Usefulness of Early Diagnosis of Sepsis-Induced Coagulopathy by Measuring Novel Platelet Activation Marker Soluble Type C Lectin-Like Receptor 2 (CLEC-2)

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Research

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Abstract

Background: C-type lectin-like receptor 2 (CLEC-2) is a platelet-activated receptor expressed on the surface of platelet membranes. Soluble CLEC-2 (sCLEC-2) has been receiving attention as a predictive marker for thrombotic predisposition, such as cerebral or myocardial infarction. In the present study, we examined the relationship between sCLEC-2 level and degree of coagulation disorder, especially platelet activation, in sepsis patients.

Methods: Seventy sepsis patients were enrolled and divided into two groups, sepsis-induced disseminated intravascular coagulation (DIC) (SID) group (*n*=44) and non-SID group (*n*=26), at the time of intensive care unit admission. In addition, 37 healthy adult volunteers were enrolled as a control group. The sCLEC-2 levels were measured and compared among the groups. Because we suspected that the sCLEC-2 level was likely to be affected by the platelet count, we also calculated the sCLEC-2/platelet count ratio (termed C2PAC index) in the groups. We further divided the sepsis patients into four groups using the Japanese Acute Medical Association (JAAM) DIC scoring system (DIC scores: 0–1, 2–3, 4–5, 6–8) and investigated the C2PAC indexes in the healthy volunteers and the four JAAM DIC score groups. Finally, we examined whether the C2PAC index could be a predictor of DIC by receiver-operating curve (ROC) analysis.

Results: The C2PAC indexes in the healthy volunteers, non-SID group, and SID group were 0.34±0.14, 1.2±0.5, and 2.6±1.7, respectively. The index was significantly higher in the non-SID and SID groups compared with the healthy volunteers and also significantly higher in the SID group compared with the non-SID group (all $P<0.001$). The C2PAC indexes in the healthy volunteers and the four JAAM DIC score groups were 0.3±0.1, 0.9±0.3, 1.1±0.3, 1.7±0.7, and 3.6±1.0, respectively. Furthermore, the C2PAC index increased significantly as the DIC score increased ($P<0.001$). According to the ROC analysis, the area under the curve and optimal cut-off value for the diagnosis of DIC were 0.80507 and 1.40 (sensitivity, 75.0%; specificity, 76.9%), respectively.

Conclusions: The present findings suggest that evaluation of the C2PAC index may be a useful early predictor of sepsis-induced coagulopathy progression and DIC diagnosis in sepsis patients.

Trial registration: This study was approved by the institutional ethics committees at Fukuoka University Hospital (U19-01-001), Yamanashi University Hospital (2289), and LSI Medience Corporation (Shindan/Narita 19-04).

Background

Platelets are involved in both hemostasis and immune responses. These mechanisms work together in a complex and synchronous manner, making the contribution of platelets to sepsis of major importance 1). The traditional roles of platelets in the circulation are to help maintain primary hemostasis and blood flow within vessels by initial clot or thrombus formation when a vascular insult or injury occurs and to cause an occlusive thrombus under pathological conditions such as arteriosclerosis or dyslipidemia.

Thrombus formation is also caused by platelet activation. Therefore, an *in vivo* biomarker for platelet activation is useful for the prediction or diagnosis of various thrombotic diseases such as hypertension, atherosclerosis, inflammation, type 2 diabetes mellitus, cardiovascular diseases, and ischemic stroke that are deeply related to blood clots 2),3).

Recently, another potential role for platelets has been proposed that is independent of hemostasis or thrombosis. This role is related to immunity. Participation of neutrophils and monocytes, as well as dendritic cells, leads to a “thrombosis-related signature” that initiates and propagates fibrin formation and triggers platelet activation during the development of thrombosis. Recent works have mentioned a phenomenon termed “immunothrombosis”. This phenomenon suggests that under certain circumstances, thrombosis is a physiological process that constitutes an effective mechanism within innate immunity in which platelets play an important part 1). Therefore, we suspect that it may be important to not only track the changes in platelet counts, but also determine the status of platelet activation to understand the severity of sepsis.

To date, several biomarkers such as P-selectin, platelet factor 4, β-thromboglobulin, and platelet microparticles have been proposed as useful markers for platelet activation 2). All of these markers indicate that platelet activation may be an important step in the development and progression of diseases. However, these markers have the disadvantage of being easily released during the minimal platelet activation that occurs during sampling, and thus no efficient markers have been developed to date 4).

