 5a, (B) mean platelet volume, (C) platelet distribution width, (D) partial thromboplastin time, (E) D−dimer, (F) C−reactive protein, (G) procalcitonin, (H) adjusted calcium, (I) urea, (J) total protein, (K) albumin, (L) calcium, (M) high−sensitivity Troponin−T, (N) alkaline phosphatase, (O) alanine aminotransferase, (P) aspartate aminotransferase.

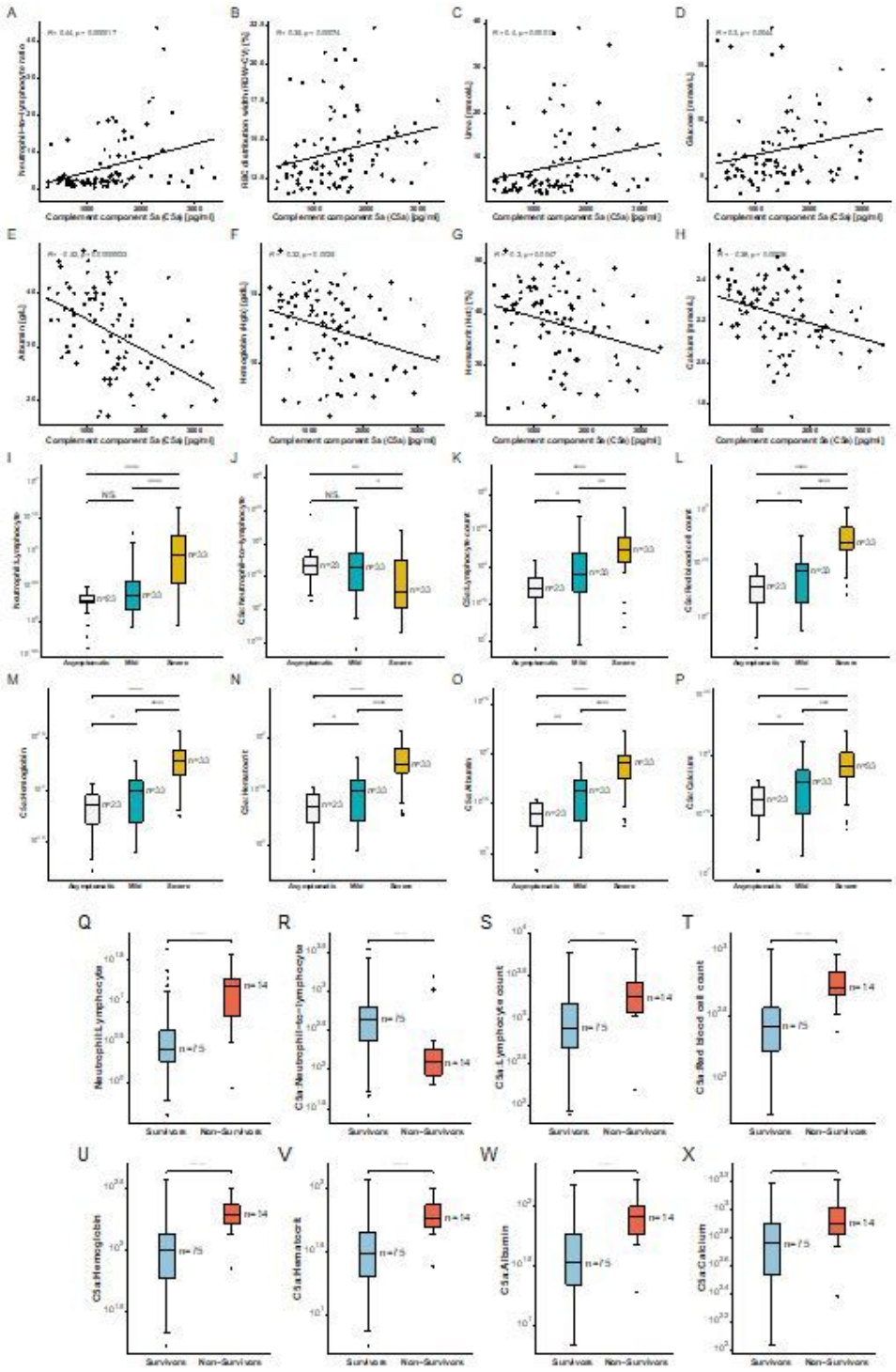

Figure 6

Correlation of complement component 5a with blood indices in SARS-CoV2 infected patients (A) Neutrophil–to–lymphocyte ratio, (B) RBC distribution width, (C) Urea, (D) glucose, (E) albumin, (F) hemoglobin, (G) hematocrit, (H) calcium. Ratios of complement component 5a to blood indices including (I, Q) neutrophil: Lymphocyte, (J, R) C5a: neutrophil–to–lymphocyte, (K, S) C5a: lymphocyte count, (L, T)

