

Prognostic significance of complement factors in severely ill patients with COVID-19

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ABSTRACT

Coagulopathy, cytokine release, platelet hyperactivity and endothelial activation are regarded as potential major contributors to COVID-19 morbidity. Complement activation might provide a bridge linking these factors in severe COVID-19 illness. In this study, we investigated the prognostic significance of selected complement factors in hospitalized patients with severe COVID-19 infection. The study included 300 hospitalized adults with severe COVID-19 infection. Complement factors (C3, C3a, C4, sC5b-9) were assessed by commercial ELISA kits. Outcome parameters included mortality, intensive care unit admission and duration of hospital stay. It was found that survivors had significantly higher serum C3 (median (IQR): 128.5 (116.3–141.0) mg/dL vs 98.0 (70.0–112.8) mg/dL, $p < 0.001$) and C4 (median (IQR): 36.0 (30.0–42.0) mg/dL vs 31.0 (26.0–35.0) mg/dL, $p < 0.001$) levels when compared with non-survivors. On the other hand, it was shown that survivors had significantly lower C3a (median (IQR): 203.0 (170.3–244.0) ng/mL vs 385.0 (293.0–424.8) ng/mL, $p < 0.001$) and sC5b-9 (median (IQR): 294.0 (242.0–318.8) ng/mL vs 393.0 (342.0–436.5) ng/mL, $p < 0.001$) levels when compared with non-survivors. Multivariate logistic regression analysis identified C3a (OR: 0.97 (95% CI 0.96 to 0.99), $p < 0.001$) and C4 (OR: 0.92 (95% CI 0.86 to 0.98), $p = 0.011$) levels as significant predictors of mortality. In conclusion, serum levels of complement factors are related to mortality in severely ill patients with COVID-19.

INTRODUCTION

COVID-19 infection caused by the newly identified SARS-CoV-2 was declared a global pandemic in early 2020, resulting in an unprecedented worldwide health crisis. The disease spectrum is markedly variable and only a small proportion of patients are severely affected.¹

Almost half of patients admitted to intensive care units (ICUs) are submitted to mechanical ventilation with high mortality rate. The most encountered causes of death are acute respiratory distress syndrome, disseminated intravascular coagulation and multiorgan failure.²

Thromboinflammation is a top cause of mortality and morbidity in patients with COVID-19.³ Clinical deterioration and rapid progressive course in some patients have been

WHAT IS ALREADY KNOWN ON THIS TOPIC

- ⇒ Severe COVID-19 pandemic is characterized by multiple systems derangements.
- ⇒ Altered immune response is involved in COVID-19 pathogenesis.
- ⇒ Dysregulation of complement factors is part of this response.

WHAT THIS STUDY ADDS

- ⇒ Complement factors are related to mortality in severe COVID-19 infection.
- ⇒ In this group of patients, survivors had significantly higher serum C3 and C4 levels.
- ⇒ In addition, survivors had significantly lower C3a and sC5b-9 levels.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- ⇒ Focus on this group of hospitalized patients with severe disease can provide a more pragmatic approach aiming to uncover better performing diagnostic markers and therapeutic approaches targeting the involved complement pathways.

attributed to immune dysregulation with massive proinflammatory response known as cytokine storm and/or procoagulant state.⁴

Despite complement system being predominantly involved in the innate immune response against miscellaneous microbial infections, excessive complement activation might result in systemic proinflammatory and procoagulant state with endothelial activation and ultimately multiorgan damage.^{5,6} In this study, we investigated the prognostic significance of selected complement factors in hospitalized patients with severe COVID-19 infection.

MATERIALS AND METHODS

This single-center cohort study was conducted on 300 adults with severe COVID-19 infection hospitalized at Kafr-Elsheikh University Hospital. Included patients or their legal guardians gave informed consent to participate in the study.

