



# Characterization and Usefulness of Clot-Fibrinolysis Waveform Analysis in Critical Care Patients with Enhanced or Suppressed Fibrinolysis

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Thromb Haemost 2024;124:40–48.

## Abstract

### Keywords

- ▶ clot-fibrinolysis waveform analysis
- ▶ coagulation and fibrinolysis
- ▶ fibrin
- ▶ tissue-type plasminogen activator
- ▶ fibrinolysis

**Introduction** Recently, clot-fibrinolysis waveform analysis (CFWA), which is a coagulation and fibrinolysis global assay based on assessing the activated partial thromboplastin time with tissue-type plasminogen activator, was developed. This study aimed to investigate the characteristics of CFWA using plasma samples from patients in the critical care unit.

**Materials and Methods** The fibrinolysis times using CFWA were measured in 298 plasma samples. These samples were divided into three groups based on the reference interval (RI) of fibrinolysis time using CFWA: shortened group, less than RI; within group, within RI; prolonged group, more than RI. The coagulation and fibrinolysis markers, including D-dimer, plasmin- $\alpha_2$  plasmin inhibitor complex (PIC), fibrin monomer complex (FMC), plasmin- $\alpha_2$  plasmin inhibitor ( $\alpha_2$ -PI), plasminogen (Plg), and fibrinogen (Fbg) were analyzed and compared among the three groups.

**Results** The FMC level decreased in the order of shortened, within, and prolonged groups, and the decrease was statistically significant among all three group pairs. The opposite tendency was observed for Fbg and fibrinolysis-related markers of  $\alpha_2$ -PI and Plg, and significant differences were recognized in all pair comparisons except for between within and prolonged groups in Plg. The mean values of the fibrinolysis markers D-dimer and PIC in all three groups were higher than the cut-off values, and the PIC value differed significantly between the within and prolonged groups.

**Conclusion** The fibrinolysis reaction was detected in all three groups, but the status differed. CFWA has the potential to reflect the fibrinolysis status in one global assay.

received

March 5, 2023

accepted after revision

July 10, 2023

accepted manuscript online

August 1, 2023

article published online

September 5, 2023

DOI <https://doi.org/10.1055/a-2145-7139>  
ISSN 0340-6245.

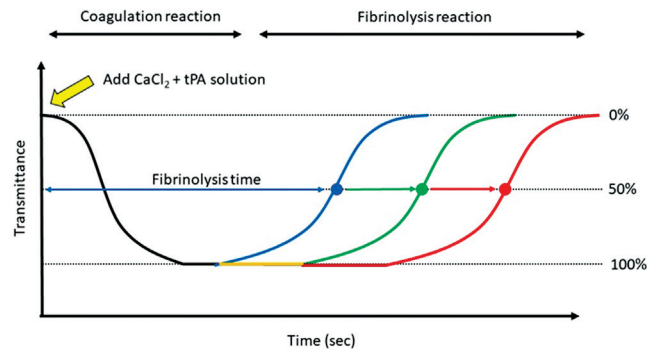
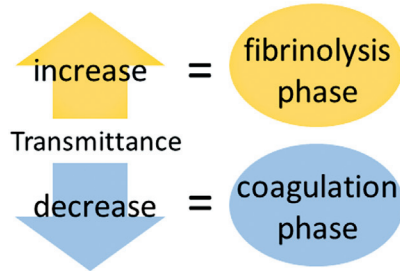
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### Clot-fibrinolysis waveform analysis (CFWA) for critical care patients

- CFWA is a global assay to monitor coagulofibrinolytic reactions using the transmittance changes.
- CFWA could potentially represent fibrinolytic status in one assay.



Fibrinolysis time	Shortened	Within normal range	Prolonged
$\alpha_2$ plasmin inhibitor	Low	Medium	High
Fibrin monomer complex	High	Medium	Low
Plasminogen	Low	High	High
Fibrinogen	Low	Medium	High

