


Update on the Clot Waveform Analysis

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Abstract

The activated partial thromboplastin time (APTT)–clot waveform analysis (CWA) was previously reported to be associated with the early detection of disseminated intravascular coagulation and was also reported to be able to measure very low levels of coagulation factor VIII activity. The software program for the analysis for the APTT-CWA allows the associated first and second derivative curves (first and second DCs) to be displayed. The first and second DC reflect the velocity and acceleration, respectively. The height of the first DC reflects the “thrombin burst” and bleeding risk, while that of the second DC is useful for detecting any coagulation factor deficiency and abnormal enhancement of coagulation by phospholipids. Activated partial thromboplastin time-CWA aids in making a differential diagnosis which is difficult to do using only the routine APTT. The CWA is currently used for many applications in the clinical setting, including the monitoring of hemophilia patients and patients receiving anticoagulant therapy and the differential diagnosis of diseases.

Keywords

CWA, APTT, hemophilia, DIC

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Introduction

Various assays are used to evaluate the blood coagulation system, including but not limited to the activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time, thromboelastography (TEG),¹ and the thrombin generation test (TGT).² As the APTT and PT are inexpensive and allow the easy performance of multiple assays, they are frequently measured as routine assays. However, the information they provide has some limitations. In contrast, TEG¹ and TGT² show a different pattern of information but are more expensive and time-consuming to be applied as routine assays at the present time. The blood coagulation system involves specific mechanisms: the cascade system,³ thrombin burst,⁴ and the enhancement of clotting activation by phospholipids (PLs)⁵ (Figure 1). In the cascade system, the activation of one molecule of coagulation factor XI (FXIa) finally generates 200 000 000 molecules of fibrin monomer. Thrombin converts FXI, coagulation factor VIII (FVIII), and FV to activated FXI (FXIa), activated FVIII (FVIIIa), and activated FV (FVa), respectively. Then, FXIa, FVIIIa, and FVa further activate the downstream coagulation factors of the coagulation system. Finally the generation of thrombin causes an activation cycle from thrombin to FXIa. Phospholipids enhance the activation of FX by activated coagulation factor IX (FIXa) and FVIIIa to generate FXa on the

surface of platelets, which is called Xase and the activation of prothrombin by FXa and FVa to generate thrombin on the surface of platelets which is referred to as prothrombinase complex.

The routinely measured APTT reflects the status of the “cascade system in plasma” but does not sufficiently reflect the “thrombin burst” or “enhancement of clotting activation by PLs. In contrast, the automatic optical end-point coagulation analyzers have the ability to show the clot reaction curve of the PT and APTT and to reflect the “thrombin burst” and “enhancement of clotting activation by PLs”; this is referred to as, a clot waveform analysis (CWA).

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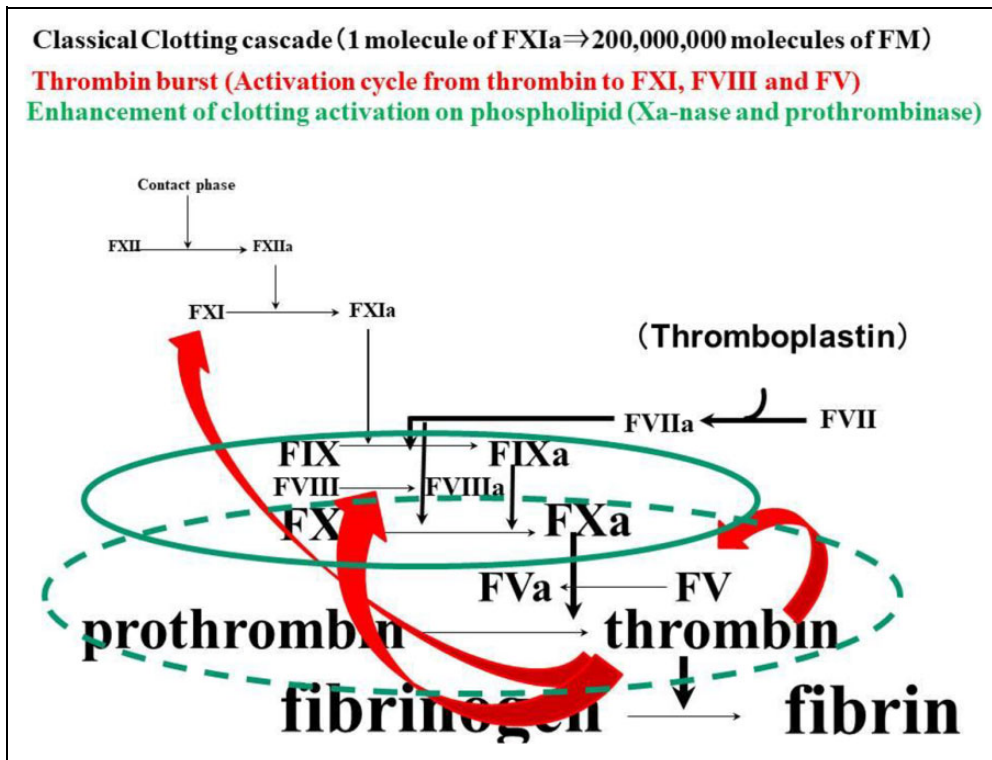


