

Overall, the data from RE-VERSE AD should be presented in a clearer and more comprehensive way; thus, enabling clinicians to understand the advantages and shortcomings of idarucizumab. Furthermore, as mentioned above, there is a need for an independent prospective randomized trial, which compares current guideline-recommended practices for reversal of dabigatran with idarucizumab in patients with life-threatening bleedings and those requiring urgent surgery.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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DOI:10.1097/MBC.0000000000000468

OPEN

Usefulness of the second-derivative curve of activated partial thromboplastin time on the ACL-TOP coagulation analyzer for detecting factor deficiencies

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Prolongation of the activated partial thromboplastin time (APTT) may be caused by a factor deficiency, inhibitor of coagulation factors, lupus anticoagulant, liver dysfunction, or anticoagulants such as unfractionated heparin and

direct oral anticoagulants. Waveform analyses of APTT assay results from automated coagulation analyzers have been used for assessing very low levels (<1 IU/dl) of factor VIII (FVIII) and factor IX (FIX) and the in-vivo clotting function in patients with various types of hemophilia A [1–3]. It was reported that the ACL-TOP series of hemostasis testing systems (Instrumentation Laboratory, Bedford, Massachusetts, USA), which monitor absorbance, can detect abnormalities not only in FVIII and FIX but also in factor XI (FXI) and factor XII (FXII) by analyzing the waveform of the second-derivative curve [4]. However, it is not clear what types of deficiency and what levels of each deficiency can be detected by the waveform of the second-derivative curve on an ACL-TOP system.

ACL-TOP analysis produces three types of curves. One is a curve showing the changes in absorbance observed during the measurement of APTT. The second is the first derivative of the absorbance, corresponding to the coagulation velocity. The third type is the second derivative of the absorbance, corresponding to the coagulation acceleration. It was reported that among APTT reagents including silica or ellagic acid as an activator of FXII, the reagent APTT-SP (Instrumentation Laboratory), which includes silica, is suitable for the detection of deficiencies of the intrinsic coagulation factors or lupus anticoagulant when using an ACL-TOP system [4]. In the present study, we sought to determine the usefulness of the second-derivative curve of APTT assayed by the combination of APTT-SP and ACL-TOP to detect deficiencies of the intrinsic coagulation factor.

We made mixtures using normal pooled plasma and deficient plasma of each coagulation factor including factor V, FVIII, FIX, FXI, and FXII, and then we analyzed the second-derivative curve of the APTT assay using APTT-SP. We used calibration plasma (Instrumentation Laboratory) as the normal pooled plasma. At the same time, we measured the levels of each coagulation factor using a one-stage APTT-based clotting assay and SynthASil (Instrumentation Laboratory) as an APTT reagent. All tests were performed on an ACL-TOP.

