Microbes and Infectious Diseases 2022; 3(4): 830-841

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Terminal complement complex C5b-9 and C5b assay in sera of COVID-19 patients with different disease severities

Asmaa Nasr El-Din [*1], ***Kamal Abd El-Sattar Ata*** [2]

1-Microbiology and Immunology Department, Faculty of Medicine, Sohag University, Egypt.

2-Chest Diseases and Tuberculosis Department, Faculty of Medicine, Sohag University.

ARTICLE INFO

Article history:

Received 4 September 2022

Received in revised form 25 September 2022

Accepted 28 September 2022

Keywords:

Complement

COVID-19

C5b

C5b-9

ABSTRACT

Objective: The aim of this study is to find out the link between the involvement of complement activation in the inflammatory reactions in COVID-19 patients, the deterioration of the clinical status and development of sever COVID-19 in those patients. **Methods:** The study included 274 COVID-19 patients, divided into three groups; group1: severe COVID-19 patients (n=37), group 2; moderate COVID-19 severity patients (n=78), group 3; mild COVID-19 severity patients (n=159). Serum levels of C-reactive protein, D-dimer, and ferritin were measured in the three patient groups, and the patients were subjected to CT chest imaging. Serum levels of the tested biomarkers were measured by ELISA at diagnosis. **Results:** Sever COVID-19 patients had higher serum levels of ferritin and D-dimer in comparison to patients with moderate and mild severity COVID-19 with statistically significant difference (*p* value 0.01 and 0.02 respectively). There was a significant elevation in the serum levels IL-6 and TNF-α in severe COVID-19 patients (488.5±112.2 and 159.6±38.3 respectively) versus moderate (206.07±53.3 and 93.5±39.5) and mild group (200.9± 52.27 and 52.9±23) respectively, (*p* value < 0.001). There was also a significant elevation of C5b and C5b-9 serum levels in severe COVID-19 patients (18.6±10.3 and 73.25±7.35) compared to moderate (14.11±15.6 and 143.6±170) and mild COVID-19 groups (76± 11.32 and 3.9± 3.03) respectively. **Conclusion:** We conclude that the severity of inflammation presented in elevated neutrophil counts and serum levels of inflammatory cytokines IL-6 and TNF in association with sever complement activation are implicated in severity and bad prognosis of COVID-19.

Introduction

The novel Coronavirus SARS-CoV-2 caused COVID-19 which complicated by a deadly form of viral pneumonia in severe cases. The virus spread rapidly from China to the whole world with considerable intensity and severity creating a ‘‘global pandemic emergency”. Till now it caused about 1.62 million deaths and 72.8 million suffered COVID-19 [1].

SARS-CoV-2 is an RNA virus that has an eighty percent similarity the genome of SARS-CoV1 responsible for the 2003 outbreak [2,3]. In vitro studies confirmed that the virus binds to angiotensin converting enzyme-2 (ACE-2) receptor,

DOI: 10.21608/MID.2022.160620.1379

* *Corresponding author:* Asmaa Nasr El-Din

E-mail address: asmaanasreldin81@gmail.com

which is part of the renin-angiotensin system (RAS) and is considered the main receptor protein [4, 5].

COVID-19 has a highly variable presentation, like being asymptomatic in 20% to 40% of cases, a mild form of common cold-like syndrome in 30% to 70%, or a severe life-threatening form of ARDS in less than 10%. Severe disease is characterized by respiratory failure, a hyperinflammatory immune reactions, and multi-organ thrombo-embolic manifestations [6].

Patients are considered to have a severe disease if they have bilateral interstitial pneumonia and a hyperactive immune-inflammatory state that affect all body tissues causing multi-organ failure [7]. Sever COVID-19 usually results from an over-reacting immune system, rather than the high viral load, the massive inflammatory reaction that occurs in sever COVID-19 is characterized by a rapid release of cytokines in high serum levels, an event called a ‘‘cytokine storm”. This storm is characterized by high levels of pro-inflammatory cytokines; IL-1 and IL-6 and also high levels of D-dimer, lactate dehydrogenase, and ferritin [8].

Impaired regulation of complement cascade is one of the main driving factors of these pathogenic events [9], Complement cascade can be activated via the classical pathway which is triggered by antibody-antigen reactions, the alternative pathway which is stimulated by specific surface antigens, and the lectin pathway, initiated by binding mannose residues on the microbial surface [10, 11]. These pathways converge into a common pathway. The common pathway includes production of C3a and C5a inflammatory mediators and C3b-initiated pathogen opsonization and ends in formation of the C5b-9 membrane attack complex [12].

The aim of this study is to find out the link between high serum levels of complement activation end-products namely; C5b and C5b9 and the severity of COVID-19 and also to test the association between complement activation and the magnitude of inflammation in COVID-19 patients presented by pro-inflammatory cytokines like IL-6, tumor necrosis factor-α (TNF-α), and neutrophil blood counts.

Patients and methods

Study population

Our cross-sectional study was conducted during the period from May 2021 to May 2022 in Central Research Laboratory and Medical Microbiology and Immunology Department, Faculty of Medicine, Sohag University. Our study included 274 patients who were diagnosed as COVID19 by PCR and attended the isolation building of Sohag University Hospitals.

Patients of the study were classified into 3 groups:

Group 1; Mild COVID-19 Patients presented with anosmia, loss of taste, GIT symptoms, or influenza-like symptoms with normal chest imaging (n=159 patients).

Group 2; Moderate COVID-19 patients (n=78) who had:

- Clinical or radiological evidence of lower respiratory tract involvement
- Oxygen saturation (SpO2) ≥ 94% on room air.

