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Optimizing Blood Collection and Storage Conditions for Soluble C-type Lectin-like Receptor 2 Measurement and Evaluating Diurnal Variation

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ABSTRACT

Platelets play a central role in hemostasis and thrombosis, with excessive activation contributing to thrombotic disease. While conventional activation markers such as platelet factor 4 (PF4), β-thromboglobulin (β-TG), and glycoprotein VI (GPVI) are measurable by ELISA, their utility is limited by pre-analytical variability associated with factors such as exercise, smoking, caffeine intake, and sample handling. Soluble C-type lectin-like receptor 2 (sCLEC-2) has recently been identified as a novel biomarker released upon platelet activation, although its stability and physiological variation have not been fully elucidated.

sCLEC-2 was measured using an automated chemiluminescence immunoassay (STACIA®). Blood from healthy volunteers into 3.2% sodium citrate (4.5 mL) and EDTA (2.0 mL) tubes was centrifuged to separate plasma and then stored in tubes at room temperature or 4℃ for up to 24 hours. The influence of residual platelets on sCLEC-2 levels was evaluated. Diurnal variation was assessed in healthy volunteers, and correlations with GPVI were analyzed in 29 patients with hematologic disorders.

sCLEC-2 levels were 1.5-fold higher in EDTA samples than in citrate ($p < 0.05$). Immediately separated plasma remained stable for 24 hours, whereas storage in tubes led to time-dependent increases. Residual platelet counts above $1.0 \times 10^4/\mu L$ significantly elevated sCLEC-2. Morning sCLEC-2 levels were higher, consistent with the di-

urnal pattern of PAI-1. In hematologic disorders likely involving platelet activation, sCLEC-2 strongly correlated with GPVI ($r = 0.809$, $p < 0.001$). These findings demonstrate that sCLEC-2 is a sensitive and stable biomarker of platelet activation and provide practical recommendations for clinical sample handling.

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Key Words

Soluble C-type lectin-like receptor 2 (sCLEC-2), Activated platelets, Plasma assay, Sample stability, Sample storage conditions

INTRODUCTION

Traditionally, platelet factor 4 (PF4) and β-thromboglobulin (β-TG) measured by enzyme-linked immunosorbent assay (ELISA), as well as glycoprotein VI (GPVI), have been widely used to quantify platelet activation [1]. However, PF4 and β-TG levels are highly susceptible to pre-analytical factors such as exercise, smoking, caffeine intake, blood collection procedures, and sample handling, which complicates data interpretation. Unless these pre-analytical conditions are strictly controlled, the assessment of platelet activation becomes unstable and may lead to misinterpretation.

Recently, Kazama et al. developed soluble C-type lectin-like receptor 2 (sCLEC-2) as a novel biomarker of platelet activation [1]. CLEC-2 is a receptor expressed on the platelet membrane, and upon platelet activation, a portion is released into the plasma either as soluble fragments (shed CLEC-2) or as microparticle-associated CLEC-2 (MP-CLEC-2) [2]. Therefore, measurement of sCLEC-2 directly and quantitatively reflects platelet activation. Indeed, sCLEC-2 has been reported to be significantly elevated in thrombotic microangiopathy, disseminated intravascular coagulation [3)-5)], and acute coronary syndrome [2)6)], demonstrating its clinical utility. Moreover, in some cases of diabetes and atherosclerosis, sCLEC-2 has also been shown to be useful for evaluating platelet activation [1]. Thus, sCLEC-2 holds promise as a biomarker for the evaluation and diagnosis of various thrombotic disorders. Furthermore, sCLEC-2 can now be measured using an automated chemiluminescence immunoassay system (STACIA®, PHC Corporation, Tokyo, Japan), providing simple and reliable results comparable to those of routine coagulation assays [7]. According to Kazama et al. [1] and Ueda et al. [8], both sodium citrate and EDTA plasma are suitable for sCLEC-2 measurement, with no significant difference observed between them [1)8)]. However, since residual platelets can affect the measurement, appropriate centrifugation and sampling are essential [7)8)]. Additionally, when plasma is stored under refrigeration or frozen after separation, stable results can be obtained for up to 28 days [8]. Based on these findings, sCLEC-2 is considered feasible marker of platelet activation in routine clinical practice.

