# The First-Derivative Curve of the Coagulation Waveform Reveals the Cause of aPTT Prolongation

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## Abstract

Clot waveform analysis based on activated partial thromboplastin time (aPTT) is reported to be a useful assay. We attempted to find beneficial parameters with the first-derivative curve. We examined 106 plasma samples with prolonged aPTT and analyzed the first-derivative curve statistically by dividing it into 6 groups (Lupus anticoagulant, Heparin, Direct oral anticoagulants, Factor VIII inhibitor, Hepatic dysfunctions and Factor deficiency). We obtained 7 coordinates for parameter measurement by analyzing the first-derivative curve and set 20 parameters including the velocity axis, the time axis, and area parameters. The distribution was checked by extracting each parameter that showed the most significant difference in the 6 groups. As a result, it was revealed that we could classify aPTT prolongation by using a combination of 3 parameters, the initial-to-peak gradient, the ratio initial-to-intermediate velocity/intermediate-to-peak velocity, and the initial-to-peak area size. We constructed a flowchart combining these 3 parameters and were able to discriminate 75% of the specimens. These parameters derived from the first-derivative curve of clot waveform analysis are useful tools to discriminate aPTT prolongation.

## Introduction

Activated partial thromboplastin time (aPTT) provided for conventional clotting testing is used as a screening test that reflects the mechanism of intrinsic coagulation activation, intrinsic coagulation factor deficiency/abnormality, and decreased synthesis functions of the liver. The aPTT reflects various coagulation disorders, and it is important in testing for factor deficiency/hemorrhagic symptoms, the liver failure, administration or contamination of anticoagulants such as unfractionated heparin, and anticoagulants such as coagulation factor VIII with inhibitor and lupus anticoagulant (LA). Clot waveform analysis (CWA) is accomplished with a curve obtained from the raw data in the aPTT analysis. CWA can monitor changes in the permeability of the plasma sample during fibrin formation and depict the entire process of coagulation as a coagulation waveform. In addition, the clot waveforms are classified to pre-coagulation phase, the coagulation phase, and post-coagulation phase.[1-3] The first-derivative curve of CWA indicates the coagulation velocity, and the second-derivative curve indicates the coagulation acceleration (Figure 1-(1)). These derivative curves are reported to be useful in the diagnosis of bleeding disorders and understanding of the pathophysiology of hemophilia, therapeutic effects, risk assessment of disseminated intravascular coagulation (DIC), risk of massive bleeding with anticoagulants and the detailed examination of factors affecting aPTT prolongation.[4-14] In particular, there are many reports on the analysis of peak time and peak height in the first- and second-derivative curves.[4-9,12-15] However, other parameters to be derived from the first- or second-derivative curve have not been regarded as important. Because the
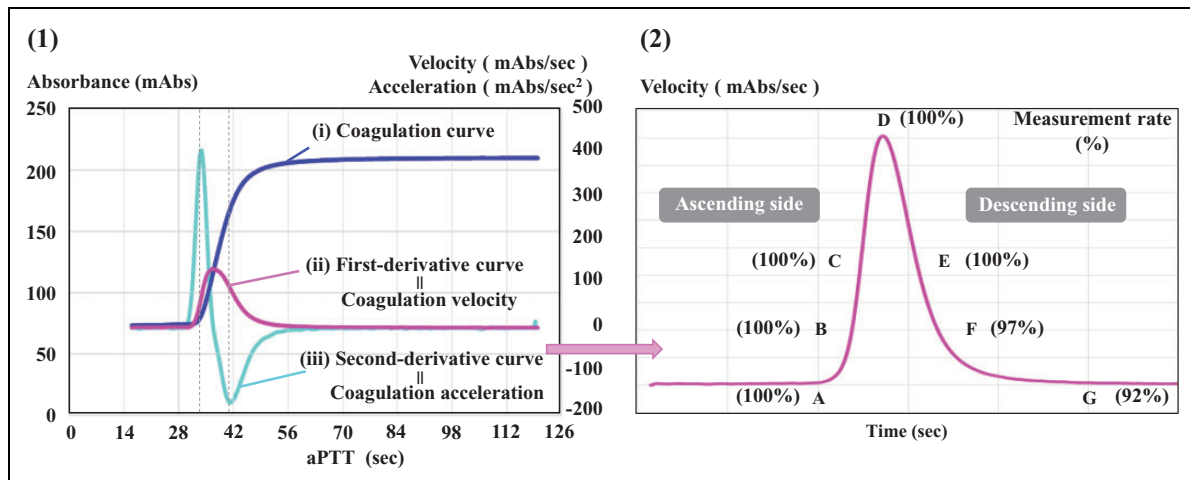
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**Figure 1.** Clot waveform analysis. (1) Activated partial thromboplastin time (aPTT)-based clot waveforms and derived parameters in normal plasma. (i) Recording of the changes in absorbance observed over time during the performance of a normal aPTT. (ii) Curves from first (dT/dt)-derivatives of the clot waveform. (iii) Curves from second ($d^2T/dt2$)-derivatives of the clot waveform. (2) Enlarged picture of the first-derivative curve. (A) baseline of ascending side, (B) 1/4 height of ascending side, (C) 1/2 height of ascending side, (D) peak, (E) 1/2 height of descending side, (F) 1/4 height of descending side, (G) baseline of descending side. Measurement rate: percentage of time and absorbance measured for coordinates A to E in 106 specimens.