Group 3; Severe COVID-19 patients (n= 37) who had:

- Oxygen saturation ≤ 94% on room air
- A ratio of partial pressure of arterial oxygen to fraction of inspired oxygen, (PaO2/FiO2) <300.
- Lung infiltrates involving >50% of both lungs.
- Respiratory rate >30 breaths/min, reflecting marked tachypnea.

Exclusion criteria

Patients who had any condition predisposing to immune suppression like HIV infection, neutropenia, blood malignancies, diabetes, renal or hepatic impairment. Patients who had chronic chest disease like asthma, bronchiectasis, and pulmonary fibrosis were also excluded.

Methods

1-Clinical examination was conducted for assessment of the severity of symptoms and provisional staging.

2-Radiological examination of the chest using Computed tomography (CT) depending on COVID-19 Reporting and Data System (CO-RADS) which is a chest CT-based system used for staging of pulmonary involvement in COVID-19 patients.

3- Laboratory investigations of all patients were recorded including serum levels of C-reactive protein, D-dimer, ferritin, liver enzymes, total and direct bilirubin, renal functions, and coagulation profile including

prothrombin time and partial thromboplastin time.

4- Complete blood counts were done for all patients to assess the number lymphocytes, granulocytes (neutrophils, basophils, and eosinophils) and monocytes/macrophages percentages.

5- Blood samples were collected from all included patients, centrifuged and the collected sera were divided into 4 parts for EIISA- measurement of IL-6, TNF-α (as indicators of inflammatory conditions that is a predilection towards severe COVID-19), C5b, and C5b-9 (two terminal complement activation components)

- **Intereukine-6 (IL-6);** using ELISA kits (Interleukine-6, Catalog: RAB0306, Sigma Aldrich, Germany, the detection range; 137 pg/ml to 1000 pg/ml)
- **Tumor necrosis factor- α (TNF-α);** using ELISA kit (tumor necrosis factor-α, Catalog; SRP3177, Sigma Adrich, Germany, the detection range of TNF-α is; 3.16 pg/ml to 1000 pg/ml).
- **Complement fragment 5b (C5b)**; using (Human C5b , Catalog; MBS8807454, Mybiosource, England, detection range; 0.79 ng/ml to 50 ng/ml)
- **Complement fragment 5b-9 (C5b-9);** using (human terminal complement complex C5b-9, Catalog; SG-14328, Sinogene, China, detection range; 62.5 ng/ml to 2000 ng/ml).

Ethical considerations

Informed written consents were taken from all the patients included or their first degree relatives in case of severe COVID-19 due to ICU admission; the study protocol was approved by The Ethics Committee of Scientific Research in faculty of medicine Sohag University.

Statistical analysis

The collected data was coded and verified prior to computerized data entry. The collected data was statistically analysed using Statistical Package for the Social Science (SPSS) version 23 program and expressed in tables and graphs. The data were tested for normality by Kolmogorov-Smirnov. Chart builder was used to get the graphs. Kruskal Wallis for non-parametric data and Post hoc test, Tukey type was used to get the p value between groups. In all analyses, $p<0.05$ indicated statistical significance. Spearman's rho test for non-parametric data was used to get correlation between parameters.

Results

This cross sectional study was conducted on 274 patients with diagnosis of COVID-19 who attended Sohag University Hospitals and cases with COVID-19 were admitted in the isolation building of the hospital. The ages of the patients were classified to the following; 42% of the patients aged from 25-<45 years, 39% aged from 55- <75 years, and 19% aged 75 years or more. 62% of the study patients were males, and 38% were females. On assessment of severity of COVID-19 by the clinical, laboratory, and radiological criteria, the patients were divided into three groups; one hundred and fifty nine patients (58%) were in the group of mild COVID-19 , 78 patients (28.4%) had moderate illness, and 37 patients (13.5%) were classified as sever COVID-19.

Patients with severe COVID-19 were found to have higher serum levels of CRP, ferritin, and D-dimer as compared to the mild and moderate COVID-19 patients groups with statistically significant difference (*p* value 0.04, 0.01, and 0.02 respectively). Based on the pulmonary involvement as detected by chest-CT, COVID-19 patients were assigned to different CO-RAD categories, 75.6 % of severe COVID-19 patients had staged as CO-RAD category (3-5), only 26.9% of patients with moderate COVID-19 disease had CO-RAD category (3-5), while only (2.5%) of patients with mild COVID-19 have abnormal CT findings and this difference was significant (*p* value 0.001) **(Table 1)**.

Interleukin-6, TNF-α were measured in serum of the three patient groups along with the previously mentioned laboratory parameters. The mean serum levels of IL-6 were higher patients with severe COVID-19 (488.5 pg/ml) than in patients with moderate disease (206.0 pg/ml) (*p* value < 0.001), and mild disease (200.9 pg/ml) (*p* value < 0.001) with a statistically significant difference. No statistically significant difference between the mean serum levels of IL-6 in mild and moderate COVID-19 patients (*p* value 0.83). TNF-α mean serum levels were also higher in severe COVID-19 patient group (159.7 pg/ml) than was measured in moderate (93.5 pg/ml) and mild (52.9 pg/ml) disease with a high statistically significant difference (*p* values 0.02, and 0.01) respectively, also there was a significant difference in the mean levels of TNF-α between

mild and moderate COVID19 patient groups (*p* value < 0.001) **(Figure1)**.