However, the studies by Kazama et al. [1] and Inoue et al. [2] primarily focused on fundamental investigations, such as the correlation between sCLEC-2 and GPVI, as well as the release mechanism of CLEC-2 during platelet activation, and thus did not directly address issues of specimen handling in routine clinical testing. Ueda et al. [8] and Kawamura et al. [7] analyzed pre-analytical factors, including anticoagulant type and residual platelets, in detail, thereby contributing to improved measurement reliability. Notably, in routine testing, immediate plasma separation after collection is not always feasible, and the stability of sCLEC-2 when whole blood is stored at room temperature or 4℃ remains unclear. Moreover, to apply sCLEC-2 as a platelet activation marker in clinical practice, it is essential to clarify its physiological variability, particularly whether diurnal variation exists. Therefore, verification of its stability under clinically relevant storage conditions, as well as assessment of physiological variability, is required to establish the clinical utility of sCLEC-2. Accordingly, in this study, we aimed to reproduce and expand upon previous basic findings, evaluate the stability of sCLEC-2 under storage conditions relevant to routine clinical practice, and investigate the diurnal variation of sCLEC-2 in healthy individuals.

MATERIALS AND METHODS

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Kanazawa University (No. 981-3) and the Ethics Committee of Tsukuba International University (No. R05-8).

1. Sample Preparation and sCLEC-2 Measurement

Plasma samples that had been frozen at –80℃ were thawed in a 37℃ water bath for 5 minutes and used for sCLEC-2 measurement. The measurements were performed on the STACIA® (PHC Corporation, Tokyo, Japan) based on the chemiluminescent enzyme immunoassay (CLEIA). The assay kit employed anti-CLEC-2

monoclonal antibodies (11D5 and 11E6). The measurement procedure was as follows: plasma samples were incubated with magnetic particles coated with solid-phase anti-CLEC-2 monoclonal antibody (11D5). After washing, alkaline phosphatase–conjugated anti-CLEC-2 monoclonal antibody (11E6) was added and incubated. Following a second wash, the chemiluminescent substrate CDP-Star (Applied BioSystems, Waltham, MA) was added to induce luminescence, and the signal intensity was measured.

2. Evaluation of the Association Between Anticoagulants, Sample Storage Conditions, Residual Platelet Count, and sCLEC-2 Levels

2.1 Analysis of sCLEC-2 Levels by Anticoagulant Type

This study used samples from four healthy volunteers who provided informed consent: two males (32 ± 4 years) and two females (38 ± 11 years). Blood was drawn using a 21-gauge winged needle (Terumo Corporation, Tokyo, Japan). The blood collection tubes used were 3.2% sodium citrate tubes (4.5 mL), commonly used for coagulation tests (Becton, Dickinson and Company, Franklin Lakes, NJ), and EDTA tubes (2.0 mL), used for blood count tests (Becton, Dickinson and Company). Each tube was gently inverted 10 times after collection, followed by centrifugation at 2,000 × g for 15 minutes to separate the plasma, which was immediately frozen and stored at –80℃. Notably, although the recommended centrifugation conditions for coagulation testing samples are typically 1,500 × g for 15 minutes or 2,000 × g for 10 minutes, centrifugation at 2,000 × g for 15 minutes was to minimize the influence of residual platelets.

2.2 Analysis of the Effect of Sample Storage Conditions on sCLEC-2 Levels

The samples and blood collection methods used in this experiment were the same as those used in the study of anticoagulant types involving four healthy volunteers. To reflect routine clinical practice, the following storage conditions were established: (1) blood samples were centrifuged immediately after collection and then stored at room temperature or 4℃ in the original tubes; (2) blood samples were centrifuged immediately after collection, and the separated plasma was transferred to secondary tubes and stored at room temperature or 4℃. To evaluate time-dependent changes under each condition, plasma samples were taken at 0 hours (immediately after centrifugation), as well as at 1, 2, 4, 8, and 24 hours, from both the samples that were centrifuged immediately after blood collection and stored in the original tubes, and the separated tubes that had been stored. All samples were stored at –80℃ until measurement.