C5a: red blood cell count, (M, U) C5a: hemoglobin, (N, V) C5a: hematocrit, (O, W) C5a: albumin, (P, X) C5a: calcium in asymptomatic, mild and severe COVID-19 patients, and in survivors versus non-survivors.

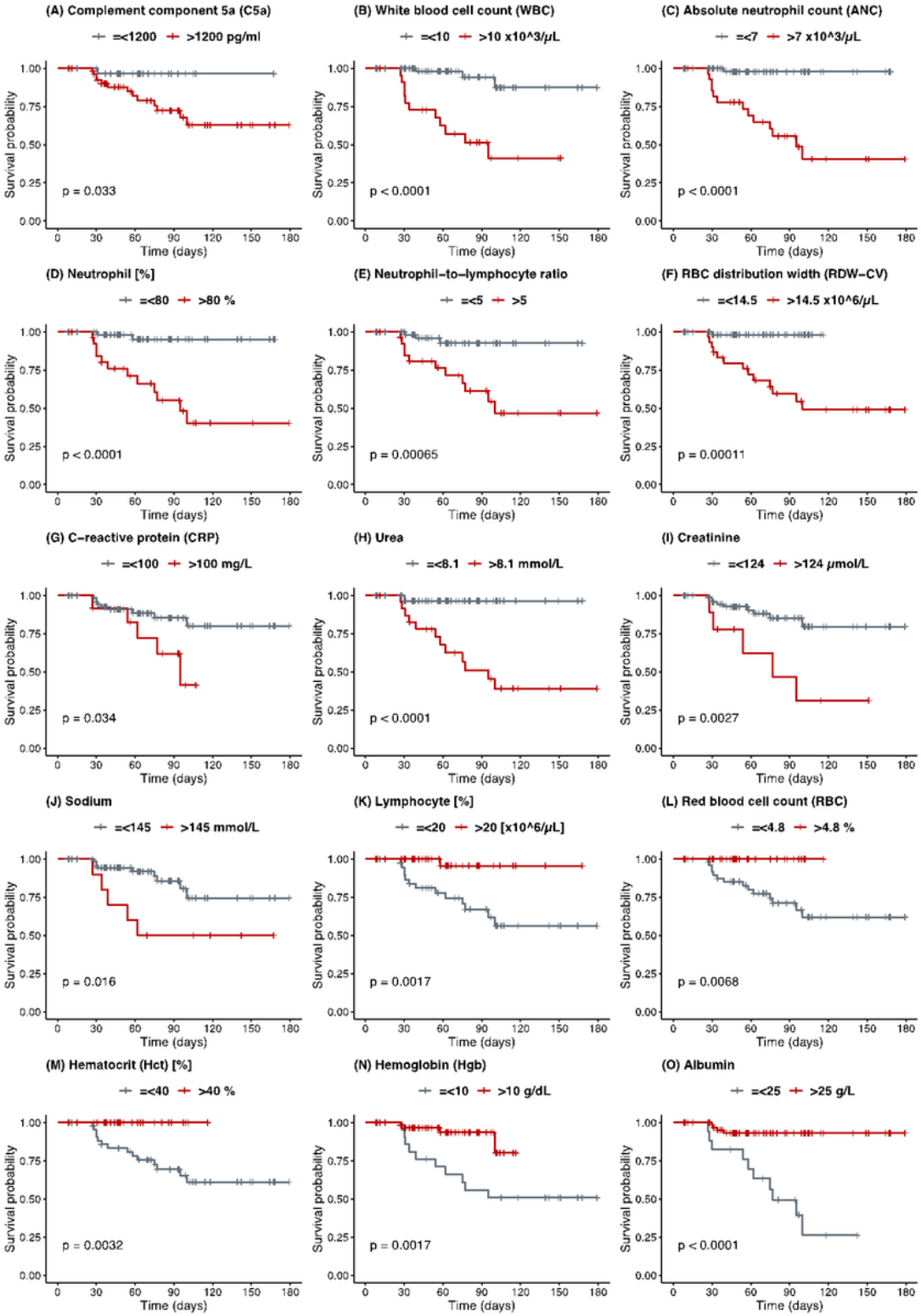

Figure 7

Kaplan–Meier estimated survival probabilities in SARS-CoV2 infected patients using the time from admission or quarantine to the time (days) of death or end of survey for multiple prognostic factors including C5a and routine laboratory investigations.