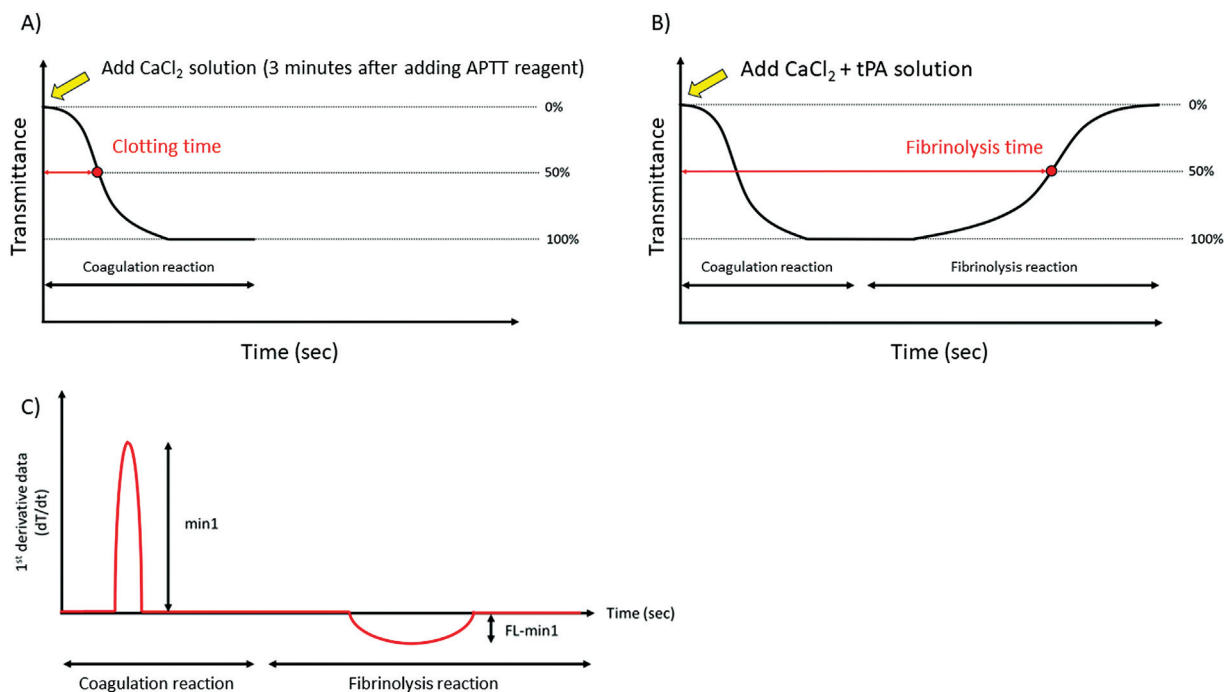
**Visual summary.** Characteristics of characterization of clot-fibrinolysis waveform analysis (CFWA)

## Introduction

A well-controlled balance between procoagulant, anticoagulant, and fibrinolytic mechanisms is critical for maintaining normal hemostasis in the circulation. If this well-controlled balance is lost, it may clinically manifest as hemorrhage or thrombosis. In patients with severe trauma, hemorrhage is usually associated with hyperfibrinolysis in the early trauma period, while thrombus formation is associated with the activation of hypercoagulability in the subacute period.<sup>1,2</sup> Moreover, severe coagulopathy immediately after trauma is a predictor of a poor prognosis.<sup>3-5</sup> In sepsis, a hypercoagulable state occurs, which is characterized by microvascular thrombi, fibrin deposition, neutrophil extracellular trap formation, and endothelial damage.<sup>6</sup> Furthermore, subsequent consumptive coagulopathy can lead to uncontrolled bleeding.<sup>7</sup> In patients with severe sepsis, repeated screening for disseminated intravascular coagulation (DIC) has, by itself, been associated with an improved prognosis.<sup>8</sup> Similarly, in patients with post-cardiac arrest syndrome, coagulation is persistently activated in the presence of the underlying disease, resulting in diffuse microthrombus formation in small blood vessels.<sup>9</sup> Coagulopathy has also been associated with a poor prognosis in post-cardiac arrest patients.<sup>10-12</sup> Thus, the variability in coagulation-fibrinolysis mechanisms is particularly large in critically ill patients, and its relationship with prognosis has been a focus of attention. It is important to understand the balance between coagulation and fibrinolysis for planning an appropriate treatment

strategy in the emergency and critical care fields, as the coagulation and fibrinolysis status can change remarkably in critically ill patients.

Various assay systems such as activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time, thromboelastography, and the thrombin generation test have been used to evaluate the blood coagulation system. Among them, the APTT-clot waveform analysis (CWA) has been developed as an automated global coagulation assay that the light transmittance changes are monitored, and the transmittance and the derivative curves are described with some parameters in the analyzer.<sup>13</sup> Several reports show the usefulness of CWA for DIC diagnosis, bleeding risk prediction, hemostatic monitoring under anticoagulant therapy, and so on.<sup>13-16</sup> Recently, it was also reported that CWA was one of the parameters for predicting coronavirus disease 2019 (COVID-19)-associated coagulopathy.<sup>17</sup> Furthermore, the improved assay called clot-fibrinolysis waveform analysis (CFWA), a global functional assay system, has been developed to assess coagulation and fibrinolysis simultaneously lately and detect the fibrinolysis time<sup>18,19</sup> (► Fig. 1). During routine APTT measurements, a sample is mixed with the APTT reagent, including an activator and phospholipids, and  $\text{CaCl}_2$  solution is added to the mixture. Once the coagulation reaction is triggered by the  $\text{CaCl}_2$  solution, the transmittance decreases until the clot is formed. The transmittance change is monitored during the reaction, and the clotting time is measured as the time taken for the reaction until 50% transmittance reduction is reached (► Fig. 1A). For CFWA measurements,



