Automated screening procedure for the phenotypes of congenital fibrinogen disorders using novel parameters, |min1|c and Ac/|min1|c, obtained from clot waveform analysis using the Clauss method

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Abstract

Introduction: Fibrinogen activity (Ac) is widely measured, but fibrinogen antigen (Ag) is measured only in specialized laboratories, so it is difficult to discriminate congenital fibrinogen disorders (CFDs) from acquired hypofibrinogenemia (aHypo). In this study, to screen for CFD phenotypes we adopted novel parameters, |min1|c and Ac/ |min1|c, and compared these with validated Ac, Ag, and Ac/Ag, and previously proposed Ac/dH and Ac/|min1|.

Materials and Methods: We calibrated |min1| using a CN-6000 instrument and investigated the correlation between Ag and |min1|c for aHypo (n=131) and CFD [18 dysfibrinogenemia (Dys), two hypodysfibrinogenemia (Hypodys) and four hypofibrinpogenemia (Hypo)]. Furthermore, we proposed a schema for screening CFD phenotypes using |min1|c and Ac/|min1|c.

Results: The |min1|c correlated well with Ag in aHypo, and Ac/|min1|c was a better parameter for screening Dys and Hypodys than Ac/dH and Ac/|min1|. With the combination of |min1|c and Ac/|min1|c parameters, 15 Dys, 2 Hypodys and four Hypo were categorized in agreement with the phenotype determined using Ag and Ac/Ag; conversely three Dys were classified as one Hypodys (AαR16C) and two Hypo (BβG15C).

Conclusion: We demonstrated that |min1|c and Ac/|min1|c are valuable parameters for

screening CFD patients and phenotypes in laboratories that do not measure Ag or perform genetic analysis.

Key Words:

fibrinogen activity, fibrinogen antigen, congenital fibrinogen disorder, clot waveform analysis, laboratory testing

Abbreviations:

Ac, fibrinogen activity; Ag, fibrinogen antigen; aHypo, acquired hypofibrinogenemia; AUC, area under the curve; CFDs, congenital fibrinogen disorders; CWA, clot waveform analysis; dH, delta H; Dys, dysfibrinogenemia; Hypo, hypofibrinogenemia; Hypodys, hypodysfibrinogenemia; min1, minimum value of the first derivative of transmittance; ROC, receiver operating characteristic

1. Introduction

Fibrinogen is a 45 nm-long plasma glycoprotein with a molecular weight of 340 kDa synthesized in hepatocytes and with a plasma concentration of 1.5–3.5 g/L [1,2]. It consists of two sets of three polypeptide chains, Aα, Bβ, and γ, which are encoded by the fibrinogen gene cluster *FGA, FGB*, and *FGG* on human chromosome 4 and form a hexamer $(A\alpha B\beta\gamma)_2$ joined at their amino-terminal regions by disulfide bonds [3]. Fibrinogen and fibrin play key roles in not only hemostasis and thrombosis but also matrix functions such as inflammation, infection, wound healing, and tumor growth and metastasis [4,5].

Congenital fibrinogen disorders (CFDs) are rare inherited abnormalities of blood coagulation. CFDs result from monoallelic or biallelic mutations in the three fibrinogen-encoding genes, and up to 800 cases have been listed in the human fibrinogen database [6]. The phenotypes of CFD are classified into quantitative (type I) and qualitative disorders (type II) according to functional and antigenic fibrinogen levels. Type I disorders include afibrinogenemia (absence of fibrinogen; homozygous state) and hypofibrinogenemia (Hypo, simultaneous decrease in functional and antigenic fibrinogen levels; heterozygous state), whereas type II disorders include dysfibrinogenemia (Dys, decreased functional and normal antigenic fibrinogen levels) and hypodysfibrinogenemia (Hypodys, discordant decrease in functional and antigenic fibrinogen levels) [7]. Patients with CFDs are at higher risk of bleeding or thrombotic complication than healthy individuals, and in some cases infertility

has also been reported [8,9]. Furthermore, several Hypo variants on the C-terminal side of the γ chain lead to intrahepatic accumulation of fibrinogen, resulting in life-threatening liver disease (fibrinogen storage disease) [10-12]. Most asymptomatic patients are identified by coagulation screening tests, and it is important to determine whether lower fibrinogen levels are due to the congenital or acquired background.