first-derivative curve is composed with only positive values, we thought that the first-derivative curve would be more suitable for quantifying the parameters than the second-derivative curve with its mixture of positive and negative values. Therefore, we sought to use the measurable values of the first-derivative curve to create useful CWA parameters and establish a practical method for identifying the causes of aPTT prolongation.

## Materials and Methods

### Sample Collection

This study was approved by the Tenri Hospital Ethics Committee (Identification number: 875). Blood was collected in polypropylene tubes with 0.109 mol/L sodium citrate from 2004 to 2018 in Tenri Hospital. Citrated blood was centrifuged at 2000×g for 10 min and the separated plasma was placed in novel polypropylene tubes. Plasma samples were kept at −80°C. 25 plasma of healthy volunteers were collected as a normal plasma (NP) group. The cut-off value for aPTT prolongation and LA positivity was based on the values exceed +2 standard deviations (SDs) in the 25 healthy volunteers. The aPTT prolongation was 39.7 sec (Average:33.5, SDs:3.075), the dRVVT Normalized Screen Ratio/Confirm Ratio was 1.13 (Average:1.00, SDs:0.0646), and the Normalized Screen Ratio/Confirm Ratio of SCT for the phospholipid neutralization method was 1.25 (Average:1.01, SDs:0.1196). We randomly selected 106 aPTT prolongation plasma, and coded personal information with non-connection anonymity. A total of 106 patients with aPTT prolongation were measured. This group included 28 LA-positive cases (LA group: positive both for diluted Russell's viper venom time (dRVVT) and silica

clotting time (SCT) [dRVVT 1.15-3.29, SCT 1.29-3.99]), 26 cases of unfractionated heparin sodium administration (Heparin group: blood concentration >0.1 (0.1-0.7) IU/mL), 24 cases of patients taking direct oral anticoagulants (DOACs group) (rivaroxaban 22, apixaban 2: blood concentration >100 (113-546) ng/mL [μg/L]), 14 cases with coagulation factor VIII inhibitor (FVIIIi group: FVIII inhibitor titer ≥1.0 Bethesda U/mL and FVIII: C <5.0 IU/dL), 9 cases with hepatic dysfunction (Hepatic dysfunctions group: albumin-bilirubin grade 2 or 3), and 5 cases of congenital intrinsic coagulation factor deficiency (Factor deficiency group: congenital hemophilia A, 1 case [FVIII activity 19.0 IU/dL]; FXI deficient, 3 cases [FXI activity 3.0-4.0 IU/dL]; and FXII deficient, 1 case [FXII activity 2.0 IU/dL]).

### Reagents and Instrument

HemosIL APTT-SP®, HemosIL dRVVT Screen®, HemosIL dRVVT Confirm® and HemosIL SCT® were provided by Instrumentation Laboratory Corporation (IL Corp.). CWA and antiphospholipid syndrome screening tests were performed on an ACL-TOP® instrument (IL Corp.).