**Fig. 1** The principle of clot-fibrinolysis waveform analysis and fibrinolysis time. The horizontal and vertical axes show the time (seconds) and transmittance, respectively. (A) Activated partial thromboplastin time (APTT) measurement: monitoring of transmittance starts with the addition of the  $\text{CaCl}_2$  solution 3 minutes after adding the APTT reagent, and the transmittance decreases because of clot formation. (B) Clot-fibrinolysis waveform analysis: monitoring of transmittance is started with the addition of the  $\text{CaCl}_2$  solution, including recombinant tissue-type plasminogen activator (r-tPA), 3 minutes after adding the APTT reagent, similarly to APTT measurements. The monitoring of transmittance is continued after clot formation. After clot formation, plasmin generated by biogenic and spiked tPA dissolves the clot, and the transmittance increases over the fibrinolysis reaction phase. The maximum and minimum values of transmittance are defined as 0 and 100%, respectively, and the fibrinolysis time is defined as the time taken to reach 50% transmittance. (C) Clot-fibrinolysis waveforms and the parameters in the first derivative data. The curves of the first derivative in the clot-fibrinolysis waveform are described. The first peak is observed in the coagulation phase, and the maximum value in the coagulation phase is defined as min1. The second peak at the negative value in the fibrinolysis phase is also observed. The absolute value is used as FL-min1. These parameters mean the velocity of coagulation and fibrinolysis reactions, respectively.

a tissue-type plasminogen activator (tPA) is included in the  $\text{CaCl}_2$  solution. The APTT reagent and  $\text{CaCl}_2$  solution are added to the sample and mixed, leading to the coagulation reaction and transmittance change, followed by clot formation, similarly to APTT measurements. However, the addition of tPA to the  $\text{CaCl}_2$  solution triggers the fibrinolysis reaction after clot formation, and the transmittance increases. During this fibrinolysis reaction, the fibrinolysis time is defined as the time taken to reach 50% transmittance. This assay is conducted using an automated coagulation analyzer with regular APTT measurements. The benefit of this assay has been reported in several articles. Oka et al showed that direct oral anticoagulants (DOACs) affect both the fibrinolysis and coagulation reactions and suggested that CFWA could be useful for the assessment of the efficacy of DOACs.<sup>18</sup> With regard to bleeding disorders, Nogami et al reported that the fibrinolysis reaction starts before clot formation in factor VIII-deficient plasma and is suppressed by tranexamic acid.<sup>19</sup> Consequently, CFWA has the potential to evaluate the functional fibrinolysis status of patients.

The basic characteristics of CFWA have been investigated during the evaluation of coagulation factor deficiency or drug-spiked samples.<sup>19–24</sup> Although several fibrinolysis bio-

markers, such as D-dimer, plasminogen, and  $\alpha_2$ -plasmin inhibitor ( $\alpha_2$ -PI), are employed in the clinical setting, few studies have shown the relationship between the fibrinolysis time detected using CFWA and fibrinolysis markers. Previous reports, especially in the emergency and critical care settings, are limited and have only included patients with COVID-19 and sepsis.<sup>25,26</sup> It is important to investigate the characteristics of CFWA by comparing the parameters with the current markers used in clinical laboratories and to understand the patient's coagulation and fibrinolysis status from the data collected. This study aimed to investigate the characteristics of CFWA using blood samples collected from critically ill patients who had disorders of both the coagulation and fibrinolytic systems due to various underlying severe acute illnesses. This was achieved by comparing the CFWA parameters with several coagulation and fibrinolysis markers.

## Materials and Methods

### Plasma Samples

Patients who were transported to a tertiary emergency facility and admitted to the intensive care unit from

September to December 2014 were included. Blood samples were collected at the time of admission to the hospital and during the hospitalization without any restriction. Whole blood samples were collected in plastic tubes containing 3.2% sodium citrate in a 9:1 ratio, and platelet-poor plasmas were obtained after the centrifugation. In total, 298 clinical samples were prepared and stored at  $-80^{\circ}\text{C}$ . As a control group, 50 healthy donor samples from CRYOcheck Normal Donor Set (Precision BioLogic Inc., Dartmouth, Canada) were used to establish the normal reference interval. All plasma samples were stored at  $-80^{\circ}\text{C}$  and thawed at  $37^{\circ}\text{C}$  immediately before the assays.

Written informed consent was obtained from all patients who provided the plasma samples. The study protocol was approved by our Institutional Review Board (approval number: 019-0354).