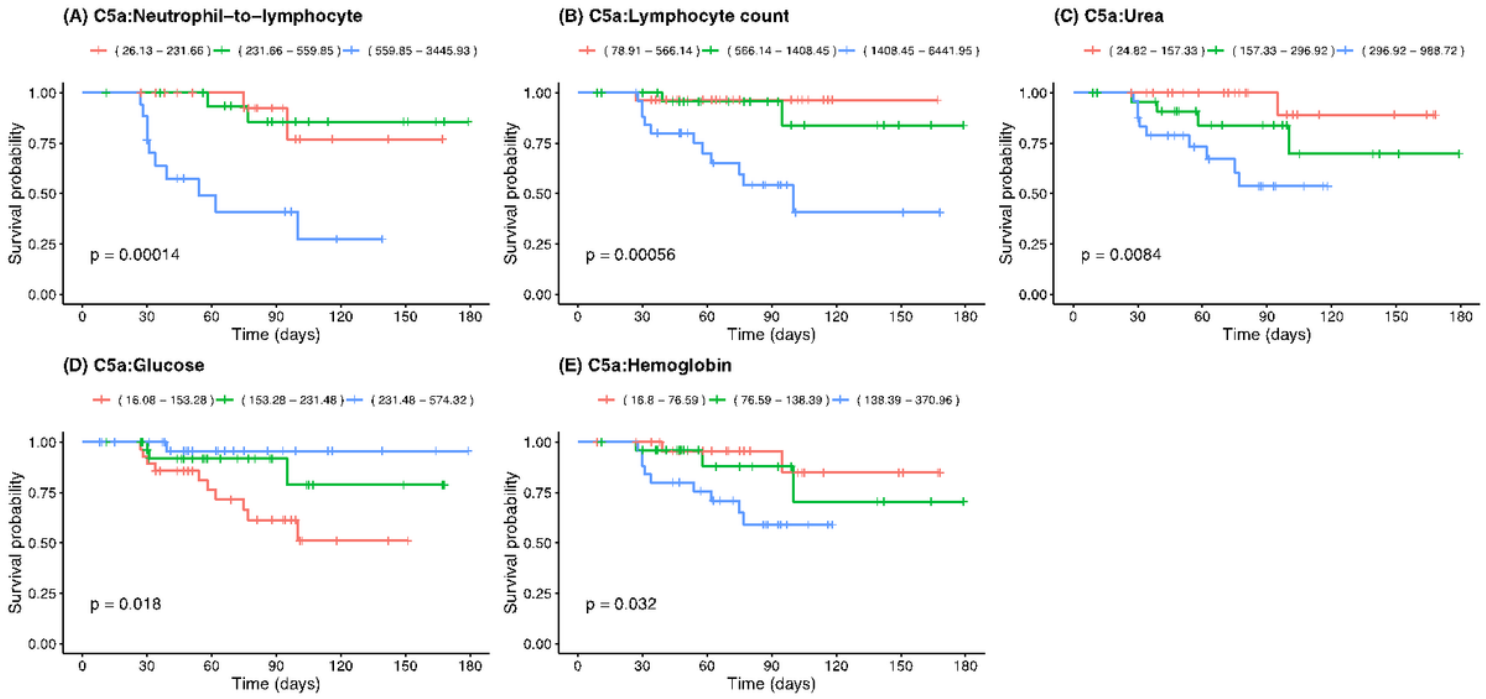

Figure 8

Kaplan-Meier survival analysis using tertiles of C5a-to blood indices including -NLR, -lymphocyte, -urea, -glucose and -Hgb ratios. The significance of the log-rank Mantel–Cox test of equality of survival distributions is shown as p-value.