### Method

Time and absorbance were measured in each specimen at 7 coordinates on the first-derivative curve (Figure 1-(2)). These 7 coordinates were baseline of ascending side (A), 1/4 height of ascending side (B), 1/2 height of ascending side (C), peak (D), 1/2 height of descending side (E), 1/4 height of descending side (F) and baseline of descending side (G). The 7 coordinates of the first-derivative curve correspond to the baseline (A), the start of coagulation (B), the initial point of coagulation (C), the
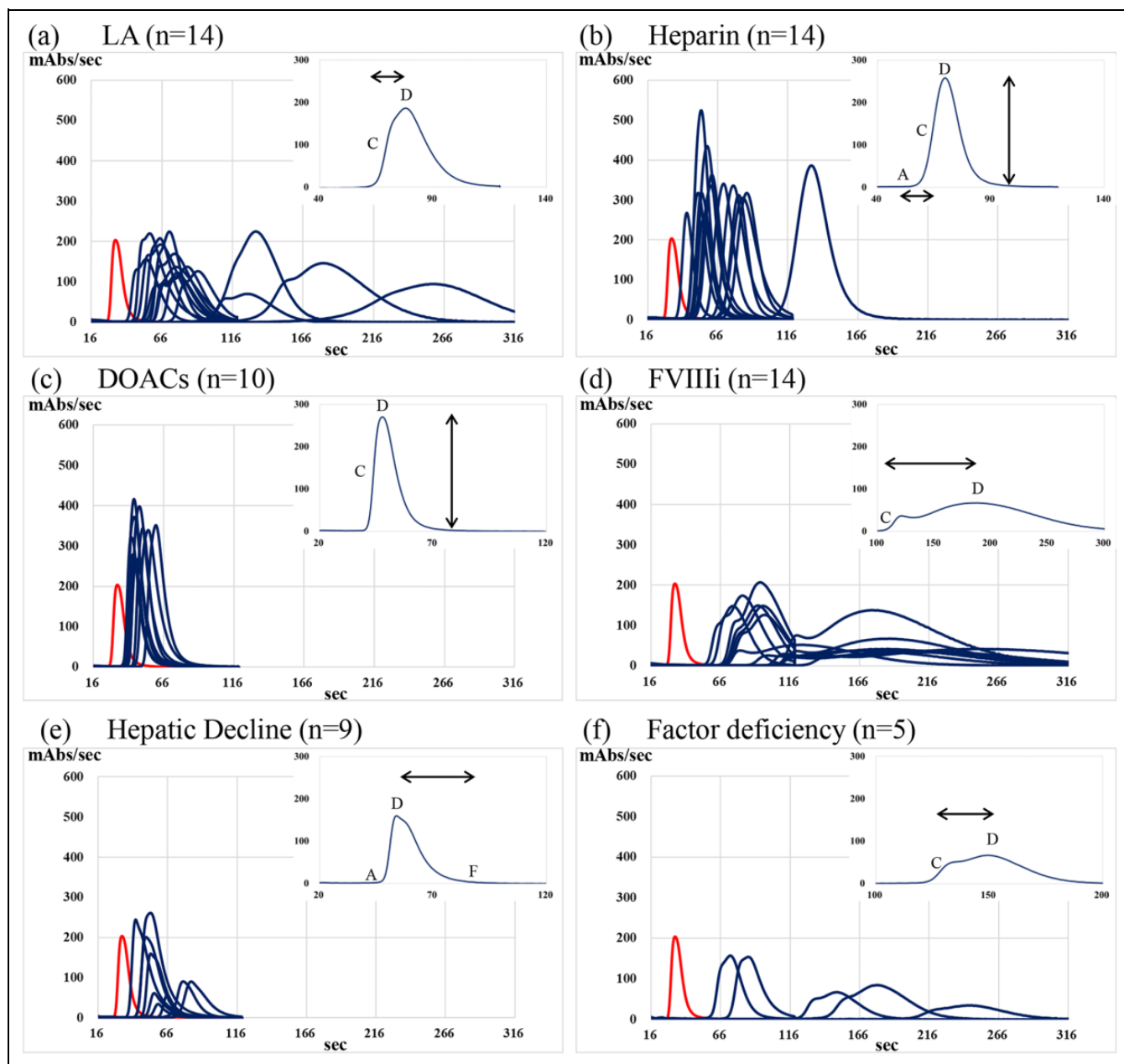
**Figure 2.** The first-derivative curve in each group with aPTT prolonged. The red line indicates the normal plasma (NP) group, and the other lines indicate the other groups. The average first-derivative curve for each group is shown in the upper right corner of the graph. (a) LA group: lupus anticoagulant-positive group. (b) Heparin group: unfractionated heparin administration group. (c) DOACs group: patients taking direct oral anticoagulants group. (d) FVIIIi group: coagulation factor VIII inhibitor possession group. (e) Hepatic dysfunctions group: dysfunctions of hepatic function group. (f) Factor deficiency group: congenital intrinsic coagulation factor deficiency group. LA group, Heparin group, and DOACs group were chosen for 14 and 10 cases to clarify the characteristics of the first-derivative curve.

midpoint of coagulation (D), the late point of coagulation (E), the near-complete coagulation point (F), and the complete coagulation point (G) of the coagulation curve for a normal plasma (Figure 1-(1)). The measurement points for the first derivative curve were chosen to cover all phases of the coagulation curve. We examined how these points changed in each group. Additionally, we selected effective parameters including the velocity axis, the time axis, and area parameters. We used these

parameters to create a flowchart that discriminated 6 clinical groups.

