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Thrombomodulin, Plasminogen Activator Inhibitor-1 and Protein C Levels, and Organ Dysfunction in Sepsis

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Objectives: Since endothelial function is closely related to organ dysfunction in sepsis and the relationship among endothelial injury, organ dysfunction, and other biomarkers remains unclear, we aimed to evaluate the correlation among endothelial injury, organ dysfunction, and several biomarkers in patients with sepsis.

Design: This was a retrospective observational study.

Setting: The study was conducted in a university hospital with 14 mixed ICU beds.

Patients: ICU patients with sepsis from June 2011 to December 2017 were enrolled in this study.

Interventions: Endothelial biomarkers (soluble thrombomodulin, plasminogen activator inhibitor-1, and protein C) and markers of inflammation and coagulation were evaluated during the ICU stay. Sequential Organ Failure Assessment scores were assessed for 7 days after ICU admission to determine organ dysfunction. Variables were compared among five stratified groups according to the Sequential Organ Failure Assessment score (0–2, 3–5, 6–8, 9–12, and 13–24). Regression analysis and 95% CIs were used to evaluate trends in biomarkers.

Measurements and Main Results: The patients were divided into five stratified groups (Sequential Organ Failure Assessment 0–2, $n = 159$ [20.5%]; Sequential Organ Failure Assessment 3–5, $n = 296$ [38.2%]; Sequential Organ Failure Assessment 6–8, $n = 182$ [23.5%]; Sequential Organ Failure Assessment 9–12, $n = 75$ [9.7%]; Sequential Organ Failure Assessment 13–24, $n = 31$ [4.0%]). Protein C activity was significantly correlated with the severity of organ dysfunction. It was lower on day 1, increased upon successful treatment, and was significantly higher in groups with lower Sequential Organ Failure Assessment scores.

Conclusions: Trends and activity of protein C were superior in predicting organ dysfunction compared with other endothelial biomarkers. Monitoring the level of protein C activity is an ideal tool to monitor organ dysfunctions in patients with sepsis.

Key Words: biomarker; plasminogen activator inhibitor-1; protein C; sepsis; Sequential Organ Failure Assessment score; thrombomodulin

Sepsis is a life-threatening complication of severe infections, where widespread inflammation can lead to multiple organ failure. Endothelial injury is a major pathophysiologic event in sepsis, and as such, damaged endothelial cells are closely linked with organ dysfunction (1, 2). Endothelial cells play a pivotal role during sepsis to maintain the balance between coagulation and anticoagulation. They generate tissue factor-mediated thrombin, inhibit fibrinolysis by producing plasminogen activator inhibitor-1 (PAI-1), and inhibit the antithrombin and thrombomodulin-protein C anticoagulant system. However, once injured, endothelial cells mainly promote coagulation, which leads to the formation of a large number of microthrombi and results in microcirculatory failure (2, 3). Thus, some biomarkers related to coagulopathy (soluble thrombomodulin [sTM], PAI-1, and protein C) might represent endothelial function and appear in the early phase of sepsis, as an indicator of organ dysfunction.

Although previous studies have reported on the relationship between endothelial biomarkers and organ dysfunction, it is still unknown whether any endothelial biomarker can separately represent the recovery of endothelial or organ function, as opposed to simply representing the severity of inflammation and endothelial

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injury. Also, the relationship between endothelial biomarkers and other inflammation or coagulation variables commonly available in the clinical situations remains unclear. If some endothelial biomarkers were predictive of recovery from endothelial dysfunction and not the presence of endothelial damage at the time of inflammation, they might prove useful in assessing the recovery from organ dysfunctions.

In this study, we aimed to determine the association of some endothelial biomarkers, especially sTM, PAI-1, and protein C pathways, which are pathophysiologically related to the recovery of endothelial function, and several inflammation and coagulation biomarkers along with the severity of organ dysfunction.

MATERIALS AND METHODS

Study Design and Setting

This was a single-center, retrospective, observational study conducted in a university hospital with 14 mixed general and surgical ICU beds (Tochigi, Japan) from June 2011 to December 2017. Laboratory tests, including those for biomarkers of endothelial injury and coagulopathy, were routinely performed at our institute and were partly used for data analysis. Clinical decisions were made at the discretion of the attending ICU physicians. The study protocol was approved by the Institutional Research Ethics Committee of Jichi Medical University Hospital (number 18-032). Informed consent was waived because of the retrospective nature of this study.