Complement components C5-b and C5b-9 were also measured in serum of the three patient groups by ELISA. The mean serum levels of C5b were higher patients with severe COVID-19 (18.5 ng/ml) than in patients with moderate disease (14.1 ng/ml) (*p* value < 0.04), and those with mild disease (4.0 ng/ml) (*p* value < 0.001) with a statistically significant difference. There was also a high statistically significant difference between the mean serum levels of C5b in mild and moderate COVID-19 patients (*p* value < 0.001). The terminal component of complement activation C5b-9 mean serum levels were also higher in severe COVID-19 patient group (391.2 ng/ml) than was measured in moderate (143.6 ng/ml) and mild (76.3 ng/ml) disease with a high statistically significant difference (*p* values <0.03, and <0.01) respectively, also there was a significant difference in the mean levels of TNF between mild and moderate COVID19 patient groups (*p* value < 0.001) **(Figures 2&3)**

The collective percentages of neutrophils and monocytes from the total leucocyte count were generally higher in COVID-19 patients than the normal individuals as the normal percentage of neutrophil in healthy adults is (40% - 60% of the total leucocyte count). We compared also the percentages of neutrophils in the three patient groups; the mean percentages of these cells in cases with sever COVID-19 was (67.6%), in moderate COVID19 was (73.2%), and in mild COVID-19 was (64.8%). The higher percentage in sever disease has a statistically significant difference with the percentages of the same cells in moderate and mild disease (*p* value <0.001 for both) **(Table 2).**

There was an imbalance of immune function observed in COVID-19 patients represented in the elevated percentages of neutrophils and lowered percentages of lymphocytes than normal values. We assessed the correlation between the serum levels of IL-6 in COVID-19 patients with different severities at one side with the serum levels of TNF-α as an inflammatory cytokine and with percentages of neutrophils from the total leucocyte count in the same patient groups. We found that; there was a highly significant Moderate positive correlation with IL6 and TNF-α in all COVID-19 patients with different disease severities (r = 0.42, *p* value < 0.001). We also found that there was a highly significant mild positive correlation between IL6 serum levels and percentages of neutrophils in all COVID-19 patients (r = 0.38, *p* value 0.001). **(Table 3)** and **(Figure 4)**

We also assessed the correlation between markers of inflammation including IL-6, TNF-α, and neutrophil counts at one side and the components of complement activation C5b and C5b-9 at the other side. There was a highly significant Moderate positive correlation with serum levels of IL6 and C5b in all COVID-19 patients (r = 0.35, *p* value < 0.001), and a highly significant Mild positive correlation between IL6 serum levels and C5b-9 in all COVID-19 patients (r = 0.34, *p* value <0.001).

We also assessed the correlation between markers of inflammation including IL-6, TNF-α, and neutrophil counts at one side and the components of complement activation C5b and C5b-9 at the other side. There was a highly significant Moderate positive correlation with serum levels of IL6 and C5b in all COVID-19 patients (r = 0.35, *p* value < 0.001), and a highly significant Mild positive correlation between IL6 serum levels and C5b-9 in all COVID patients (r = 0.34, *p* value <0.001). **(Table 3)** and **(Figure 5)**

As regards to TNF-α there was a statistically significant Moderate positive correlation with serum levels of TNF-α and C5b in all COVID patients (r = 0.54, *p* value < 0.001), and a highly significant moderate positive correlation between TNF-α serum levels and C5b-9 in all COVID patients (r = 0.51, *p* value <0.001).

For the variable of neutrophil percentage, there was also a statistically significant moderate positive correlation between neutrophil counts in blood of COVID-19 patients and serum levels of C5b in the same patients with different disease severities (r = 0.48, *p* value < 0.001), and a highly significant moderate positive correlation between neutrophil counts and C5b-9 serum levels (r = 0.52, *p* value <0.001) **(Table 4)** .

Table 1. Radiological, clinical features of sever COVID-19 patients versus patients with moderate disease.

Variable	Sever COVID-19 N=37	Moderate COVID-19 N=78	Mild COVID19 N=159	*P* value
Ferritin serum level (ng/mL)				
Mean ± SD	572.95±314.05	318.83±80.87	298.83±80.87	0.01
Median (range)	435 (265:1500)	313 (205:650)	261 (205:411)	
D-dimer level (ng/mL)				
Mean ± SD	628.23±375.11	456.98±112.69	96.98±112.69	0.02
Median (range)	509 (341:2000)	415.5 (230:661)	85.5 (53:123)	
CO-RADS Category 3 Upto CO-RADS category 5	28 (75.6%)	21 (26.9%)	5 (2.5%)	0.001

Table 2. Mean difference of IL6, TNF-α, C5b, C5b-9 and percentages of neutrophils in mild, moderate, and severe COVID-19 patients

Parameter	Mild N=159	Moderate N=78	Severe N=37	*P* value by Tukey test		
				between groups	Between mild & severe	Between moderate & severe
IL6				$p < 0.001$	$p < 0.001$	$p < 0.001$
(mean±SD)	200.9± 52.27	206.07±53.3	488.5±112.2			
(median and IQ)	200(150-250)	208.5(154.5-254.5)	460(415-573)			
TNF				$p < 0.001$	$p < 0.01$	$p < 0.02$
(mean±SD)	52.9±23	93.5±39.5	159.6±38.3			
(median and IQ)	48 (34-75)	86.5 (50-128)	160(140-187)			
C5b				$p < 0.001$	$p < 0.001$	$p < 0.04$
(mean±SD)	3.9± 3.03	14.11±15.6	18.6±10.3			
(median and IQ)	2.9(1.8-5.1)	12.5(7.8-17.1)	16.7(11.05-22.7)			
C5b9				$p < 0.001$	$p < 0.01$	$p < 0.03$
(mean±SD)	76± 11.32	143.6±170.8	73.25±7.35			
(median and IQ)	77(66.7-88.5)	123.7(88.7-135.9)	70.8(68.2-78.2)			
Percentage of neutrophils (mean±SD) (median and IQ)	64.8± 9.7 61.2(57.9-68.9)	391.2±152.1 399(324-504)	87.6±4.29 87.2(84.4-91.3)	$p < 0.001$	$p < 0.001$	$p < 0.001$