## Statistical Analysis

The distribution of each group was expressed as the median (10th-90th percentiles). The differences between the groups were examined using the Mann-Whitney *U* test. A *p* value of

**Table 1.** Number of Significant Differences Between the 6 Groups of Parameters Created With the First-Derivative Curve and the Peak Height of the First- and Second-Derivative Curve.

| Velocity axis parameters | Number * |
|---|---|
| Slope AD | 11 |
| Slope BD | 10 |
| Slope CD | **12** |
| Slope DE | 10 |
| Angle AD | 10 |
| Angle BD | 10 |
| Angle CD | **12** |
| Angle DE | 10 |
| **Time axis parameters** | |
| D−A | 11 |
| D−B | 12 |
| D−C | 12 |
| E−C | 12 |
| E−D | 11 |
| (C−A) / (D−C) | **14** |
| (D−C) / (E−D) | **14** |
| **Area parameters** | |
| Area [1] | **12** |
| Area [2] | 7 |
| Area [3] | 11 |
| Area [4] | 11 |
| Area [5] | **12** |
| **Peak value of the derivative curve** | |
| First | 10 |
| Second | 11 |

Number: Count the number of significant differences obtained between the 6 groups in the statistics of each parameter.

$< 0.05$ was considered to indicate statistical significance. All of the statistical analyses were performed using the Stat Flex software package (version 6; Artec Co., Ltd., Osaka, Japan).

## Results

Patterns of the First-derivative Curve of the Coagulation Disorders with Prolonged aPTT The median (10th-90th percentiles) aPTT in the NP group was 33.1 (30.6-37.0) sec and was as follows in the 6 groups: LA: 61.1 (49.0-108.2), Heparin: 60.4 (44.6-105.3), DOACs: 43.5 (40.4-53.4), FVIIIi: 97.6 (67.5-140.8), Hepatic dysfunctions: 53.7(46.0-74.5) and Factor deficiency: 128.8 (69.0-186.3). The first-derivative curve for all groups is shown in Figure 2, in which the median NP is indicated by a red line. Additionally, the average first-derivative curve for each group is shown in the upper right corner of the graph. In the LA group, the height of the peak was equal to or slightly lower than normal, the range of C and D tended to be wider than in groups Heparin, DOACs and Hepatic dysfunctions. In the heparin group, the height of peak was high, and the range of A and C detected to be wide. In the DOACs groups, the height of peak was high, and the range of C and D observed to be shorter. In FVIIIi group, the height of peak was mildly to noticeably lower and the range of C and D tended to be markedly wider. In Hepatic dysfunctions group, the height of peak varied

and the range of D and F observed to be significantly wider than the range of A and D. In Factor deficiency group, the height of peak was moderately to noticeably lower and the range of C and D confirmed to be wider, but shorter than that of FVIIIi group. Although some groups showed characteristic patterns, it was difficult to distinguish coagulation disorders using these relatively simple convex forms of the first-derivative curve.

### Examination of the Useful Parameters of the First-Derivative Curve

The percentages of time and absorbance that could be measured for the 7 coordinates on the first-derivative curve were examined. In ACL-TOP, the coagulation detection point is defined as the time of the peak height in the second-derivative curve. Thereby, 2 of 7 coordinates of the first-derivative curve were not used for the presence of the case that could not clarify the endpoint of the coagulation reaction. Consequently, the rates of each measurement were 100% for A, B, C, D, and E, 97% for F, and 92% for G (Figure 1-(2)), so that the parameters were set at 5 points except for G and F near the endpoint. In total, 20 parameters were set: 8 parameters using the velocity axis, 7 parameters using the time axis, and 5 area parameters (Table 1). The area parameter represents the velocity-related magnitude multiplied by time and velocity. Area [1] shows the magnitude between 1/2 height of ascending side and peak, Area [2] presents the magnitude between starting and 1/2 height of ascending side, Area [3] shows the magnitude between peak and 1/2 height of descending side, Area [4] presents the magnitude between width of 1/2 height and peak, and Area [5] shows the magnitude of the ascending side. To evaluate the usefulness of the 20 parameters, significant differences between the 6 groups were determined, and the number was compared. The number of significant differences was higher for Slope CD and Angle CD for parameters using the velocity axis, (C-A) / (D-C) and (D-C) / (E-D) for parameters using the time axis, and Area [1] and Area [5] for area parameters, respectively (indicated by bold type in the table). These parameters exceeded the number of significant differences in the peak height of the first- and second-derivative curves (Table 1). We checked the distribution of each parameter and chose the one of Slope CD and Angle CD, (C-A) / (D-C) and (D-C) / (E-D), Area [1] and Area [5] to be the combination with the highest discrimination rate by the flowchart discrimination described below. The combination of Slope CD, (C-A) / (D-C), and Area [5] gave the highest discrimination rate (Figure 3).