Participants

Patients were eligible for enrollment if they were 20 years old or older and had sepsis at the time of ICU admission. Sepsis and septic shock were defined according to The Third International Consensus Definitions for Sepsis and Septic Shock (4). Exclusion criteria included patients with end-stage renal disease requiring dialysis, those with an ICU length of stay of less than 24 hours, or those with missing body weight records. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

The following biomarkers were evaluated during the ICU stay: endothelial biomarkers (sTM, PAI-1, and protein C), the coagulation biomarker (antithrombin III), and inflammation biomarkers (C-reactive protein [CRP], WBC count, procalcitonin, and lactate). Patient baseline characteristics including age, sex, body weight, infection site, and the premorbid creatinine level were collected from electronic medical records. The underlying medical history was also obtained, including information on hypertension, ischemic heart disease, chronic heart failure, chronic obstructive pulmonary disease, cerebrovascular accidents, diabetes mellitus, hepatic diseases, and an immunocompromised state. Antithrombin III and sTM values were excluded from the dataset just after antithrombin III, or recombinant thrombomodulin was administered for the treatment of disseminated intravascular coagulation (DIC). The Acute Physiology and Chronic Health Evaluation (APACHE) II (5) and Sequential Organ Failure Assessment (SOFA) (6) scores were used to assess organ dysfunction. Laboratory data were collected for 7 days after ICU admission. SOFA scores for all patients were reevaluated on ICU day 7 ("day-7 SOFA," hereafter), and patients were divided into five groups based on this score (SOFA 0–2, 3–5, 6–8, 9–12, and 13–24). If the patient was discharged from the ICU before day 7, the last SOFA score recorded in the ICU was used. The 7-, 28-, and 90-day mortality rates were calculated.

Definitions

Chronic kidney disease was defined as an estimated glomerular filtration rate of less than 60 mL/min/1.73 m^2 (7), at the patient's baseline creatinine level. To define the baseline creatinine level, we used a stable value within 1 year prior to the indexed hospital admission as the premorbid creatinine. If baseline creatinine data were not available, the baseline estimated glomerular filtration rate of 75 mL/min per 1.73 m^2 was used based on the Modification of Diet in Renal Disease equation (8). Overt DIC was defined according to the criteria of the International Society on Thrombosis and Haemostasis (9).

Biomarker Measurement and Laboratory Analysis

Markers of endothelial injury (sTM, PAI-1, and protein C) and coagulation (antithrombin III) were measured during the ICU stay. A chemiluminescent enzyme immunoassay was used to determine sTM levels (STACIA CLEIA; LSI Medience, Tokyo, Japan). PAI-1 levels were measured using the tPAI test (Mitsubishi Chemical Medience, Tokyo, Japan). Berichrom assays (Siemens Healthcare Diagnostics, Tokyo, Japan) were used to determine the activities of protein C and antithrombin III. Markers of inflammation (CRP, WBC, procalcitonin, and lactate) were also measured at ICU admission. The detection limits and normal values of the endothelial biomarkers are listed in **Table s1** (Supplemental Digital Content 1, http://links.lww.com/CCX/A41). If PAI-1 level was less than 10 ng/mL, it was considered 10 ng/mL.

Statistical Analysis

Variables were compared between groups using the Wilcoxon/Kruskal-Wallis test, Pearson's chi-square test, or Tukey honestly significant difference test. Regression analyses and 95% CIs were used for evaluating trends in biomarkers. The area under the receiver operating characteristic (AUROC) curve was used to evaluate biomarkers for predicting the SOFA score on ICU day 7. All analyses were performed using JMP 13 (SAS Institute, Cary, NC). Data were presented as medians and interquartile ranges (25–75th percentiles) or as percentages. p values of less than 0.05 were considered statistically significant.

RESULTS

Enrollment and Baseline Characteristics

In total, 743 patients with sepsis were included (**Fig. 1**). Patients were divided into SOFA 0–2 (n = 159, 20.5%), 3–5 (n = 296, 38.2%), 6–8 (n = 182, 23.5%), 9–12 (n = 75, 9.7%), and 13–24 (n = 31, 4.0%) groups according to their day-7 SOFA score. The APACHE II (18 vs 21 vs 26 vs 29 vs 36; $p < 0.0001$) and SOFA scores (4 vs 6