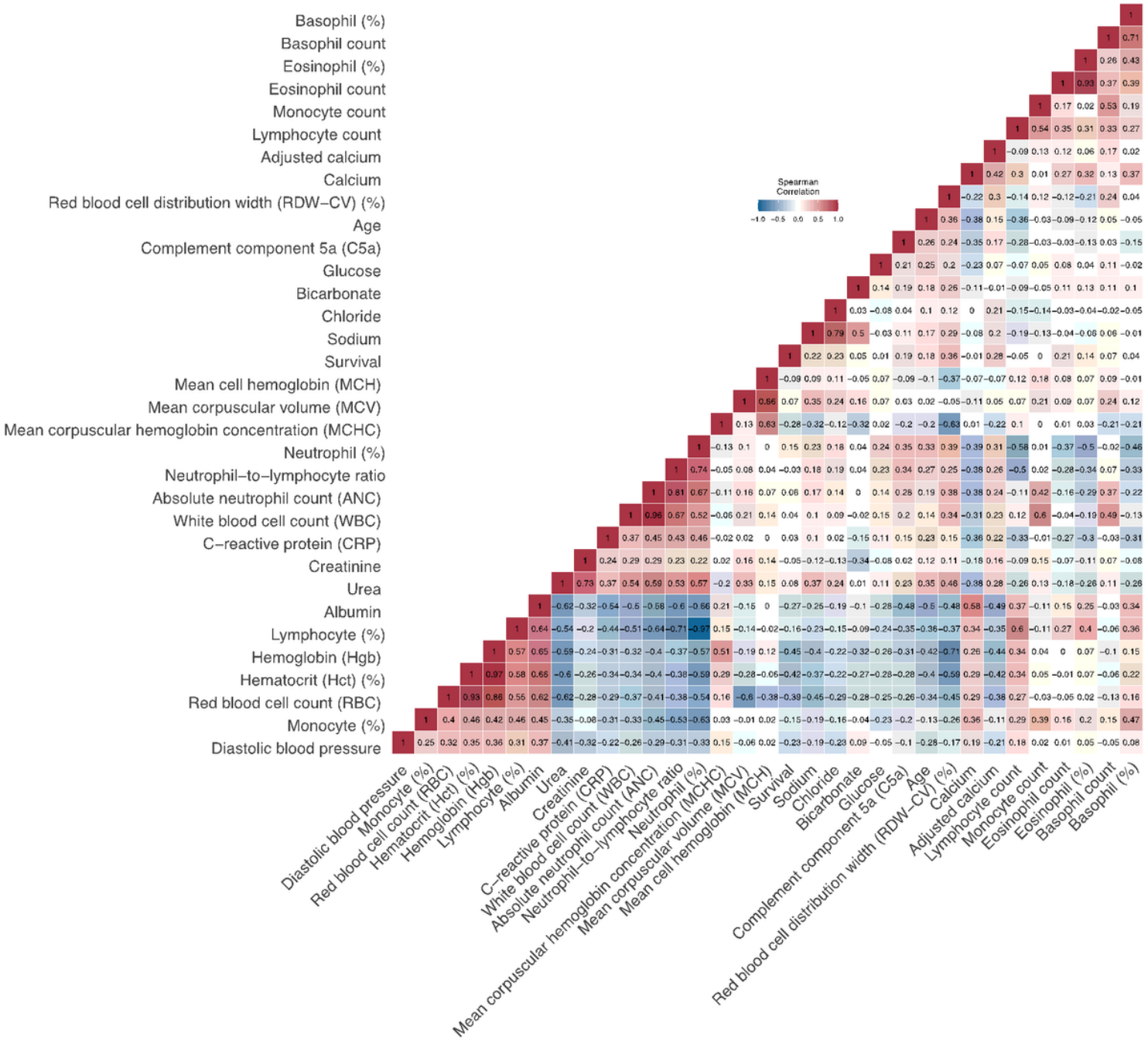

Figure 9

Spearman correlation matrix for investigated covariates showing R values.