Figure 1. The hemostatic system including the clotting cascade, thrombin burst, and the enhancement of clotting activation by phospholipid. FM indicates fibrin monomer; FV, coagulation factor V; FVa, activated FV; FVII, coagulation factor VII; FVIIa, activated FVII; FVIII, coagulation factor VIII; FVIIIa, activated FVIII; FIX, coagulation factor IX; FIXa, activated FIX; FX, coagulation factor X; FXa, activated FX; FXI, coagulation factor XI; FXIa, activated FXI; FXII, coagulation factor XII; FXIIa, activated FXII.

The History of CWA/APTT

The APTT is a clotting time assay that is useful for diagnosing deficiencies in the coagulation factors of the intrinsic pathway, such as hemophilia,⁶ the presence of inhibitors, such as lupus anticoagulant (LA),⁷ and acquired hemophilia and for monitoring heparin treatment.⁸ All coagulation tests were in the beginning performed by hand and so the timing was done using a stop watch; thus multiple assays and standardization of APTT were difficult to carry out with this manual method. The development of automatic optical coagulation analyzers has made it easy to perform multiple assays and enabled the standardization of the APTT reagents for LA and heparin.

Furthermore, optical coagulation analyzers are capable of showing the clot reaction curve of APTT. The MDA automated coagulation analyzer, was first able to show the clot reaction curve to detect a biphasic form in disseminated intravascular coagulation (DIC) or very low levels of FVIII activity. Previous reports have highlighted the usefulness of the visual inspection of APTT clot reaction curves, and abnormal biphasic clot reaction curves have been reported to be associated with the early detection of DIC.^{9,10} The biphasic clot reaction curve was reported to be caused by a complex with C-reactive protein, very low-density lipoprotein, and calcium ion.¹¹ Additionally, the clot reaction curves with an abnormal biphasic pattern have been linked to morbidity and mortality in patients with DIC.¹² The APTT-CWA has been also reported to detect very low

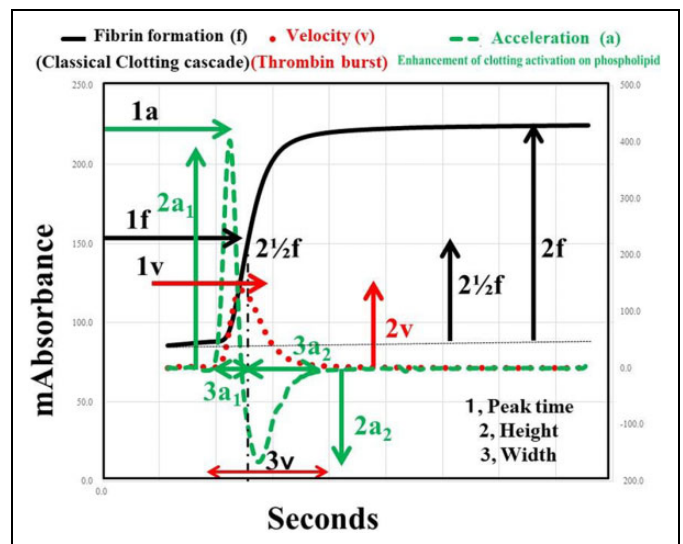


Figure 2. Parameters of CWA (APTT). a indicates acceleration (second derivative curve, DC); APTT, activated partial thromboplastin time; CWA, clot waveform analysis; F, fibrin formation curve; v, velocity (first derivative curve; DC); 1, peak time; 2, peak height; 3, peak width.

levels of FVIII activity in patients with hemophilia A.¹³ It was reported that the wavelengths above 650 nm are recommended to perform CWA and most of the commercialized reagents can be used for CWA.¹⁴