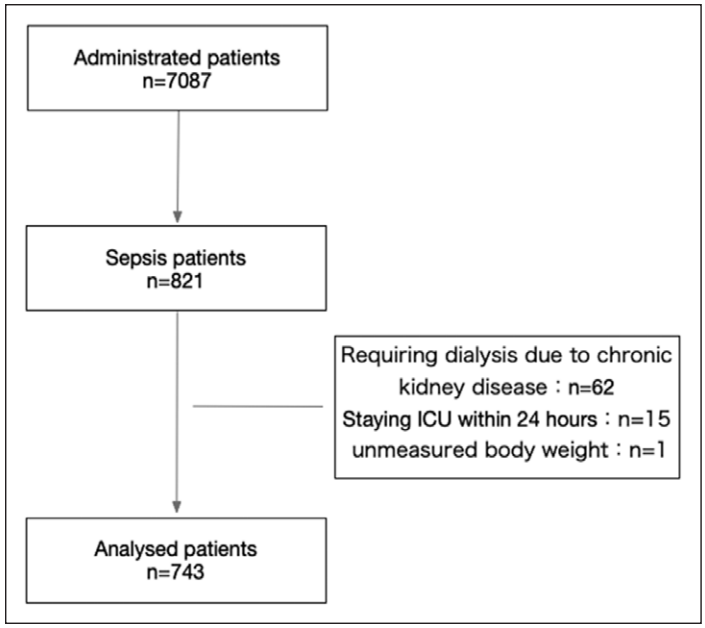

Figure 1. Flowchart of patient enrollment.

vs 8 vs 10 vs 12; $p < 0.0001$) on day 1 significantly differed between the groups. The 7-day (0.6% vs 0.0% vs 0.6% vs 4.0% vs 51.6%; $p < 0.0001$), 28-day (3.1% vs 2.8% vs 7.1% vs 20.3% vs 77.4%; $p < 0.0001$), and 90-day (5.9% vs 11.5% vs 26.9% vs 54.4% vs 85.7%; $p < 0.0001$) mortality rates were also significantly correlated with day-7 SOFA score, as expected (**Table 1**). **Supplemental Figure 1** (Supplemental Digital Content 2, http://links.lww.com/CCX/A42; **legend**, Supplemental Digital Content 7, http://links.lww.com/CCX/A47) shows the trends of the SOFA scores for each group. All groups had significantly different scores throughout the study period.

Laboratory Tests and Endothelial Biomarkers

Table 2 shows the measured variables upon ICU admission among groups. The markers of endothelial function were significantly correlated with day-7 SOFA scores. Protein C levels were significantly lower when day-7 SOFA scores were higher (62.8% vs 53.1% vs 46.5% vs 37.8% vs 38.5%; $p < 0.0001$), whereas sTM levels (15.5 vs 19.7 vs 22.3 vs 36.2 vs 33.0 U/mL; $p < 0.0001$) and PAI-1 levels (61 vs 94 vs 184 vs 182 vs 987 ng/mL; $p < 0.0001$) were significantly increased with SOFA scores. In addition, the coagulation marker antithrombin III significantly decreased with the increase in day-7 SOFA scores (62.6% vs 53.9% vs 51.5% vs 48.4% vs 40.0%; $p < 0.0001$). Finally, the presence of overt DIC (4.5% vs 15.4% vs 26.7% vs 57.3% vs 76.7%; $p < 0.0001$) and septic shock (21.4% vs 36.8% vs 57.5% vs 69.3% vs 90.3%; $p < 0.0001$) significantly differed among the groups.

Trends to Predict Day-7 SOFA Severity

Figure 2 and **Supplemental Figure 2** (Supplemental Digital Content 4, http://links.lww.com/CCX/A44; legend, Supplemental Digital Content 7, http://links.lww.com/CCX/A47) show the time course for each biomarker in accordance with the day-7 SOFA scores. Among these variables, protein C was the only variable that significantly correlated with day-7 SOFA scores. Inflammation variables, including CRP and WBC, showed relatively similar changes among the groups during the first 3 days and then varied according to SOFA score. However, procalcitonin followed the trends of SOFA scores from day 2 to day 4. As for the endothelial biomarkers, PAI-1 was significantly correlated with SOFA scores during the first 2 days. However, this effect converged to no significant correlation by day 5. Levels of sTM significantly differed among all groups. However, this trend was not as obvious as the correlation between protein C activity and SOFA score. Antithrombin III levels were not significantly different between groups. Lactate levels significantly differed in accordance with the SOFA scores on days 1–5. The AUROC of all biomarkers was also evaluated with the results presented in **Table s2** (Supplemental Digital Content 3, http://links.lww.com/CCX/A43).

Relationships Between Protein C and Day-7 SOFA Components

Supplemental Figure 3 (Supplemental Digital Content 5, http://links.lww.com/CCX/A45; legend, Supplemental Digital Content 7, http://links.lww.com/CCX/A47) shows the relationships between protein C and individual components of day-7 SOFA scores. Among the six organ-specific variables of SOFA, the time course of protein C was significantly associated with platelet counts, liver function, and circulation on day 7. However, for respiratory function, Glasgow Coma Scale, and kidney function, there was no significant correlation.

Relationship Between Protein C and 7-Day and 28-Day Mortalities

Figure 3 shows the time course for the level of protein C divided by 7-day and 28-day mortalities. In nonsurvivors, the levels of protein C decreased continuously till day 7. However, although 28-day mortality did not differ relative to day 1 levels of protein C, protein C activity significantly differed between survivors and nonsurvivors on day 28.

