Original Article

Association between plasma complement factor H concentration and clinical outcomes in patients with sepsis

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Aim: The complement system is important for defending against pathogens, however, excessive complement activation is associated with a poor prognosis and organ dysfunction in sepsis. Complement factor H (CFH) acts to prevent excessive complement activation and damage to the self through the regulation of the complement alternative pathway. We investigated the association between plasma CFH levels on admission to the intensive care unit (ICU) and 90-day mortality, severity scores, and organ dysfunction in patients with sepsis.

Methods: We assessed the relationship between the plasma CFH on admission to the ICU and 90-day mortality, severity scores such as the Acute Physiology and Chronic Health Evaluation II score, Sequential Organ Failure Assessment score, and Simplified Acute Physiology Score 2, and organ dysfunction.

Results: This analysis included 62 patients. The plasma CFH levels were significantly lower in 90-day non-survivors than in survivors (70.0 μg/mL [interquartile range, 51.2–97.6] versus 104.8 μg/mL [interquartile range, 66.8–124.2]; $P = 0.006$) . The plasma CFH levels were associated with 90-day mortality (odds ratio 0.977; 95% confidence interval, 0.957–0.994; $P = 0.01$). The plasma CFH levels were negatively correlated with severity scores. The Sequential Organ Failure Assessment scores for the coagulation and neurological components were negatively correlated with the CFH concentration.

Conclusion: Lower plasma levels of CFH were associated with increased severity and mortality in patients with sepsis on admission to the ICU and were correlated with central nervous system dysfunction and coagulopathy.

Key words: complement, complement factor H, sepsis, coagulopathy, central nervous system dysfunction

INTRODUCTION

SEPSIS IS A leading cause of morbidity and mortality among critically ill patients and a major global medical problem.[1] Recently, sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[2]

The complement system is an essential component of the innate immune system. Activation of the complement system occurs through three pathways: the classical pathway, the lectin pathway, and the alternative pathway.[3] Complement factor H (CFH) is the major negative regulator of the alternative pathway.[4] Complement factor H acts to prevent excessive complement activation and damage to the self through the repression of the complement alternative pathway. Abnormalities in CFH causing the dysregulation of the alternative pathway have been involved in the pathogenesis of atypical hemolytic uremic syndrome (aHUS), which is a type of thrombotic microangiopathy.[5]

The rapid activation of the complement system plays an important role in defending against pathogens; however, the excessive complement activation in sepsis has been reported to produce the detrimental effects, including neutrophil dysfunction,[6] coagulopathy,[7] and organ failure, leading to a poor outcome.[8] However, it is unclear whether CFH is associated with severity, mortality, and organ dysfunction in patients with sepsis. The objective of this study was to assess the association of CFH with 90-day mortality, sepsis

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severity, and organ dysfunction, including coagulopathy, in patients with sepsis on admission to the intensive care unit (ICU).

METHODS

Study design and population

WE UNDERTOOK A single-center prospective observational study from July 2016 to March 2019 in the surgical and medical ICU of Shiga University of Medical Science Hospital (Otsu, Japan). Subjects were sepsis patients admitted to that ICU. Sepsis was defined according to the Third International Consensus Definitions for Sepsis and Septic Shock.[2] The exclusion criteria were age less than 18 years, patients with aHUS, and patients with a history of aHUS. The patients were followed for 90 days.

Measurements and outcome

The following data were collected: age, sex, platelet count, prothrombin (PT) activity, activated partial thromboplastin time (APTT), fibrinogen level, white blood cell (WBC) count, C-reactive protein (CRP) level, lactate level, Acute Physiology and Chronic Health Evaluation (APACHE)-II score, Sequential Organ Failure Assessment (SOFA) score, Simplified Acute Physiology Score (SAPS) 2, and 90-day mortality. The main prognostic outcome was 90-day mortality. The correlations of CFH with each SOFA score and the correlations of CFH with the coagulation test results were investigated.

Blood collection

Blood samples were collected within 24 h of admission to the ICU. The blood platelet count and white blood cell count were assessed using samples collected in EDTA tubes, and CRP was measured in samples collected in vacuum blood collection tubes with coagulation accelerators and serum separators. Coagulation tests including PT activity, APTT, and fibrinogen were undertaken with samples collected in sodium citrate tubes. Lactate was measured with blood gas analysis. These blood sample measurements were undertaken in the hospital's central laboratory.