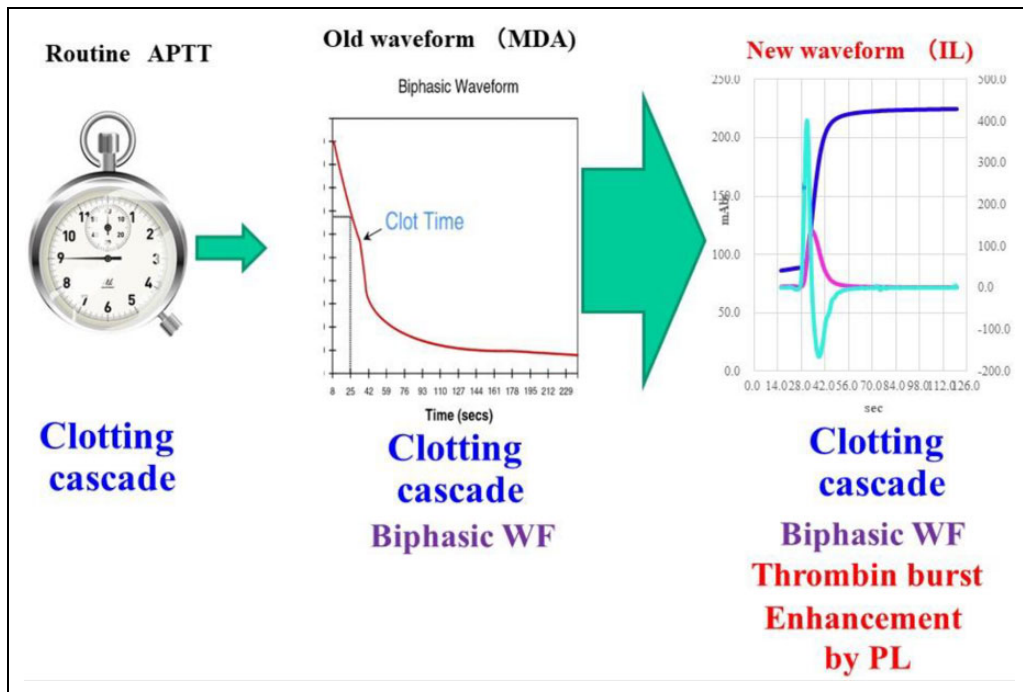


Figure 3. Development of CWA (APTT). New waveform shows fibrin formation curve, first derivative curve and second derivative curve. APTT indicates activated partial thromboplastin time; CWA; clot waveform analysis; IL, Instrumentation Laboratory; PL, phospholipid; WF, waveform.