DISCUSSION

In this study, we found that depending on the extent of endothelial injury, several endothelial biomarkers uniquely correlated with organ dysfunction. In particular, protein C activity was the best predictor of the degree of organ dysfunction after 7 days in the ICU. Based on these results, we think that changes in protein C values may be superior to other biomarkers of endothelium, inflammation, or coagulation to evaluate organ dysfunction in septic patients.

Previous studies that evaluated the relationship between protein C activity and organ dysfunction (10–12) or mortality (13, 14) did not clarify the usefulness of protein C compared with other common clinical biomarkers. In this study, we found that protein C activity was significantly correlated with the severity of organ dysfunction at day 7 in the ICU. This finding may support the theory that activated protein C depicts the extent of endothelial recovery in addition to its known anticoagulant, anti-inflammatory, cytoprotective, and antiapoptotic activities (15–19). If this is the case, the level of protein C can represent the degree of recovery

TABLE 1. Patient Characteristics

Characteristics	All Patients (*n* = 743)	Day-7 SOFA 0–2 (*n* = 159)	3–5 (*n* = 296)	6–8 (*n* = 182)	9–12 (*n* = 75)	13–24 (*n* = 31)	*p*
Age, yr	69 (59–78)	66 (52–74)	72 (60–79)	70 (62–78)	68 (59–76)	61 (51–71)	< 0.0001
Height, cm	159 (151–166)	158 (150–165)	159 (150–166)	158 (150–165)	158 (152–167)	164 (155–168)	0.284
Body weight, kg	55 (48–65)	57 (48–67)	54 (47–63)	57 (46–65)	56 (50–65)	69 (55–80)	0.001
Body mass index	22.2 (19.6–25.3)	22.4 (20.0–25.4)	21.5 (19.4–24.5)	22.9 (19.2–25.6)	22.7 (20.0–25.5)	24.2 (21.8–29.8)	0.012
Male	413 (55.6%)	73 (45.9%)	162 (54.7%)	109 (59.9%)	49 (65.3%)	20 (64.5%)	0.023
Acute Physiology and Chronic Health Evaluation II score	23 (18–29)	18 (14–24)	21 (17–26)	26 (20–31)	29 (25–34)	36 (31–42)	< 0.0001
SOFA (day 1)	7 (4–9)	4 (3–6)	6 (4–8)	8 (6–10)	10 (8–12)	12 (11–15)	< 0.0001
Chronic kidney disease	201 (51.0%)	29 (42.0%)	77 (51.0%)	65 (58.6%)	21 (47.7%)	9 (47.4%)	0.282
Premorbid creatinine, mg/dL	0.75 (0.60–0.96)	0.72 (0.56–0.91)	0.74 (0.60–0.96)	0.77 (0.62–1.12)	0.77 (0.61–0.96)	0.76 (0.58–0.99)	0.258
Baseline creatinine, mg/dL	0.62 (0.58–0.78)	0.62 (0.57–0.73)	0.62 (0.57–0.76)	0.63 (0.58–0.87)	0.62 (0.58–0.81)	0.64 (0.58–0.85)	0.229
Hypertension	47.5%	44.7%	50.7%	50.6%	37.3%	38.7%	0.167
Ischemic heart disease	8.3%	5.7%	8.5%	11.5%	9.3%	0.0%	0.144
Chronic heart failure	8.9%	6.9%	8.8%	11.5%	9.3%	3.2%	0.464
Chronic obstructive pulmonary disease	5.7%	1.9%	7.1%	7.1%	6.7%	0.0%	0.087
Cerebrovascular accident	11.6%	8.8%	11.5%	14.8%	10.7%	9.7%	0.516
Diabetes mellitus	26.1%	22.6%	28.4%	26.4%	28.0%	16.1%	0.474
Hepatic diseases	8.9%	6.3%	8.8%	9.9%	10.7%	12.9%	0.651
Immunocompromised	26.8%	19.5%	25.0%	30.2%	33.3%	45.2%	0.012
Infection site							
Intra-cranial	1.3%	2.5%	1.4%	1.1%	0.0%	0.0%	0.530
Head and neck	5.2%	12.6%	3.7%	3.3%	1.3%	3.2%	0.0002
Thoracic	22.9%	14.5%	23.7%	28.0%	28.0%	16.1%	0.025
Abdominal	52.6%	59.8%	56.1%	46.7%	45.3%	35.5%	0.014
Soft tissue	4.3%	3.8%%	3.4%	4.4%	5.3%	12.9%	0.166
Urinary tract infection	4.3%	3.1%	5.4%	5.0%	2.7%	0.0%	0.485
Catheter-related blood stream infection	1.2%	0.0%	1.0%	1.7%	4.0%	0.0%	0.108
Other	8.3%	3.8%	5.4%	10.4%	14.7%	32.3%	< 0.0001
Mechanical ventilation	81.7%	78.0%	76.7%	86.8%	94.7%	96.8%	< 0.0001
Use of antithrombin III	24.6%	4.4%	14.9%	38.5%	56.0%	64.5%	< 0.0001
Use of recombinant thrombomodulin	8.7%	3.2%	3.4%	11.5%	26.7%	29.0%	< 0.0001
ICU days	8 (5–12)	6 (4–7)	7 (4–10)	10 (7–15)	16 (10–20)	7 (4–16)	< 0.0001
7-d mortality	2.8%	0.6%	0.0%	0.6%	4.0%	51.6%	< 0.0001
ICU mortality	5.4%	0.6%	0.3%	5.0%	12.0%	64.5%	< 0.0001
Hospital mortality	14.3%	2.5%	6.1%	18.7%	34.7%	77.4%	< 0.0001
28-d mortality	9.4%	3.1%	2.8%	7.1%	20.3%	77.4%	< 0.0001
90-d mortality	22.9%	5.9%	11.5%	26.9%	54.4%	85.7%	< 0.0001