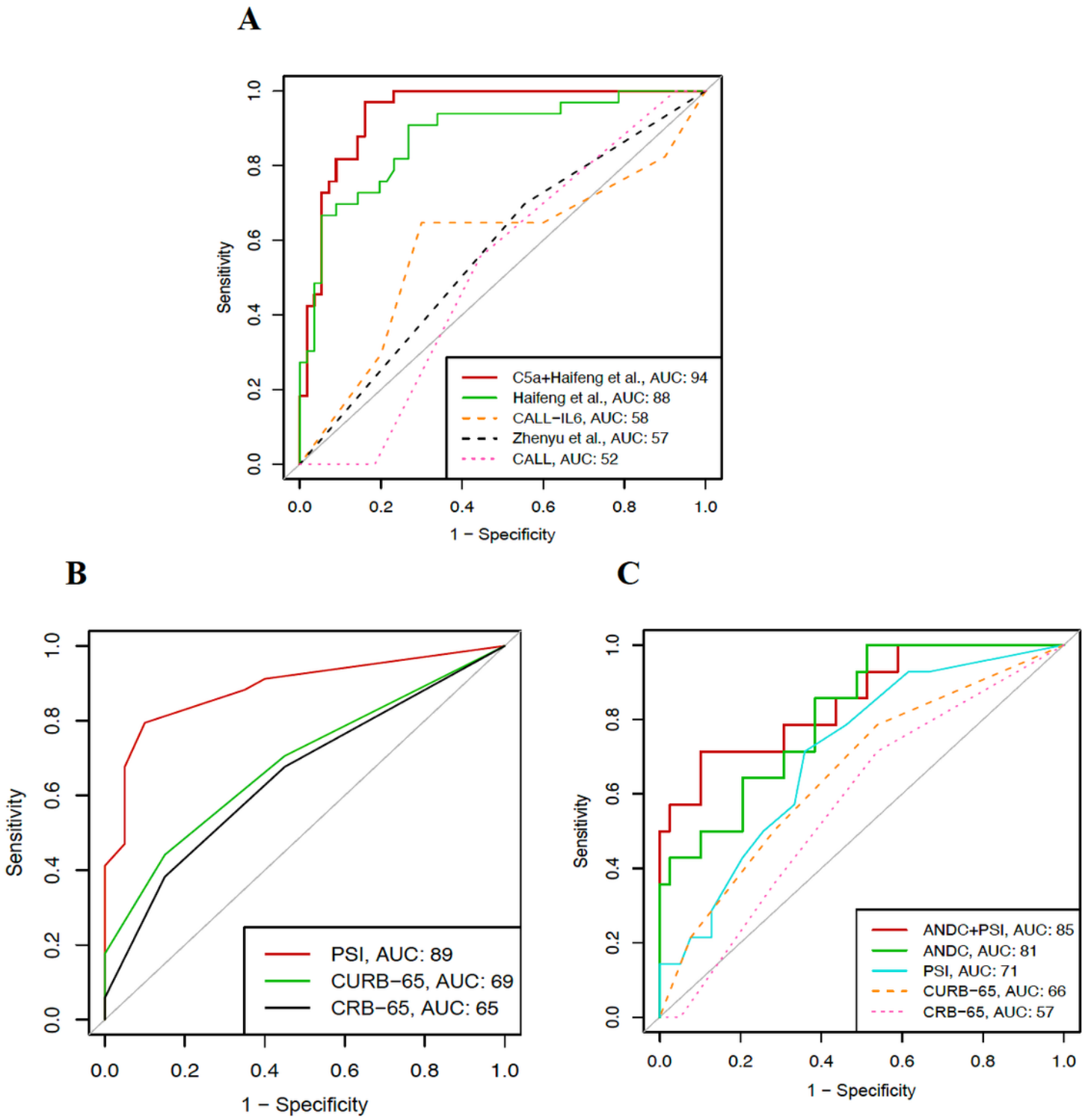

Figure 10

ROC curves showing the comparison between CAL (C5a, albumin, and lymphocyte count) and previously described severity models (A) including Haifeng et al. (lymphocyte count, albumin), CALL-interleukin-6 (IL-6), Zhenyu et al. (age, albumin, comorbidity, CRP), and CALL (comorbidity, age, lymphocyte count, lactate dehydrogenase). (B) ROC curves for CRB-65, CURB-65, and PSI in predicting ICU admission. (C)

ROC curves for CRB-65, CURB-65, and PSI alone, ANDC alone and PSI with ANDC combined in predicting mortality.