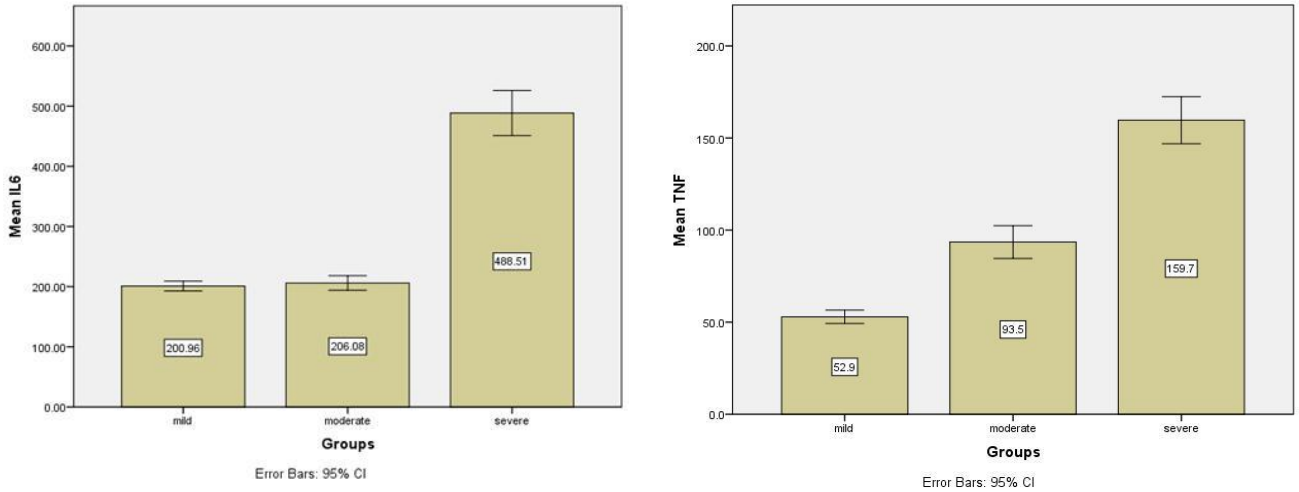
Table 3. Correlation of IL-6 with TNF-α serum levels and neutrophil blood counts in COVID-19 patients with different severities.

Variable	**Correlation Co-efficient**	***P*-value**
Correlation between IL-6 and TNF (n=274)	r=0.422	<0.001**
Correlation between IL-6 and Percentages of neutrophils (n=274)	r=0.382	<0.001**

Table 4. Correlation of IL-6, TNF-α, and neutrophil percentages from one side with C5b and C5b-9 serum levels from the other side in COVID-19 patients with different severities.

variable	**Correlation Co-efficient**	***P*-value**
Correlation between IL-6 and C5b serum levels	r=0.353	<0.001**
Correlation between IL-6 and C5b-9 serum levels	r=0.345	<0.001**
Correlation between TNF-α and C5b serum levels	r=0.543	<0.001**
Correlation between TNF-α and C5b-9 serum levels	r=0.516	<0.001**
Correlation between Percentages of neutrophils and C5b serum level	r=0.480	<0.001**
Correlation between Percentages of neutrophils and C5b-9 serum level	r=0.529	<0.001**

Figure 1. Mean serum levels of: a) IL-6 and b) TNF-α in sever- versus mild- and moderate COVID-19.



a) **b)**

Figure 2. C5b mean serum levels in severe versus mild and moderate COVID-19 patients.

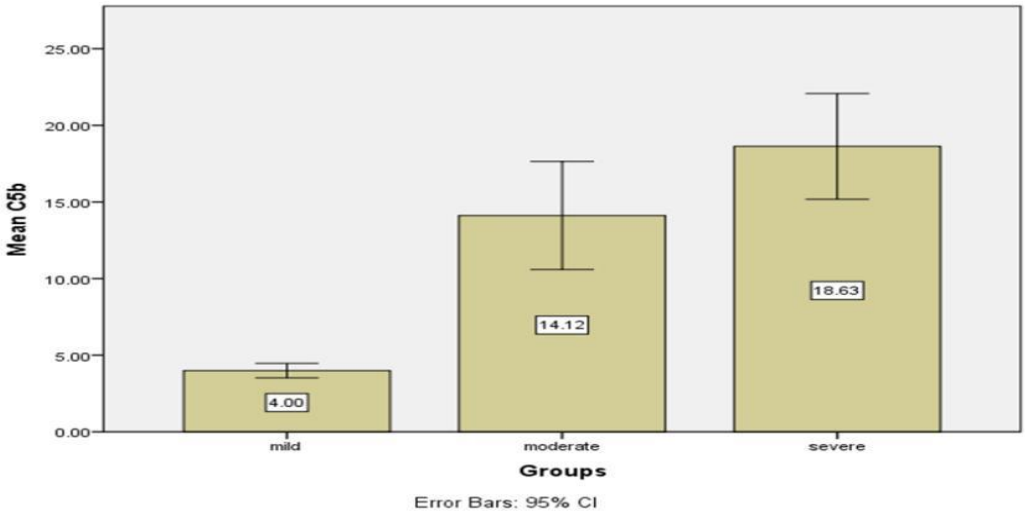


Figure 3. C5b-9 mean serum levels in severe versus mild and moderate COVID-19 patients.