### Initial-to-Peak Gradient: Slope CD

Slope CD indicates the amount of change per unit time between the 1/2 height and peak of coagulation velocity (Figure 3-(1)). This means that Slope CD is the acceleration from 1/2 height to the peak. The DOACs group and Heparin group showed high values, the Hepatic dysfunctions group and LA group showed widely distributed values, and the Factor deficiency group and FVIIIi group had low values. This parameter showed notable increases in the DOACs group and Heparin group compared
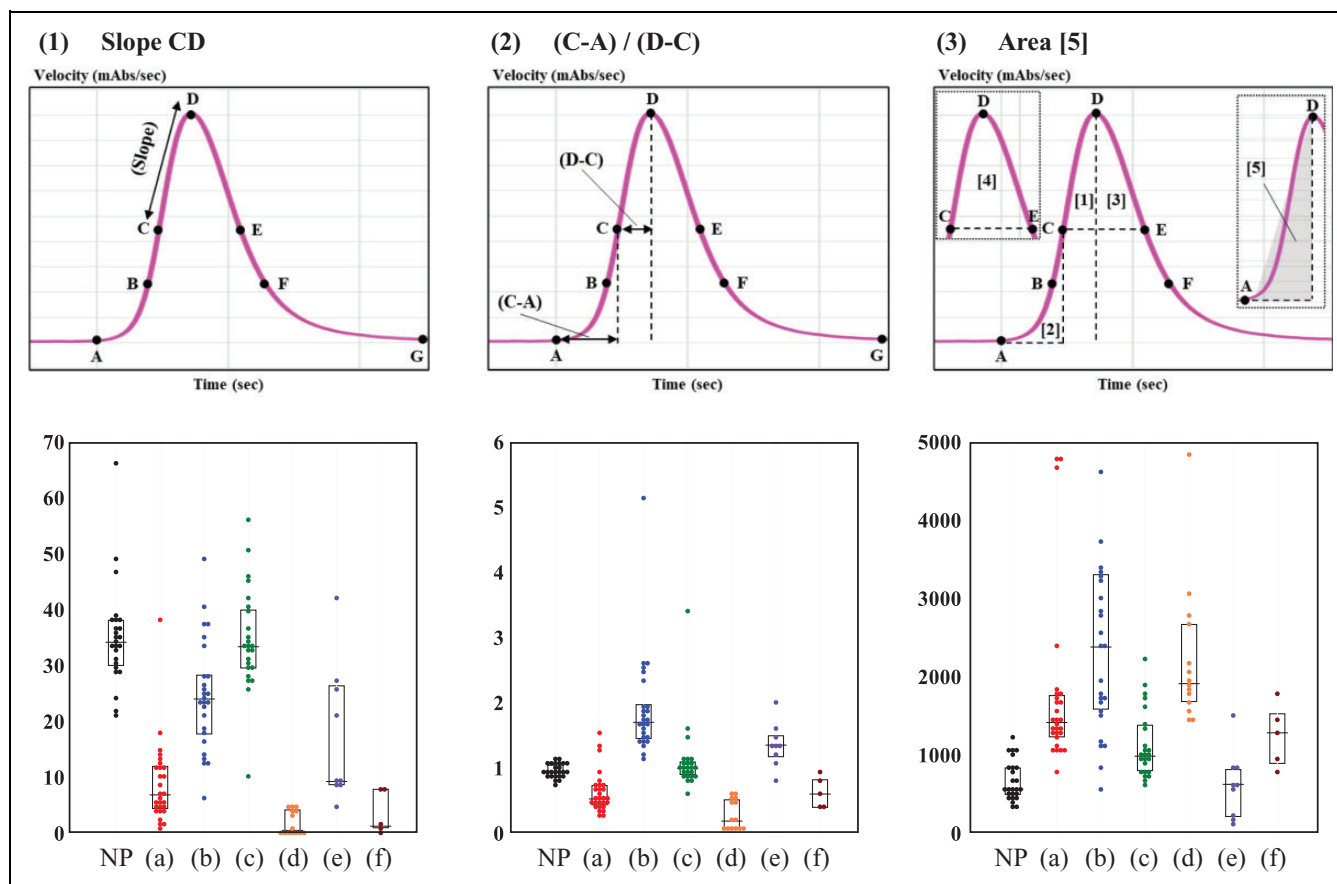
**Figure 3.** Distribution of 3 parameters derived with the first-derivative curve (1) (C-A)/(D-C). (2) Area [5]. (3) Slope CD. NP: NP group, (a): LA group, (b): Heparin group, (c): DOACs group, (d): FVIII i group, (e): Hepatic dysfunctions group, (f): Factor deficiency group.