2.3 Relationship Between Residual Platelet Count in Plasma After Centrifugation and sCLEC-2 Levels

The samples and blood collection methods used in the experiment were the same as those used in the study of anticoagulant types involving four healthy volunteers. After blood collection, platelet-rich plasma (PRP) obtained by centrifugation at 200 × g for 10 minutes and platelet-poor plasma (PPP) obtained by centrifugation at 2,000 × g for 15 minutes were mixed, and the platelet count in the measurement samples was adjusted using an automated hematology analyzer, XN-300 (Sysmex, Kobe, Japan). The residual platelet counts in the measurement samples were adjusted to three levels: less than 1.0 × 10^4/μL, 1.0–2.9 × 10^4/μL, and 3.0–5.0 × 10^4/μL, and sCLEC-2 levels were measured for each. These three platelet count ranges were defined as group I (<1.0 × 10^4/μL), group II (1.0–2.9 × 10^4/μL), and group III (3.0–5.0 × 10^4/μL), respectively.

3. Evaluation of Diurnal Variation in Platelet Activation Using sCLEC-2

Blood samples were collected from seven healthy volunteers (three males, 32 ± 13 years; four females, 26 ± 6 years) using 3.2% sodium citrate tubes (4.5 mL). Blood collection was performed at 6:00, 12:00, and 18:00, and all collections were conducted after the participants had fasted for at least two hours prior to each time point. After centrifugation at 2,000 × g for 15 minutes, plasma was separated and frozen at –80 ℃ until sCLEC-2 measurement. Notably, plasminogen activator inhibitor-1 (PAI-1) has been reported to exhibit higher levels in the morning and lower levels in the afternoon. In this study, PAI-1 was measured as a positive control for the evaluating the diurnal variation in sCLEC-2[9)10)]. PAI-1 levels were measured using the LPIA tPAI test (PHC Corporation) on the STACIA® system.

4. Measurement of GPVI in Plasma from Patients with Hematologic Disorders and Its Correlation with sCLEC-2

To evaluate the correlation between GPVI and sCLEC-2, we analyzed samples from patients in whom high platelet activation was expected. The study included 29 patients (74 ± 12 years; 12 males, 17 females) from the Department of Hematology at Kanazawa University. The disease distribution of hematologic disorders was as follows: acute leukemia (2 cases), malignant lymphoma (11 cases), myelodysplastic syndrome (3 cases), multiple myeloma (4 cases), immune thrombocytopenia (3 cases), myeloproliferative neoplasm (1 case), and others (5 cases). Blood samples were collected using 3.2% sodium citrate tubes (4.5 mL). After centrifugation at 2,000

× g for 15 minutes, plasma was separated following routine coagulation testing and immediately frozen at –80℃ until measurement of sCLEC-2 and GPVI. All blood samples were collected in the morning. GPVI levels were measured using the Human GPVI ELISA Kit (Thermo Fisher Scientific Inc., Waltham, MA), according to the manufacturer's instructions.

5. Statistical Analysis

Graphs were generated using GraphPad Prism 10 (GraphPad Software, San Diego, CA), and statistical analyses were performed using StatFlex V7 (ver. 7, Medical Watch Institute, Ube, Japan). The Mann-Whitney test was used to compare sCLEC-2 levels by anticoagulant type. For comparisons based on sample storage conditions after blood collection, Dunn's test (multiple comparison vs. control group) was applied. sCLEC-2 and PAI-1 levels were expressed as median (minimum - maximum), whereas Age was expressed as mean ± SD. The association between GPVI and sCLEC-2 was evaluated using simple linear regression analysis. P-values < 0.05 were considered statistically significant.

RESULTS

1. Evaluation of Anticoagulants, Sample Storage Conditions, Residual Platelet Count, and Their Impact on sCLEC-2 Levels

1.1 Comparison of sCLEC-2 Levels by Anticoagulant Type

The sCLEC-2 levels by anticoagulant type were as follows: 3.2% sodium citrate (4.5 mL), 63.9 pg/mL (30.7–83.1); EDTA (2.0 mL), 94.2 pg/mL (66.3–118.2). The Mann-Whitney test revealed that sCLEC-2 levels in EDTA tubes were significantly higher than those in 3.2% sodium citrate tubes (**Fig. 1**, $p < 0.05$).

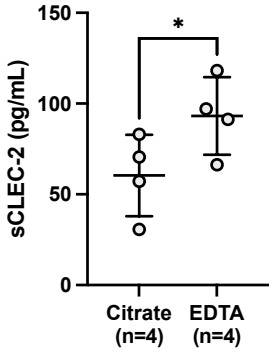

Figure 1 Comparison of sCLEC-2 levels in citrate or EDTA blood collection tubes
Comparison of sCLEC-2 levels based on the type of anticoagulant used. The anticoagulants compared were 3.2% sodium citrate (4.5 mL) and EDTA (2.0 mL). * $p < 0.05$.