Diagnosis of COVID-19 infection was confirmed by testing nasopharyngeal swabs using PCR assays. Severe infection was defined according to the WHO recommendations by



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Table 1 Comparison between survivors and non-survivors with regard to clinical and laboratory parameters

	All patients N=300	Survivors n=172	Non-survivors n=128	P value
Age (years), median (IQR)	58.0 (46.0–66.8)	55.0 (43.3–66.0)	60.0 (49.0–70.0)	0.26
Male/female, n	180/120	92/80	88/40	0.008
Comorbidities, n (%)				
Obesity	66 (22.0)	22 (12.8)	44 (25.6)	<0.001
Hypertension	116 (38.7)	46 (26.7)	70 (40.7)	<0.001
Diabetes mellitus	132 (44.0)	78 (45.4)	54 (31.4)	0.59
Ischemic heart disease	46 (15.3)	24 (14.0)	22 (12.8)	0.44
Liver cirrhosis	32 (10.7)	14 (8.1)	18 (10.5)	0.1
Venous thromboembolism	65 (21.7)	17 (9.9)	48 (37.5)	<0.001
Complete blood count, median (IQR)				
Hb (g/L)	130.0 (120.0–140.0)	130.0 (120.0–140.0)	130.0 (115.0–140.0)	0.36
WCC ($\times 10^9$ /mL)	6.6 (4.3–10.0)	6.6 (5.0–10.0)	6.7 (3.9–12.0)	0.9
Platelets ($\times 10^9$ /mL)	209.0 (169.0–250.0)	220.0 (183.3–259.0)	189.0 (131.5–223.0)	<0.001
Inflammatory markers, median (IQR)				
CRP (mg/dL)	88.5 (47.3–130.0)	52.0 (35.0–82.3)	127.5 (97.3–173.0)	<0.001
Procalcitonin (μ g/L)	0.2 (0.1–0.2)	0.1 (0.08–0.2)	0.2 (0.1–0.5)	0.002
LDH (U/L)	603.5 (410.3–913.0)	448.5 (367.3–598.0)	911.5 (713.5–1289.8)	<0.001
Ferritin (μ g/L)	679.0 (419.5–1322.8)	518.5 (348.5–667.0)	1318.5 (858.3–1925.0)	<0.001
Coagulation profile, median (IQR)				
PT (s)	14.3 (13.3–16.0)	13.3 (13.0–14.5)	16.0 (14.8–17.3)	<0.001
APTT (s)	35.0 (31.0–44.0)	32.0 (30.0–36.0)	43.0 (36.0–50.0)	<0.001
D-dimer (ng/mL)	1000.0 (740.0–2083.3)	790.0 (689.0–942.8)	2178.5 (1370.0–3288.5)	<0.001
Fibrinogen (g/dL)	3.8 (3.2–5.1)	3.2 (3.0–3.7)	5.2 (4.5–5.5)	<0.001
Complement protein, median (IQR)				
C3 (mg/dL)	119.0 (98.0–129.0)	128.5 (116.3–141.0)	98.0 (70.0–112.8)	<0.001
C3a (ng/mL)	272.5 (195.3–363.8)	203.0 (170.3–244.0)	385.0 (293.0–424.8)	<0.001
C4 (mg/dL)	33.0 (29.0–41.0)	36.0 (30.0–42.0)	31.0 (26.0–35.0)	<0.001
sC5b-9 (ng/mL)	318.0 (286.3–389.0)	294.0 (242.0–318.8)	393.0 (342.0–436.5)	<0.001
Other laboratory data, median (IQR)				
Creatinine (mg/dL)	1.0 (0.9–1.3)	0.9 (0.8–1.1)	1.2 (1.1–1.6)	<0.001
Urea (mg/dL)	51.0 (39.0–80.0)	43.0 (35.0–55.8)	72.5 (53.0–94.8)	<0.001
Albumin (g/dL)	3.5 (3.1–3.9)	3.7 (3.5–4.0)	3.2 (2.9–3.5)	<0.001
AST (U/L)	47.0 (32.0–75.0)	41.0 (27.0–55.0)	68.0 (42.0–92.8)	<0.001
ALT (U/L)	56.0 (36.0–84.0)	46.0 (33.0–61.0)	76.0 (51.0–106.0)	<0.001
O ₂ saturation (%), median (IQR)	80.0 (70.0–85.0)	84.0 (80.0–86.0)	70.0 (60.0–80.0)	<0.001
O ₂ support, n (%)				
Mask reservoir	142 (47.3)	140 (81.4)	2 (1.6)	<0.001
Face mask	28 (9.3)	28 (16.3)	–	
Invasive	130 (43.3)	4 (2.3)	126 (98.4)	
Hospital stay (days), median (IQR)	10.0 (7.0–13.0)	9.0 (7.0–12.0)	10.5 (8.0–14.0)	0.047
ICU admission, n (%)	130 (43.3)	4 (2.3)	126 (98.4)	<0.001

ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CRP, C reactive protein; Hb, hemoglobin; ICU, intensive care unit; LDH, lactate dehydrogenase; O₂, oxygen; PT, prothrombin time; WCC, white cell count.

the presence of one of the following conditions: (1) shortness of breath, respiratory rate ≥ 30 breaths per minute; (2) oxygen saturation (resting state) $\leq 93\%$; and (3) PaO₂/FIO₂ ≤ 300 mm Hg.

Patients were excluded from the study if they received any treatment except for antipyretics before admission, had any documented coinfection, died less than 2 days after admission or had immunological disorders or malignancies.

Blood samples were collected from all patients on EDTA (1.2 mg/mL), 0.129 M trisodium citrate and plain tubes within 48 hours of admission. Routine laboratory tests were

determined using automated chemistry analyzer. Serum and citrated plasma aliquots were saved at -80°C for further analysis. Complete blood count was done on XN-550 five-part differential hematology analyzer followed by blood film examination for accurate differential white cell count and morphology assessment. All hemostasis tests were performed on platelet-poor plasma obtained after double centrifugation of citrate tubes at 2000g for 15 min at room temperature. Assays including prothrombin time, activated partial thromboplastin time, von Willebrand factor antigen

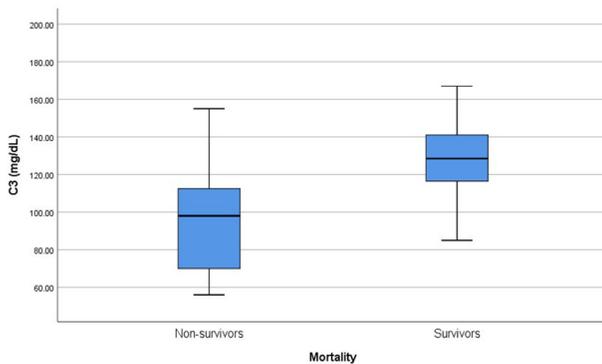


Figure 1 C3 level and mortality.

and fibrinogen were assessed using automated coagulation analyzer.

Complement factors C3 and C4 are consumed during complement activation. They were measured using turbidimetry assays (Cobas C311, Roche Diagnostics, Germany). According to the manufacturer, the normal reference ranges for C3 and C4 were 90–180 mg/dL and 10–40 mg/dL, respectively. C3a and sC5b-9 are complement activation products that are increased with complement activation. They were detected by a commercial ELISA kit (Human C3a ELISA Kit/sC5b-9 ELISA Kit, Invitrogen, Thermo Fisher Scientific, Austria). According to the manufacturers, the normal reference ranges for C3a and sC5b-9 were 70–270 ng/mL and 110–252 ng/mL, respectively. Outcome parameters included mortality, ICU admission and duration of hospital stay.

Data obtained from the present study were presented as number and per cent or median and IQR. Categorical data were compared using Fisher's exact test or χ^2 test as appropriate and numerical data were compared using Mann-Whitney U test. Spearman's correlation coefficient was used to detect correlations among numerical variables. Logistic regression was used to identify predictors of mortality. All statistical procedures were processed using SPSS v. 25, with p value less than 0.05 considered statistically significant.