Plasma CFH measurement

The plasma CFH level was measured with a commercially available enzyme-linked immunosorbent assay kit (Hycult Biotech, Pennsylvania, PA, USA) in accordance with the manufacturer's instructions. Blood samples were collected in vacutainers containing EDTA. The samples were then centrifuged at 1,000 *g* for 10 min at room temperature. Immediately after centrifugation, the plasma was collected and stored at −80°C until measurement.

Statistical analysis

Data are presented as either frequencies and percentages for categorical variables or medians and interquartile ranges (IQRs) for continuous variables. Fisher's exact test was used for the comparison of categorical variables, and the Mann–Whitney *U*-test was used for the comparison of continuous variables. Univariate and multivariate analyses were undertaken using logistic regression analysis. Receiver operating characteristic (ROC) curve analysis was carried out to evaluate the predictive accuracy. Spearman's test was used for correlation analysis. All tests were two-tailed, and *P*-values <0.05 indicated statistical significance. Data were analyzed using SPSS software, version 25 (IBM, Armonk, NY, USA).

RESULTS

Study group

A TOTAL OF 109 patients met the inclusion criteria. Written informed consent was obtained from 62 of those patients and 10 healthy controls and those were included in the analysis. The characteristics of the patients included in this study are shown in Table 1. The median age was 75 (interquartile range [IQR], 68–80) years, and APACHE-II score, SOFA score, and SAPS 2 were 23 (IQR 16–28), 9 (7–12), and 55 (41–63), respectively. Eighteen patients (29.0%) died within 90 days. In the comparison of 90-day survivors and non-survivors, age and all severity scores were significantly higher in 90-day non-survivors. Inflammatory markers such as the WBC count and levels of CRP and lactate were not different between the two groups on admission to the ICU; however, the plasma level of fibrinogen was significantly lower in the non-survivors than in the survivors. The other coagulation markers did not differ between the two groups.

Plasma levels of CFH and 90-day mortality

Overall, the median plasma CFH level was 114.8 μg/mL (IQR, 100.0–158.8), 104.8 μg/mL (IQR, 68.8–124.2), and 70.0 μg/mL (IQR, 51.2–97.6) in healthy controls, survivors, and 90-days non-survivors, respectively. The CFH level was significantly lower in non-survivors than in survivors and healthy controls (Fig. 1). In univariate logistic regression analysis, age, SOFA score, and CFH level were associated

Table 1. Characteristics of patients with sepsis

Patient characteristic	Total (n = 62)	90-day survivors (n = 44)	90-day non-survivors (n = 18)	P-value
Age, years	75 (68–80)	72 (67–77)	77 (72–82)	0.022
Male sex, n (%)	42 (67)	29 (66)	13 (72)	0.629
APACHE-II score	23 (16–28)	18 (14–27)	27 (22–34)	0.003
SOFA score	9 (7–12)	9 (6–10)	12 (9–14)	0.001
SAPS 2	55 (41–63)	49 (35–57)	63 (55–68)	0.002
Platelet count, $\times 10^9$/L	154 (103–231)	158 (119–269)	121 (68–195)	0.166
PT activity, %	63 (53–76)	61 (53–73)	64 (53–87)	0.375
APTT, s	39 (33–48)	39 (33–45)	41 (32–52)	0.633
Fibrinogen, g/L	378 (274–524)	417 (308–540)	320 (172–421)	0.026
White blood cell count, /μL	14.7 (9.3–19.9)	14.2 (9.9–19.5)	15.3 (8.5–19.9)	0.816
CRP, mg/dL	8.6 (5.4–18.7)	9.5 (6.1–20.4)	7.6 (5.0–17.8)	0.174
Lactate, mg/dL	18.0 (12.0–37.0)	17.0 (12.5–33.5)	22.5 (11.0–52.0)	0.433
Infection site, n (%)				
Lung	18 (29.0)	11 (25.0)	7 (38.9)	0.357
Abdomen	28 (45.2)	20 (45.5)	8 (44.4)	1.000
Bile duct, cholangitis	5 (8.0)	3 (6.8)	2 (11.1)	0.622
Urinary tract	1 (1.6)	1 (2.3)	0	1.000
Skin, soft tissue	6 (9.7)	6 (20.5)	0	0.165
Blood stream	2 (3.2)	1 (2.3)	1 (5.6)	0.500
Others	2 (3.2)	2 (4.5)	0	1.000

APACHE, Acute Physiology and Chronic Health Evaluation; APTT, activated partial thromboplastin time; CRP, C-reactive protein; PT, prothrombin time; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment.