1.2 Effect of Post-Centrifugation Storage in Original Blood Collection Tubes on sCLEC-2 Levels

sCLEC-2 levels were measured over time in two types of blood collection tubes, 3.2% sodium citrate (4.5 mL) and EDTA (2.0 mL), with samples stored at room temperature or 4 ℃ after centrifugation without plasma separation. In samples stored at room temperature in 3.2% sodium citrate tubes, a sharp increase in sCLEC-2 was observed only in Sample C after 8 hours; however, no statistically significant differences were observed up to 24 hours (**Fig. 2A**). In samples stored at 4 ℃ in 3.2% sodium citrate tubes, a significant increase in sCLEC-2 levels was observed at 24 hours (**Fig. 2B**, $p < 0.01$). In samples stored at room temperature in EDTA tubes, sCLEC-2 levels were significantly elevated at 24 hours (**Fig. 2C**, $p < 0.01$). In samples stored at 4℃ in EDTA tubes, sCLEC-2 levels showed a significant increase beginning at 8 hours (**Fig. 2D**; at 8 hours, $p < 0.05$; at 24 hours, $p < 0.01$).

1.3 Effect of Storage After Plasma Separation on sCLEC-2 Levels

Plasma was separated immediately after centrifugation and stored at room temperature or 4 ℃ , and sCLEC-2 levels were measured over time. In plasma samples from 3.2% sodium citrate tubes (4.5 mL), no statistically significant differences in sCLEC-2 levels were observed at either room temperature or 4℃ up to 24 hours (**Fig. 2E**, **F**). Similarly, in plasma samples collected using EDTA tubes (2.0 mL), no statistically significant differences were observed under either storage condition up to 24 hours (**Fig. 2G**, **H**).

1.4 Relationship Between Residual Platelet Count in Plasma and sCLEC-2 Levels

Blood samples from four volunteers (Sample A–D) were centrifuged, and the residual platelet count in plasma and sCLEC-2 levels were measured. The definitions of platelet count groups were as described in the Methods section. In sodium citrate tubes (4.5 mL), the sCLEC-2 levels were as follows: group I, 101.4 pg/mL (51.3–164.5); group II, 507.4 pg/mL (345.6–542.4); group III, 513.7 pg/mL (361.4–584.7). A significant increase in sCLEC-2 levels was observed in group III compared to group I (**Fig. 3A**, $p < 0.05$). Similarly, in EDTA tubes (2.0 mL), sCLEC-2 levels were as follows: group I, 93.7 pg/mL (58.9–100.1); group II, 161.3 pg/mL (145.7–209.6); group III, 1364.8 pg/mL (1110.0–1952.4). A significant increase in sCLEC-2 levels was also observed in group III compared to group I (**Fig. 3B**, $p < 0.05$).