RESULTS

The present study included 300 adult patients with severe COVID-19 infection, comprising 180 men (60.0%) and 120 women (40.0%). At the end of the study, 128 patients (42.7%) had died. Comparison between survivors and

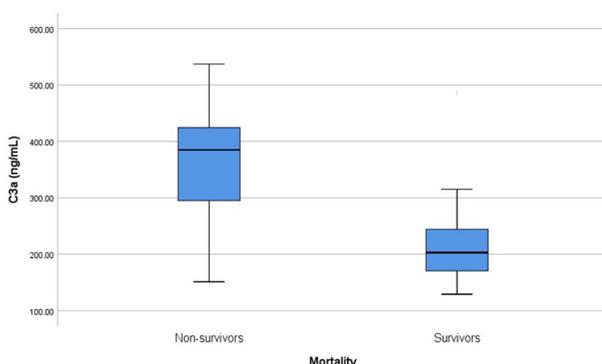


Figure 2 C3a level and mortality.

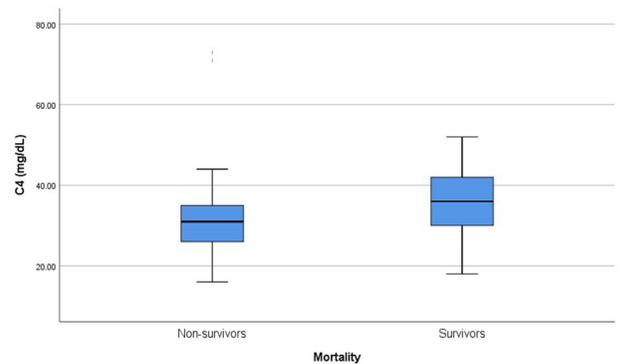


Figure 3 C4 and mortality.

non-survivors with regard to clinical and laboratory data is shown in [table 1](#). It was found that survivors had significantly higher serum C3 (median (IQR): 128.5 (116.3–141.0) vs 98.0 (70.0–112.8), $p < 0.001$) and C4 (median (IQR): 36.0 (30.0–42.0) vs 31.0 (26.0–35.0), $p < 0.001$) when compared with non-survivors. On the other hand, it was shown that survivors had significantly lower C3a (median (IQR): 203.0 (170.3–244.0) vs 385.0 (293.0–424.8)) and sC5b-9 (median (IQR): 294.0 (242.0–318.8) vs 393.0 (342.0–436.5), $p < 0.001$) levels when compared with non-survivors ([table 1](#), [figures 1–4](#)).

Besides, it was shown that non-survivors had significantly worse coagulation profile and more elevated inflammatory markers when compared with survivors. Moreover, non-survivors expressed significantly marked affection of some vital organs' functions, including the kidney (higher creatinine levels) and the liver (lower albumin levels) ([table 1](#)).

Correlation analysis revealed significant correlation between complement factors and many laboratory findings. C3 levels were inversely correlated with inflammatory markers (C reactive protein (CRP) ($r = -0.42$), lactate dehydrogenase (LDH) ($r = -0.41$) and ferritin ($r = -0.41$)) and coagulation/fibrinolysis factors (D-dimer ($r = -0.54$) and fibrinogen ($r = -0.46$)). Also, C3 levels were inversely correlated with serum creatinine levels ($r = -0.23$) and duration of hospital stay ($r = -0.13$). Similar correlations were found between C4 and laboratory data, including CRP ($r = -0.17$), LDH ($r = -0.18$), ferritin ($r = -0.16$), D-dimer ($r = -0.17$) and fibrinogen ($r = -0.14$).

On the other hand, there were significant direct correlations between C3a and laboratory findings, including LDH

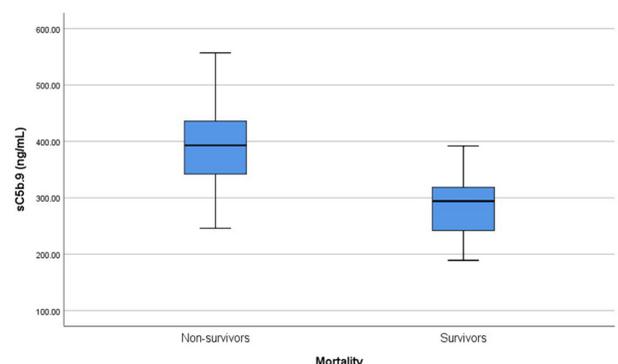


Figure 4 sC5b-9 and mortality.