C-type lectin-like receptor 2 (CLEC-2) is a one of the platelet-activated receptors expressed on the surface of platelet membranes. Recent reports have described that soluble CLEC-2 (sCLEC-2) is elevated in patients with cardiovascular diseases 5), ischemic stroke 6), blunt traumatic brain injury 7), and thrombotic microangiopathy 8). Therefore, sCLEC-2 may be a predictive marker for thrombotic predisposition and is currently receiving attention.

However, to our knowledge, no previous studies have evaluated the relationship between sCLEC-2 level and severity of sepsis-induced coagulopathy (SIC) and/or disseminated intravascular coagulation (DIC).

In the present study, we measured the levels of sCLEC-2 in patients with SIC or sepsis-induced DIC (SID), and examined the relationship between plasma sCLEC-2 level and degree of coagulation disorder.

Methods

This retrospective single-center observational study was conducted at the Department of Emergency and Critical Care Medicine, Fukuoka University Hospital, Fukuoka, Japan, a 915-bed referral and tertiary hospital, from April 2015 to March 2018. The study was approved by the institutional ethics committees at Fukuoka University Hospital (U19-01-001; registered on 19 January 2019), Yamanashi University Hospital (2289; registered on 17 June 2020), and LSI Medience Corporation (Tokyo, Japan) (Shindan/Narita 19-04; registered on 19 May 2019). All participants provided informed consent prior to

participation. Patients aged ≥18 years who were diagnosed with sepsis were enrolled in the study. The exclusion criteria were: post cardiopulmonary arrest; liver cirrhosis (Child–Pugh grade C or above); chronic hemodialysis; pregnancy; death within 48 hours of hospitalization; continuing antibiotic use; traumatic injury; determination by the doctor in charge that entry was inappropriate. We also excluded patients who lacked concentrations of biomarkers or apparent clinical manifestations. Patients were evaluated for the presence of sepsis according to the Sepsis-3 diagnosis criteria 9). The DIC scoring system of the Japanese Association for Acute Medicine (JAAM) was used for the diagnosis of DIC. The JAAM DIC diagnostic algorithm for scoring DIC includes the following variables: platelet count, prothrombin time (PT), fibrin/fibrinogen degradation product (FDP) level, and systemic inflammation response syndrome criteria 10). The details of the algorithm were published elsewhere 11). DIC was defined as a score of ≥4. We also evaluated two other major DIC scoring systems: the International Society on Thrombosis and Haemostasis (ISTH) overt DIC criteria 12) and the Japanese Ministry of Health and Welfare (JMHW) DIC criteria 13). Illness severity was evaluated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score 14). The APACHE II score assesses illness severity in critical patients admitted to an intensive care unit (ICU) on the basis of routine physiologic measurements, age, and previous health status. It is used to predict the outcomes of critical illnesses. Organ failure was assessed by the Sequential Organ Failure Assessment (SOFA) score 15). The SOFA score estimates organ dysfunction related to various disease statuses, especially sepsis, and is calculated using readily available measurements to quantify the dysfunction of the six major organs. It is also useful for evaluating the morbidity and mortality of critical illnesses.

The sepsis patients were divided into a sepsis-induced DIC (SID) group and a non-SID group at the time of ICU admission. The non-SID group was also divided into a late-SID group that was diagnosed with DIC within 5 days of admission and a non-diagnosed SID group that was not diagnosed with DIC during 5 days from the time of admission. In addition, 37 healthy adult volunteers aged 20–60 years were included as a control group. These healthy volunteers were selected from the previously described cohort 4) by excluding three individuals of incomplete data set. The data for the healthy volunteers were provided by Yamanashi University Hospital.

Study procedures

In the sepsis patients, blood samples for measurement of various markers were collected on admission. In the present study, we measured coagulation and fibrinolysis molecular markers such as platelet count, prothrombin time-international normalized ratio (PT-INR), activated partial thromboplastin time (APTT), FDP, D-dimer, thrombin-antithrombin complex (TAT), plasmin α2-plasmin inhibitor complex (PIC), antithrombin (AT), protein C (PC), thrombomodulin (TM), soluble fibrin (SF), and plasminogen activator inhibitor-1 (PAI-1), and inflammatory molecular markers such as white blood cell (WBC) count, C-reactive protein (CRP), presepsin (P-SEP), and procalcitonin (PCT). Platelet and WBC counts were measured in whole blood using an XT-1800i (Sysmex Co., Kobe, Japan). PT, APTT, FDP, D-dimer level, AT activity, PC activity, and SF were measured in plasma using a CP 3000 (Sekisui Medical, Tokyo, Japan). TM and PAI-1 were measured using a STACIA (LSI Medience Co., Tokyo, Japan). CRP concentrations were measured