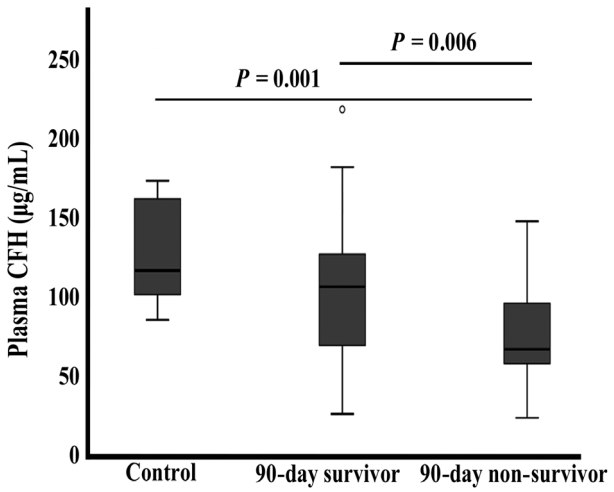

Fig. 1. Plasma complement factor H (CFH) levels in patients with sepsis were significantly lower in non-survivors than in survivors and healthy controls.

with 90-day mortality (odds ratio [OR] 1.097, 95% CI, 1.016–1.185, P = 0.02; OR 1.415, 95% CI, 1.140–1.755, P = 0.02; and OR 0.977, 95% CI, 0.957–0.994, P = 0.01, respectively). A multivariable logistic regression analysis with age and SOFA score as confounders showed that the plasma CFH levels were not independently associated with 90-day mortality (Table 2). To assess the diagnostic accuracy of CFH for the prediction of 90-day mortality, ROC analysis comparing CRP and WBC was carried out. The areas under the ROC curves for CFH, CRP, and WBC were 0.724 (95% CI, 0.587–0.862), 0.611 (95% CI, 0.466–0.756), and 0.484 (95% CI, 0.319–0.650), respectively (Fig. 2). The CFH levels had superior predictive value to CRP and WBC.

Correlation analysis between CFH and severity scores and coagulation factors

The plasma CFH levels were negatively correlated with the severity scores, namely the APACHE-II score, SOFA score, and SAPS 2 (r = −0.35, P = 0.005; r = −0.28, P = 0.026; and r = −0.30, P = 0.019, respectively) (Fig. 3). In the individual SOFA scores for each organ, the scores for the coagulation and neurological components were negatively correlated with the CFH level (r = −0.33, P = 0.01 and r = −0.25, P = 0.046, respectively) (Fig. 4). Plasma CFH levels were positively correlated with the fibrinogen level

Table 2. Univariable and multivariable logistic regression analyses for 90-day mortality among patients with sepsis

	Univariable analysis			Multivariable analysis		
	OR	95% CI	*P*-value	aOR	95% CI	*P*-value
Age, years	1.097	1.016–1.185	0.020	1.124	1.005–1.256	0.040
SOFA score	1.415	1.140–1.755	0.020	1.432	1.099–1.865	0.010
CFH (μg/mL)	0.977	0.957–0.994	0.010	0.986	0.965–1.008	0.205

aOR, adjusted odds ratio; CFH, complement factor H; CI, confidence interval; OR, odds ratio.