In routine laboratory testing, the Clauss method is widely used to measure fibrinogen activity (Ac) [13]. This method is based on the detection of fibrin polymerization and subsequent clot formation. Although, the Clauss method is well-optimized with automated coagulation analyzers and suitable for screening tests, it cannot distinguish between the patients with CFDs and acquired hypofibrinogenemia (aHypo), both showing lower plasma Ac level. Measurement of fibrinogen antigen (Ag) is needed to evaluate the phenotype of quantitative and qualitative disorders, and genomic analysis is required for definitive diagnosis of CFDs. However, they are carried out only in specialized laboratories, so it is difficult for the majority of laboratories to distinguish between CFDs and aHypo.

Clot waveform analysis (CWA) using an automated coagulation analyzer is a recently developed procedure for monitoring fibrin formation [14-16]. CWA can be performed during the monitoring of turbidity changes in clotting assays and provides useful information concerning the coagulation process. Recently, Suzuki et al. analyzed CWA

parameters obtained using the Clauss method, dH and |min1|, and revealed that |min1| values were well correlated with Ag in normal plasma [17]. Our previous study demonstrated that both the parameters of Ac/dH and Ac/|min1| ratios obtained from Dys were lower than those from aHypo [18]. Based on these results, |min1| was integrated as a CWA parameter into the newly developed automated coagulation analyzer, CN-6000 (Sysmex, Kobe, Japan).

In this study, to screen CFDs and classify CFD phenotypes, Dys, Hypo, and Hypodys, we propose a schema using novel CWA parameters, |min1|c and Ac/|min1|c, and evaluated by validated Ac, Ag and Ac/Ag, and compared them to previously proposed parameters, Ac/dH and Ac/|min1|.

2. Materials and methods

This study was approved by the Ethics Review Board of Shinshu University School of Medicine (Identification number: 4791) and was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

2.1. Sample collection

Blood samples were collected into evacuated anticoagulant tubes with a blood to 0.109 mol/L sodium citrate ratio of 9:1. Platelet poor plasma was obtained after centrifugation of

citrated whole blood for 10 minutes at 2000×g. All plasma samples were separated into polypropylene tubes, stored at −80°C, and then thawed at 37°C immediately prior to performing assays. Plasma samples of acquired hypofibrinogenemia were collected from 131 patients with plasma Ac levels below lower limit of the reference interval (1.5 g/L) and these were described as aHypo. Plasma samples from patients with CFD were collected from 24 patients with Dys (n=18), Hypodys (n=2), or Hypo (n=4). The phenotypes of CFD were classified as quantitative disorder with less than 1.5 g/L of Ag level and as qualitative disorder with an Ac/Ag ratio of less than 0.7 [7]. The definitive diagnosis for CFD and its phenotype was determined using the fibrinogen level and genomic analysis.

2.2. Genomic analysis

Genomic DNA purification, PCR-amplification, and DNA sequencing were performed as described previously [19].

2.3. Measurement of plasma fibrinogen activity and antigen

Coagulation tests for sodium citrate plasma were measured using an automated coagulation analyzer CN-6000 (Sysmex, Kobe, Japan). Plasma Ac levels were determined using the Clauss method with thrombin reagents, Thrombocheck Fib (L) (Sysmex), in

accordance with the manufacturer's instructions. Ten microliters of plasma were diluted with 90 μL of Owren's veronal buffer (Sysmex), and the diluted plasma was incubated at 37℃ for 190 sec, and then mixed with 50 μL of Thrombin reagent. The reaction mixture was incubated at 37℃ and the absorbance was measured at 405 nm for 300 sec. A calibration curve was generated by serial dilution of Coagtrol N (Sysmex). Plasma Ag levels were determined using a latex agglutination immunoassay with FactorAuto Fibrinogen (Q-may, Ohita, Japan) and FactorAuto fibrinogen multi-point standard plasma (Q-may).

2.4. Parameters of clot waveform analysis (CWA) and calculation value using CWA

CWA was performed on the CN-6000. The CN-6000 detects transmitted light intensity every 0.1 seconds at 405 nm wavelength in the Clauss method for fibrinogen assay. The representative clot waveform of normal plasma is shown in **Fig. 1A**. Transmittance decreased depending on fibrin formation, and the total difference in the transmittance level is shown as delta H (dH). The minimum value of the first derivative of transmittance (*dT/dt*, min1) was indicated as maximum velocity of fibrin formation process. The minimum value of min1 was minus, then the data were expressed as |min1| in this study (**Fig. 1B**). Furthermore, we calibrated |min1| using a serial dilutions of fibrinogen calibrator (**Fig. 1C**) and the fibrinogen concentration obtained from calibrated |min1| was termed as |min1|c and

used as novel parameter.