2. Diurnal Variation in sCLEC-2 Levels

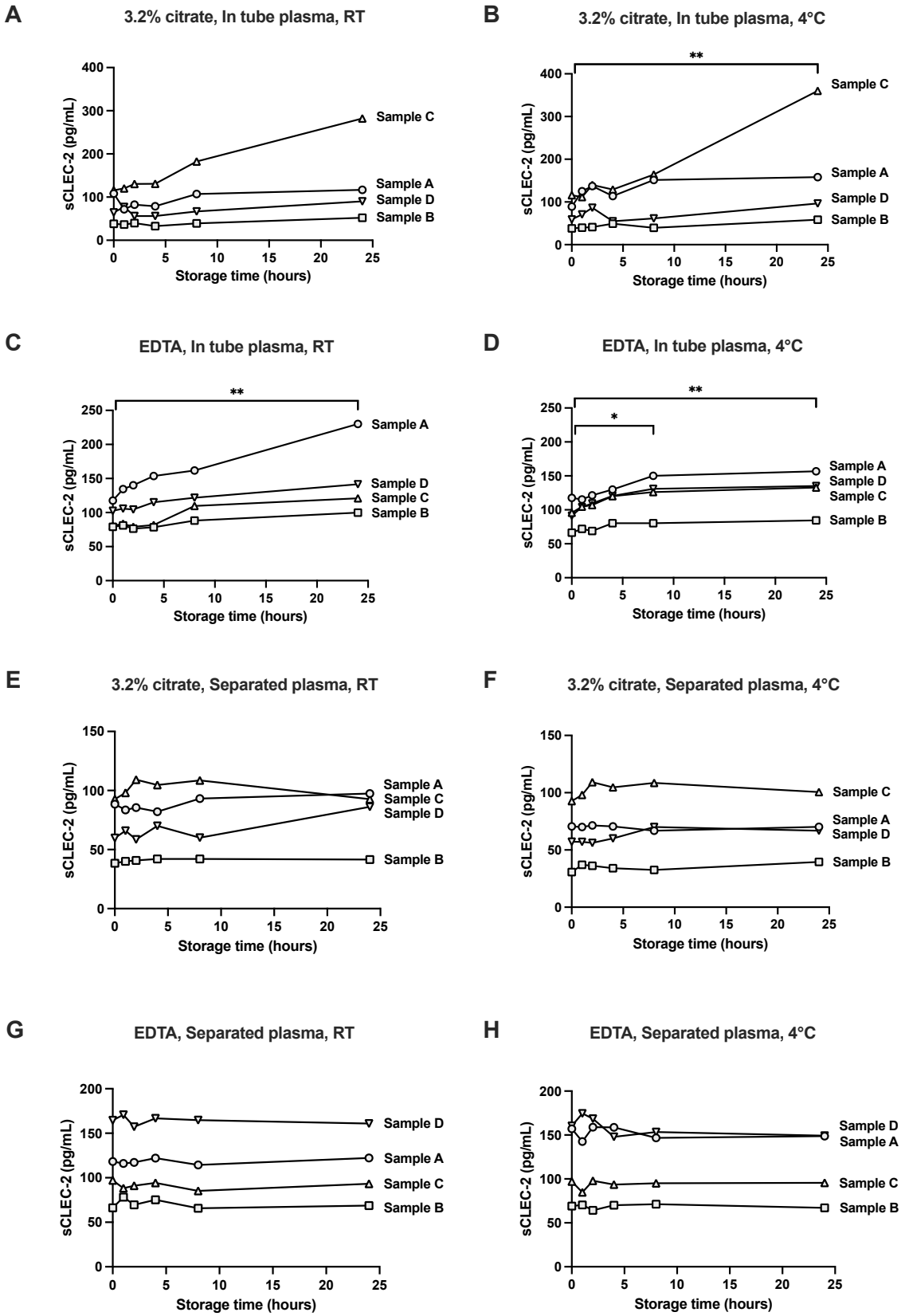

Figure 2 Effects of post-centrifugation storage in blood collection tubes and separated plasma, as well as storage temperature, on time-dependent changes in sCLEC-2 levels

Changes in sCLEC-2 levels in sodium citrate (A, B) and EDTA (C, D) blood collection tubes after centrifugation, with samples stored at room temperature (A, C) or 4℃ (B, D). Changes in sCLEC-2 levels in sodium citrate (E, F) and EDTA (G, H) plasma after centrifugation and plasma separation, with samples stored at room temperature (E, G) or 4℃ (F, H). * $p < 0.05$, ** $p < 0.01$.