by CRP-LATEX (II) X2 “SEIKEN” (Denka Seiken Co. Ltd., Tokyo, Japan). P-SEP concentrations were measured using a compact automated immunoanalyzer, PATHFAST, based on a chemiluminescent enzyme immunoassay (CLEIA) (LSI Medience Co.). PCT concentrations were measured by the Elecsys BRAHMS PCT assay (Roche Diagnostics, Tokyo, Japan). PT-INR was calculated using the following formula: INR=(patient PT/normal PT)^{ISI}, where normal PT represents the average of the mean normal PT range in laboratory results and ISI is the International Sensitivity Index, a correction coefficient for thromboplastin in commercial kits calculated according to international reference samples.

Measurement of plasma sCLEC-2 levels by enzyme-linked immunosorbent assay (ELISA)

We measured plasma sCLEC-2 levels by ELISA using previously described methods. Briefly, a 96-well F8 Maxisorp plate (Nunc, Roskilde, Denmark) was coated with F(ab')$_2$ of anti-CLEC-2 monoclonal antibody 11D5 (10 μg/mL) in coating buffer (0.05 M bicarbonate, pH 9.5) overnight at 4°C. The wells were washed six times with 100-μL aliquots of 0.1 M borate-buffered saline pH 8.0 (BBS) containing 0.1% (v/v) Tween 20 (BBS-T), blocked with 1% (w/v) bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 1 hour at room temperature, and washed with BBS-T six more times prior to addition of samples. Experiments were performed in duplicate and each plate routinely included standards consisting of recombinant human CLEC-2 extracellular domain (hCLEC-2ex; final concentration, 0–5 ng/mL) in 0.3% BSA, 0.1% sodium octanoate, and 0.14 M NaCl in 25 mM sodium phosphate buffer (pH 7.2; sample diluent buffer). Test samples of plasma were diluted 4–8 times with sample diluent buffer. After a 1.5-hour incubation at room temperature, the plates were washed six times with BBS-T and 100 μL (1 μg/mL) of biotin-labeled F(ab')$_2$ of anti-CLEC-2 monoclonal antibody 11E6 was added to each well. After 1 hour of incubation and three washes with BBS-T, AMDEX High-Performance Conjugate (RPN4401V; GE Healthcare, Little Chalfont, UK) was added to the plates (100 μL/well; 1:6000 dilution of stock) for 1 hour, followed by another five washes with BBS-T. Next, 100 μL of 3,3',5,5'-tetramethylbenzidine Liquid Substrate System for ELISA (Sigma-Aldrich) was added to each well and the mixture was incubated for 20–30 minutes at room temperature in the dark. To stop the reaction, 100 μL of 2 N H_2S0_4 was added to each well and the absorbance was measured within 30 minutes at 450 nm following excitation at 630 nm 16).

Statistical analysis

Unless otherwise indicated, all data were expressed as mean ± standard deviation (SD). SPSS 15.0 J (SPSS Inc., Chicago, IL, USA) and StatFlex version 7 (Artech Co., Osaka, Japan) was used for statistical analyses. Comparisons between two groups were performed using an unpaired Student t-test and either the χ^2 test or Fisher’s exact test if necessary. Comparisons between three or more groups were carried out using the Kruskal–Wallis test. A receiver-operating curve (ROC) analysis including the area under the curve (AUC) was performed to compare the prognostic methods as predictors of sepsis and DIC. The standard error of the ROC analysis was calculated using the formula described by Hanley and McNeil 17). The level of significance was set at $P<0.05$.

Results

Population characteristics

Seventy sepsis patients were enrolled during the observation period and all patients were included in the analysis (Table 1). The mean age of the patients (36 men; 34 women) was 67.2±15.6 years (median, 71 years; range, 22–89 years). Among all 70 patients, 26 and 44 patients were classified into the non-SID group and SID group according to the JAAM DIC criteria, respectively (Table 1). The SID group was significantly older than the non-SID group. Moreover, the SID group had a significantly higher SOFA score than the non-SID group. When we calculated the DIC scores using the JAAM DIC, ISTH overt DIC, and JMHW DIC scoring systems, the scores for all scoring systems were significantly higher in the SID group compared with the non-SID group (Table 1). We also calculated the DIC positivity rate using the ISTH overt DIC and JMHW DIC scoring systems, and found that the DIC positivity rate was significantly higher in the SID group compared with the non-SID group (Table 1). The infection focuses in the sepsis patients with and without DIC are shown in Table 2.