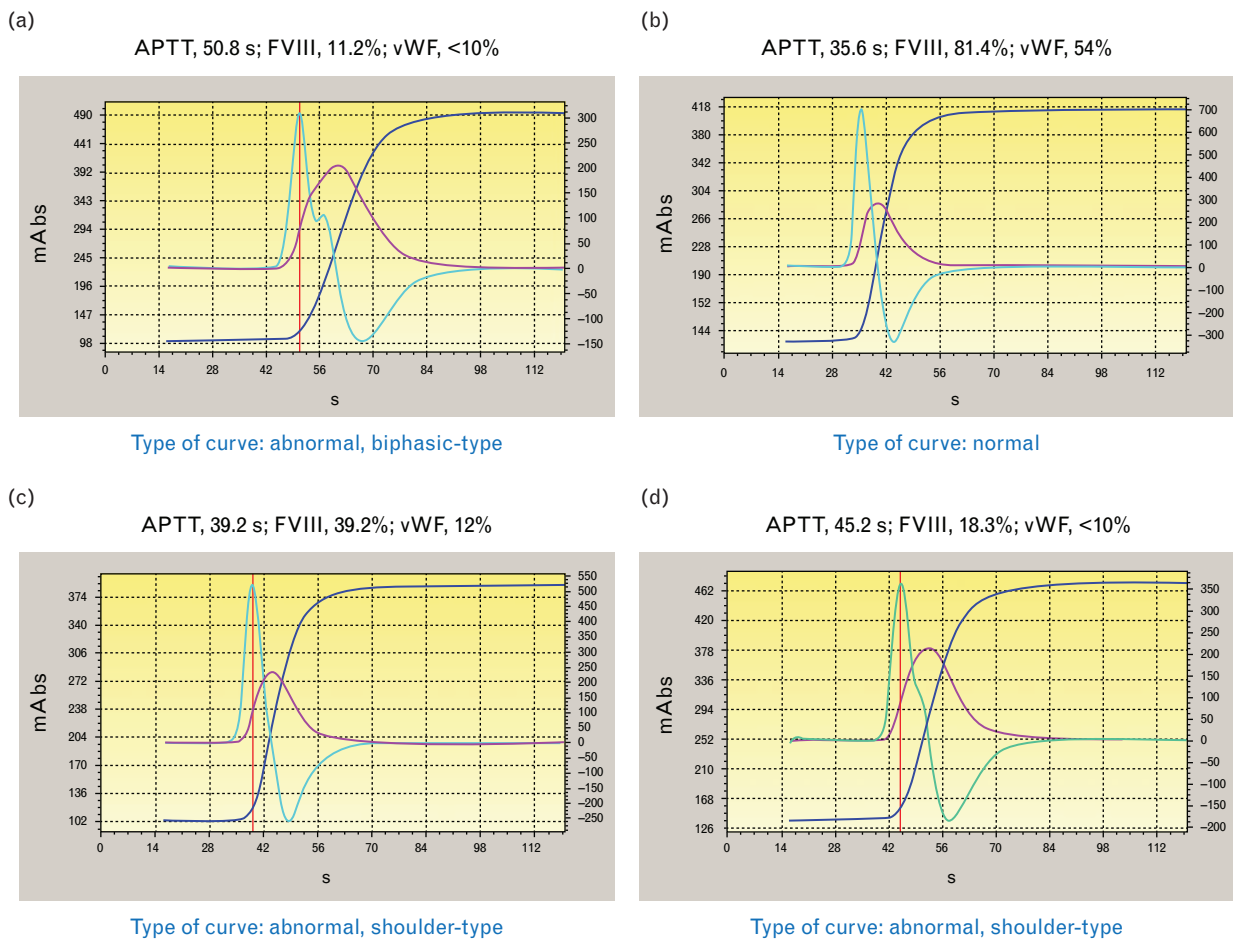
The results revealed two types of abnormal second-derivative curve: a shoulder type in which the peak appears as a notch, and a biphasic type with a double peak. The measured levels of each mixture and the types of the second-derivative curve are shown in Table 1. We detected biphasic abnormal curves for plasma deficient in factor V, FVIII, FIX, and FXI. We also detected a shoulder-type abnormal curve in plasma with FVIII and FIX levels of 2–10%. The shoulder-type abnormal curve was also found for plasma with calculated levels of factor V and FXI of 2–4 and 2%, respectively. We detected only a shoulder-type abnormal curve in

Table 1 Factor levels and type of second-derivative curve in the activated partial thromboplastin time assay

Calculated level of factor (%)	Factor									
	V		VIII		IX		XI		XII	
	Measured level of factor (%)	Type of curve	Measured level of factor (%)	Type of curve	Measured level of factor (%)	Type of curve	Measured level of factor (%)	Type of curve	Measured level of factor (%)	Type of curve
0	0.6	ABN-B	0.3	ABN-B	0.4	ABN-B	0.5	ABN-B	0.4	ABN-S
2	2.5	ABN-S	3.6	ABN-S	5.5	ABN-S	4.1	ABN-S	2.3	N
4	3.9	ABN-S	5.8	ABN-S	6.7	ABN-S	5.8	N	3.8	N
10	10.2	N	11.7	ABN-S	15.1	ABN-S	10.8	N	11.4	N
20	17.7	N	17.9	N	28.5	N	20.9	N	22.7	N
50	46.3	N	48.3	N	64.3	N	46.6	N	52.8	N
80	72.3	N	70.3	N	90.8	N	69.0	N	72.4	N
100	92.8	N	80.7	N	115.7	N	85.0	N	86.4	N

Factor activity was determined in a standard one-stage assay using activated partial thromboplastin time (APTT) SynthASil. APTT assay for waveform analysis was performed using APTT-SP. 'Type of curve' indicates the status of the second-derivative curve in the APTT assay. ABN-B, abnormal biphasic-type; ABN-S, abnormal shoulder-type; N, normal.

Fig. 1



Serial changes of the ACL-TOP second-derivative curve and the levels of factor VIII and von Willebrand factor. (a) Before the desmopressin loading test. (b) After 15 min. (c) After 2 h. (d) After 6 h. An abnormal biphasic curve on the second-derivative curve (light blue) was detected in a patient with von Willebrand factor deficiency. A shoulder-type abnormal curve was detected at 2 and 6 h after the desmopressin loading test. APTT, activated partial thromboplastin time; FVIII, factor VIII; vWF, von Willebrand Factor.