SOFA = Sequential Organ Failure Assessment score.

Data are presented as the median and interquartile ranges (25–75th percentiles).

p values were determined using Wilcoxon/Kruskal-Wallis or Pearson correlation tests.

TABLE 2. Baseline Laboratory Results Upon ICU Admission

Variables	All Patients ($n = 743$)	Day-7 Sequential Organ Failure Assessment 0–2 ($n = 159$)	3–5 ($n = 296$)	6–8 ($n = 182$)	9–12 ($n = 75$)	13–24 ($n = 31$)	p
Laboratory test							
WBC, 10^3/μL	9.7 (4.4–14.8)	9.9 (5.0–13.4)	10.5 (5.8–15.4)	9.5 (3.4–15.6)	6.8 (2.2–15.1)	5.1 (2.7–12.6)	0.026
Hemoglobin, g/dL	10.5 (8.9–12.1)	11.0 (9.8–12.4)	10.5 (9.1–11.9)	10.1 (8.6–12.0)	10.0(8.1–12.2)	9.7 (7.4–11.9)	0.002
C-reactive protein, mg/dL	11.9 (5.4–22.2)	12.9 (5.5–23.0)	11.6 (6.1–21.0)	10.2 (4.8–22.3)	13.1 (5.7–22.8)	16.4 (5.9–24.4)	0.445
Procalcitonin, ng/mL	6.9 (0.9–38.2)	3.8 (0.6–12.1)	6.0 (0.7–31.8)	10.8 (1.6–65.8)	12.0 (1.3–63.6)	33.4 (8.3–91.3)	< 0.0001
Albumin, g/dL	2.4 (2.0–2.8)	2.4 (2.1–2.9)	2.4 (2.0–2.8)	2.4 (2.0–2.8)	2.3 (1.8–2.5)	2.4 (1.9–2.8)	0.0495
Total bilirubin, mg/dL	0.9 (0.6–1.5)	0.8 (0.6–1.2)	0.9 (0.6–1.4)	1.0 (0.6–1.7)	1.2 (0.6–3.0)	1.1 (0.7–2.1)	0.001
Lactate, mmol/L	2.2 (1.4–3.6)	1.6 (1.2–2.6)	2.0 (1.3–2.9)	2.4 (1.5–4.0)	3.0 (2.0–5.5)	6.2 (3.7–9.5)	< 0.0001
Brain natriuretic peptide, pg/mL	133 (49–362)	56 (26–187)	117 (50–272)	179 (55–532)	291 (97–609)	394 (141–1025)	< 0.0001
Renal variable							
Blood urea nitrogen, mg/dL	24 (15–41)	18 (12–26)	23 (15–36)	30 (18–49)	36 (22–69)	44 (30–56)	< 0.0001
Creatinine, mg/dL	0.98 (0.67–1.76)	0.76 (0.60–1.07)	0.91 (0.61–1.58)	1.29 (0.78–2.37)	1.60 (0.96–2.59)	1.93 (1.22–3.45)	< 0.0001
Cystatin C, mg/L	1.28 (0.90–2.01)	0.97 (0.78–1.40)	1.24 (0.87–1.79)	1.46 (1.01–2.66)	2.28 (1.57–3.15)	1.87 (1.26–2.42)	< 0.0001
Coagulation variable							
Platelet count, 10^4/μL	14.6 (9.3–21.9)	18.8 (13.7–25.4)	16.7 (11.1–24.3)	12.1 (8.0–17.59	8.4 (5.3–14.1)	5.4 (3.8–12.2)	< 0.0001