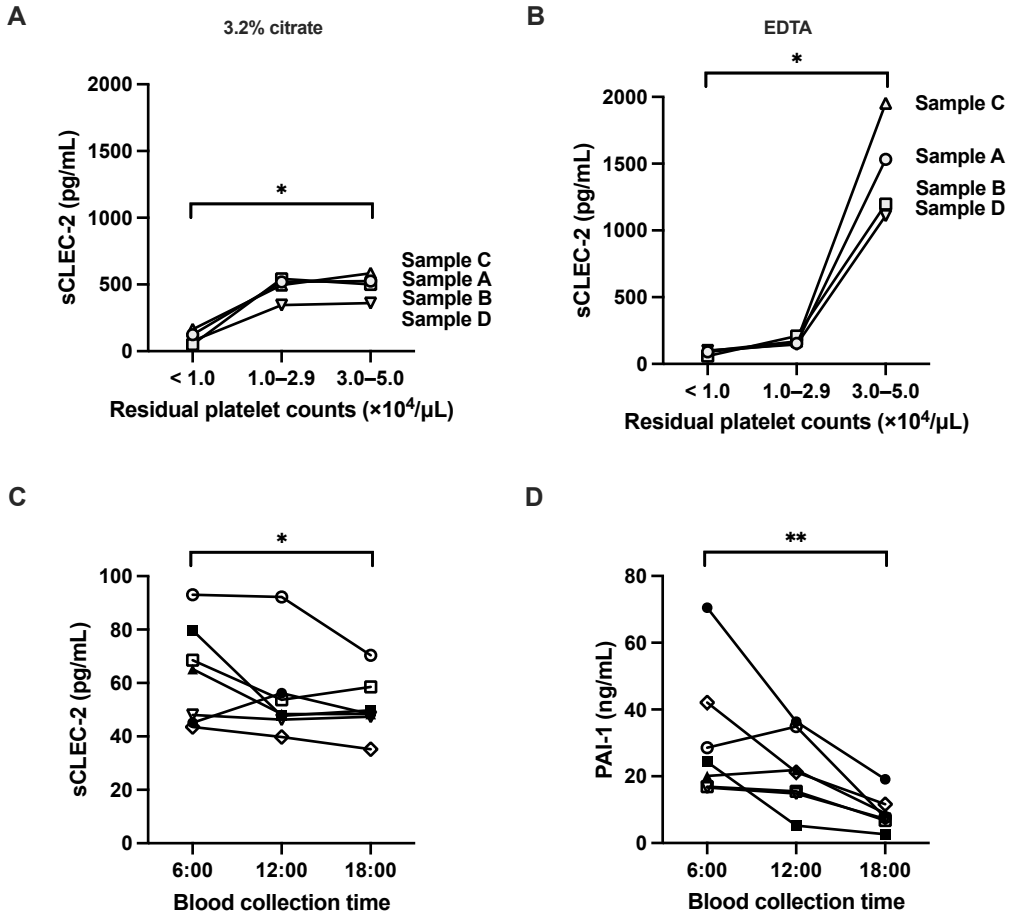

Figure 3 The relationship between residual platelet counts and sCLEC-2 levels, and the diurnal variation of sCLEC-2 and PAI-1

Residual platelet counts and sCLEC-2 levels are displayed according to anticoagulant type. (A) Sodium citrate tube and (B) EDTA tube. Diurnal variation of sCLEC-2 (C) and PAI-1 (D) levels in sodium citrate plasma is also presented. * $p < 0.05$, ** $p < 0.01$.

The diurnal variation of platelet activation was evaluated using sCLEC-2 levels in sodium citrate plasma. The sCLEC-2 levels were as follows: 65.2 pg/mL (43.5–93.0) at 6:00, 48.4 pg/mL (39.8–92.2) at 12:00 ($p = 0.1537$), and 48.4 pg/mL (35.2–70.4) at 18:00 ($p = 0.0414$), showing a statistically significant decrease at 18:00 compared with 6:00 (**Fig. 3C**). PAI-1 also demonstrated the expected diurnal variation, with levels of 24.3 ng/mL (16.6–70.5) at 6:00, 21.2 ng/mL (5.2–36.4) at 12:00 ($p = 0.8450$), and 7.6 ng/mL (2.7–19.1) at 18:00 ($p = 0.0027$), indicating a significant decrease at 18:00 compared with 6:00 (Fig. 3D).

3. Correlation Between GPVI and sCLEC-2 in Plasma of Patients with Hematologic Disorders

Sodium citrate plasma samples from 29 patients with hematologic disorders, in whom platelet activation was expected, were used to measure GPVI, a well-established marker of platelet activation , and sCLEC-2 levels. Simple linear regression analysis revealed a significant positive correlation between GPVI and sCLEC-2 (**Fig. 4**, $r = 0.809$, $p < 0.001$).

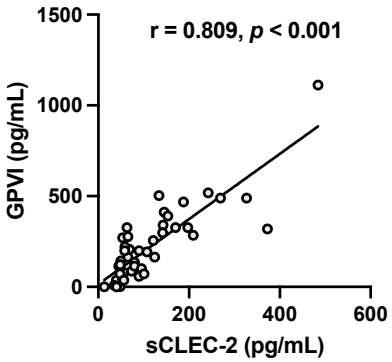

Figure 4 Correlation analysis of GPVI and sCLEC-2 levels

Correlation between GPVI and sCLEC-2 levels in sodium citrate plasma from patients with hematologic disorders.