### CFWA Method

The detailed method of CFWA has been described elsewhere.<sup>19</sup> Briefly, alteplase (Kyowa Kirin, Tokyo, Japan), which is recombinant tPA (r-tPA), was diluted with distilled water and then added to Thrombocheck  $\text{CaCl}_2$  solution (Sysmex Corporation, Kobe, Japan) to achieve a final concentration of  $4.1\text{ }\mu\text{g/mL}$  in the Thrombocheck  $\text{CaCl}_2$  solution. The  $\text{CaCl}_2$  solution with r-tPA was prepared every 2 hours according to the previous study.<sup>16</sup> Each plasma sample ( $50\text{ }\mu\text{L}$ ) was mixed with  $50\text{ }\mu\text{L}$  of Thrombocheck APTT-SLA (Sysmex Corporation), including the activator and phospholipids, and incubated for 3 minutes at  $37^{\circ}\text{C}$ . Then, the  $\text{CaCl}_2$  solution with r-tPA was added to the sample, and the transmittance change was monitored at  $660\text{ nm}$  wavelength. All measurements were conducted on an automated coagulation analyzer CS-5100 (Sysmex Corporation). For determining the fibrinolysis time, the maximum and minimum values of transmittance were defined as 0 and 100% in the clot-fibrinolysis waveform, respectively; the time taken to reach 50% of the transmittance at the middle point between 0 and 100% on the curve was defined as the fibrinolysis time in accordance with previous studies<sup>6,7</sup> (►Fig. 1). Besides the fibrinolysis time, two kinds of parameters were calculated from the derivative curve in the transmittance results according to the previous study.<sup>16</sup> The first derivative curve of the transmittance data was described, and the absolute maximum values in the coagulation and fibrinolysis phases were calculated (►Fig. 1C). These absolute maximum values, defined as min1 and fibrinolysis min1 (FL-min1), indicate the maximum velocity in the coagulation and fibrinolysis reactions, respectively. The ratios of min1/FL-min1 were also calculated to express the balance between coagulation and fibrinolysis reactions.

### Laboratory Measurements

Various examinations were performed on all 298 samples with the following reagents: Revohem PT (PT), Thrombocheck Fib(L) (fibrinogen, Fbg), Auto LIAFM (fibrin monomer complex, FMC), Revohem Plasminogen (plasminogen, Plg), Lias Auto D-Dimer Neo (D-dimer), Lias Auto PIC (plasmin- $\alpha_2$  plasmin inhibitor complex, PIC), and Revohem  $\alpha_2$ -antiplasmin  $\alpha_2$ -PI),

**Table 1** Components of coagulation and fibrinolytic system measured in the present study

Components	Description
D-dimer	It is the degradation of stabilized fibrin by plasmin, indicating an enhanced secondary fibrinolytic reaction
Fibrinogen (Fbg)	It is a precursor to fibrin, which forms fibrin under the action of thrombin
Fibrin monomer complex (FMC)	It is an indicator that trace amounts of thrombin have been produced, an indicator of hypercoagulability
Plasminogen (Plg)	It is a zymogen of plasmin, which is the major enzyme that degrades fibrin clots
$\alpha_2$ -Plasmin inhibitor ( $\alpha_2$ -PI)	It is a primary and fast inhibitor of plasmin, which is an important enzyme to degrade fibrin clots
Plasmin $\alpha_2$ -PI complex (PIC)	It is a complex of plasmin and $\alpha_2$ -PI. Its elevation indicates production of plasmin

which were all obtained from Sysmex Corporation. The reference values recommended by the manufacturer were 9.6 to 13.1 seconds for PT, 200 to 400 mg/dL for Fbg,  $\leq 6.1\text{ }\mu\text{g/mL}$  for FMC, 80 to 130% for Plg,  $\leq 1.0\text{ }\mu\text{g/mL}$  for D-dimer, and  $\leq 0.8\text{ }\mu\text{g/mL}$  for PIC. All measurements were conducted using the CS-5100 instrument (Sysmex Corporation). As additional testing, tPA and plasminogen activator inhibitor-1 (PAI-1) were also measured for 237 samples with enough volume; the remaining 61 samples did not have enough volume for the tests. Human Tissue-type Plasminogen Activator (tPA) Chromogenic Assay-Sense Activity Assay Kit and Human Plasminogen Activator Inhibitor-1 (PAI-1) Chromogenic Assay-Sense Activity Assay Kit were purchased from ASSAYPRO (St. Charles, Missouri, United States) and used. tPA/PAI-1 ratios and their concentrations were also calculated to express the balance between enhanced and suppressed fibrinolysis status. The descriptions of the measurement components of the present study are presented in ►Table 1.

### Statistical Analysis

The normal reference interval range of fibrinolysis time was established from the data of 50 healthy donor samples, and the lower and upper limits of mean  $\pm 2$  standard deviation (SD) were defined as the cut-off values, respectively. The measurement data of the 298 clinical samples were divided into three groups based on two kinds of cut-off values: less than the lower limit of the normal reference interval (shortened group), within the normal reference interval (within group), and higher than the upper limit of the normal reference interval (prolonged group). In the shortened group, the clot was dissolved earlier than the normal range from coagulation activation. In contrast, the clot dissolved later than the normal range from coagulation activation in the prolonged group. The values were compared among these

**Table 2** Baseline characteristics of patients by group

	Shortened group (n = 61)	Within group (n = 102)	Prolonged group (n = 135)
Age, y	63 (37–78)	66 (38–76)	67 (48–75)
Gender; male (%)	30 (49.2)	66 (64.7)	114 (84.4)
Causative disease, n (%)			
Post-cardiac arrest syndrome	15 (24.6)	38 (37.3)	51 (37.8)
Hemorrhagic shock	11 (18.0)	5 (4.9)	5 (3.7)
Trauma	13 (21.3)	31 (30.4)	21 (15.6)
Poisoning	10 (16.4)	4 (3.9)	7 (5.2)
Sepsis/septic shock	3 (4.9)	15 (14.7)	21 (15.6)
Aortic diseases	3 (4.9)	1 (1.0)	2 (1.5)
Cardiogenic shock	4 (6.6)	4 (3.9)	7 (5.2)
Others	2 (3.3)	4 (3.9)	21 (15.6)

Note: Data presented as median (25th–75th percentile), percentage, or numbers.

three groups using the Kruskal–Wallis test and Bonferroni's multiple comparison test, and  $p < 0.05$  was used to denote statistical significance.