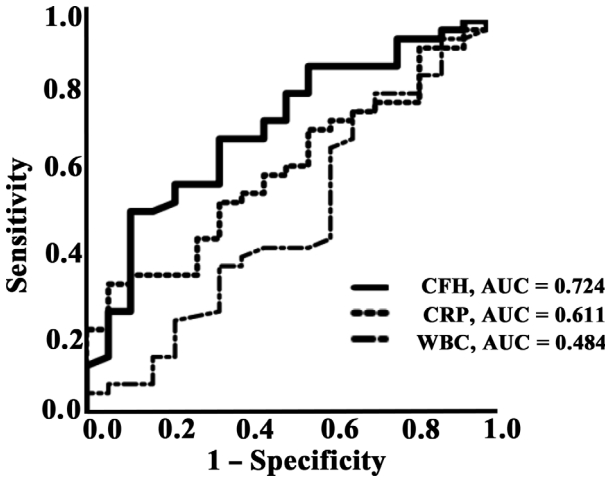

Fig. 2. Receiver operating characteristic curve analysis assessing the diagnostic accuracy of complement factor H (CFH) compared with C-reactive protein (CRP) and white blood cell count (WBC) for the prediction of 90-day mortality among patients with sepsis showed significant predictive value. AUC, area under the receiver operating characteristic curve.

and PT activity ($r = 0.31$, $P = 0.015$ and $r = 0.32$, $P = 0.012$, respectively) and negatively correlated with the APTT ($r = -0.35$, $P = 0.007$) (Fig. 5).

DISCUSSION

IN THIS STUDY, plasma levels of CFH on admission to the ICU were related to severity and mortality in patients with sepsis. The CFH level was correlated with the coagulation and neurological components of the SOFA score and with the fibrinogen level, PT activity, and APTT. To our knowledge, this is the first report showing an association between the CFH level and the prognosis of and organ damage in sepsis in a clinical setting.

Our study showed that the plasma levels of CFH were lower in the 90-day non-surviving group than in the surviving group and healthy controls and were negatively correlated with severity score. Previous studies have reported organ damage due to excessive complement activation in sepsis.[8,9] Among the complement components, C3a, C4a, C5a, and membrane attack complex have been reported to be associated with coagulopathy, organ dysfunction, and prognosis.[9–13] These complement components and terminal complement complexes are regulated by CFH.[4] Taken together, the decreased plasma CFH concentrations could have resulted in dysregulated complement activation, leading to a worse prognosis.

Concerning coagulation abnormalities, our study showed that lower plasma concentrations of CFH were associated with lower PT activity, lower fibrinogen levels, prolonged APTT, and the coagulation components of the SOFA score. Recently, immunothrombosis and immunohemostasis processes involving the innate immune system have been identified as helping prevent the dissemination of, and tissue invasion by, pathogens;[14,15] however, excessive activation leads to the development of disseminated intravascular coagulation, which is characterized by systemic coagulation activation and organ dysfunction due to disordered microcirculation.[16] These coagulation systems have been reported to interact with the complement system,[17,18] and excessive complement activation has been reported to be associated with sepsis-associated disseminated intravascular coagulation and poor prognosis.[12,19] Mutations of CFH or CFH autoantibodies are causes of aHUS, which is known to cause thrombocytopenia and could cause coagulation disorders.[5,20,21] In this context, the association of lower plasma concentrations of CFH with lower PT activity, lower fibrinogen levels, prolonged APTT, and the coagulation components of the SOFA score shown in this study could suggest abnormal coagulation due to excessive complement activation.

This study found a correlation between the plasma CFH concentration and the neurological components of the SOFA score. Patients with sepsis are known to have complications involving the central nervous system, such as sepsis-