2.5. Statistical analysis

All statistical analyses were performed with EZR software, which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [20]. The relationship between two variables were analyzed using Pearson and Spearman rank tests. We evaluated differences among patient phenotypes using one-way ANOVA with Dunnett post hoc test. A difference was considered significant when the *p* value was lower than 0.05. We analyzed receiver operating characteristic (ROC) curves and cut-off values were predicted using Youden's index [21]. The accuracy of CWA parameters in the diagnosis of CFD was evaluated using the area under the curve (AUC).

3. Results

3.1 Correlation between Ag and |min1|c in aHypo

For plasma samples from 131 patients with aHypo, Ac was measured using thrombin reagent, and the |min1|c was obtained from calibrated CWA parameter |min1|. The levels of Ac and Ag were 0.62–1.50 and 0.59–1.86 g/L, respectively, and the Ac/Ag ratios were 0.75–1.44 (data not shown). A good correlation was obtained between Ac and Ag for plasma from

aHypo (r = 0.735, data not shown). Furthermore, |min1|c correlated well with Ag for patients with aHypo (r = 0.822) (**Fig. 2A)**.

3.2 Correlation between Ag and |min1|c in CFD

Next, we measured and analyzed plasma samples from patients with CFD: 18 patients with Dys, four patients with Hypo and two patients with Hypodys, and the results are summarized in **Table 1**. Ac/Ag of Dys and Hypodys was 0.13–0.53 and 0.35–0.48, respectively, and both were markedly lower than that of Hypo (1.15–1.25). Furthermore, there was a positive coefficient of correlation between Ag and |min1|c (r = 0.689); however, six cases in the Dys group showed a marked discordance between Ag and |min1|c (namely |min1|c was more than 1.00 g/L lower than Ag) [case IDs 1 to 6 (variants AαR16C, AαR16H, BβG15C, or γR275C)]. Excluding these cases, the coefficient of correlation increased to r = 0.881 (**Fig. 2B**).

Next step, we investigated whether Ac divided by |min1|c, namely Ac/|min1|c, distinguish CFD from aHypo. As shown in **Fig. 3A**, Ac/|min1|c of Dys (median, 0.40) and Hypodys (median, 0.49) were significantly lower than those of the aHypo (median, 0.79) and Hypo (median, 0.84) (no significant difference between aHypo and Hypo). These results suggested that the Ac/|min1|c is a useful parameter to distinguish Dys and Hypodys

from aHypo. To compare the CFD discrimination using Ac/|min1|c, previously proposed parameters, Ac/dH and Ac/|min1|, were calculated and are shown in **Fig. 3B** and **Fig. 3C**, respectively. Only Dys (Ac/dH; median, 0.0037 and Ac/|min1|; median, 2.764) showed significantly lower values than aHypo (Ac/dH; median, 0.0117 and Ac/|min1|; median, 6.224); however, there were no significant difference between Hypo (Ac/dH; median, 0.0127 and Ac/|min1|; median, 7.131) or Hypodys (Ac/dH; median, 0.0072 and Ac/|min1|; median, 6.799) and aHypo.

3.3 ROC analysis for the classification of type II disorders using Ac/|min1|c ratio

We further analyzed and compared cut-off values of the Ac/|min1|c, Ac/dH, and Ac/|min1| using ROC analysis. We determined the cut-off values of each parameter for distinguish type II qualitative disorders (Dys and Hypodys) from aHypo (Table not shown). The cut-off value of Ac/|min1|c was 0.55, and the sensitivity was 0.90, whereas the specificity was 1.00 and AUC was 0.90. On the other hand, the cut-off values, sensitivity, specificity, and AUC of Ac/dH and Ac/|min1|, previously proposed parameters, were 0.0090 and 5.072, 0.85 and 0.80, 0.97 and 0.95, and 0.90 and 0.84, respectively. These results indicated that the newly adopted Ac/|min1|c was the best parameter for distinguishing Dys and Hypodys from aHypo, whereas Hypo could not be detected even when using all parameters.

3.4 Determination of CFD phenotype using |min1|c and Ac/|min1|c

Subsequently, we classified CFD cases into type I or type II disorders using the cut-off values of three parameters, Ac/|min1|c, Ac/dH, and Ac/|min1|, calculated from ROC analysis (Table 2). For each parameter, all Hypo cases were classified as type I disorder, and 16 of 18 Dys cases were classified as type II disorder, whereas two cases were misclassified as type I disorders [case ID 3 and 4 (BβG15C)]. For two cases of Hypodys, Ac/|min1|c classified these as type II disorders, but Ac/dH or Ac/|min1| misclassified one and two cases as Type I disorders, respectively.