## Results

The normal reference interval of fibrinolysis time calculated using 50 healthy donor samples was 225.7 to 317.8 seconds. The number of samples in the shortened, within, and prolonged groups was 61, 102, and 135, respectively. In addition, the mean  $\pm$  SD values of fibrinolysis time in the same three groups were  $199.1 \pm 21.6$ ,  $274.0 \pm 27.0$ , and  $434.4 \pm 105.4$  seconds, respectively. Background data on age, sex, and causative disease of the patients in each group are presented in ►Table 2.

Comparisons of the coagulation and fibrinolysis markers among the three groups are shown in ►Fig. 2. For the coagulation reaction markers, the Fbg level increased in the order of shortened, within, and prolonged groups, and the increase was statistically significant among all three group pairs. The opposite tendency was observed for the FMC; the values significantly decreased as the CFWA was prolonged in all pair comparisons among the three groups. Among the fibrinolysis markers, the level of  $\alpha_2$ -PI, a serine protease inhibitor of plasmin, also significantly increased as the CFWA was prolonged when comparing the shortened and within groups and shortened and prolonged groups. Furthermore, the level of Plg, the precursor of plasmin with the ability to dissolve Fbg, exhibited a similar tendency to that of  $\alpha_2$ -PI; the changes were statistically significant among all three pair comparisons. PIC, which usually increases during the fibrinolysis reaction after plasmin is inhibited by the  $\alpha_2$ -PI, showed a statistically higher value in the prolonged group than in the within group, and no other significant differences were detected among other group pairs. Additionally, no statistically significant differences were observed in D-dimer measurements among all three

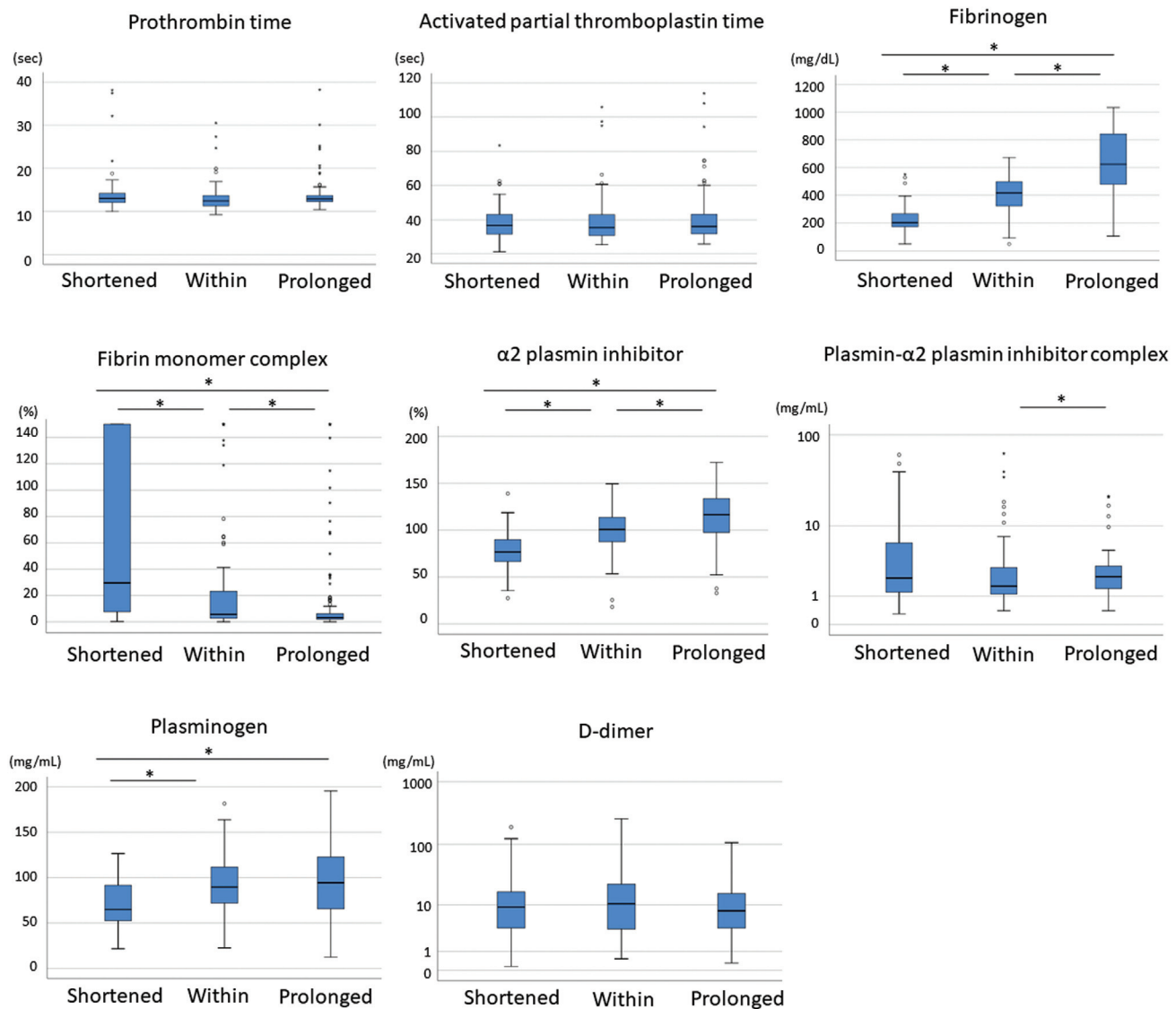
group pairs. As for the coagulation screening test, no significant difference was noted in PT, indicating that the coagulation backgrounds were similar among the three groups. The values of APTT were also similar among the three groups, indicating that the prolongation of fibrinolysis time was derived from only fibrinolysis time prolongation and not from the clotting time.