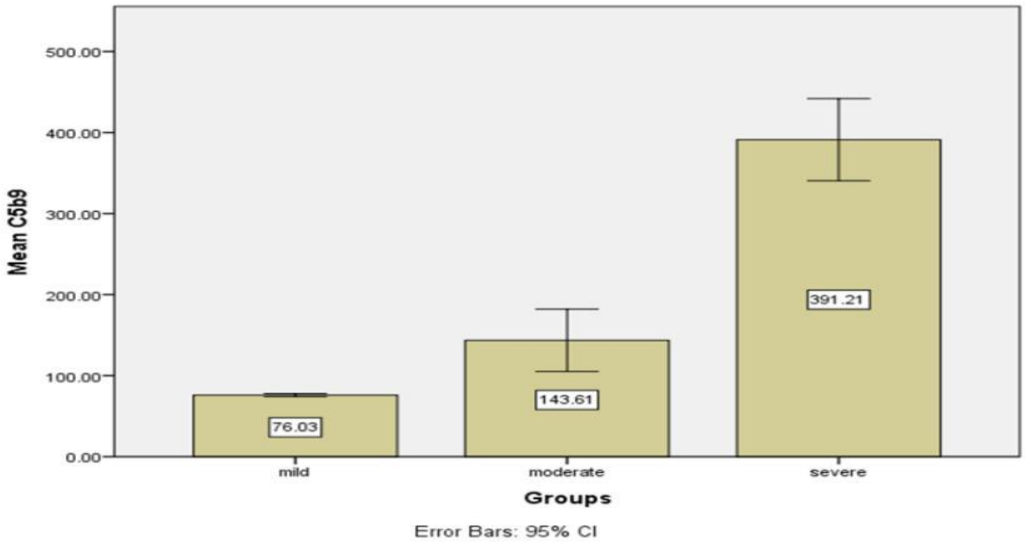
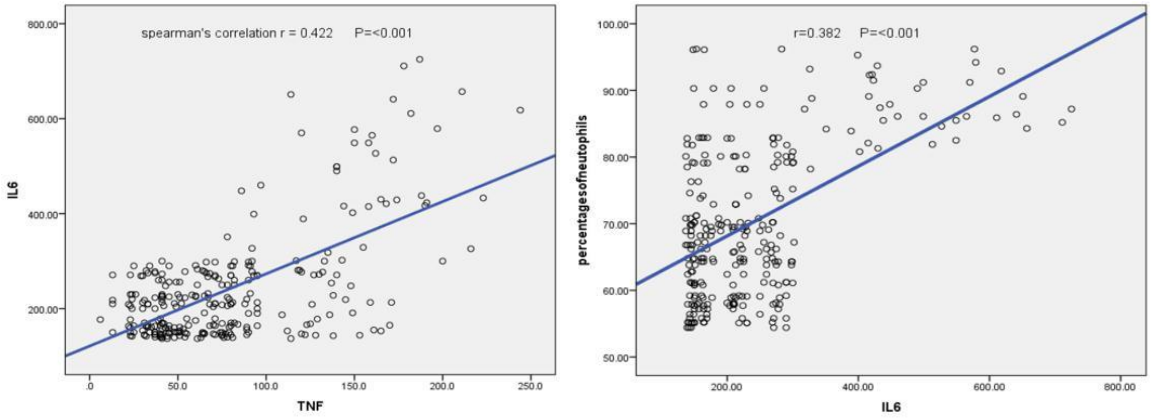
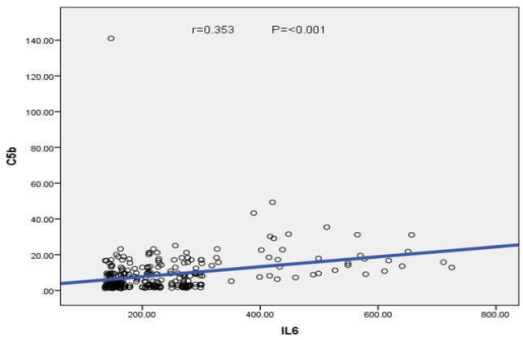


Figure 4. a): Correlation of IL-6 with TNF-α serum levels and, b) Correlation of IL-6 with neutrophil blood counts in COVID-19 patients with different severities.

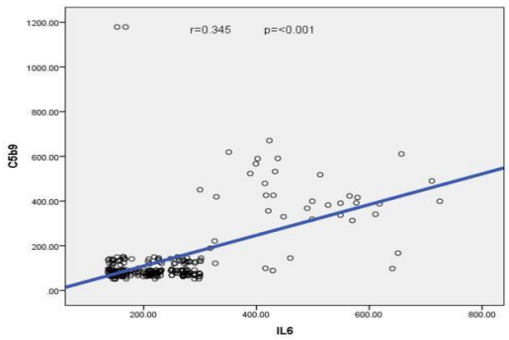


a) **b)**

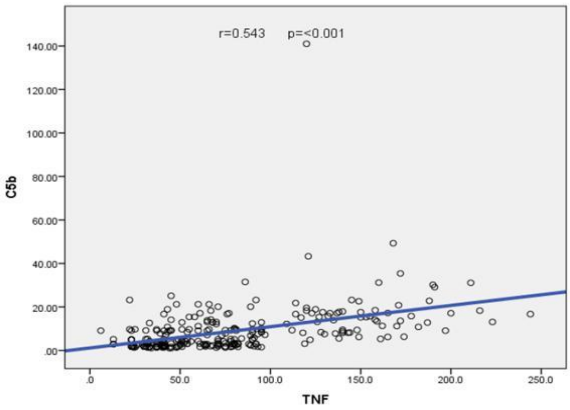
Figure 5. (a, b): Correlation of IL-6 with C5b and C5b-9 serum levels, (c, d): Correlation of TNF-α with C5b and C5b-9 serum levels, (e. f): Correction of neutrophil blood counts with C5b and C5b-9 serum levels in COVID-19 patients with different severities.