DISCUSSION

In this study, we comprehensively evaluated the clinical utility of sCLEC-2 as a biomarker of platelet activation by investigating its analytical stability and reliability under pre-analytical conditions that reflect routine clinical practice. Specifically, we examined the effects of anticoagulant type, sample storage conditions, residual platelet count, and diurnal variation on sCLEC-2 measurement. In addition, we assessed the correlation between sCLEC-2 and GPVI, a conventional marker of platelet activation, in patients with hematologic disorders. These findings provide valuable insights into the applicability and limitations of sCLEC-2 in real-world clinical settings.

First, we examined the influence of anticoagulants on sCLEC-2 measurement under pre-analytical conditions. Our results demonstrated that sCLEC-2 levels in EDTA tubes (2.0 mL) were approximately 1.5 times higher than those in 3.2% sodium citrate tubes (4.5 mL). Ueda et al. [8] compared EDTA (2.0 mL), 3.2% sodium citrate (2.0 mL), and 3.2% sodium citrate (5.0 mL) tubes, reporting no difference between EDTA (2.0 mL) and 3.2% sodium citrate (2.0 mL), whereas a significant decrease was observed in 3.2% sodium citrate (5.0 mL) tubes compared with both. Although our study was limited to only four samples, which is insufficient to ensure statistical reliability, it is likely that the use of 4.5 mL sodium citrate tubes and the absence of dilution correction for citrate contributed to the lower sCLEC-2 levels observed compared with EDTA tubes. Furthermore, although plasma was carefully collected after centrifugation, strict control of the sampling position, such as maintaining a constant distance from the buffy coat, was not implemented. Therefore, the potential influence of tube volume and sampling depth on sCLEC-2 levels cannot be completely ruled out. Ueda et al. also investigated several pre-analytical factors, including centrifugal force and negative pressure differences among collection tubes [8]; however, the precise mechanisms remain unclear, and further investigation is warranted. For the clinical application of sCLEC-2, both EDTA and sodium citrate tubes appear to be feasible. Nevertheless, when platelet activation is assessed as part of hemostasis and thrombosis testing, or when consistency in pre-analytical processing is prioritized, the use of 2.0 mL 3.2% sodium citrate tubes may be preferable. However, further validation is needed to ensure its practical implementation in clinical laboratories.

It has been reported that arterial thrombosis, such as cerebral infarction and myocardial infarction, occurs more frequently in the early to late morning hours [11–15]. In this study, we evaluated the diurnal variation of sCLEC-2 in seven healthy volunteers and found that, in six out of seven participants, sCLEC-2 levels were higher in the morning and decreased toward the evening. This pattern was consistent with the known diurnal variation of PAI-1, and the sCLEC-2 level at 18:00 was significantly lower than that at 6:00 ($p < 0.01$). Although previous studies have reported that platelet activation tends to be higher in the morning [9,10], our results show a similar trend for sCLEC-2, further supporting the utility as a marker of platelet activation. These findings highlighted the importance of considering the timing of blood collection when clinically evaluating sCLEC-2 levels.

Blood samples used in clinical testing are typically stored at room temperature or refrigerated for a certain period to accommodate requests for retesting or additional analyses. Upon arrival at the laboratory, blood collected in EDTA tubes for complete blood count (CBC) testing is analyzed directly as whole blood. In contrast, blood collected in 3.2% sodium citrate tubes for coagulation testing is immediately centrifuged and used for coagulation assays, including prothrombin time (PT) and activated partial thromboplastin time (APTT). Ueda et al. investigated the stability of sCLEC-2 in plasma samples after centrifugation and separation, reporting that sCLEC-2 levels remained stable for up to 28 days [8]. In this study, in addition to evaluating plasma samples separated after centrifugation as described by Ueda et al., we also investigated the stability of sCLEC-2 levels in samples that were stored in the original blood collection tubes without plasma separation following centrifugation. Specifically, we evaluated the impact of storage time and temperature after centrifugation on sCLEC-2 levels, simulating a situation in which sCLEC-2 measurement is additionally requested as a marker of platelet activation following CBC (EDTA tube) or coagulation testing (3.2% sodium citrate tube). For both anticoagulants, stable results were observed when samples were stored at room temperature for up to 4 hours. However, under refrigerated conditions at 4℃, considerable individual variability was noted; two participants (Sample A and C) exhibited an increasing trend in sCLEC-2 levels after 2 hours of storage in the blood collection tube (**Fig. 2B**). It has been reported that refrigerated storage of samples can affect coagulation assays such as PT and APTT with prolonged storage [16]. This alteration is thought to result not only from decreased activity of coagulation factors but also from residual platelets present in the buffy coat. Although the precise mechanism remains unclear, studies have