Coagulation/fibrinolysis and inflammation molecular marker distributions

Sepsis patients with and without DIC

The data for the coagulation/fibrinolysis molecular markers and inflammatory molecular markers in the non-SID group and SID group are shown in Table 3. In the non-SID group, almost all coagulation/fibrinolysis markers such as PT-INR, fibrinogen, FDP, D-dimer, TAT, PIC, TM, SF, and PAI-1 and inflammatory markers such as CRP, WBC count, P-SEP, and PCT were outside their normal ranges. In addition, the SID group had significantly higher PT-INR, FDP, D-dimer, TAT, PIC, SF, P-SEP, and PCT than the non-SID group. In contrast, the SID group had significantly lower platelet count, AT activity, and PC activity than the non-SID group.

Platelet count and sCLEC-2 level

Healthy volunteers and sepsis patients with and without DIC

In the present study, we enrolled 37 healthy volunteers as a control group. The platelet counts and sCLEC-2 levels in the healthy volunteers and sepsis patients on ICU admission were 263±63 and 177±122 ($\times 10^3$/μL) and 87.2±38.9 and 286±205 (pg/mL), respectively (Table 4). The sepsis patients had significantly lower platelet count and significantly higher sCLEC-2 level compared with the healthy volunteers (P<0.001). We further investigated the relationships for platelet counts and sCLEC-2 levels between the healthy volunteers and the non-SID and SID groups. The SID group had significant lower platelet count (134±87$\times 10^3$/μL) and significantly higher sCLEC-2 level (299±234 pg/mL) than the healthy volunteers (platelet count, 263±63$\times 10^3$/μL; sCLEC-2, 87.2±38.9 pg/mL) (P<0.001) (Table 4). Meanwhile, the non-SID group had a significantly higher sCLEC-2 level (253±140 pg/mL) than the healthy volunteers (P<0.001),

but the platelet count (227±102×10^3/μL) did not differ significantly from that in the healthy volunteers and remained within the normal range (Table 4).

sCLEC-2/platelet count ratio (C2PAC index)

We suspected that the reason why there was no significant difference in the sCLEC-2 levels between the non-SID group and the SID group was that the platelet count in the SID group was significantly lower than that in the non-SID group. Because we considered that the sCLEC-2 level was likely to be affected by the platelet count, we calculated the sCLEC-2 (pg/mL)/platelet count ratio (×10^3/μL) (termed the C2PAC index) in the groups.

Healthy volunteers and sepsis patients

The C2PAC indexes in the healthy volunteers and sepsis patients on ICU admission were 0.34±0.14 and 2.0±1.6, respectively (Table 4). The C2PAC index in the sepsis patients was significantly higher than that in the healthy volunteers (P<0.001).

Sepsis patients with and without DIC

Among the sepsis patients, the C2PAC indexes in the non-SID group and SID group on ICU admission were 1.2±0.5 and 2.6±1.7, respectively (Table 4). The C2PAC index in the SID group was significantly higher than that in the non-SID group (P<0.001).

Relationship between JAAM DIC score and C2PAC index

We further divided the sepsis patients into four groups using the JAAM DIC scores on ICU admission: 0–1, 2–3, 4–5, and 6–8. As a result, the C2PAC indexes in the healthy volunteers and the four JAAM DIC score groups were 0.3±0.1, 0.9±0.3, 1.1±0.3, 1.7±0.7, and 3.6±1.0, respectively. We confirmed that the C2PAC index increased significantly as the DIC score increased (Figure 1).

Relationship between diagnosis of DIC and C2PAC index

We examined whether the C2PAC index could be a predictor of SID by performing a ROC analysis including the AUC. According to the ROC analysis, the AUC and optimal cut-off value of the C2PAC index for the diagnosis of DIC were 0.80507 and 1.40 (sensitivity, 75.0%; specificity, 76.9%), respectively.