Table 2 Correlations between complement proteins and clinical and laboratory data

	C3		C3a		C4		sC5b-9	
	r	P value	r	P value	r	P value	r	P value
Age	-0.14	0.017	0.17	0.003	-0.06	0.31	0.13	0.03
Hb	0.15	0.009	-0.1	0.1	0.007	0.91	-0.09	0.12
WCC	0.04	0.55	0.01	0.81	0.1	0.077	-0.03	0.58
Platelets	0.21	<0.001	-0.27	<0.001	0.088	0.13	-0.19	<0.001
CRP	-0.42	<0.001	0.48	<0.001	-0.17	0.004	0.36	<0.001
Procalcitonin	-0.1	0.076	0.11	0.066	-0.09	0.14	0.048	0.41
LDH	-0.41	<0.001	0.5	<0.001	-0.18	0.002	0.35	<0.001
Ferritin	-0.41	<0.001	0.45	<0.001	-0.16	0.007	0.3	<0.001
PT	-0.38	<0.001	0.39	<0.001	-0.16	0.006	0.35	<0.001
APTT	-0.44	<0.001	0.43	<0.001	-0.11	0.05	0.37	<0.001
D-dimer	-0.54	<0.001	0.56	<0.001	-0.17	0.003	0.43	<0.001
Fibrinogen	-0.46	<0.001	0.51	<0.001	-0.14	0.014	0.44	<0.001
C3	-	-	-0.8	<0.001	0.34	<0.001	-0.74	<0.001
C3a	-0.8	<0.001	-	-	-0.41	<0.001	0.84	<0.001
C4	0.34	<0.001	-0.41	<0.001	-	-	-0.37	<0.001
sC5b-9	-0.74	<0.001	0.84	<0.001	-0.37	<0.001	-	-
Creatinine	-0.23	<0.001	0.31	<0.001	-0.09	0.12	0.24	<0.001
Urea	-0.3	<0.001	0.43	<0.001	-0.18	0.002	0.33	<0.001
Albumin	0.35	<0.001	-0.44	<0.001	0.17	0.004	-0.32	<0.001
AST	-0.25	<0.001	0.33	<0.001	-0.16	0.003	0.25	<0.001
ALT	-0.24	<0.001	0.32	<0.001	-0.19	0.001	0.27	<0.001
O ₂ saturation	0.39	<0.001	-0.49	<0.001	0.22	<0.001	-0.4	<0.001
Hospital stay	-0.13	0.025	0.21	<0.001	-0.1	0.078	0.27	<0.001

ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CRP, C reactive protein; Hb, hemoglobin; LDH, lactate dehydrogenase; O₂, oxygen; PT, prothrombin time; WCC, white cell count.

($r=0.5$), ferritin ($r=0.5$), D-dimer ($r=0.56$) and fibrinogen ($r=0.51$). C3a levels were also significantly correlated with duration of hospital stay ($r=0.21$). sC5b-9 levels showed similar correlations with clinical and laboratory data, including LDH ($r=0.35$), ferritin ($r=0.3$), D-dimer ($r=0.43$), fibrinogen ($r=0.44$) and creatinine ($r=0.24$) (table 2).

Multivariate logistic regression analysis identified C3a (OR: 0.97 (95% CI 0.96 to 0.99), $p<0.001$) and C4 (OR: 0.92 (95% CI 0.86 to 0.98), $p=0.011$) levels as significant predictors of mortality (table 3).

DISCUSSION

The present study identified significant contribution of the studied complement factors as prognostic markers in

hospitalized patients with severe COVID-19 infection. In comparison with survivors, deceased patients had significantly higher levels of C3a and sC5b-9. In multivariate analysis, however, elevated level of C3a remained a significant predictor of mortality. On the other hand, survivors had significantly higher levels of C3 and C4.