Fibrin degradation product, μg/mL	16.7 (10.4–28.0)	13.5 (9.1–22.1)	15.6 (9.3–26.5)	19.3 (11.9–28.8)	25.1 (13.6–45.1)	35.4 (14.7–79.5)	< 0.0001
Fibrinogen, mg/dL	342 (234–480)	389 (270–558)	349 (246–480)	311 (199–445)	305 (154–420)	252 (170–455)	< 0.0001
α2-plasminogen inhibitor, %	75.2 (57.7–95.1)	83.2 (68.4–102.8)	75.3 (60.0–93.9)	71.5 (49.2–96.0)	70.6 (41.2–90.7)	68.7 (40.5–86.2)	< 0.0001
Antithrombin III, %	54.3 (42.8–69.1)	62.6 (50.0–75.2)	53.9 (44.2–69.5)	51.5 (51.1–62.6)	48.4 (33.2–62.1)	40.0 (30.6–56.5)	< 0.0001
Plasminogen, %	62.2 (46.6–80.5)	70.1 (55.6–91.1)	64.1 (49.1–82.6)	59.0 (43.7–74.3)	54.6 (35.9–69.0)	50.8 (38.8–75.0)	< 0.0001
D-dimer, μg/mL	6.8 (3.2–12.6)	4.5 (2.3–7.9)	5.8 (2.9–11.9)	8.5 (3.8–15.2)	9.8 (5.7–25.6)	13.3 (7.1–22.0)	< 0.0001
Thrombin-antithrombin complex, ng/mL	10.6 (6.1–19.5)	7.9 (5.5–13.4)	9.9 (5.9–16.6)	13.6 (7.7–24.7)	13.4 (6.0–25.0)	20.8 (11.0–49.8)	< 0.0001
Plasmin–α2-plasmin inhibitor complex, μg/mL	1.3 (0.8–2.1)	1.4 (0.9–1.9)	1.3 (0.8–2.0)	1.3 (0.7–2.2)	1.2 (0.7–3.2)	1.2 (0.5–3.2)	0.902
Endothelial activation							
Protein C, %	50.8 (37.1–68.0)	62.8 (48.8–74.9)	53.1 (40.2–68.2)	46.5 (35.2–63.5)	37.8 (26.2–50.2)	38.5 (19.3–44.3)	< 0.0001
Soluble thrombomodulin, U/mL	20.5 (14.9–31.1)	15.5 (12.4–20.8)	19.7 (14.7–27.5)	22.3 (16.3–35.0)	36.2 (21.9–44.4)	33.0 (23.2–49.9)	< 0.0001
Plasminogen activator inhibitor-1, ng/mL	112 (45–245)	61 (37–164)	94 (41–214)	184 (79–264)	182 (43–402)	987 (243–1239)	< 0.0001
Other definitions, %							
Overt disseminated intravascular coagulation	22.6	4.5	15.4	26.7	57.3	76.7	< 0.0001
Septic shock	44.1	21.4	36.8	57.5	69.3	90.3	< 0.0001

Results were analyzed based on grouping patients by day-7 Sequential Organ Failure Assessment severity. Data are presented as the median and interquartile ranges (25–75th percentiles). *p* values were determined using Wilcoxon/Kruskal-Wallis or Pearson correlation tests.