For the CFWA parameters of min1, FL-min1, and min1/FL-min1, the values were compared among three groups with the normal reference ranges calculated as the control groups (►Table 3). The median values of min1 and min1/FL-min1 were increased in the order of shortened, within, and prolonged groups, although the value of FL-min1 in the within group was equivalent to that of the prolonged group. Moreover, the medians of fibrinolysis time, min1, and min1/FL-min1 in the within group were close to the normal reference level. However, the FL-min1 value was higher than the normal reference level, indicating that the fibrinolysis reaction in the within group differed from that of normal samples. Overall, statistical differences were observed in all parameters, and it was recognized that the balance between coagulation and fibrinolysis reactions was different among the three groups. The fibrinolysis reaction would be enhanced as the fibrinolysis time is shortened. For tPA and PAI-1, 51, 81, and 105 samples were used for the tests in the shortened, within, and prolonged groups, respectively (►Fig. 3). Although the significant difference was recognized in only tPA between shortened and prolonged groups, the median values of tPA/PAI-1 were 2.440, 2.174, and 1.214, respectively. The values were decreased as the fibrinolysis times were prolonged, indicating that the shortened and prolonged groups have high and low tPA activity, respectively.

## Discussion

In this study, we investigated the relationship of CFWA with the related coagulation and fibrinolysis parameters in





**Fig. 2** Distribution of coagulation and fibrinolysis parameters among the three groups classified according to the fibrinolysis time in clot-fibrinolysis waveform analysis. Samples were divided into three groups based on the reference interval (RI) established as mean  $\pm$  2 SD of the fibrinolysis time in the healthy plasma samples as follows: less than the lower limit of RI (shortened group), within the RI (within group), and higher than the upper limit of RI (prolonged group).  $p$ -Value  $<0.05$  was used to define statistical significance. SD, significant difference.

critically ill patients. For comparison, we defined three groups, shortened, within, and prolonged, according to the fibrinolysis time determined using CFWA. In the shortened group, we observed an increase in the FMC level and a

decrease in Fbg, Plg, and  $\alpha_2$ -PI levels. This indicates the activation of coagulation and fibrinolysis because FMC is generated from Fbg by thrombin in the coagulation reaction, and the decrease in Plg and  $\alpha_2$ -PI levels indicates their

