Advances and Innovations in Diagnosis and Hemostasis Monitoring on Hemophilia

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Profile

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Hemophilia A and B is diagnosed, based on the coagulation factor (F) VIII and IX activity, respectively. This disorder is classified into three groups, severe (< 1 IU/dL), moderate (1 - < 5 IU/dL), and mild type (5 - < 40 IU/dL), since FVIII or FIX activity correlates with the clinical severity. Although this activity is measured by conventional one-stage clotting assay based on activated partial thromboplastin time (aPTT), we often experience cases where the severity of the disease based on the activity level does not correlate with the clinical symptoms. Also, it is difficult to monitor hemostasis potentials when alloantibodies (inhibitors) have appeared, which is one of the serious unmet needs in hemophilia therapy. In addition, although innovative advances have been made in terms of hemostatic products for hemophilia, there are limitations to conventional aPTT-base hemostatic monitoring, as it is not always possible to accurately measure coagulation potential depending on the products. To solve these issues, comprehensive coagulation function measurement has been developed to evaluate hemostatic coagulation function. In particular, coagulation waveform analysis (CWA) can monitor the changes of permeability in plasma sample during fibrin formation in the prothrombin time (PT) and aPTT measurement systems and depict the entire coagulation process as coagulation waveforms. Computer analysis of the data enables quantitative evaluation of the dynamic process of coagulation and fibrinolysis from this waveform and the obtained parameters. Innovations in coagulation measurement devices have made it possible to more accurately reflect the coagulation assessment of patients with hemophilia and are now leading to improvements in hemophilia treatment care.

Key Words：Hemophilia A, Factor VIII, Inhibitor, Clot Waveform Analysis, Coagulation, Fibrinolysis

Introduction

Hemophilia is a quantitative and qualitative disorder of blood coagulation factor VIII (FVIII) and factor IX (FIX) caused by genetic abnormalities, which develops in two types, namely, hemophilia A and B, respectively. It is the most prevalent of congenital coagulation disorders. The two types are nearly the same in clinical pathology; serious hemorrhagic symptoms, mainly deep bleeding in joints and muscles, develop because of disorders of the reaction to activate endogenous factor X (FX) in the FX complex, which represents the axis of the mechanism of blood coagulation reactions. In the clinical practice for hemophilia, it is a common approach to measure factor activities to determine its clinical severity and the hemostatic effect of treatment with relevant factor products. Nevertheless, some cases exist where hemostatic (prophylactic) management is difficult simply by assessing the factor activity. For this reason, there have been advances not only in simple assessments of factor activity alone but also in comprehensive assessments of coagulation function as a whole, making it possible to more extensively understand the hemostatic potentials of individual patients in terms of blood coagulation using a conventional factor activity assay in combination with a comprehensive coagulation functional assay.

In this paper, we describe the present status of comprehensive coagulation functional assays and their applicability to clinical practice for hemophilia.

Comprehensive coagulation functional assessments

1. Developmental background

In hemophilia, FVIII and FIX activity levels are generally correlated with clinical severity. Regarding factor activity as measured using the one-step coagulation technique, levels of < 1 IU/dL are defined as severe; 1 to < 5 IU/dL as moderate; and 5 to 40 IU/dL as mild[1]. In reality, however, clinicians often encounter cases of severe hemophilia diagnosed as such but with very mild hemorrhagic symptoms, and reverse cases of moderate or mild hemophilia diagnosed as such but with severe hemorrhagic symptoms[2]. These discrepancies between the factor activity and clinical severity are considered to involve a wide variety of aspects, including the choice of assay. The one-step coagulation technique may be essentially subject to limitations because of the concept that the body's coagulation/hemostatic potential as a whole is well reflected solely by FVIII and FIX activities as determined using the technique, because it is based on measurements of activated partial thromboplastin time (aPTT) taken under nonphysiological conditions. Other coagulation factors are known to influence the clinical severity[2].

In hemostatic treatment for patients with hemophilia, conversely, repeated replenishment of a coagulation factor product leads to the development of a homogeneous antibody (inhibitor) for the factor. Since the hemostatic effect of adjunctive therapy with the factor product lessens or disappears, hemostatic management with a bypass hemostatic agent is performed; nevertheless, assessing its effect in terms of factor activity is difficult. Contrary to this background, an assay for assessing the comprehensive coagulation/hemostatic potential has traditionally been performed, in place of factor activity assays, exclusively at medical institutions specializing in hemophilia. In particular, thromboelastography, clot waveform analysis, and thrombin production testing are currently in practical applications[3]. All these methods are computerized to enable qualitative assessments of coagulation processes and even quantitative assessments using various calculation parameters. Clot waveform analysis is described in detail below.

2. Clot waveform analysis (CWA)

The commonly used coagulation functional testing is based on prothrombin time (PT) and aPTT, and it assesses only the precoagulation phase from the addition of $CaCl_2$ to the start of coagulation. The coagulation reaction to cause fibrin formation at a constant rate after the start of coagulation proceeds through a sequence of processes, from the precoagulation phase to the postcoagulation phase occurring after the end of the coagulation reaction; thus, it is important to assess all these processes. CWA enables us to delineate all the processes of coagulation in the form of clot waveforms by monitoring changes in the transmittance of plasma samples in the fibrin formation in an ordinary PT or aPTT assay reaction system.

The resulting data are computer-analyzed to determine the first-order differential

coagulation velocity from the clot waveform and the second-order differential coagulation acceleration, and their maximum parameters are calculated to obtain the maximum coagulation velocity (|min1|) and maximum coagulation acceleration (|min2|), respectively (**Fig 1**). The dynamic processes of coagulation can be described by the clot waveform and parameters to enable quantitative assessments[4]. Recently, there has been an increasing availability of measuring equipment capable of CWA[5].