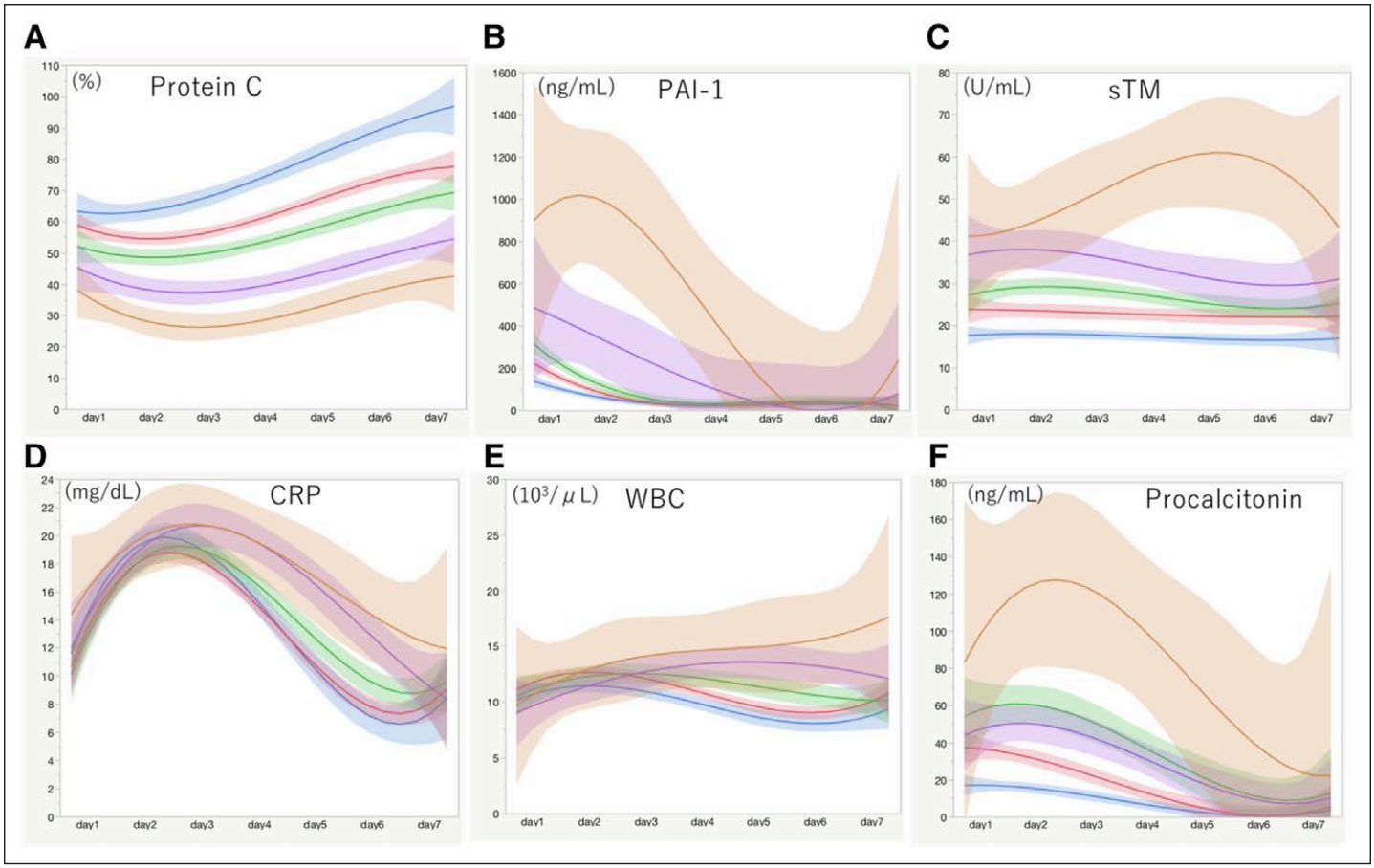

Figure 2. Relationship between biomarkers and day-7 Sequential Organ Failure Assessment (SOFA) score. Among the variables, protein C activity was the only variable that significantly correlated with day-7 SOFA scores. The *colored area* represents the 95% CI. The SOFA score on day 7 is plotted against protein C activity (**A**), plasminogen activator inhibitor-1 (PAI-1) (**B**), thrombomodulin (**C**), C-reactive protein (CRP) (**D**), WBC count (**E**), and procalcitonin (**F**). *Blue* = SOFA scores 0–2, *Red* = SOFA scores 3–5, *Green* = SOFA scores 6–8, *Purple* = SOFA scores 9–12, and *Orange* = SOFA scores 13–24. sTM = soluble thrombomodulin.

from organ dysfunction. Decreases in protein C activity correlated with some components of the SOFA scores, including platelet counts, liver function, and circulation. However, protein C activity did not correlate with variables known to depict kidney function.