with the other 4 groups ($p < 0.01$). In particular, it was distributed most clearly between the DOACs group and LA group, FVIIIi group and Factor deficiency group ($p < 0.001$). The FVIIIi group included patients with congenital hemophilia A and acquired hemophilia. In the 7 patients with FVIII: C $\geq 0.5$ IU/dL, Slope CD was reduced to 1.2-5.4, whereas in the 7 patients with FVIII: C $< 0.5$ IU/dL, Slope CD was remarkably reduced to 0.2-0.5.

## Ratio of Initial-to-Intermediate Velocity/ Intermediate-to-Peak Velocity: (C-A)/(D-C)

(C-A)/(D-C) indicates the ratio of the time from the rise of the curve to the 1/2 height and the time from the 1/2 height to the peak (Figure 3-(2)). In other words, (C-A)/(D-C) indicates the alteration of the velocity anteroposterior at the intermediate point on the ascending curve. This parameter was clearly higher in the Heparin group ($p < 0.01$) and clearly lower in the FVIIIi group ($p < 0.05$) compared with that in the other groups. Velocity in the Heparin group was faster in the 1/2 height−peak, and velocity in the FVIIIi group was faster in the rise−1/2 height but slower in the 1/2 height−peak. Especially, this parameter was most clearly divided between the heparin and FVIIIi group, the Factor deficiency group (p < 0.001).

## Initial-to-Peak Area Size: Area [5]

Area [5] represents an area of reaction from the start to the point maximum velocity is reached and is calculated by (D-A (time) × D-A (mAbs/sec)) / 2 (Figure 3-(3)). Area [5] shows the velocity-related magnitude of the ascending side. This parameter was clearly smaller in the Hepatic dysfunctions group than that in the other groups ($p < 0.05$). In the Hepatic dysfunctions group, once coagulation started, the time to reach the peak was fast. Furthermore, this parameter in the Heparin group and FVIIIi group was significantly larger than that on the other 4 groups ($p < 0.05$). Area [5] was large because Heparin group had a higher peak and FVIIIi group had a longer time to reach peak. Especially, this parameter was distributed most clearly between the Hepatic dysfunctions group and LA group, Heparin group and FVIIIi group ($p < 0.001$).