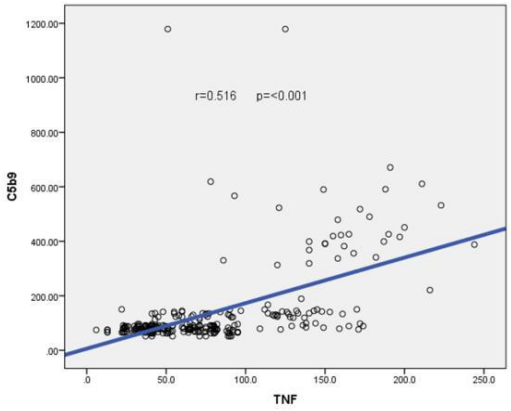
a)



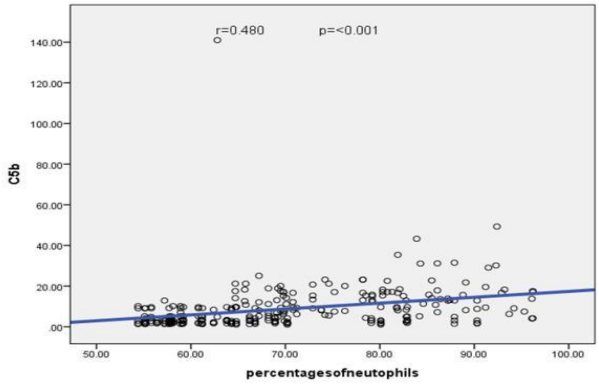
b)



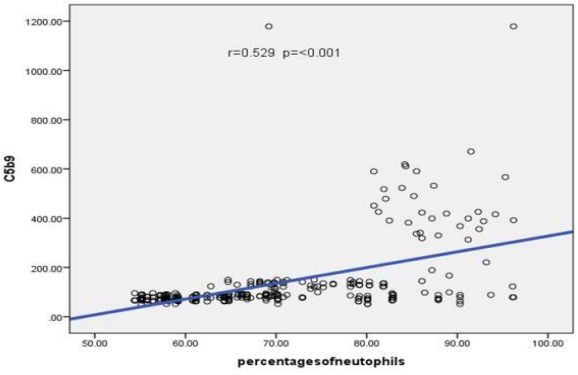
c)



d



e)



f)

Discussion

The complement cascade is now gaining an interest as a contributing factor in certain pathogenic criteria of severe COVID-19 infection, particularly the thrombotic micro angiopathy (TMA) [7, 8]. In the early phases of the innate immune response against COVID-19, a rapid antiviral response to SARS-CoV-2 occurs in which the complement system plays a fundamental role. Natural and cross-reacting antibodies and lectins trigger complement activation via classical pathway to destroy complement-coated virus and block the virus entry into the target cell.

A prominent role for the complement cascade activation in severe COVID-19 came from early clinic-pathologic studies, where C5b-9, C4d, and MASP-2 deposition was noted in affected tis-

sues, including the skin in and the lung microvasculature in patients who died as with respiratory failure [7].

C5a and C3a are considered potent anaphylatoxin and both act as strong activators of neutrophils, monocytes, and macrophages, leading to the release of pro-inflammatory cytokines and induction of severe inflammation [13]. The ability to measure these anaphylatoxins, especially C5a, is technically difficult due to the presence of its high affinity receptors (C5aR) on circulating neutrophils, which will bind free C5a resulting in short half-life in circulation (approximately 1 minute). Complement activation will lead to cleavage of C5 into the split products C5a and C5b. In turn, Terminal Complement Complex (TCC) is composed of the C5b subunit together with C5b-9 molecules, also C5b-9 complex formation without simultaneous release of C5a has thus far never been demonstrated [26, 27]. We demonstrated instead the terminal complement components C5b and C5b-9 in patients with COVID-19 of different severities.

In this study we measured the serum levels of IL-6 and TNF-α in patients with mild, moderate, and sever COVID-19, and found that there was highly significant elevation of IL-6 serum levels in patients with severe COVID-19 (488.5 pg/ml) than in patients with moderate disease (206.0 pg/ml) (*p* value < 0.001), and mild disease (200.9 pg/ml) (*p* value < 0.001).

No statistically significant difference between the mean serum levels of IL-6 in mild and moderate COVID-19 patients (*p* value 0.83). TNF-α mean serum level was also higher in severe COVID-19 patient group (159.7 pg/ml) than was measured in moderate (93.5 pg/ml) and mild (52.9 pg/ml) disease with a high statistically significant difference (*p* values 0.02, and 0.01) respectively, also there was a significant difference in the mean levels of TNF-α between mild and moderate COVID19 patient groups (*p* value < *0.001*).

Our results were in accordance with **Zhou et al.** [22] demonstrated that concentrations of biomarkers, such as D-dimer, serum ferritin, and interleukin 6 (IL-6), were significantly elevated in non-survivors of COVID-19 compared to survivors. Another study in China observed higher plasma concentrations of interleukin 6 and TNF- α in severe COVID-19 cases admitted in ICU compared to non- ICU patients [4].

A hyper-inflammatory state was also observed in patients with severe COVID-19, In a study by **Huang et al.** [4] on 41 hospitalized patients, elevated serum levels of pro-inflammatory cytokines like IL-2, IL-6, IL-7and TNF-α were associated with adverse clinical outcomes, such as ARDS, shock, organ failure, and death.