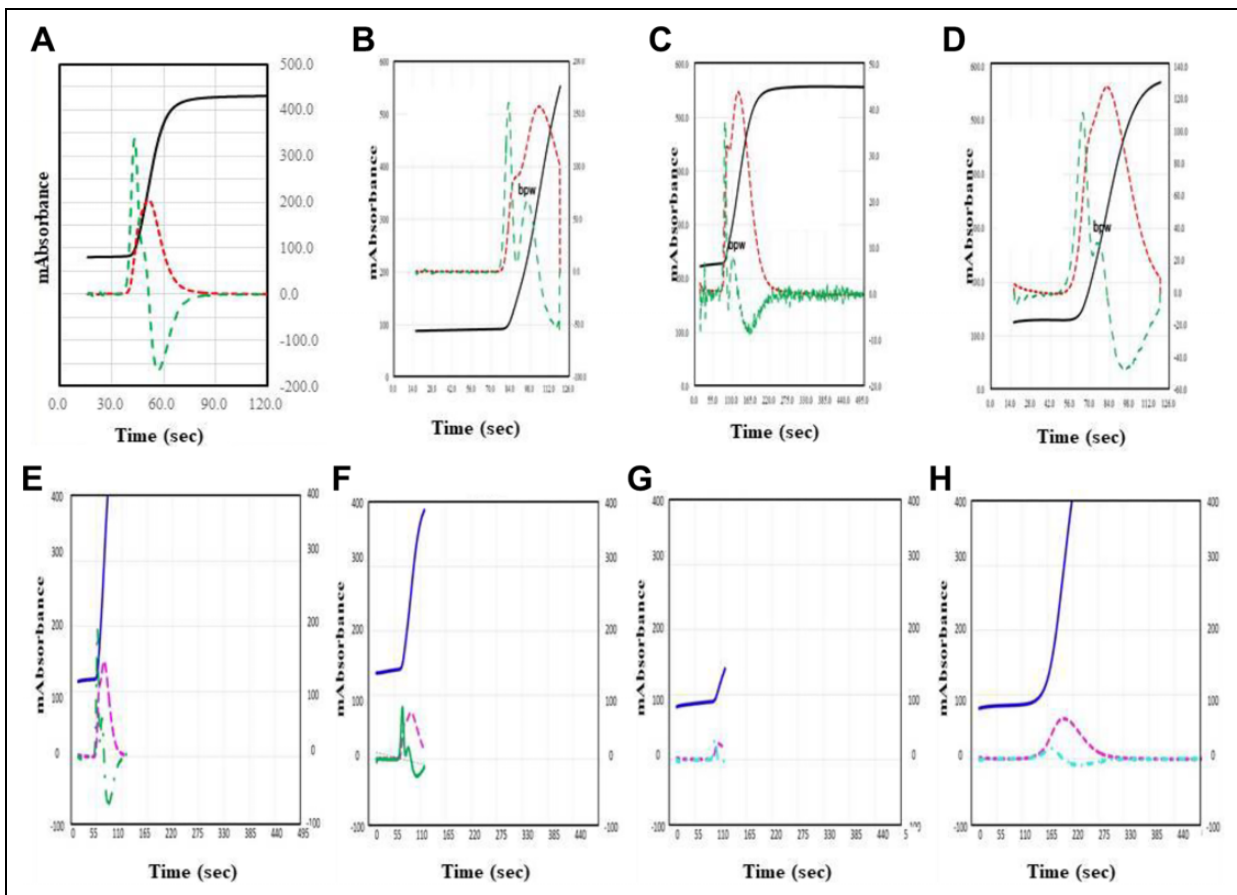


Figure 4. The CWA (APTT) in mild hemophilia (A, E), severe hemophilia (B, F), hemophilia with inhibitor (C, G), and lupus anticoagulant (D, H). Upper column (A-D), automatic enlargement of the waveform; Lower column (E-H), without the automatic enlargement of the waveform. APTT indicates activated partial thromboplastin time; CWA; clot waveform analysis.