Finally, we determined the CFD phenotype using Ac/|min1|c combined with |min1|c and compared the phenotype classification using validated Ag and Ac/Ag. In this study, we classified less than 1.5 g/L of |min1|c was as a quantitative disorder (namely Hypo or Hypodys). As shown in **Table 2**, using |min1|c and Ac/|min1|c we determined 15 out of 18 cases as Dys, and three cases were misclassified as Hypodys [case ID 1 (AαR16C)] or Hypo [case ID 3 and 4 (BβG15C)]. On the other hand, for four cases of Hypo and two cases of Hypodys each classification was completely coincident with confirmed CFD phenotype.

4. Discussion

In the present study, to screen the CFD phenotype, Dys, Hypodys and Hypo, we adopted the novel parameter, Ac/|min1|c, calculated with Ac and |min1|c, which were obtained from fibrinogen measurement using the Clauss method, and compared with the confirmed CFD phenotype using Ac, Ag, and Ac/Ag.

First, we evaluated the coefficient of correlation between Ag and |min1|c. Our results showed that Ag and |min1|c correlated well, and the Ac/|min1|c for Dys and Hypodys were lower than for Hypo and aHypo. Therefore, we concluded Ac/|min1|c is a useful parameter for the classification of type I or type II disorders. Subsequently, we compared cut-off values of three parameter, Ac/|min1|c, Ac/dH, and Ac/|min1|, using ROC analysis. Ac/|min1|c had a higher sensitivity and specificity in the classification of type II disorders compared with the other two parameters. Finally, we propose a laboratory testing flowchart for the determination of CFD phenotypes using |min1|c and Ac/|min1|c (**Fig. 4**).

Our proposed screening schema found that variants with Dys, AαR16C, and two BβG15C fibrinogen, were misclassified as Hypodys and Hypo, respectively (**Table 3**). Therefore, we examined the coagulation curves of these patients. As shown in **Fig. 5**, for AαR16C fibrinogen, the start of increasing turbidity was markedly slower and the turbidity changing velocity during coagulation was markedly lower (markedly reduced lateral aggregation of protofibrils), resulting in markedly lower |min1|c (0.87 g/L, less than 1.5 g/L) and

Ac/|min1|c (0.40, less than 0.55), which was eventually classified as Hypodys. We have performed thrombin-catalyzed fibrin polymerization for purified AαR16C fibrinogen and observed turbidity changing velocity during coagulation was markedly lower than normal controls.

For two BβG15C fibrinogen coagulation curves were depicted almost same as lower concentration control plasma (1.30 g/L) (**Fig. 5**). Namely, markedly lower |min1|c (0.58 and 0.80 g/L, respectively, and less than 1.5 g/L) and Ac/|min1|c (1.53 and 1.14, respectively, and more than 0.55) was finally classified as Hypo. In our previous report, purified BβG15C fibrinogen showed a markedly lower turbidity change after 30 minutes of thrombin-catalyzed fibrin polymerization (markedly reduced lateral aggregation of protofibrils) than wild-type [22,23].

In addition to the above two variants, AαR16H (Case ID 2) and γR275H (Case ID 5 and 6) also showed lower |min1|c than Ag (Fig. 2B). Our previous studies have demonstrated that these variants also showed impaired fibrin polymerization [24,25]. During coagulation, thrombin cleaves fibrinogen, releasing fibrinopeptide A and fibrinopeptide B from the N-terminus of the Aα chain (residues of Aα.1–16) and Bβchain (residues of Bβ.1–14), respectively, and converting fibrinogen to fibrin monomers [26,27]. Thus, the AαR16 residue is crucial for thrombin cleavage for fibrin monomer formation. Furthermore, when

the fibrin monomers forms a double-stranded protofibril, it has been reported that residue γR275 is an important site for the binding of the D regions between one molecule and another, the so-called “D:D” interaction [26,28]. For variants of these residues, which are extremely important for the fibrin polymerization, there is a possibility that |min1|c might be lower than Ag. Even if our proposed schema is used, it is necessary to determine the Ag level in order to prevent misclassification of CFD phenotypes.

This study demonstrated that the novel parameters |min1|c, and Ac/|min1|c were beneficial for screening the CFD phenotypes without Ag level and genomic analysis. However, there are some limitations for adoption of the |min1|c. First, because the patient sample used in this study did not include healthy subjects, the exact reference range of |min1|c is unknown. In order to determine the decrease in antigenic fibrinogen levels using |min1|c, it is necessary to determine the lower limit of the reference interval. In addition, to obtain the common cut-off value of |min1|c and Ac/|min1|c, large numbers of samples are needed for analysis. Furthermore, in this study, only one thrombin reagent was used for verification. Fibrin clot formation differs depending on the thrombin activity, dissolving matrix, or derived animal species; therefore, coagulation waveform and values of the analyzed CWA parameter must be predicted for the variation among reagents, instruments and laboratories.