Time course of C2PAC index in sepsis patients

Finally, we investigated the time course of the C2PAC index in the sepsis patients, using the SID group diagnosed with DIC on the day of ICU admission, the late-SID group that was not diagnosed DIC on the day of admission but was diagnosed within 5 days, and the non-diagnosed SID group that was not diagnosed with DIC during 5 days from the time of ICU admission (Figure 2). The C2PAC index in the SID group remained above the DIC cut-off value of 1.4, while the C2PAC index in the non-diagnosed SID group

remained below 1.4 throughout the 5 days after admission. In contrast, the C2PAC index in the late-SID group was about 1.4 on ICU admission, but gradually increased over time.

Discussion

Sepsis, defined as life-threatening organ dysfunction arising from a dysregulated host response to infection 18), is a complex inflammatory syndrome and an important cause of worldwide morbidity and mortality in ICU settings. It affects between 47 and 50 million people every year, and causes at least 11 million deaths, representing one death every 2.8 seconds. Depending on the country involved, mortality varies from 15% to > 50% 19).

Sepsis survivors have an increased long-term risk of thromboembolic events, including myocardial infarction and venous thromboembolism 20). Therefore, many surviving patients suffer from the consequences of sepsis for the rest of their lives 19).

Platelets have received increasing attention for their role in the pathophysiology of infectious diseases, inflammation, and immunity. In sepsis, a low platelet count is a well-known biomarker for disease severity. Recently, attention has been focused on the active role of platelets in the pathogenesis of multiorgan failure. Because of their placement at the crossroads between the immune system, clotting cascade, and endothelial cells, platelets appear to be an appealing central mediator and possible therapeutic target in sepsis 21).

In recent years, increasing numbers of studies have shown that platelets contribute to the pathophysiological processes involved in sepsis and play an important role in organ damage. When pathogens invade the body, activation of the coagulation system at the site of infection and thrombus formation in local capillaries serve as defense mechanisms that limit the infection to the lesions by a process known as immunothrombosis. In sepsis, these local reactions spread to the entire body, and the resulting loss of control of the “inflammation-coagulation” interaction leads to platelet activation, followed by DIC and subsequent multiorgan dysfunction syndrome (MODS) 22). Infections lead to decreases in platelet counts through effects on both platelet production and platelet survival 23). Consequently, early recognition and diagnosis of sepsis is the key to achieving improved outcomes. Therefore, it is important to measure platelet counts daily and recognize the signs of thrombocytopenia in sepsis patients admitted to the ICU.

We conducted the present study under the hypothesis that recognizing the stage of platelet activation before the onset of thrombocytopenia could lead to earlier detection of SIC or SID. This is the first report to examine the relationship between SID and platelet activation using sCLEC-2 as a marker for platelet activation.

In this study, almost all coagulation/fibrinolysis markers and inflammatory markers were outside their normal ranges in the non-SID group. Through these results, we confirmed that inflammation and coagulation exhibit close cross-talk, and that sepsis patients simultaneously show not only an elevated

inflammatory condition, but also an enhanced abnormal coagulation/fibrinolysis condition 24). In other words, we suggest that non-SID patients can be considered an SIC group or pre-SID group. Furthermore, when sepsis patients developed DIC, both their hyper-inflammatory state and abnormal coagulation/fibrinolysis condition became even more pronounced.

The platelet count in the non-SID group (227 ± 102×10^3/μL; normal range, 158–348×10^3/μL) did not differ significantly from that in the healthy volunteers, and remained within the normal range. Meanwhile, the sCLEC-2 level in the non-SID group was approximately three times higher than that in the healthy volunteers with a significant difference. Therefore, we suspect that measurement of sCLEC-2 can help toward understanding of pre-DIC or SIC states in patients who have coagulopathy but have not reached DIC. However, there was no difference in the sCLEC-2 levels between the non-SID group and SID group. Based on these results, we speculated that the sCLEC-2 level was likely to be affected by the platelet count and investigated the C2PAC index. We confirmed that the C2PAC index was not only significantly higher in sepsis patients compared with healthy volunteers, but also significantly higher in the SID group compared with the non-SID group. Therefore, by measuring not only the platelet count but also the sCLEC-2 level and calculating the C2PAC index, it may be possible to recognize the stage of SIC or pre-SID earlier than measurement of the platelet count alone.

In this study, we confirmed that the C2PAC index increased significantly as the DIC score increased, and that a C2PAC index of ≥ 1.4 was likely to result in DIC. These results strongly suggested that the C2PAC index is useful for identifying DIC. Moreover, we investigated the time course of the C2PAC index in sepsis patients. Interestingly, the C2PAC index in the SID group already complicated with DIC at ICU admission remained above 1.4, while the C2PAC index in the non-diagnosed SID group not complicated with DIC did not exceed 1.4 over time. Meanwhile, in the late-SID group that developed DIC after admission, the C2PAC index gradually increased and eventually exceeded 1.4. Considering these results, we need to carefully monitor patients with sepsis whose daily C2PAC index exceeds 1.4 and rises over time, because they may be likely to develop DIC.