In this study we measured also the neutrophil/monocytes count in blood of COVID-19 cases and we found that the collective percentages of neutrophils and monocytes from the total leucocytic count were generally higher in COVID-19 patient than the normal individuals. We also compared the percentages of neutrophils in the three patient groups; it represented (67.6%) of the total leucocyte count in cases with sever COVID-19 was, in moderate COVID19 was (73.2%), and in mild COVID-19 was (64.8%). The higher percentage in sever disease has a statistically significant difference with the percentages of the same cells in moderate and mild disease (*p* value <0.001 for both). One mechanism by which complement may be over-activated in severe COVID-19 lies in the interplay between neutrophils and complement. Activated neutrophils generate extracellular chromatin-rich structures called neutrophil "traps," which bind pathogenic material. Neutrophil traps promote alternative pathway activation as they contain C3, factor B, and properdin. Exuberant neutrophil activation and formation of neutrophil traps have been noted in patients with severe COVID-19 [8]

We also measured the serum levels of terminal complement-activation components C5-b and C5b-9 in the three patient groups, C5b was higher patients with severe COVID-19 than in patients with moderate disease ((*p* value < 0.04) and mild disease (*p* value < 0.001). There was also a high statistically significant difference between the mean serum levels of C5b in mild and moderate COVID-19 patients(*p* value < 0.001). C5b-9 mean serum levels were also higher in severe COVID-19 patient group than in moderate and mild disease with a high statistically significant difference (p values <0.03, and <0.01) respectively.

According to **Gao et al.** [12], Infection of cells by SARS-CoV-2 may promote C3 activation, as suggested in a preprint publication demonstrating that the N protein of SARS-CoV-2 activated the mannan-binding lectin-associated serine protease (MASP-2). In another report by **Yu et al.** [13], utilizing an in vitro system, spike proteins 1 and 2 led to activation primarily of the alternative pathway on human cells. These types of innate immune responses coupled with a rapid adaptive response

lead to either no clinical signs of infection or a common cold-like or influenza-like syndrome.

Our results were in accordance with **Cugno et al.** [15] who reported that there is an increased levels of plasma C5a and sC5b-9 were noted in patients with moderate (patients requiring continuous positive airway pressure) and severe (mechanically ventilated) COVID-19. However, elevated circulating concentrations of complement factors were associated with disease severity criteria like ICU admission, thrombo-embolic tendencies, and possibly mortality in COVID-19.

Complement fragment deposition has also been reported in a study by Magro et al [7] in multiple organs in patients with COVID-19. **Diao B, et al.** [14] noticed also the occurrence of septal capillary injury in the lungs of those who died of respiratory failure is accompanied by extensive deposits of C5b-9, C4d, and MASP-2 in the microvasculature. **Shen B et al.** [17] documented a significant up-regulation of mannose in sera of severe COVID-19 patients, which may lead to complement activation upon binding of MBL to mannose.

On the other hand **Ramlall et al.** [16] found that the alternative and the classical pathways of complements were of lower activity due complement consumption. In the same study by **Ramlall et al.** [16] they found that classical pathway, lectin pathway activity and mannose binding lectin concentrations correlated weakly with CRP, IL-1, IL-6, TNF-α, which was different from our results where a strong association was found between those parameters. Other results were in line with observations of **Ramlall et al.** [16] from smaller cohorts that have not found an association of MBL or FCN-3 serum concentrations [20], [21], [22] with outcome or severity in COVID-19.

We assessed the correlation between the serum levels of IL-6 and (TNF-α and neutrophil counts) in COVID-19 patients with different severities. We found that; there was a significant positive correlation of Moderate strength between IL6 and TNF-α in all COVID patients (r = 0.42, *p* value < 0.001). We also found that there was a highly significant mild positive correlation between IL6 serum levels and percentages of neutrophils in all COVID-19 patients (r = 0.38, *p* value 0.001).

Because activation of the complement system has a potent inflammatory effect, we assessed the relationship between inflammatory cytokines (IL-6 and TNF-α) and complement activation components. The correlations were clearly present where, a highly significant positive correlation of moderate strength was found between the serum levels of IL6 and C5b in all COVID patients (r = 0.35, *p* value < 0.001), and a highly significant mild positive correlation between IL6 serum levels and C5b-9 in all COVID patients (r = 0.34, *p* value <0.001). For TNF-α there was a statistically significant Moderate positive correlation with C5b and C5b-9 in all COVID patients (r = 0.54, *p* value < 0.001) and (r = 0.51, *p* value <0.001) respectively. Our results agreed with those of **Klok et al.** [27], who demonstrated an increase in the concentrations of complement markers C5b and C5b-9 in COVID-19 patients with positive correlation between the levels of these markers and the inflammatory cytokines like IL-6 and TNF-α.

Different components of the complement system can be targeted by therapy, because both inhibition of C3 and C5 cleavage have therapeutic potential for COVID-19 by reducing the formation of anaphylatoxins C3a and C5a and the end-product terminal complement components. Moreover, the activation fragments C3a and C5a have robust pro-inflammatory effects, contributing to tissue damage, reported in COVID-19. Interestingly, **Cugno et al.** [15] suggest the therapeutic use of C5 inhibitors based on the observation of elevated concentrations of C5a and TCC in patients with COVID-19. Previous studies have reported that complement blockade could alleviate pulmonary complications in mouse models of Middle Eastern respiratory syndrome coronavirus and severe acute respiratory syndrome–related coronavirus (SARS-CoV), other coronaviruses that caused outbreaks in humans before [11, 25].

Our study did not include a control population of SARSCoV- 2 negative patients with similar disease severity (e.g. influenza). This is an important limitation; also the significance of our results is limited by the small sample size of study groups. Collectively, our findings suggest that complement factors may be useful biomarkers of disease severity, while the possibility of targetting the complement system could be a useful therapeutic option in hospitalized patients with COVID-19.

Conflict of interest

The authors declare that they have no financial or non- financial conflicts of interest related to the work reported in the manuscript. Each author listed in the manuscript saw and approved the submission of this version of the manuscript and takes full responsibility for it. This article had not been published elsewhere and is not currently under consideration by another journal or a publisher.

Funding

None

Authors contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, agreed to the submitted journal, and agree to be accountable for all aspects of the work.

References

1-World health organization (WHO) (Situation Reports Dic 2020). Available athttps://www.who.int/emergencies/ diseases/novel-coronavirus2019/situation-reports.