## Flowchart Construction and Evaluation

To effectively use numerical parameters to identify coagulation disorders, it is suitable to construct a flowchart. The order of the 3 parameters used in the flowchart was determined so that as much as possible, the distributions of the primary and secondary parameters and the distributions of the secondary and tertiary parameters did not overlap. We defined Slope CD as the primary
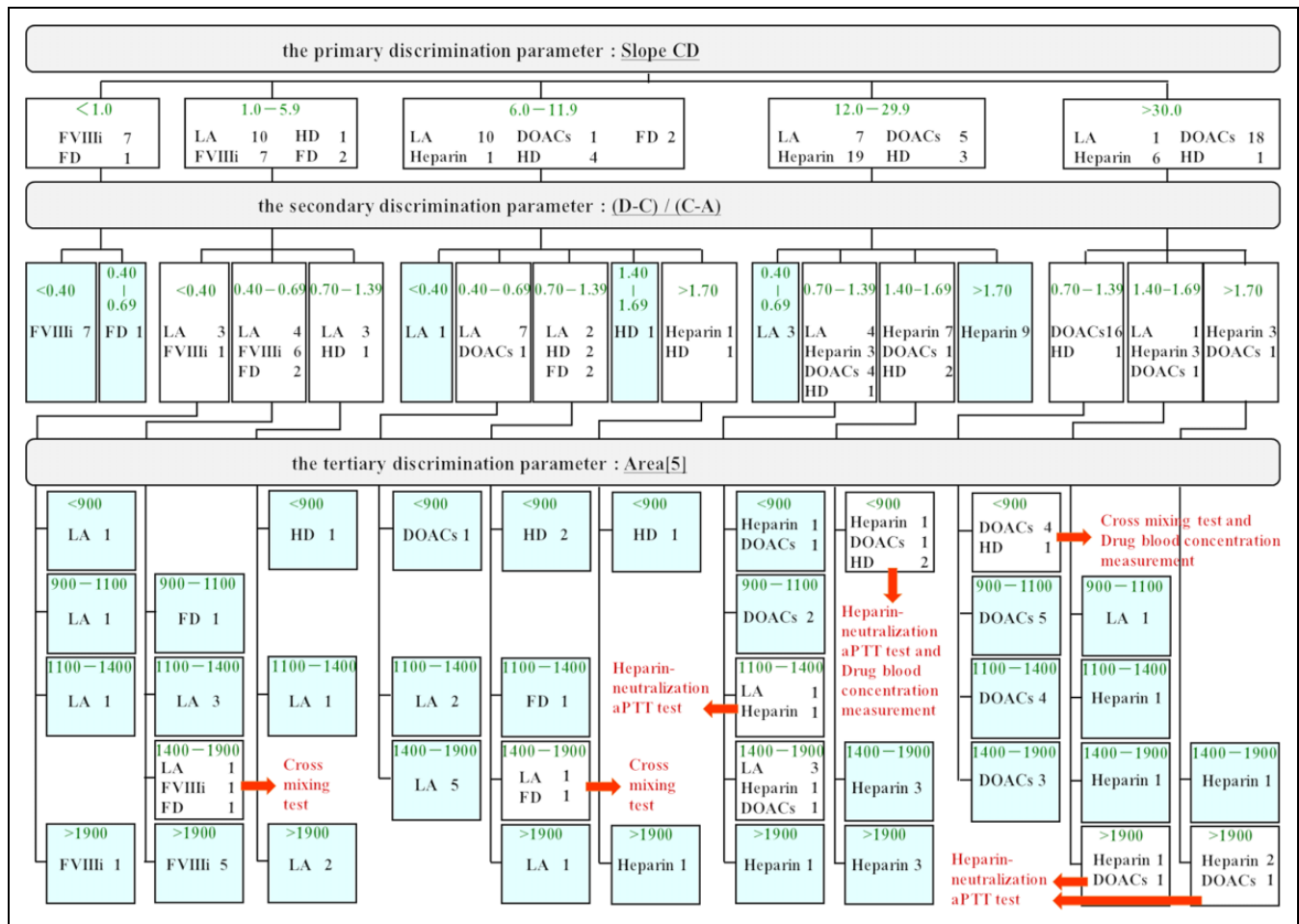
**Figure 4.** A flowchart for distinguishing prolonged aPTT with the 3 parameters. HD: Hepatic dysfunctions, FD: Factor deficiency. Blue squares indicate distinguishable categories and white squares indicates indistinguishable categories. The indistinguishable categories describe following tests (red arrow): Cross-mixing test or Heparin-neutralization (protamine-supplemented) aPTT test or Drug blood concentration measurement.

discrimination parameter, (C-A) / (D-C) as the secondary parameter and Area [5] as the tertiary parameter (Figure 4). Slope CD was divided into 5 groups: <1.0, 1.0-5.9, 6.0-11.9, 12.0-29.9 and >30.0, but it was not discernible. (C-A) / (D-C) was separated into 5 groups: <0.40, 0.40-0.69, 0.70-1.39, 1.40-1.69 and >1.70. These 2 parameters discriminated 20.8% (22/106 samples) of the samples. Area [5] was also classified into 5 groups: <900, 900-1099, 1100-1399, 1400-1899, and >1900. The categorization remained in 20.8% only by using Slope CD and (C-A)/(D-C), but the discrimination increased to 75.4% (80/106) by adding the parameter Area [5]. Furthermore, when Heparin group and DOACs group were identified as drug groups, the discrimination rate was 82.1% (87/106 samples). The order of the 3 parameters showed the highest discrimination rate for this order. Use of this flowchart provides clear guidelines by which to evaluate coagulation disorders.