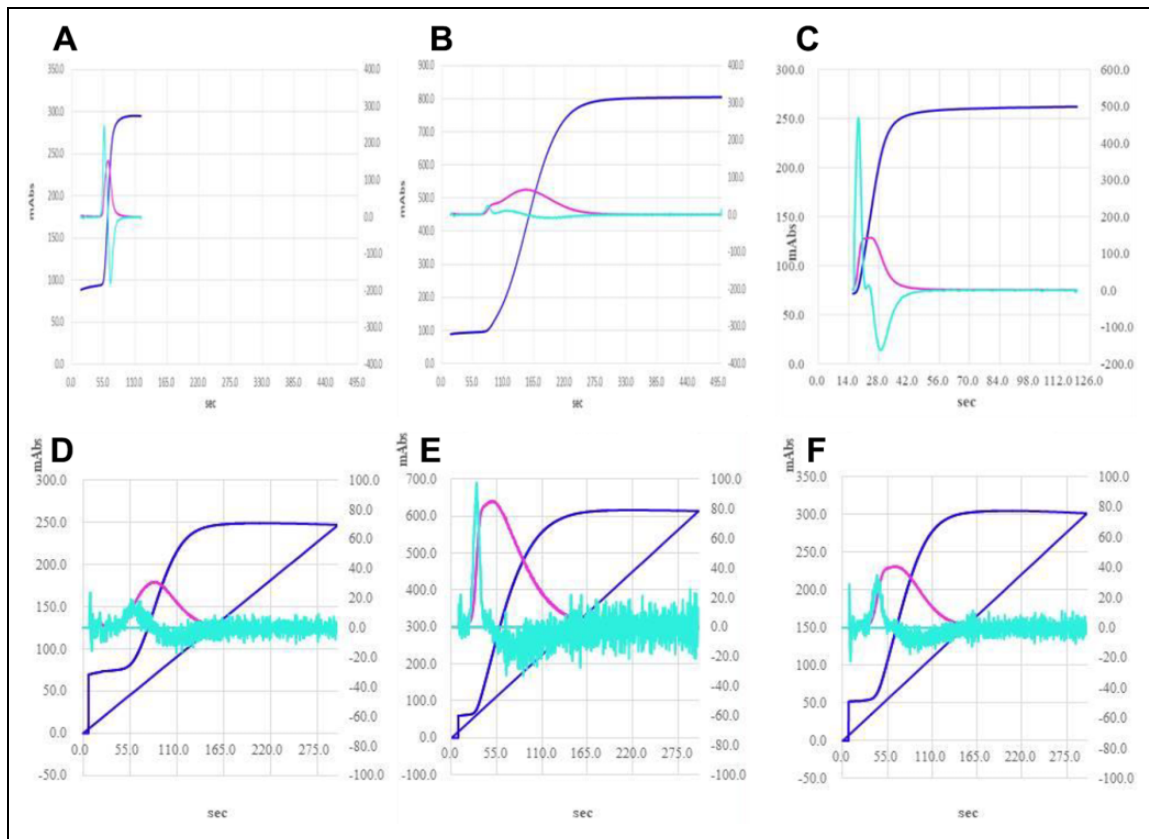


Figure 5. The CWA (APTT and small amount of tissue factor; TF/coagulation factor IX; FIX assay) in patients with inhibitor treated with activated prothrombin complex concentrate (APCC, A and D), recombinant human activated coagulation factor VII (rhVIIa, B and E) or emicizumab (C and F). Upper column (A-C), APTT/CWA; Lower column (D-F), small amount of TF/FIX assays (D-F), APTT indicates activated partial thromboplastin time; CWA; clot waveform analysis.

The ACL TOP¹⁵ and CS 2000i¹⁶ analyzers have special software program which automatically calculates the first and second derivative plots from the absorbance data. These automated photo-optical coagulation analyzers, which are used for the measurement of the APTT not only makes it possible to display the clot reaction curves but also allows the associated first and second derivative curves (first and second DCs) to be displayed. The delta absorbance plots are divided by delta time to obtain the first DCs corresponding to the velocity. The delta velocity plots are divided by delta time to obtain the second DC corresponding to the acceleration. The peak time, height, and width are obtained in each clot reaction curve (fibrin formation curve [FFC]), first DC and second DC (Figure 2). The height of the first DC in the APTT-CWA is considered to reflect to the “thrombin burst” as real hemostatic ability. Low height of the first DC in the APTT-CWA suggests the bleeding risk. The height of the second DC in the APTT-CWA is useful for detecting any coagulation factor deficiency¹⁷ and the abnormal enhancement of coagulation activity by PLs such as LA. The differential diagnosis between coagulation factor deficiency and LA or between hemophilia and acquired hemophilia¹⁰ is difficult to make based on the results of a clotting time assay without the CWA (Figure 3). Several reports^{6,18} have also recommended the

peak height of first DC and second DC of the APTT-CWA for the evaluation of hemophilia.

Differential Diagnosis by CWA

Regarding the pattern of the APTT waveform, a biphasic pattern of acceleration (second DC) or velocity curve (first DC) was observed in hemophilic patients with and without inhibitor, patients positive for LA, and patients with DIC (Figure 4B-D). The peak time of the first DC, second DC, and FFC were significantly prolonged in these patients. The routine APTT-CWA (Figure 4A-D) is automatically enlarged to visualize biphasic pattern, which allows hemostatic abnormalities to be easily recognized; however, it is not able to further differentiate among these diseases.¹⁹ Although the FVIII activity is measured based on the peak time of FFC in APTT, better correlation with FVIII activity is observed in the peak height of the first and second DC than in the peak time of the first and second DC and FFC.²⁰ Other reports^{6,16} have also recommended the peak height of first and second DC for evaluating hemostatic abnormalities in hemophilia. Although we routinely measure the peak time of FFC in the measurement of APTT, these findings suggest that the peak height of the first and second DC in APTT may be more useful for evaluating the FVIII