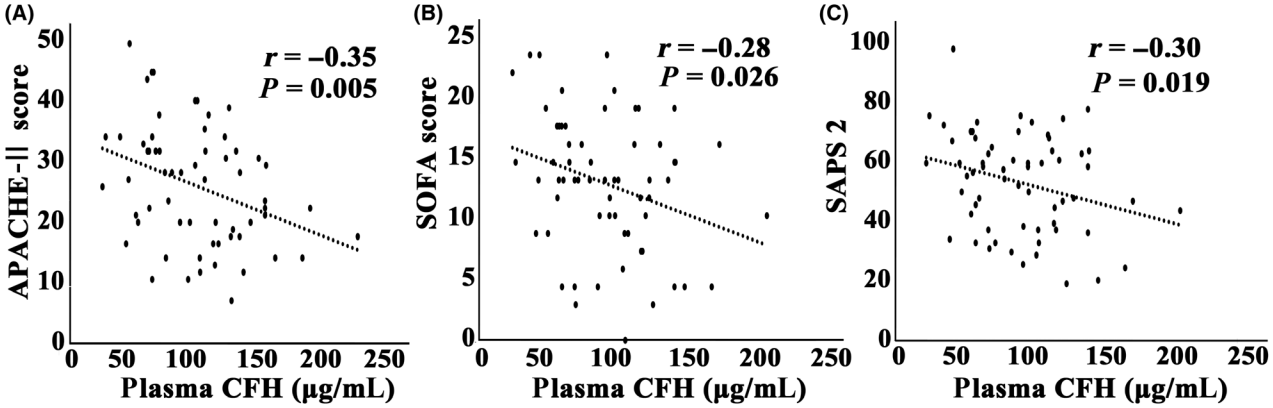

Fig. 3. Plasma complement factor H (CFH) levels were negatively correlated with severity scores in patients with sepsis. (A) Acute Physiology and Chronic Health Evaluation (APACHE)-II score. (B) Sequential Organ Failure Assessment (SOFA) score. (C) Simplified Acute Physiology Score (SAPS) 2.

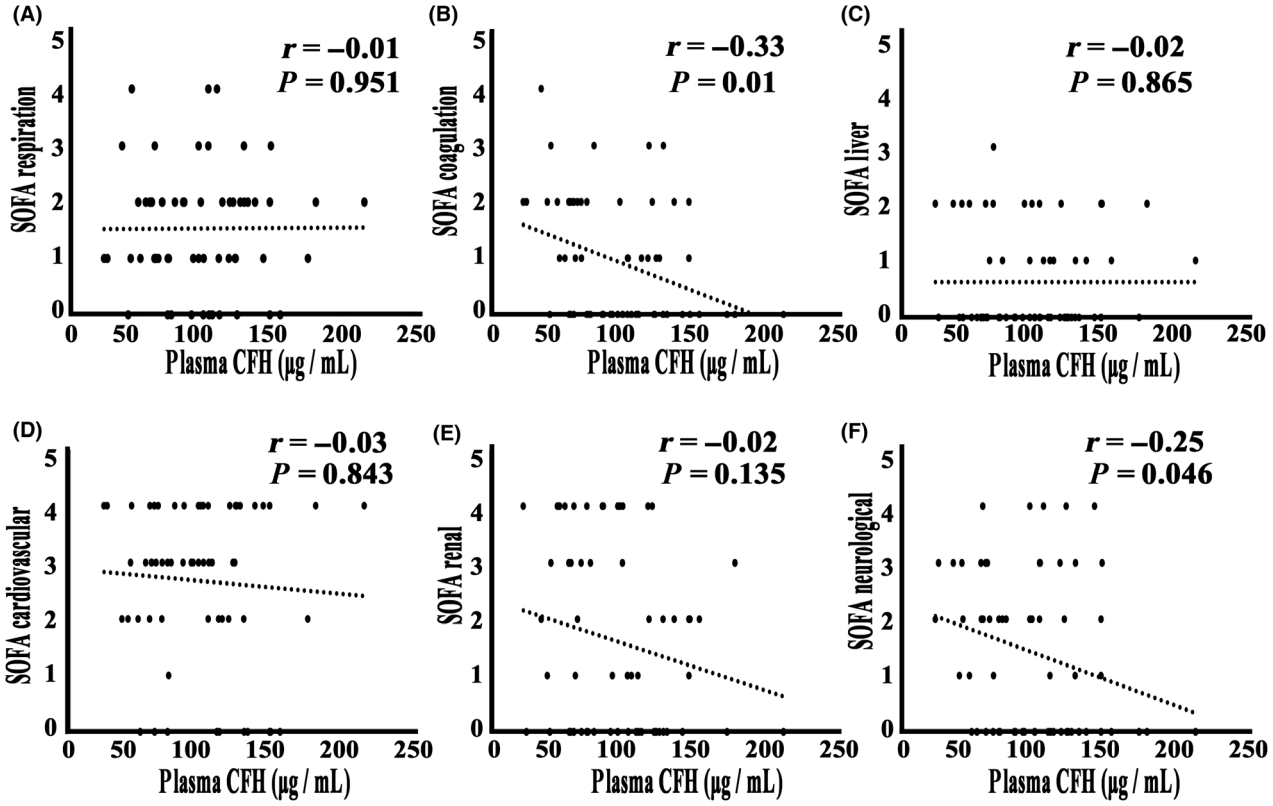

Fig. 4. Correlation analysis between complement factor H (CFH) and Sequential Organ Failure Assessment (SOFA) score for each organ in patients with sepsis showed that the plasma CFH levels were negatively correlated with the coagulation and neurological components of the SOFA score.