**Table 3** Comparison of CFWA parameters among shortened, within RI, and prolonged groups with normal reference ranges

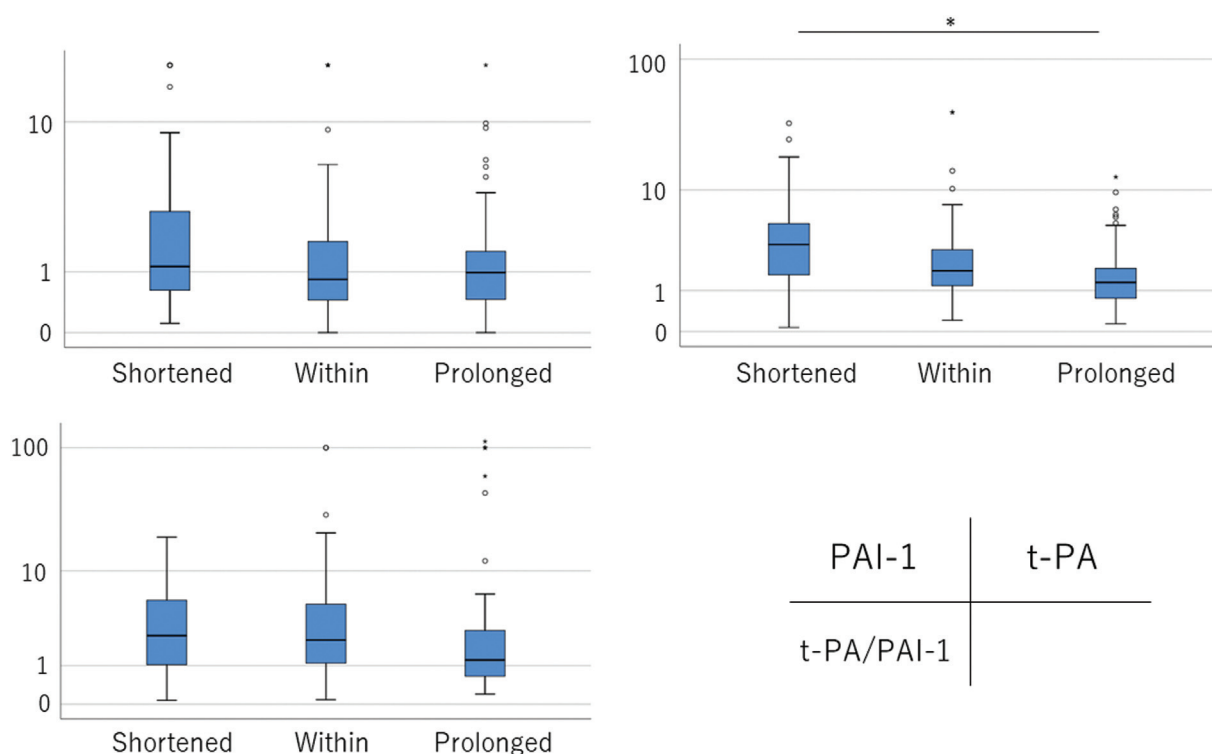
	Control	Shorten ( <i>n</i> = 61)	Within ( <i>n</i> = 102)	Prolonged ( <i>n</i> = 135)	<i>p</i> -Value
Fibrinolysis time	274.4 (249.5–283.7)	205.2 (188.0–214.6)	273.9 (252.4–294.1) <sup>a</sup>	404.4 (362.9–481.8) <sup>a,b</sup>	$<0.001$
Min1	4.75 (4.24–5.39)	2.85 (2.31–3.80)	5.68 (4.33–6.86) <sup>a</sup>	7.30 (5.69–8.47) <sup>a,b</sup>	$<0.001$
FL-min1	0.27 (0.26–0.28)	0.28 (0.19–0.34)	0.37 (0.31–0.41) <sup>a</sup>	0.35 (0.24–0.47) <sup>a</sup>	$<0.001$
Min1/FL-min1	17.80 (16.75–18.56)	10.78 (8.34–12.91)	15.49 (13.55–17.57) <sup>a</sup>	20.16 (16.64–25.51) <sup>b</sup>	$<0.001$

Abbreviation: CFWA, clot-fibrinolysis waveform analysis; FL-min1, fibrinolysis min1; RI, reference interval.

Note: Data presented as median (25th–75th percentile). Control groups are shown as reference and not statistically compared.

<sup>a</sup>Statistically significant difference between the shortened group (Bonferroni method).

<sup>b</sup>Statistically significant difference between the within group (Bonferroni method).



**Fig. 3** Distribution of PAI-1 and tPA among the three groups classified according to the fibrinolysis time in clot-fibrinolysis waveform analysis. The distributions of PAI-1 and tPA in the shortened ( $n = 51$ ), within ( $n = 81$ ), and prolonged ( $n = 105$ ) groups were described. There was a significant difference in tPA between the shortened and prolonged groups; there were no significant differences in PAI-1 or tPA/PAI-1 ratio among the three groups. tPA, tissue plasminogen activator.

consumption by conversion of Plg to plasmin and inhibition of plasmin in the fibrinolysis reaction.<sup>27–29</sup> The values of Fbg,  $\alpha_2$ -PI, and Plg in the prolonged group were higher than those in the shortened group, while the value of FMC was lower than that in the shortened group. This suggests that the coagulation and fibrinolysis status of patients in the prolonged group was the opposite of that of the patients in the shortened group while also being different from that of the within group. The mean values of PIC and D-dimer were higher than the cut-off values in all three groups, indicating the generation of plasmin and fibrin degradation products in most of the samples due to the fibrinolysis reaction. Although the fibrinolysis reaction was noted in all three groups, the status was different among the three groups because the levels of fibrinolysis markers were significantly different. The fibrinolysis situation with low and high values of Plg and  $\alpha_2$ -PI markers indicates the consumption and suppression of these proteins due to several factors, respectively. Therefore, the shortened and prolonged groups were characterized by enhanced and suppressed fibrinolysis compared to the within group, respectively.<sup>6</sup> Although several methods have been proposed to define the status of enhanced and suppressed fibrinolysis, specific markers are still required for the classification.<sup>6</sup> Alternatively, the groups divided according to the CFWA results showed enhanced and suppressed fibrinolysis situations in the shortened and prolonged groups, respectively. In addition, the min1/FL-min1 ratios increased in the order of shortened, within,