Table 2 Effect of increased level of factor VIII on the second-derivative curve in the activated partial thromboplastin time assay in plasma derived from patients with acquired von Willebrand disease or hemophilia A

Added concentration of FVIII (FC; IU/ml)	Patients							
	Patient with acquired vWD				Patient with hemophilia A			
	vWF:Ag (%)	FVIII:C (%)	APTT (s)	Type of curve	vWF:Ag (%)	FVIII:C (%)	APTT (s)	Type of curve
0.0	7.1	6.7	45.2	ABN-B	147.5	7.5	45.1	ABN-S
0.1	7.4	18.9	38.2	ABN-S	142.8	17.8	39.7	ABN-S
0.2	6.6	30.0	36.0	ABN-S	112.3	27.9	38.3	ABN-S
0.3	6.8	41.8	35.5	ABN-S	108.6	36.6	37.5	ABN-S
0.4	7.6	57.8	34.9	ABN-S	104.5	48.5	36.8	N
0.5	5.9	68.4	34.6	ABN-S	98.3	57.3	36.2	N

Factor VIII concentrate was added to the plasma derived from patients with acquired von Willebrand disease (vWD) or hemophilia A. The level of factor VIII (FVIII) was increased by FVIII concentrates in both patients' plasmas, but the abnormal curve was not disappeared only in plasma derived from a patient with acquired vWD. Type of curve indicates the status of the second-derivative curve in the activated partial thromboplastin time assay. N, normal; ABN-B, abnormal, biphasic-type; APTT, activated partial thromboplastin time; ABN-S, abnormal, shoulder-type; FC, vWF, and FVIII:C, final concentration, antigen level of von Willebrand factor, and factor VIII activity, respectively. Supplementary data, <http://links.lww.com/BCF/A24>.

the FXII-deficient plasma. In the same manner, we confirmed that there was nothing abnormal about the second-derivative curves for mixtures of FII, FVII, and factor X or in plasma derived from 30 healthy individuals. These results suggest that the analysis of the second-derivative curves of APTT on an ACL-TOP might be useful for detecting factor deficiencies in factor V, FVIII, FIX, FXI, and FXII – especially FVIII and FIX.

To investigate the effect of von Willebrand factor (vWF) on the second-derivative curve, we monitored the serial changes of waveforms in an APTT assay after desmopressin (1-deamino-8-D-arginine vasopressin; DDAVP) infusion treatment for a patient with acquired von Willebrand syndrome showing less than 10% of both vWF ristocetin cofactor activity and vWF antigen (Fig. 1) [5]. DDAVP is the treatment of choice not only for type I von Willebrand disease, but also for acquired von Willebrand syndrome [6] because it can induce the release of normal vWF from cellular compartments. After 15 min of DDAVP infusion for this patient, the levels of vWF and FVIII were greatly increased to 54 and 84%, respectively. In accord with the increase of vWF and FVIII levels, the abnormal biphasic peaks on the second-derivative curve for the patient's plasma disappeared. Also, in accord with the decreases in the vWF and FVIII levels, an abnormal curve (shoulder type) reappeared at 2 and 6 h after the DDAVP loading test.

To assess the direct effects of vWF on the waveform analysis, we added FVIII concentrates (ADVATE, a recombinant, human, full-length coagulation factor VIII) into the plasma derived from patients with acquired vWD or hemophilia A (Table 2). In case of acquired vWD, the abnormality in the curve did not disappear despite the increased level of FVIII. On the contrary, that for the plasma of hemophilia A disappeared with an increase in FVIII. These results indicate that the plasma level of vWF directly influences the second-derivative

curve in the APTT assay. Moreover, the strength might be higher than that of FVIII.

These results suggest that the waveform analysis of second-derivative curves of an APTT assay might be a useful tool not only to detect the deficiency of vWF, but also to assess the effect of DDAVP infusion treatment.

In conclusion, a waveform analysis of the second-derivative curve of an APTT assay with the combined use of APTT-SP and an ACL-TOP system might be useful not only for detecting deficiencies of intrinsic coagulation factor and vWF, but also for assessing the results of DDAVP infusion treatment for patients with acquired von Willebrand syndrome. However, further investigations using plasma derived from patients with factor deficiencies are necessary to assess the usefulness of waveform analyses by this system.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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