Some limitations of the present study should be noted, including the fact that it was a small, retrospective, single-center observational study, making it difficult to interpret the findings globally. However, the study is being continued, and a prospective multicenter study has been planned.

Conclusions

In patients with sepsis, platelet activation occurred before the platelet count decreased below the lower limit of the normal range and may indicate the preparatory phase for DIC. The findings further suggested that a sustained rise in the C2PAC index may be a predictor of progression to DIC. Therefore, we conclude that sCLEC-2, especially the C2PAC index, is a useful predictive marker for early assessment of coagulopathy and DIC diagnosis in sepsis.

Abbreviations

APACHE II Acute Physiology and Chronic Health Evaluation II

APTT activated partial thromboplastin time

AT antithrombin

AUC area under the curve

BBS borate-buffered saline

BSA bovine serum albumin

CLEC-2 C-type lectin-like receptor 2

CRP C-reactive protein

DIC disseminated intravascular coagulation

ELISA enzyme-linked immunosorbent assay

FDP fibrin/fibrinogen degradation product

ICU intensive care unit

ISTH International Society on Thrombosis and Haemostasis

JAAM Japanese Acute Medical Association

JMHW Japanese Ministry of Health and Welfare

MODS multiorgan dysfunction syndrome

PAI-1 plasminogen activator inhibitor-1

PC protein C

PCT procalcitonin

PIC plasmin α2-plasmin inhibitor complex

P-SEP presepsin

PT prothrombin time

PT-INR prothrombin time-international normalized ratio

ROC receiver operating curve

sCLEC-2 soluble C-type lectin-like receptor 2

SD standard deviation

SF soluble fibrin

SIC sepsis-induced coagulopathy

SID sepsis-induced disseminated intravascular coagulation

SOFA Sequential Organ Failure Assessment

TAT thrombin-antithrombin complex

TM thrombomodulin

TMB tetramethylbenzidine

WBC white blood cell

Declarations

Ethics approval and consent to participate

The following ethics review boards approved the protocol for this study: Fukuoka University Hospital (U19-01-001; registered on 19 January 2019); Yamanashi University Hospital (2289; registered on 17 June 2020); LSI Medience Corporation (Shindan/Narita 19-04; registered on 19 May 2019).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

MK is an employee of LSI Medience Corporation. The other authors declare that they have no competing interests.

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Authors' contributions

HI contributed to the study design, statistical analysis, interpretation of the results, drafting of the manuscript, and critical revision of the manuscript for intellectual content. YI and MK participated in the study design, statistical analysis, and interpretation of the results. KH and YN were involved in data acquisition and performed the statistical analysis. MM, JM and MN were involved in data acquisition. KSI carried out the ELISA assays and participated in the development and methodology. TK helped to draft the manuscript. All authors have read and approved the final manuscript.

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References

1. Vardon-Bounes F, Ruiz S, Gratacap MP, Garcia C, Payrastre B, Minville V. Platelets Are Critical Key Players in Sepsis. Int J Mol Sci. 2019;20:3494.
2. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet Activation: The Mechanisms and Potential Biomarkers. Biomed Res Int. 2016:9060143.
3. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovasc Diabetol. 2018;17:121.
4. Inoue O, Osada M, Nakamura J, Kazama F, Shirai T, Tsukiji N, et al. Soluble CLEC-2 is generated independently of ADAM10 and is increased in plasma in acute coronary syndrome: comparison with soluble GPVI. Int J Hematol. 2019;110:285–94.
5. Fei M, Xiang L, Chai X, Jin J, You T, Zhao Y, et al. Plasma soluble C-type lectin-like receptor-2 is associated with the risk of coronary artery disease. Front Med. 2020;14:81–90.
6. Zhang X, Zhang W, Wu X, Li H, Zhang C, Huang Z, et al. Prognostic Significance of Plasma CLEC-2 (C-Type Lectin-Like Receptor 2) in Patients With Acute Ischemic Stroke. Stroke. 2018 Dec 7:STROKEAHA118022563.
7. Guo M, Zhang H, Lv QW, Huang HB, Shen LJ. Higher plasma C-type lectin-like receptor 2 concentrations for prediction of higher risk of 30-day mortality in isolated severe blunt traumatic brain injury. Clin Chim Acta. 2019;496:1–6.
8. Yamashita Y, Suzuki K, Mastumoto T, Ikejiri M, Ohishi K, Katayama N, et al. Elevated plasma levels of soluble C-type lectin-like receptor 2 (CLEC-2) in patients with thrombotic microangiopathy. Thromb Res. 2019;178:54–8.
9. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315:801–10.
10. No authors listed. **American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of**