## Discussion

Recently, completely automatic coagulation analyzers have tended to include a CWA function, and accordingly, many

findings about CWA have been reported.[4-14] The second-derivative curve is known to present shapes of an atypical shoulder or double peak (biphasic) in coagulation disorders.[10,11] It was reported that the second-derivative curve of LA-positive plasma or factor VIII- and IX-deficient plasma frequently showed atypical shapes. However, in terms of the numerical parameters that can be evaluated, no differences were shown in these 3 groups.[10] This study only reported that the shape of the second-derivative curve varied in the compositions of aPTT reagents,[10] whereas we can evaluate alterations of the coordinates because the first-derivative curve does not take negative value. Therefore, the first-derivative curve is characteristically used to evaluate time and velocity numerically. We aimed to determine numerical parameters by analysis with the first-derivative curve and found that these parameters were effective scores for discrimination of coagulation disorders.

We extracted Slope CD, (C-A)/(D-C) and Area [5] and from the 20 parameters based on the velocity, time, and area, respectively, which showed characteristic distributions in each group coagulation disorder group. These parameters had a greater

distributional gap in each group than the peak heights of the first- and second-derivative curves.

Slope CD was high in the DOACs groups, suggesting that the onset of coagulation was delayed for the DOACs that inhibited or suppressed the FXase complex, but once coagulation had begun, it appeared to continue at normal velocity. In contrast, The FVIIIi and Factor deficiency groups had the slowest velocity and took the longest time to reach their fastest velocity, so Slope CD was thought to be smaller. Matsumoto et al. reported that the slope-|min1| parameter was lower in cases of FVIIIi than in cases of severe hemophilia (all CWA performed by a Sysmex CS-2000i automated blood coagulation analyzer).[9] Similarly, in the present study, in FVIIIi cases with factor VIII activity <0.5 IU/dL, the values of Slope CD were significantly low. Therefore, we suggest that Slope CD can be a beneficial index to evaluate bleeding risk.

(C-A)/(D-C) was low in the FVIIIi group due to the slow rise time from 1/2 height to peak, despite the fast time from rise to 1/2 height. FVIII is a cofactor involved in the amplification of the coagulation reaction, and a severe decrease in FVIII delayed amplification, which was thought to slow down the period of 1/2 height−peak. In contrast, heparin inhibits activated factor X and thrombin, so the velocity of the rise was slow, but it increased from the 1/2 height phase. Therefore, (C-A)/(D-C) was higher in the Heparin group than in the FVIIIi group.

Area [5], which indicates a delay in the onset of the coagulation reaction but shows a faster time to reach peak once clotting begins, was smaller in the Hepatic dysfunctions group due to the moderate reduction with multiple coagulation factors. Conversely, Area [5] was larger for heparin because heparin had a faster velocity, FVIIIi had a slower velocity, and LA had a slower time from 1/2 height to peak. Because LA antibodies for phospholipids are involved in the amplification of the coagulation reaction, the time of 1/2 height−peak was slower than that from the initial−1/2 height.

The most practical way to carry out a uniform differentiation of coagulation disorders by CWA is through the deployment of a flowchart with a hierarchy of useful numerical parameters (Slope CD, (C-A) / (D-C), Area [5]). With this flowchart, the coagulation disorder in approximately 75% of the specimens (80 of 106 specimens) could be distinguished. In addition, if the Heparin group and DOACs group were considered as the drug administration group, 82% (87/106 samples) of the specimens could be discriminated. We showed that this practical flowchart can be useful in determining the initial diagnosis of a coagulation disorder. Conventionally, a cross-mixing test is required to identify the cause of aPTT prolongation.[16,17] However, the cross-mixing test requires normal plasma and multiple measurements of aPTT, which is labor intensive. In contrast, CWA can be used to draw the first and second-derivative curves just by measuring aPTT, so it would be easier to save time and effort if the CWA were matched to the flowchart constructed in this study. Thus, the flowchart derived in this study could potentially contribute to a reduction in the use of the cross-mixing test. Also, specimens that cannot be discriminated should be examined in

the following tests: cross-mixing test, heparin-neutralization (protamine-supplemented) aPTT test,[18-20] and drug blood concentration measurement. The flowchart shows the following tests to be performed in each section.

As limitations of this study, the number of cases examined was small, this study was conducted within a single facility, and lot-to-lot differences in aPTT reagents were not examined. Accordingly, we intend to increase the number of specimens, carry out testing with multiple lots of aPTT reagents, and conduct joint research with other institutions.

Laboratory analysis is the quickest to recognize aPTT prolongation in a patient specimen. We propose that the flowchart in this study identify the causes of the prolongation of aPTT rapidly and accurately.

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