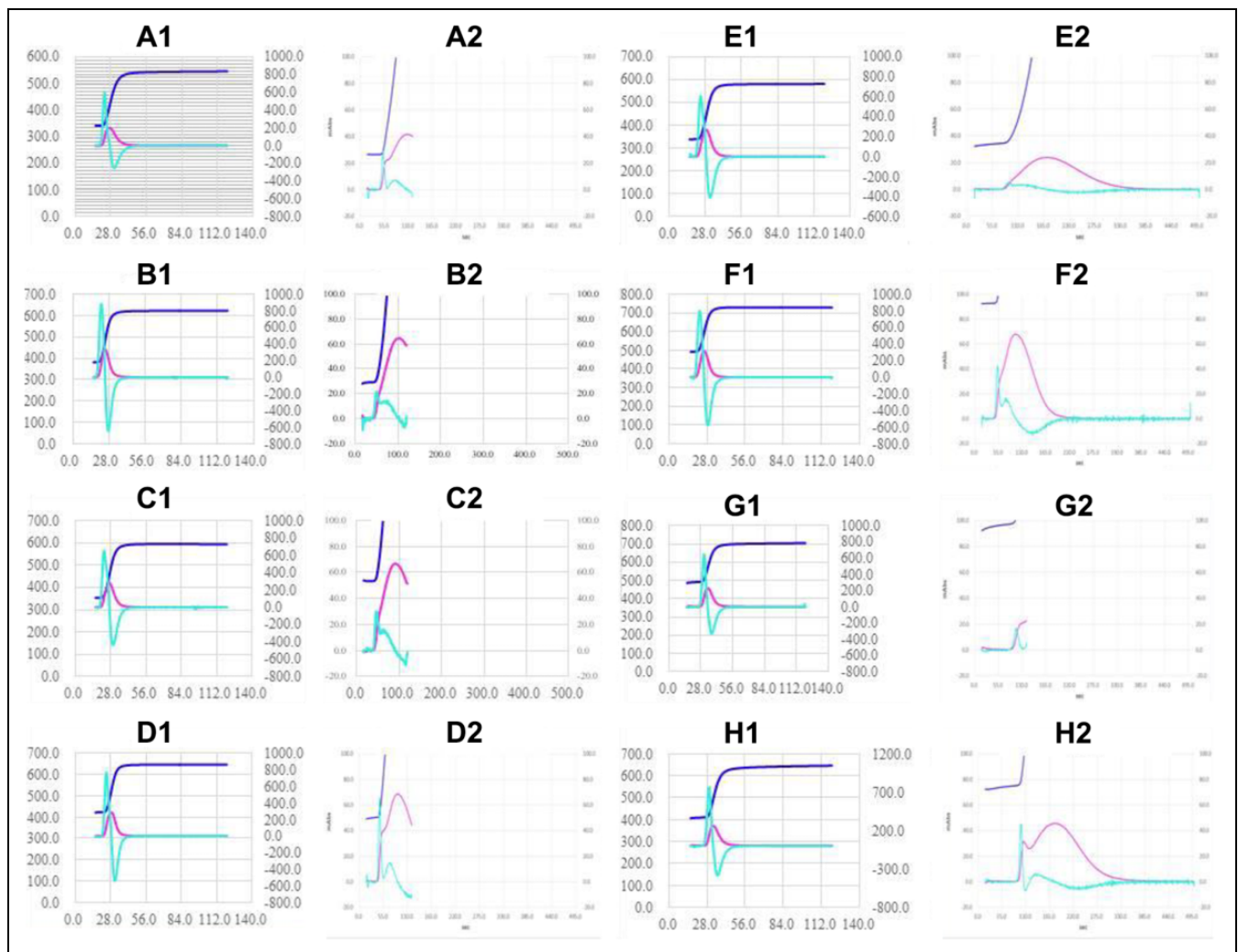


Figure 6. The APTT-CWA in various APTT reagents for normal plasma and 1% of FVIII plasma. 1, normal plasma; 2, 1% of FVIII plasma; (A) Sekisui Coagpia APTT-N; (B) Sysmex Thrombocheck APTT; (C), Sysmex Thrombocheck APTT-SLA; (D) Datafei APTT(FS). Actine FS; (E) Datafei APTT(FSL) Actine FSL; (F) Datafei APTT Actine; (G) Stago; (H) IL Synthetic. APTT indicates activated partial thromboplastin time; CWA; clot waveform analysis; FVIII, coagulation factor VIII.

activities in hemophilia. The automatic enlargement of the APTT wave size allows for the easy detection the hemostatic abnormalities, but the evaluation of the peak height of APTT cannot be used to make a differential diagnosis at a glance. Thus, a software program without automatic enlargement of CWA allows the peak height to be easily recognized would be recommended for the differential diagnosis of hemostatic abnormalities²¹ (Figure 4E and F). The peak heights of the first and second DCs are significantly low in hemophilia patients with inhibitor and patients with acquired hemophilia A.¹⁹⁻²¹