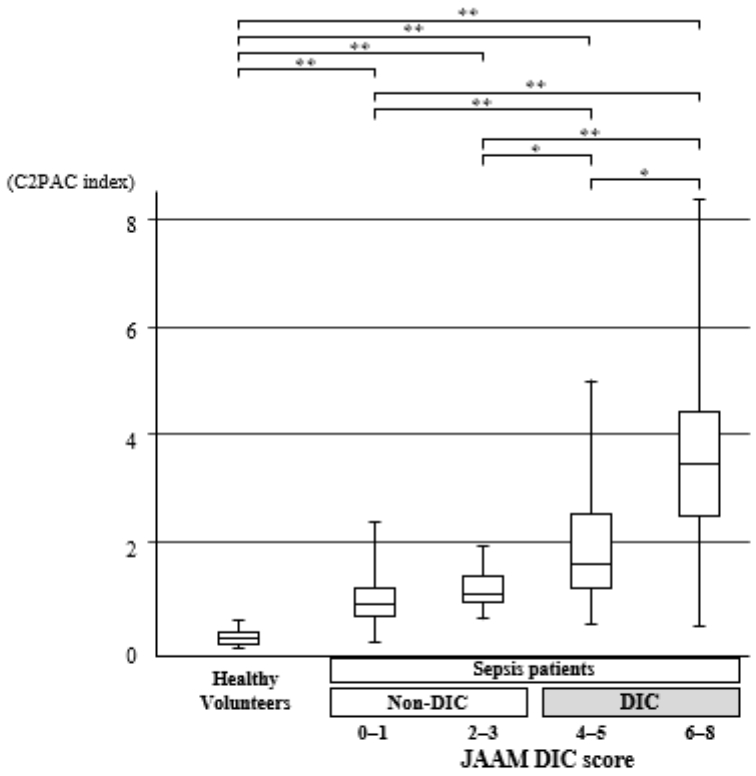
innovative therapies in sepsis. *Crit Care Med*. 1992;**20**:864 – 74.

11. Gando S, Iba T, Eguchi Y, Ohtomo Y, Okamoto K, Koseki K, et al. A multicenter, prospective validation of disseminated intravascular coagulation diagnostic criteria for critically ill patients: comparing current criteria. Crit Care Med. 2006;34:625–31.
12. Taylor FB Jr, Toh CH, Hoots WK, Wada H, Levi M, Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Haemostasis (ISTH). Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost. 2001;86:1327–30.
13. Kobayashi N, Maegawa K, Takada M, Tanaka H, Gonmori H. Criteria for diagnosis of DIC based on the analysis of clinical and laboratory findings in 345 DIC patients collected by the Research Committee on DIC in Japan. Bibl Haematol. 1987;49:265–75.
14. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med. 1985;13:818–29.
15. Vincent JL, de Mendonça A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. **Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine***Crit Care Med*. 1998;26:1793–800.
16. Kazama F, Nakamura J, Osada M, Inoue O, Oosawa M, Tamura S, Tsukiji N, Aida K, Kawaguchi A, Takizawa S, Kaneshige M, Tanaka S, Suzuki-Inoue K, Ozaki Y. Measurement of soluble C-type lectin-like receptor 2 in human plasma. Platelets. 2015;26:711–9.
17. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology. 1982;143:29–36.
18. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315:801–10.
19. Title of subordinate document. In: Sepsis. Global Sepsis alliance. https://www.global-sepsis-alliance.org/sepsis (Accessed 10 March 2021).
20. Middleton EA, Rowley JW, Campbell RA, Grissom CK, Brown SM, Beesley SJ,et al. Sepsis alters the transcriptional and translational landscape of human and murine platelets. Blood. 2019;134:911–23.
21. Greco E, Lupia E, Bosco O, Vizio B, Montrucchio G. Platelets and Multi-Organ Failure in Sepsis. Int J Mol Sci. 2017;18:2200.
22. Wang Y, Ouyang Y, Liu B, Ma X, Ding R. Platelet activation and antiplatelet therapy in sepsis: A narrative review. Thromb Res. 2018;166:28–36.
23. Parikh F. Infections and Thrombocytopenia. J Assoc Physicians India. 2016;64:11–2.
24. Levi M, van der Poll T. Inflammation and Coagulation. Crit Care Med. 2010;38(2 Suppl):26–34.