In conclusion, we obtained the novel parameter |min1|c from the CWA using the Clauss method and revealed that |min1|c and Ac/|min1|c are valuable parameters for determining the CFD phenotypes. These parameters are available in the laboratories that do not measure Ag or perform genetic analysis, allowing for easy and prompt screening of CFD patients and determination of the phenotype.

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Conflict of interests

S. Shinohara, T. Suzuki and N. Arai are employees of Sysmex Corporation. S. Arai, T. Kamijo, T. Kaido, M. Yoda, M. Sugano, T. Uehara and N. Okumura have no conflicts of interest to disclose.

Author contributions

S. Arai, T. Kamijo, T. Kaido and M. Yoda performed the research, analyzed the data. S. Arai

wrote the manuscript. S. Shinohara, T. Suzuki, N. Arai, M. Sugano, T. Uehara, and N. Okumura designed the research and discussed the data, and N. Okumura reviewed the manuscript.

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Table 1. Characteristics of patients with congenital fibrinogen disorders

Case ID	Age (years)	Sex	Symptom	Variant	Type	Subtype[a]	Ac	Ag	Ac/Ag	|min1|c	Ac/|min1|c	dH	Ac/dH	|min1|	Ac/|min1|
1	35	F	A	AαR16C	Dys	3A	0.35	2.64	0.13	0.87	0.40	95	0.0037	0.069	5.072
2	31	F	A	AαR16H	Dys	3A	0.97	4.70	0.21	3.19	0.30	480	0.0020	0.720	1.347
3	47	F	H	BβG15C	Dys	3A	0.89	1.82	0.49	0.58	1.53	35	0.0254	0.033	26.970
4	14	F	A	BβG15C	Dys	3A	0.91	1.92	0.47	0.80	1.14	48	0.0190	0.059	15.424
5	34	F	I	γR275C	Dys	3A	0.50	2.84	0.18	1.50	0.33	184	0.0027	0.181	2.762
6[b]	1	M	A	γR275C	Dys	3A	0.59	2.90	0.20	1.82	0.32	206	0.0029	0.259	2.278
7[b]	n.d	M	A	γR275C	Dys	3A	0.61	1.80	0.34	1.54	0.40	165	0.0037	0.191	3.194
8	33	F	A	γR275C	Dys	3A	0.74	2.11	0.35	1.90	0.39	244	0.0030	0.280	2.643
9	10	F	A	γR275C	Dys	3A	0.80	2.00	0.40	1.86	0.43	220	0.0036	0.269	2.974
10	2	M	A	γR275H	Dys	3A	0.73	2.32	0.31	2.16	0.34	266	0.0027	0.354	2.062
11	57	M	A	γR275H	Dys	3A	0.75	2.46	0.30	1.82	0.41	83	0.0090	0.259	2.896
14	n.d	M	A	γR275H	Dys	3A	0.60	2.13	0.28	1.65	0.36	199	0.0030	0.217	2.765
12	7	M	H	γR275H	Dys	3A	0.62	2.06	0.30	1.62	0.38	203	0.0031	0.210	2.952
13	n.d	F	A	γR275H	Dys	3A	0.81	2.61	0.31	2.20	0.37	284	0.0029	0.367	2.207
15	38	F	A	γN308K	Dys	3A	1.23	2.97	0.41	3.13	0.39	308	0.0040	0.699	1.760
16[c]	n.d	F	A	γY354H	Dys	3A	0.94	1.78	0.53	1.96	0.48	208	0.0045	0.297	3.165
17[c]	n.d	F	A	γY354H	Dys	3A	1.28	2.79	0.46	2.86	0.45	336	0.0038	0.591	2.166
18[c]	20	F	A	γY354H	Dys	3A	1.42	2.95	0.48	3.08	0.46	365	0.0039	0.678	2.094
19	38	F	H	γC326R	Hypodys	4B	0.41	0.85	0.48	0.75	0.55	43	0.0095	0.053	7.736
20	86	F	H	γR275H +AαD502N	Hypodys	4B	0.34	0.98	0.35	0.79	0.43	71	0.0048	0.058	5.862
21	31	F	H	BβN140K	Hypo	2C	1.25	1.09	1.15	1.50	0.83	60	0.0208	0.181	6.906
22	36	F	H	BβP204L	Hypo	2C	1.43	1.14	1.25	1.71	0.84	122	0.0117	0.232	6.164
23	n.d	M	A	BβW403X	Hypo	2B	1.15	0.95	1.21	1.35	0.85	91	0.0126	0.150	7.667
24	30	F	A	γR375W	Hypo	2C	1.14	1.01	1.13	1.37	0.83	90	0.0127	0.155	7.355

Note: All variants were heterozygous for carrier status. The gray highlight shows dysfibrinogenemia in which |min1|c was lower than Ag.