Monitoring for the Treatment of Hemophilia-Related Diseases by CWA

Hemophilia patients with inhibitors are frequently treated with bypass therapy using agents, such as recombinant human activated FVII (rhFVIIa), activated prothrombin complex concentrate (APCC), and emicizumab.²² In patients with FVIII

inhibitor, the effect of APCC is clear on the APTT-CWA, but it is difficult to monitor other bypass therapies, especially rhFVIIa and emicizumab²³ (Figure 5A-C). Recombinant human activated coagulation factor VII (rhVIIa) seems to be significantly effective for patients with inhibitors for that show small values in a tissue factor (sTF)/FIX assay (Figure 5E and F). The FVIII activity can also be measured by an sTF/FIX assay.²⁴ Indeed, the FVIII activity measured by APTT methods were found to be well correlated with those measured by sTF/FIX methods; however, the FVIII activity measured by the sTF/FIX method was significantly higher than that measured by the APTT method in patients treated with rhVIIa.²⁴ This was due to the efficacy of rhVIIa. The modified APTT-CWA was reported to be useful for evaluating the efficacy of emicizumab.²⁵ These findings suggest that APTT-CWA may be useful for monitoring APCC therapy and that the sTF/FIX-CWA may be useful for monitoring rhVIIa therapy, but that further modification of the CWA may be required for monitoring emicizumab therapy.

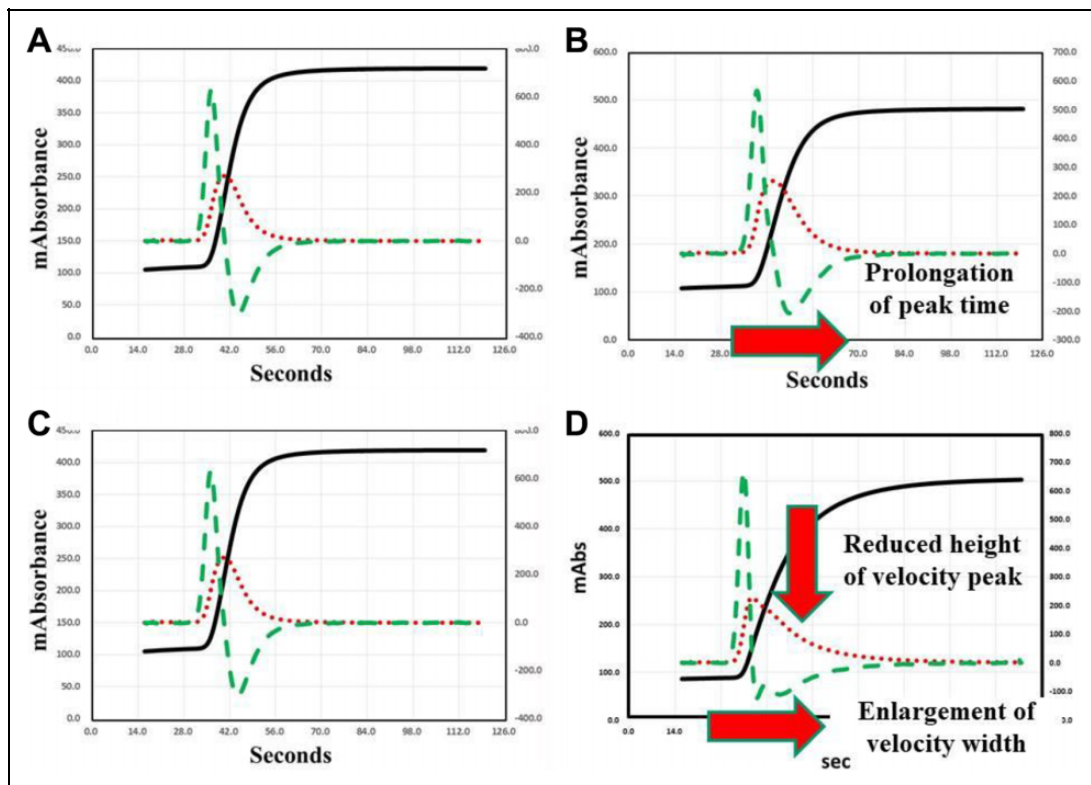


Figure 7. Difference in the APTT-CWA between edoxaban and warfarin treatment, A and C, Before treatment with edoxaban or warfarin; (B) treatment with edoxaban; (D) treatment with warfarin. APTT-CWA indicates activated partial thromboplastin time–clot waveform analysis.