2-**Wu Y**. Compensation of ACE2 Function for Possible Clinical Management of 2019-nCoV-Induced Acute Lung Injury, Virol. Sin 2020; 35: 256–258.

3-**Vitiello A, Ferrara F, Pelliccia C, Granata G, La Porta R**. Cytokine storm and colchicine potential role in fighting SARS-CoV-2 pneumonia, Italian J Med 202; 14 (2): 88-94.

4-**Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y.** Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China Lancet 395 (10222) 2020; 497–506: (20)30183-5.

5-**Vitiello A, La Porta R, Ferrara F.** Sacubitril, valsartan and SARS-CoV-2 published online ahead of print, 2020. BMJ Evid. Based Med. 2020; bmjebm-2020-111497.

6-**Toshiaky I, Levy J, Levi M, Thachil J.** Coagulopathy in COVID-19, J. Thromb. Haemost (2020); 18: (9) 2103–2109, jth.14975.

7-**Magro C, Mulvey JJ, Berlin D.** Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res 2020; 220:1–13.

8-**Java A, Apicelli AJ, Liszewski MK, Anuja M, Liszewski K, Coler-Reilly A, et al.** The complement system in COVID-19: friend and foe? JCI Insight 2020; 5(15): e140711.

9-**Kulasekararaj A, Lazana I, Large J, Posadas K, Eagleton H, Villajin J, et al.** Terminal complement inhibition dampens the inflammation during COVID-19. Br J Haematol 2020; 190(3):e141–e143.

10-**Mastellos D, Pires da Silva B, Fonseca N, Martins M, Mastaglio S, Sironi M, et al.** Complement C3 vs C5 inhibition in severe COVID-19: early clinical findings reveal differential biological efficacy. Clin Immunol 2020; 220:108598.

11-**Nesargikar P, Spiller B, Chavez R.** The complement system: history, pathways, cascade and inhibitors. Eur J Microbiol Immunol 2012; 2:103–11.

12-**Gao T, Hu M, Zhang X, Li H, Zhu L, Liu H, et al.** Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2-mediated complement over-activation. medRxiv 2020.

13-**Yu J, Yuan X, Chen H, Chaturvedi S, Braunstein E, Brodsky R, et al.** Direct activation of the alternative complement pathway by SARS-CoV-2 spike proteins is blocked by factor D inhibition Blood 2020; blood.2020008248.

14-**Diao B, Wang C, Wang R, Liu L, Liu Y, Wang G, et al.** Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. medRxiv. Published April 10, 2020. Accessed June 29, 2020.

15-**Cugno M, Meroni P, Gualtierotti R, Griffini S, Grovetti E, Torri A, et al.** Complement

activation in patients with COVID-19: A novel therapeutic target. J Allergy Clin Immunol 2020; 146(1):215–217.

16-**Ramlall V, Thangaraj PM, Meydan C, Foox J, Butler D, Kim J, et al.** Immune Complement andCoagulationDysfunction inAdverse Outcomes of SARS-CoV-2 Infection. Nat Med 2020; 26(10):1609–15.

17-**Shen B, Yi X, Sun Y, Bi X, Du J, Zhang C, et al.** Proteomic and Metabolomic Characterization of COVID-19 Patient Sera. Cell 2020; 182(1):59–72 e15.

18-**Holter J, Pischke S, de Boer E, Lind A, Jenum S, Holten AR, et al.** Systemic Complement Activation is Associated With Respiratory Failure in COVID-19 Hospitalized Patients. Proc Natl Acad Sci USA 2020; 117(40):25018–25.

19-**Eriksson O, Hultstrom M, Persson B, Lipcsey M, Ekdahl KN, Nilsson B, et al.** Mannose-Binding Lectin is Associated With Thrombosis and Coagulopathy in Critically Ill COVID-19 Patients. Thromb Haemost 2020; 120(12):1720–4.

20-**Medjeral-Thomas N, Troldborg A, Hansen A, Gisby J, Clarke C, Prendecki M, et al.** Plasma Lectin Pathway Complement Proteins in Patients With COVID-19 and Renal Disease. Front Immunol 2021;12:671052.

21-**Sinkovits G, Mezo B, Reti M, Muller V, Ivanyi Z, Gal J, et al.** Complement Overactivation and Consumption Predicts In-Hospital Mortality in SARSCoV- 2 Infection. Front Immunol 2021; 12:663187.

22-**Zhou F, Yu T, Du R, Fan G, Liu Y, Wei Y, et al.** Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395:1054–62.

23-**Jiang Y, Zhao G, Song N, Li P, Chen Y, Guo Y, et al.** Blockade of the C5a-C5aR axis alleviates lung damage in hDPP4-transgenic mice infected with MERS-CoV. Emerg Microbes Infect 2018; 7:77.

24-**Krisinger M, Goebeler V, Lu Z, Meixner S, Myles T, Pryzdial E, et al.** Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. Blood 2012; 120:1717–25.

25-**Nilsson P, Thomas A, Bergseth G, Gustavsen A, Volokhina E, Heuvel A, et al.** Eculizumab-C5 complexes express a C5a neoepitope in vivo: consequences for interpretation of patient complement analyses. Mol Immunol 2017; 89:111–4

26- **Levi M, Thachil J, Iba T, Levy JH.** Coagulation abnormalities and thrombosis in patients with COVID-19. Lancet Haematol 2020; 7:e438–40.

27-**Klok F, Kruip M, van der Meer N, Arbous M, Gommers D, Kant K, et al.** Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis. Thromb Res 2020; 191:148–50

Thabet AN, Ata KA. Terminal complement complex C5b-9 and C5b assay in sera of COVID-19 patients with different disease severities. Microbes Infect Dis 2022; 3(4): 830-841.