Abbreviations: A, asymptomatic; Ac, fibrinogen activity, g/L; Ag, fibrinogen antigen, g/L; Dys, dysfibrinogenemia; F, female; H, hemorrage; Hypo, hypofibrinogenemia; Hypodys, hypodysfibrinogenemia; I, infertility; M, male; n.d, not determined.

[a]Classification according to recommendation [7].

[b]Family with γR275C.

[c]Family with γY354H.

Table 2. Classification of CFD phenotypes using Ac/|min1|c, Ac/dH, Ac/|min1|, or the combination of |min1|c and Ac/|min1|c

	Ac/|min1|c		Ac/dH		Ac/|min1|		|min1|c + Ac/|min1|c		
	Type I	Type II	Type I	Type II	Type I	Type II	Dys	Hypodys	Hypo
Dys (n=18)	2	16	2	16	2	16	15	1	2
Hypodys (n=2)	0	2	1	1	2	0	0	2	0
Hypo (n=4)	4	0	4	0	4	0	0	0	4

Abbreviations: Ac, fibrinogen activity; Dys, dysfibrinogenemia; Hypo, hypofibrinogenemia; Hypodys, hypodysfibrinogenemia; Type I, type I quantitative disorder; Type II, type II qualititative disorder.

Figure legends

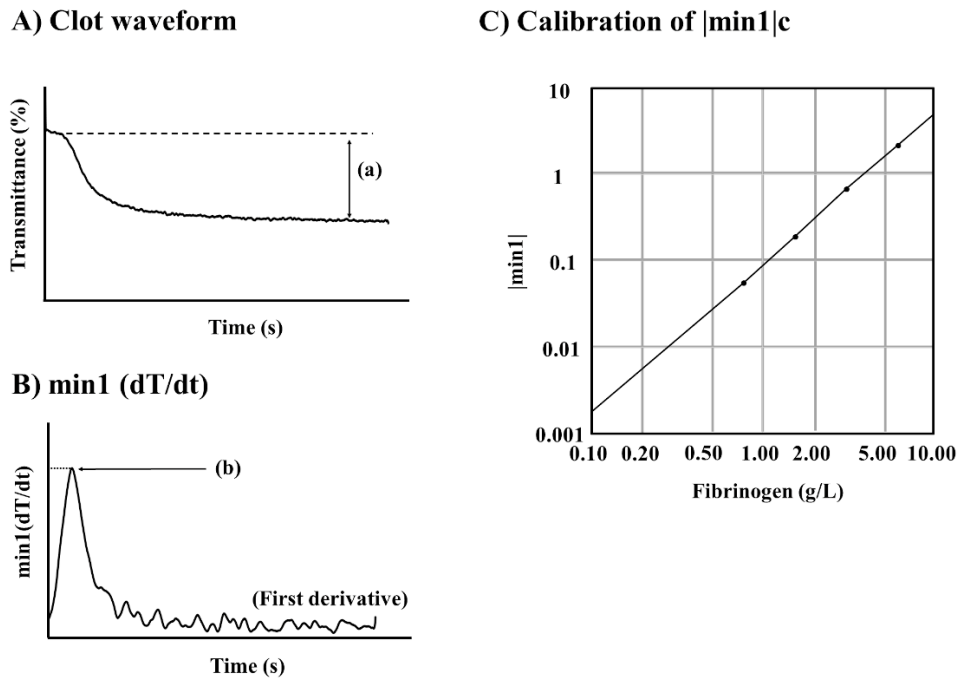

Figure 1. Clot waveform analysis using the Clauss method and calibration of |min1|c

Representative clot waveform monitored as transmittance using Clauss method with an automated coagulation analyzer, CN-6000. (A) Changes in light transmittance (%) observed over time during the recording period. (B) Curves from first derivatives (dT/dt) of the clot waveform in panel (A). (a) and (b) indicate dH and |min1|, respectively. (C) Calibration of |min1|c, performed using a fibrinogen calibrator kit. The plots indicate the mean of |min1| values from duplicate assays.