In support of our conclusions, Sinkovits *et al*⁷ observed a continuous rise of complement factors C3a and sC5b-9 in patients with COVID-19, with the highest levels reported in critically ill patients. In contrast, there was notable decrease in C3 levels in non-survivors. They also noted lower C4 levels in non-survivors. Likewise, in a single-center case series from Italy, increased levels of C5a and sC5b-9 were noted in patients with moderate and severe COVID-19.⁸

In addition, Alosaimi *et al*⁹ noted a significant association between elevated C3a levels and disease severity and mortality in their study on 53 patients with mild-to-critical COVID-19 illness. Moreover, another study reported higher circulating sC5b-9 in most patients with COVID-19, which was related to the severity of illness.¹⁰

In accordance with our findings, a large meta-analysis by Zinellu and Mangoni¹¹ reported lower serum levels of C3 and C4 in patients with COVID-19 with more severe disease and in those who died during follow-up.

Against our results, Cheng *et al*¹² found that elevated levels of C3 are particularly associated with severe COVID-19 particularly in young patients. The authors attributed this discrepancy to the immunosenescence caused by aging

Table 3 Predictors of mortality in the studied patients

	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age	0.98	0.96 to 0.99	0.016	1.01	0.98 to 1.05	0.48
Sex	0.52	0.32 to 0.34	0.008	0.54	0.17 to 1.68	0.29
CRP	0.97	0.96 to 0.97	<0.001	0.97	0.96 to 0.98	<0.001
D-dimer	0.99	0.99 to 1.0	<0.001	0.99	0.99 to 1.0	<0.001
C3	1.08	1.06 to 1.1	<0.001	0.99	0.95 to 1.02	0.36
C3a	0.97	0.96 to 0.98	<0.001	0.97	0.96 to 0.99	<0.001
C4	1.03	1.0 to 1.05	0.045	0.92	0.86 to 0.98	0.011
sC5b-9	0.98	0.97 to 0.98	<0.001	0.99	0.98 to 1.01	0.27

in contrast to the more active immune function in young patients.

In our study, complement activation showed significant association with augmented proinflammatory state. The anaphylatoxin C3a is capable of activating neutrophils, mast cells, monocytes/macrophages, basophils, eosinophils, T cells and β cells. This drives a potent proinflammatory response, especially by macrophages and neutrophils, promoting the expression of tumor necrosis factor alpha, interleukin 1 beta and interleukin 6.¹³

In addition, the investigated complement factors in the present study were well correlated with many coagulation parameters, including D-dimer and fibrinogen, reflecting a decisive involvement of complement in COVID-19-related coagulopathy.

In fact, complement activation products (eg, C3a) are able to increase tissue factor activity,^{14,15} form activated thrombin from prothrombin,^{16,17} increase platelet activity and aggregation,^{18,19} increase prothrombinase activity, enhance the release of platelet-derived procoagulant granules, and stimulate endothelial cells to release von Willebrand factor and express P-selectin.²⁰ Such impaired complement regulation could be implicated in both microvascular and macrovascular thrombotic events.⁹ Thus, in COVID-19, the crosstalk between the complement, vascular endothelium and coagulation cascades creates a prothrombotic environment associated with adverse outcomes.²¹

The association between complement activation and elevated D-dimer levels was reported in patients with COVID-19 and was related to the associated state of exaggerated thromboinflammation.²² Also, complement activation was associated with higher fibrinogen levels in those patients.²³

In the present study, complement activation was also associated with deteriorated kidney function, expressed as elevated creatinine levels. These findings are in accordance with previous reports documenting a link between elevated sC5b-9²⁴ levels and serum creatinine in patients with COVID-19.

Findings of this study may have therapeutic implications. One report including three patients with COVID-19 recalcitrant to multiple interventions found that administration of anticomplement C5 monoclonal antibody eculizumab resulted in marked improvement in D-dimer levels and neutrophil counts in the three patients and normalization of liver and kidney functions in two.²⁵

In conclusion, the present study identified a significant contribution of complement factors to clinical outcome in patients with COVID-19. This contribution is probably mediated through complement involvement in multiple inflammatory and coagulative alterations.

Contributors AEH: planning and design. MMF: analysis. DES: conception and design. EMH: acquisition of data. MHA: interpretation of data. NAN: supervision. NF: review and editing. All authors equally shared in formulating the idea, conception, and data collection statistics, writing and drafting the manuscript. AEH acts as a study guarantor.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by Kafr-Elsheikh Faculty of Medicine (approval number: MKSU-48126). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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