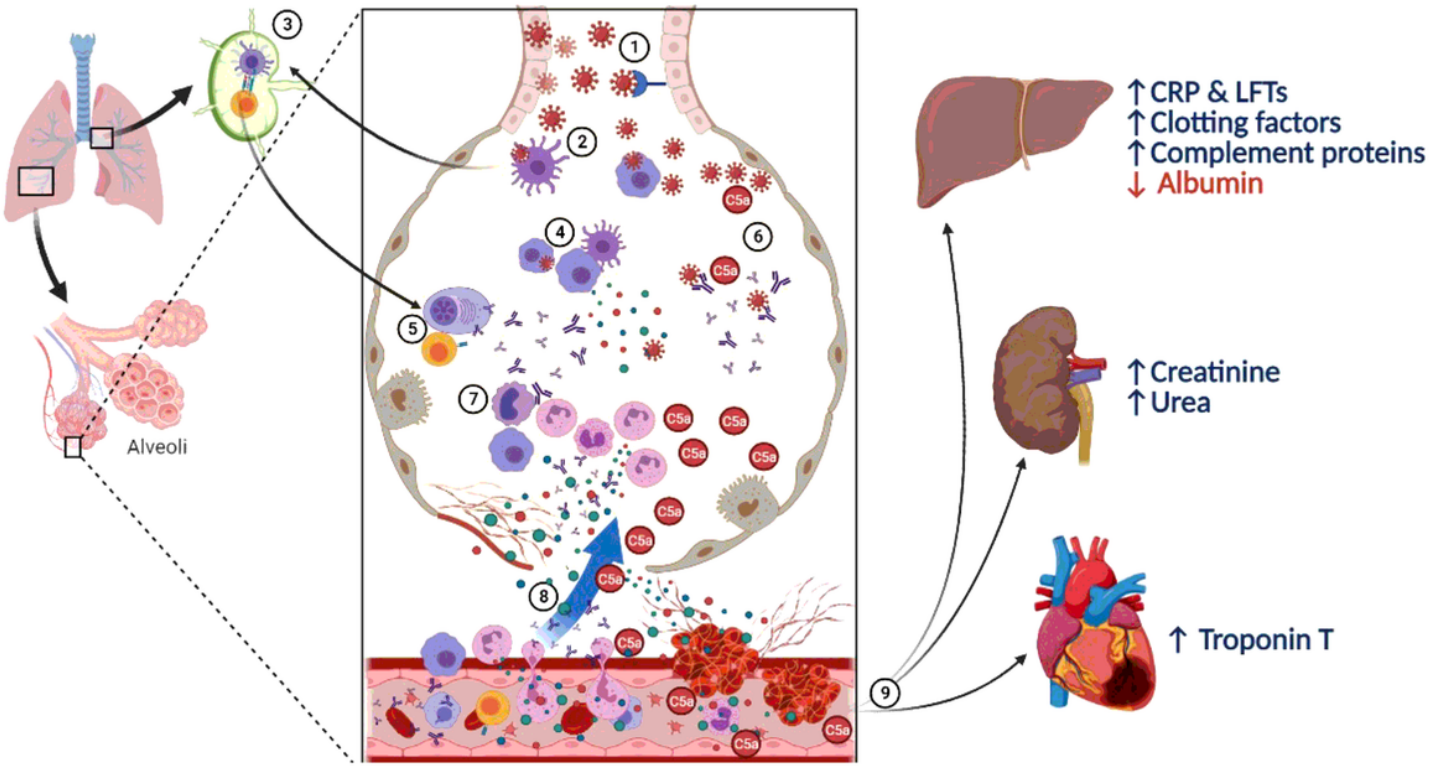

Figure 11

A proposed mechanistic role of complement C5a in lung injury during SARS-CoV-2 infection. (1) SARS-CoV-2 virus infects respiratory epithelial cells by binds to the angiotensin-converting enzyme 2 (ACE-2) receptor, followed by viral shedding. (2) Tissue resident macrophages and dendritic cells take up the virus or virus-infected cells. (3) They carry out antigen presentation in the hilar lymph nodes activating virus specific T and B cells. (4) Innate immune cells in the pulmonary microenvironment release cytokines to recruit immune cells to the site of infection. (5) T lymphocytes help B cells and plasma cells release antibodies into circulation and in pulmonary microenvironment. (6) Meanwhile, complement activation can take place directly via lectin pathway or via alternative and classical pathway in the presence of antigen-antibody complexes. (7) C5a acts as a potent chemotactic agent recruiting innate immune cells, in particular neutrophils and further activating the immune system leading to cytokine storm. (8) Damaged alveoli and leaky blood vessels promote local clotting with C5a and IL-6 stimulating platelet activation along with release of pro-inflammatory cytokines like IL-6 and C5a into circulation. (9) Systemic action of inflammatory mediators and micro-thrombi cause general tissue hypoxia along with stress on the liver to synthesise acute phase reactants, and proteins from the complement and clotting cascade on the expense of albumin with sequestration of ferritin thereby elevating liver enzymes and promoting the pro-coagulant state. The inflammatory mediators (including immune complexes and C5a) cause renal injury and cardiac stress.