associated encephalopathy and sepsis-associated delirium.[22,23] Central nervous system complications are also common in aHUS, which is caused by abnormalities in CFH.[24,25] It has been reported that complement activation, including C3 and C5a, is associated with central nervous system dysfunction in sepsis.[26,27] C5a is reported to play an important role in the blood–brain barrier breakdown in septic encephalopathy.[28] These previous reports support our

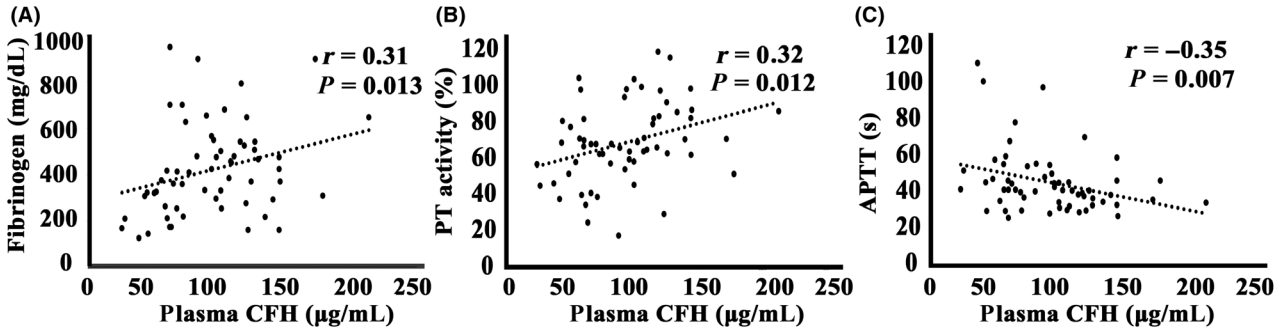

Fig. 5. Complement factor H (CFH) levels in patients with sepsis was positively correlated with the (A) fibrinogen level and (B) prothrombin time (PT) activity, and negatively correlated with (C) activated partial thromboplastin time (APTT).

finding that a lower plasma CFH concentration would be correlated with neurological dysfunction.

The results of the present study have several potential implications for future research. Patients with low CFH levels could have a worse prognosis of sepsis. Complement factor H is one of the most abundant complement components in human blood;[7] therefore, CFH might represent a potential candidate biomarker for excessive complement activation. The pharmacological enhancement of CFH and the administration of CFH could be therapeutic options for patients with sepsis who present with organ dysfunction or coagulopathy due to excessive complement activation.

A major limitation of this study was the small sample size and the single-center nature of the study. We did not measure any complement components other than CFH. Therefore, it was not possible to determine whether the low levels of CFH actually led to excessive complement activation. The CFH level was correlated with severity scores; therefore, CFH is not a prognostic factor for 90-day mortality independent of these severity scores. It took 3 or 4 h from the collection of the sample from the arterial line to centrifugation and storage at −80°C, and it is possible that the CFH concentration had changed by the time of measurement. However, all measurements were carried out under the same conditions with regard to the time from blood sample collection to measurement, and we believe that the results of the study are reliable.

Lower plasma levels of CFH were associated with increased severity and mortality in patients with sepsis on admission to the ICU and were correlated with central nervous system dysfunction and coagulopathy. Further large-sample studies are needed.

DISCLOSURE

Ethical approval: This study was approved by the Scientific-Ethical Committees of Shiga University of Medical Science (protocol ID R2015-220).

Informed consent: Written informed consent to participate in this study was obtained from the patients or their relatives.

Registry and the registration no. of the study/trial: N/A.

Animal studies: N/A.

Conflict of interest: None.

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