Tables

Due to technical limitations, table 1, 2, 3 and 4 are only available as a download in the Supplemental Files section.

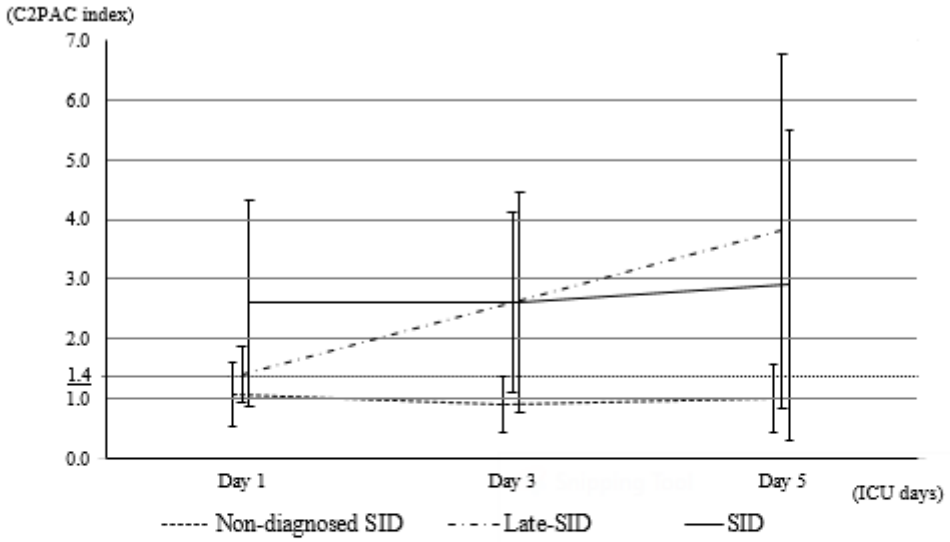
Figures



Healthy Volunteers (n=37)	JAAM DIC scores for sepsis patients (n=70)			
	0–1 (n=14)	2–3 (n=12)	4–5 (n=31)	6–8 (n=13)
0.3±0.1	0.9±0.3	1.1±0.3	1.7±0.7	3.6±1.0

Figure 1

Relationship between the JAAM DIC score and the C2PAC index. The C2PAC index in the healthy volunteers was the lowest among the groups. In the sepsis patients, the C2PAC index increased significantly as the JAAM DIC score increased. *P<0.05, **P<0.01, between the linked groups. JAAM, Japanese Association for Acute Medicine; C2PAC, sCLEC-2/platelet count ratio; sCLEC-2, soluble type C lectin-like receptor 2; DIC, disseminated intravascular coagulation.



	C2PAC index		
	Day 1	Day 3	Day 5
Non-diagnosed SID	1.08 ± 0.55	0.91 ± 0.49	0.97 ± 0.57
Late-SID	1.35 ± 0.49	2.61 ± 1.60	3.75 ± 3.16
SID	2.59 ± 1.74	2.56 ± 1.87	2.92 ± 2.62

Figure 2

Time course of the C2PAC index in sepsis patients. The dotted horizontal line at 1.4 indicates the cut-off value for DIC diagnosis in the C2PAC index. The non-diagnosed SID group, which was not diagnosed with DIC during 5 days of ICU admission, had a C2PAC index below 1.4 throughout the course, while the SID group, which was diagnosed with SID on the first day of ICU admission, had a C2PAC index above 1.4 throughout the course. Meanwhile, the C2PAC index in the late-SID group, which was not diagnosed with SID at the time of ICU admission but was diagnosed with SID after admission, increased over time from the first day of admission. In this study, the cut-off value of the C2PAC index for DIC diagnosis was 1.4. C2PAC index, sCLEC-2/platelet count ratio; sCLEC-2, soluble type C lectin-like receptor 2; SID, sepsis-induced DIC; DIC, disseminated intravascular coagulation; ICU, intensive care unit; sCLEC-2, soluble type C lectin-like receptor 2; SID, sepsis-induced DIC.

Supplementary Files

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