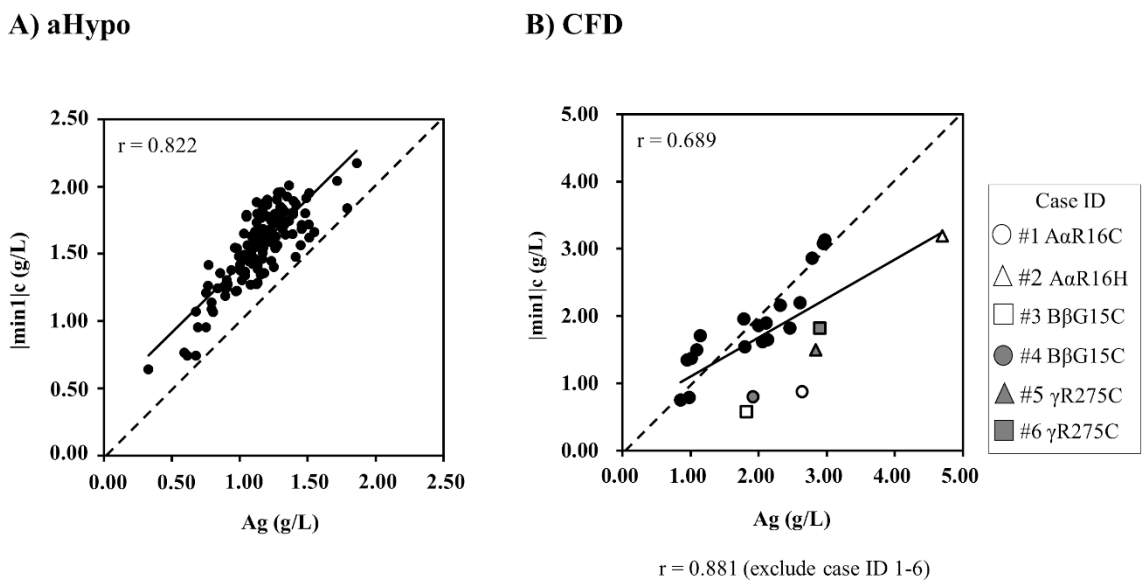

Figure 2. Correlation between Ag and |min1|c

Scatterplot showing the correlation between fibrinogen antigen and |min1|c in plasma for acquired hypofibrinogenemia (A) and congenital fibrinogen disorders (B). The respective correlation coefficients (r) are shown in each plot. Ag, fibrinogen antigen.

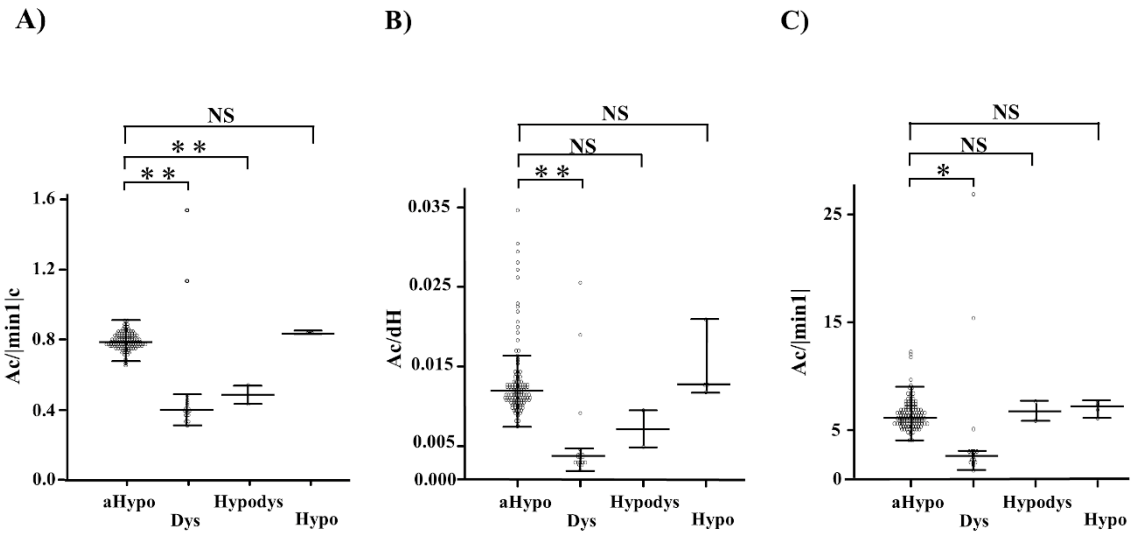

Figure 3. Distribution of Ac/|min1|c, Ac/dH, or Ac/|min1| for patients with congenital fibrinogen disorders

Comparison of the distributions of Ac/|min1|c (A), Ac/dH (B), and Ac/|min1| (C) from acquired hypofibrinogenemia and congenital fibrinogen disorders. The bottom and top lines indicate the first and third quartiles of the data, respectively, and the horizontal line indicates the median value. The whiskers extend out to the most extreme data point that is at most 1.5 times the interquartile range above the third quartile or below the first quartile. Ac, fibrinogen activity; aHypo, acquired hypofibrinogenemia; Dys, dysfibrinogenemia; Hypodys, hypodysfibrinogenemia; Hypo, hypofibrinogenemia; NS, non-significant. * and ** indicate significant differences (P<0.05 and P<0.01, respectively) between acquired hypofibrinogenemia and congenital fibrinogen disorders.