and prolonged groups, in which the tendency is consistent with that of fibrinolysis times. This indicates that the ratios and fibrinolysis times may reflect the fibrinolysis status. It was confirmed that the ratio parameter calculated from min1 and FL-min1 expressed the balance of comprehensive coagulation and fibrinolytic potential.<sup>25</sup> Thus, CFWA has the potential to classify patients according to the fibrinolysis situation by using only one assay. Recently, Onishi et al suggested that min1 and FL-min1 parameters were related to the severity in patients with COVID-19, and the assay could provide information about the hemostatic changes and disease status in patients with COVID-19.<sup>25</sup> Furthermore, it was also reported that CFWA reflected the effects of some drugs like argatroban, thrombomodulin, and tranexamic acid dose-dependently.<sup>19</sup> The drug concentrations and the effects of these drugs in blood might be useful in some cases, in which CFWA results also have the potential to estimate the drug effects and contribute to the decision-making in the therapeutic intervention.

Understanding the fibrinolysis situation is important for the diagnosis and treatment of critically ill patients because strong fibrinolytic activation is observed in various clinical settings. For example, in patients with trauma and out-of-hospital cardiac arrest, strong fibrinolytic activation is frequently observed on arrival at the emergency department.<sup>30–34</sup> The fibrinolytic activation in patients with out-of-hospital cardiac arrest is induced by the massive release of tPA, with a level up to 250 times that of healthy individuals.<sup>31</sup>

In patients with severe trauma, marked fibrinolytic activation is observed, but the total tPA concentration is increased by only approximately 30 times that in healthy individuals.<sup>33</sup> However, in the CFWA protocol used in this study, a large amount of tPA was added to the sample at the time of measurement, and the final concentration of tPA in the reaction sample was 1.37 µg/mL. The physiological tPA concentration in vivo is 720 pg/mL<sup>35</sup>; thus, the final concentration of tPA in the reaction solution was approximately 1,900 times the physiological concentration. Therefore, the patient-derived tPA amounts in the CFWA reaction solution were considered negligibly small, and this CFWA measurement system was considered not to reflect the patient-derived tPA.

One of the important markers of the fibrinolysis reaction is PAI-1, which inhibits plasminogen activators such as tPA and urokinase-type plasminogen activator. It has been reported that the PAI-1 concentration increases in critically ill patients, and it is thought that the elevated PAI-1 inhibits the tPA generated in the patients and also suppresses the fibrinolysis reaction.<sup>36–38</sup> However, it has also been shown that PAI-1 is not elevated in patients with out-of-hospital cardiac arrest and trauma in the early phase.<sup>1,39</sup> Inflammation induces the activation of coagulation and increases the levels of several coagulation markers, including PAI-1 and Fbg.<sup>40–42</sup> In this study, tPA was significantly higher in the shortened group, and both PAI-1 and tPA tended to decrease from shortened to prolonged. The decrease in these levels may be due to changes in the pathophysiology of the patients. However, to clarify this, it is necessary to include the causative disease and investigate the pathophysiology of the patients in the time course as independent variables, which requires a very large sample size and is a subject for future research.

### Limitations

This study had some limitations. First, this study was conducted at a single institution, and the number of patients may not be sufficiently large. Second, the patient samples were collected without any restriction during the hospitalization, and the sample collection timing might affect the results among the three groups. Third, the fibrinolysis situation in each patient was not defined and classified. Therefore, the evaluation of the defined samples should be planned in future studies.

### Conclusion

In the intensive care setting, CFWA is considered a global fibrinolytic assay that reflects the Plg and  $\alpha_2$ -PI levels in patients. Furthermore, CFWA is not affected by endogenous tPA because r-tPA is added during the measurement. CFWA can sensitively detect the activation of the fibrinolytic reaction that is associated with the coagulation reaction, suggesting that CFWA may be a useful marker for the classification of the fibrinolysis status of patients.

### What is known about this topic?

- Clot-fibrinolysis waveform analysis (CFWA) evaluates coagulation and fibrinolysis.
- The coagulo-fibrinolytic balance can drastically change in critically ill patients.

### What does this paper add?

- The fibrinolysis time of CFWA reflects plasminogen and  $\alpha_2$ -plasmin inhibitor levels.
- CFWA is not affected by the endogenous tissue-plasminogen activator.
- CFWA has the potential to reflect the fibrinolysis status in one global assay.

### Data Availability Statement

The corresponding author can disclose the data on request.

### Authors' Contribution

T.T. contributed to the conception of the study and manuscript preparation. O.K. contributed to the sample measurements, data analysis, creation of figures, and revision of the intellectual content. M.H. contributed to the sample collection, manuscript preparation, and revision of the intellectual content. All authors have read and approved the final manuscript version before submission.

### Conflict of Interest

T.T. and M.H. declare that they have no competing interests. O.K. was an employee of the Sysmex Corporation at the time the work was carried out.

### Acknowledgment

We would like to thank Editage (<https://online.editage.jp/>) for English language editing.

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