shown that when platelets are stored at 4 ℃ , inhibitory signals that normally suppress platelet activation become attenuated, thereby inducing platelet activation [17]. Therefore, when samples are stored in blood collection tubes, progressive platelet activation may occur, potentially resulting in a time-dependent increase in sCLEC-2 levels. Therefore, in clinical applications, sCLEC-2 values measured from plasma stored in blood collection tubes under refrigerated conditions following routine testing should be interpreted with caution, as inter-individual variability may lead to artificially elevated results. On the other hand, in samples where plasma was separated after centrifugation, stable sCLELC2 levels were obtained for up to 24 hours, regardless of the anticoagulant used or whether the samples were stored at room temperature or under refrigerated conditions, consistent with the findings of Ueda et al. [8]. Depending on the operational protocols of the laboratory, promptly separating plasma following coagulation testing and storing it under refrigerated or frozen conditions may allow for accurate measurement of sCLEC-2 levels when additional testing is requested.

Kawamura et al. also investigated residual platelets and demonstrated that when the number of residual platelets in plasma is high, sCLEC-2 levels exhibit a positive bias [7]. In the present study, PRP was added to PPP to adjust the residual platelet count, and the effect on sCLEC-2 levels was evaluated. As shown in Fig. 3A and B, in both EDTA-anticoagulated and 3.2% sodium citrate-anticoagulated blood, sCLEC-2 levels increased with rising residual platelet counts. According to the literature, approximately 2,000 to 4,000 copies of CLEC-2 are present on the surface of platelet membranes [18], and minimizing the number of residual platelets in plasma is essential for accurate measurement of sCLEC-2. As demonstrated in both this study and the report by Kawamura et al. [7], samples with residual platelet counts ≥ $1.0 \times 10^4/\mu L$ showed higher sCLEC-2 levels compared to those with counts below this threshold. In coagulation testing, including PT and APTT, it is also recommended that the residual platelet count be less than $1.0 \times 10^4/\mu L$; therefore, applying the same threshold in sCLEC-2 measurement contributes to obtaining stable results. In this study, centrifugation at 2,000 × g for 15 minutes at room temperature was performed to minimize residual platelets in plasma. According to the consensus guidelines published by the Japanese Society for Laboratory Hematology (JSLH) regarding the handling of coagulation testing samples, centrifugation at 1,500 × g for 15 minutes or 2,000 × g for 10 minutes is recommended. Therefore, it is important for each facility to establish appropriate centrifugation protocols that consider residual platelet counts to ensure the accuracy of sCLEC-2 measurement.

We observed a strong positive correlation between sCLEC-2 and GPVI, a well-established marker of platelet activation ($r = 0.809$, $p < 0.001$). Although correlation analysis was not performed in healthy individuals due to the anticipated clustering of their values within the lower range, the present findings in patients with disease are consistent with those reported by Yamashita et al. [5], further supporting the utility of sCLEC-2 as a marker reflecting platelet activation.

CONCLUSION

sCLEC-2 enables the measurement of platelet activation levels using plasma samples without the need for special blood collection tubes, in contrast to well-established markers of platelet activation. In addition, stable measurement results were obtained using 3.2% sodium citrate blood, which is commonly used in routine coagulation testing, when stored at room temperature for at least 4 hours. Furthermore, sCLEC-2 levels exhibited diurnal variation, being higher in the morning and lower in the evening, indicating the timing of blood collection should be considered when interpreting results. This study confirmed that sCLEC-2 is a reliable marker of platelet activation and can be stably measured in routine laboratory conditions. Currently, sCLEC-2 can be measured using automated chemiluminescent immunoassay systems, with stable results achieved. However, standardization, particularly with regard to pre-analytical processing and the influence of residual platelets, remains necessary. In the future, sCLEC-2 is expected to be increasingly utilized as a clinically valuable marker of platelet activation, particularly in conditions associated with enhanced platelet activity.

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Conflict of Interest

M.K. and S.M. are employees of PHC Corporation. E.M. has received research funding from PHC Corporation. The other authors declare no conflicts of interest.

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