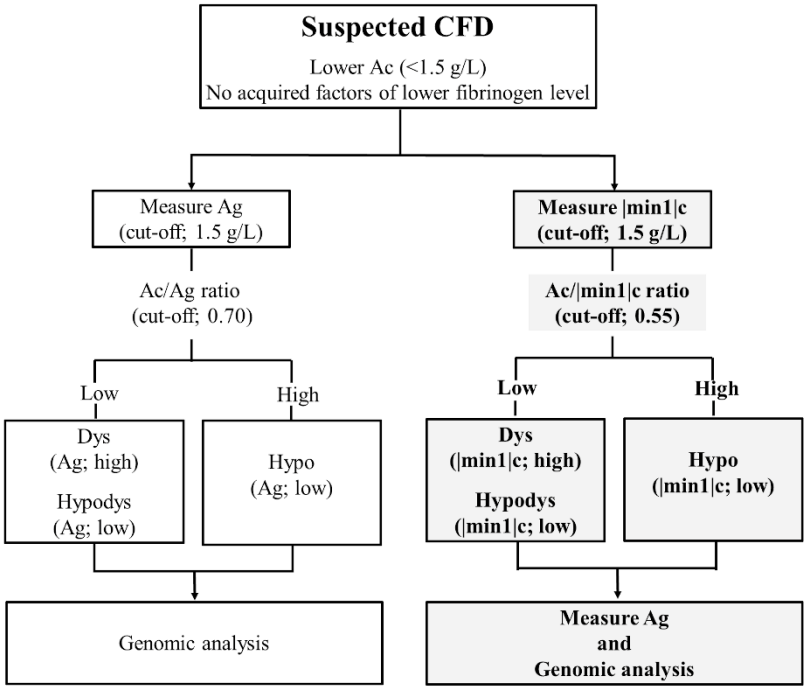

Figure 4. Laboratory flowchart for the screening of congenital fibrinogen disorders using the proposed new parameters

Ac, fibrinogen activity; Ag, fibrinogen antigen; CFD, congenital fibrinogen disorder; Dys, dysfibrinogenemia; Hypo, hypofibrinogenemia; Hypodys, hypodysfibrinogenemia.

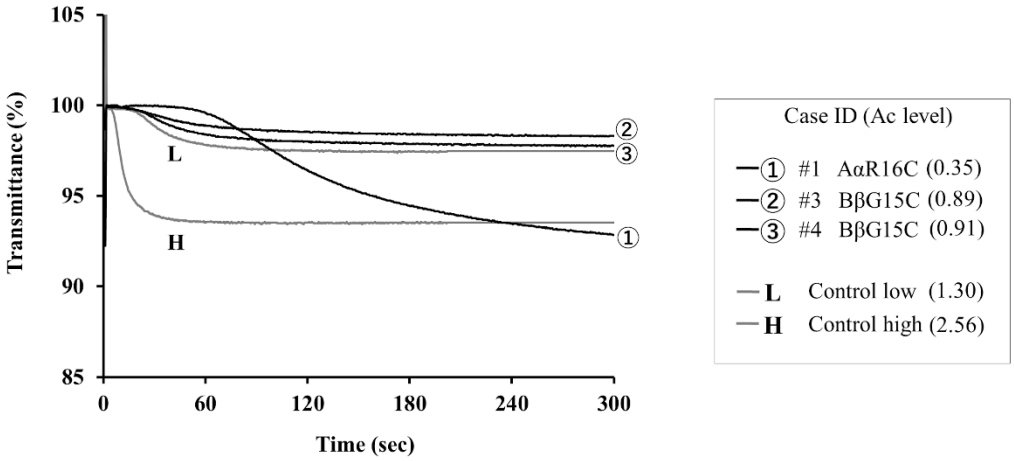

Figure 5. Clot waveforms of dysfibrinogenemia misclassified as hypodysfibrinogenemia or hypofibrinogenemia.

The clot waveforms of three cases with dysfibrinogenemia misclassified as hypodysfibrinogenemia or hypofibrinogenemia using |min1|c and Ac/|min1|c: one with AαR16C, two with BβG15C.