Prophylactic treatment with FVIII concentrate is preferred to prevent bleeding and joint damages in children with severe hemophilia.^{26,27} Extending the half-life FVIII (EHL-FVIII) would substantially improve treatment options for patients with hemophilia A.^{27,28} The attachment of polyethylene glycol (PEG) has been considered an effective method for prolonging the half-life of recombinant FVIII.^{29,30} However, it was recently reported that the FVIII activity in patients treated with EHL-FVIII, including PEG-FVIII, varied among various APTT reagents.³¹ An APTT-CWA may be able to reveal the differences among various APTT reagents, as the difference in the APTT-CWA was enlarged in patients with lower concentrations of FVIII (Figure 6).

Monitoring for Anticoagulant Therapy

There are no routine assays that are useful monitoring in patients receiving direct oral anticoagulants (DOACs) such as anti-Xa inhibitors. In addition, the monitoring of DOAC treatments is important for determining the appropriate blood sampling time after the administration of DOACs, as their half-life in blood is shorter than that of warfarin. Although it is considered unnecessary to monitor patients receiving DOACs, major bleeding is sometimes observed in the patients treated with DOACs.³² Low levels of anti-Xa activity may be risk of the onset of deep vein thrombosis^{33,34} and a prolong APTT-CWA peak time may be a risk factor for major bleeding³⁵ in orthopedic patients treated with anti-Xa inhibitors. Warfarin

decreased the height of the first and second DC in the APTT-CWA and prolonged the width of the first DC in the APTT-CWA, while edoxaban prolonged the peak time of the first and second DC in the APTT-CWA (Figure 7).³⁵ These differences in the APTT-CWA of patients treated with anti-Xa inhibitors and those treated with warfarin reflect to the difference in the effects of these drugs. That is, patients with anti-Xa inhibitors are considered to have a lower bleeding tendency.^{36,37} The basic analysis of the APTT-CWA demonstrated pharmacological evidence of the blockade of thrombin-positive feedback by antithrombin and anti-Xa inhibitors and differences in anticoagulant cooperativity between them.^{38,39}

Clot Waveform Analysis in Other Diseases

The detection of abnormal biphasic clot reaction curves on the APTT-CWA has been reported to facilitate the early detection of DIC.^{9,10} Recently, the APTT-CWA of infectious disease patients with DIC have been reported; while the heights of the first and second DCs of the APTT-CWA were significantly high in infectious disease patients without DIC, they were significantly reduced in patients with DIC, especially bleeding-type DIC.⁴⁰ These findings suggest that the reduction in the height of the first and second DCs of the APTT-CWA is more important than the biphasic waveform in the diagnosis of DIC. The APTT-CWA has also been reported to be useful for the diagnosis of DIC and the prediction of major bleeding and a poor outcome.⁴⁰

As thrombophilia associated with factors such as protein C, protein S, and antithrombin abnormalities, antiphospholipid antibody syndrome, and pregnancy is frequently associated with thrombosis,⁴¹ the evaluation of these patients to detect hypercoagulability is important. However, there were no significant differences in the results of routine APTT and PT assays between healthy individuals and thrombophilia patients with hypercoagulability. The routine APTT and PT which are based on the peak time of the FFC may tend to be decreased; however, the difference is not statistically significant. The heights of the first and second DCs of the APTT-CWA were reported to be increased in pregnant women and postoperative patients.^{19,35} It was considered that pregnant women and postoperative patients are at increased risk of developing thrombosis and hypercoagulability.⁴² The increased heights of the first and second DCs of the APTT-CWA may be a marker of hypercoagulability. The hypercoagulability in Kawasaki disease was also reported using CWA assay.⁴³ Recently the clot-fibrinolysis waveform analysis was reported in hemorrhagic disorders,⁴⁴ suggesting hyperfibrinolysis could be evaluated by modified CWA which is added with tissue plasminogen activator.

Conclusion

The CWA is useful for the differential diagnosis of diseases and the monitoring of hemophilia patients undergoing treatment and thrombophilic patients on anticoagulation and expected to be developed for more informative applications.

Authors' Note

Wada H fully wrote this manuscript. Matsumoto T and Ohishi K reviewed these references. Shiraki K and Shimaoka M discussed and gave the suggestions for this manuscript. The APTT-CWF assay was supported by the Instrumentation Laboratory. The study protocol was approved by the Human Ethics Review Committee of the Mie University School of Medicine, and a signed consent form was obtained from each participants. This study was faithfully carried out in accordance with the principles of the Declaration of Helsinki.


Declaration of Conflicting Interests

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