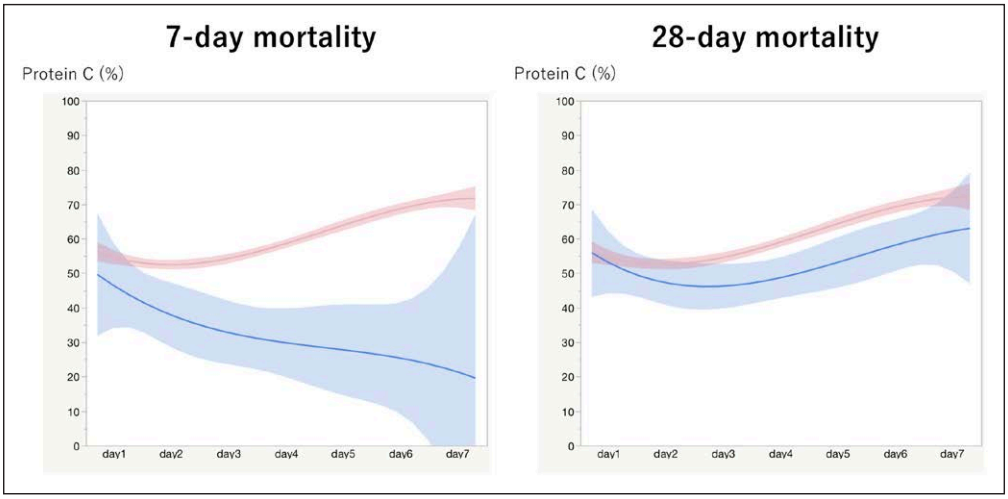

Figure 3. Relationship between protein C activity and mortality (7-d and 28-d). In nonsurvivors, protein C activity decreased continuously till day 7. Also, protein C activity significantly differed between survivors and nonsurvivors on day 28. *Red* = survivors, *Blue* = nonsurvivors. *Colored area* represents the 95% CI.

There were several studies focusing on the relationship between protein C activity and acute kidney injury (AKI), revealing that protein C activity decreased significantly in accordance with AKI severity (10, 12, 20). However, protein C activity was not shown to be an independent factor in the multivariable analysis (21). Based on our current findings, we believe that protein C activity was better related to the coagulation system, cardiovascular system, and hepatic function and may not be a sufficient predictor of kidney function.

One of the endothelial biomarkers, PAI-1, has a role in regulating fibrinolysis by inhibiting PAI-1. Previous studies have reported a relationship between PAI-1 levels and DIC (22, 23), multiple organ failure (22, 24, 25), and mortality (26, 27). Our previous study showed that PAI-1 levels peaked around the onset of sepsis and might reflect the severity of inflammation and

coagulopathy in the early phase of sepsis (23). This study supports those results. PAI-1 levels correlated with the intensity of inflammation and coagulopathy in the early phase of sepsis; however, these trends did not predict organ dysfunction 7 days after ICU admission. Thus, PAI-1, CRP, WBC, and procalcitonin seem to correlate merely with the intensity of inflammation and cannot be predictive of organ dysfunction.

Protein C and sTM are closely linked. Once thrombin binds with endothelial thrombomodulin, protein C is activated and sTM is released into the bloodstream. This process inactivates the procoagulant function of thrombin. There are several studies that have shown that thrombomodulin indicates endothelial injury and correlates with DIC, multiple organ failure, and mortality (1, 24, 28). In our previous study, we found that sTM was an independent, predictive biomarker for the development of AKI and that it outperformed other coagulation and inflammation biomarkers associated with organ function in patients with sepsis (21). In this study, sTM was closely related to organ dysfunction; however, protein C activity was more obvious. This indicates that although sTM reveals the severity of the endothelial injury and the presence of AKI, it is not as specific an indicator as protein C activity when determining systemic organ functions. Since the number of patients in the SOFA 13–24 group was relatively small, the statistical significance of this biomarker might be underestimated.

One study suggested that antithrombin III predicts organ dysfunction (29); however, our study showed that protein C had superior accuracy for predicting the SOFA score 7 days after ICU admission. This indicates that endothelial biomarkers may be better predictors of organ dysfunction than other common coagulation markers. This supports the idea that endothelial injury is caused during the early phase of inflammation in sepsis and thus directly relates to organ dysfunction.

Our study had several limitations. First, this was a single-center, retrospective, observational study with a more homogenous patient population. In addition, not all biomarkers in every patient could be measured completely throughout the study. However, protein C was measured in 86.4% of this population; therefore, although this study might not represent a new area of investigation, we believe that the sample size was large enough to make our findings more reliable. Finally, antithrombin III and recombinant thrombomodulin were sometimes administered to patients with the most severe organ dysfunctions, and this could have had an influence on several biomarker readings in this study. We excluded potentially altered values after administration of these drugs, and thus, the number of patients that could be influenced by the administration of these drugs was relatively small. Further studies are warranted to evaluate the ability of these biomarkers to predict organ dysfunction in more severe cases.

Despite these limitations, this study presented several strengths. One is that this study had a considerable number of patients. Furthermore, this is the first study to evaluate several biomarkers (depicting endothelial function, coagulation, and inflammation variables) in relation to organ dysfunction after 7 days of ICU treatment. Although the recombinant form of human activated protein C failed as a treatment measure for sepsis, possible treatment to increase the level of protein C activity might decrease sepsis-related mortality in the future.

CONCLUSIONS

Protein C was superior for the prediction of organ dysfunction after 7 days of ICU treatment when compared with other biomarkers of endothelial function, inflammation, and coagulation. CRP and WBC did not significantly differ in accordance with the severity of organ dysfunction, especially within the first 3 days. PAI-1 and procalcitonin might be the best biomarkers to represent the extent of septic injury rather than predicting organ dysfunction. Thus, we conclude that protein C readings are an ideal tool to monitor organ dysfunction in sepsis patients.

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