Facts of comprehensive coagulation functional analysis

1. Assessing trace FVIII activity

The detection limit for FVIII and FIX activity is reportedly 1 IU/dL. Nevertheless, aPTT CWA of plasma samples from patients with severe hemophilia A having FVIII activity levels of < 1 IU/dL showed that waveform patterns differed among different patients[6]. Although all those patients had FVIII activity levels of < 1 IU/dL, some had a longer precoagulation phase and an extremely slow gradient following the start of coagulation, and others had a shorter precoagulation phase and a sharp gradient in the coagulation phase; patients with severe hemophilia were shown to have individually variable coagulation function. Thus, we generated a standard curve by adding sequential dilutions of recombinant FVIII to FVIII-deficient plasma and demonstrated that the curve could detect FVIII at very low levels of < 1.0 IU/dL in CWA, with a detection sensitivity of < 0.2 IU/dL. When plasma samples from 32 patients with severe hemophilia A having FVIII activity of < 1 IU/dL were analyzed using CWA, 27 patients were found to have FVIII activity of < 0.2 IU/dL, with extremely low levels of |min2|. The remaining five patients had activity levels of 0.2–1.0 IU/dL. Of the 27 patients, 24 were severely symptomatic, whereas the other three were mildly

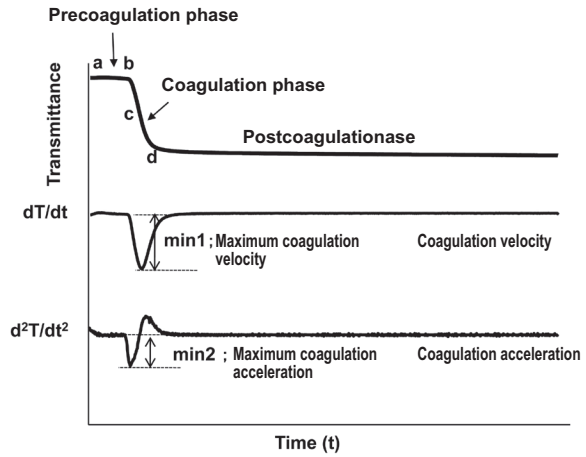

Fig 1. Clot waveform analysis in normal plasma

This figure shows a clot waveform, first-order differential waveform, second-order differential waveform, and their parameters, obtained from normal plasma.

symptomatic; the majority of hemophilia A cases with < 0.2 IU/dL were clinically severe, but some cases were only mildly affected. The above findings showed that CWA could well reflect trace amounts of FVIII.

2. Assessing patients with hemophilia in terms of blood coagulation—"Coagulotype"

In clinical settings, we concurrently assess the factor activity and comprehensive coagulation function in not only severely but also moderately or mildly affected patients with hemophilia to obtain comprehensive assessments of the essential coagulation and hemostatic potential of the patient, thus helping draw up a hemostatic (prophylactic) management plan. We call this comprehensive coagulation and hemostatic potential of the patient "coagulotype". Particularly, moderate or mild cases of hemophilia are often caused due to point mutations of the FVIII (FIX) gene, sometimes showing discrepancies between their factor activity and comprehensive coagulation potential. The following presents our case of severe hemophilia A with a moderate coagulotype rating[7]. A patient with severe hemophilia A (R1781H point mutation). The patient in childhood experienced difficulty with hemostasis for a forehead cut, had an FVIII activity of < 1 IU/dL, and was diagnosed with severe hemophilia A. Since then, the patient has experienced only a few hemorrhagic events, has received an extremely low total dose of FVIII product, and has not undergone periodic adjunctive therapy. The latest FVIII activity was < 1.0 IU/dL (one-step coagulation technique), and the antigen level was 1.8 IU/dL. Little hemophilic arthropathy was noted. Since a discrepancy was found between the activity level and clinical symptoms, a comprehensive coagulation functional assessment was performed. Parameters of the patient's coagulation/hemostatic potential were determined at FVIII activity levels of 5–10 IU/dL in rotational thromboelastometry and 10 IU/dL in CWA. The above findings showed that the patient's actual coagulation potential was not at a severe level but at a mild level of coagulotype of approximately 10 IU/dL. Further extensive experimentation showed that an enhanced FX binding potential due to R1781H point mutations influenced the clinical severity.

3. Monitoring of bypass hemostatic therapy in inhibitor-carrier patients with hemophilia

Anticoagulation factor antibodies (inhibitors) develop in 20%–30% of patients with hemophilia A and 3%–5% of patients with hemophilia B, reducing or eliminating the hemostatic effect of the adjunctive therapy with the coagulation factor product. Hemostatic management is performed using a bypass agent (BPA), which, however, makes it difficult for ordinary assessments based on factor activity. Nevertheless, since no established method of hemostatic monitoring by BPA is available, currently available measures are based on symptoms, imaging, hemoglobin levels, and the like. There have been a gradually increasing number of dedicated facilities where hemostatic monitoring is possible by comprehensive functional testing based on rotational thromboelastometry (ROTEM). However, whole blood samples are required, and measurements must be taken

within 4–5 h after sampling. In this situation, we reported on the utility of hemostatic monitoring in BPA therapy with CWA of high applicability for general purposes. Since aPTT-based CWA does not well reflect the effect of BPA, we established a new approach to CWA with a trigger reagent prepared by adding ellagic acid to a trace amount of tissue factor to reflect both the endogenous and exogenous systems (hereinafter referred to as the mixed method)[8]. It became possible to monitor the infusion effect of BPA using the mixed method. This method was successfully used to monitor the parameters clot time (CT) and |min2| to achieve good hemostatic management during the perioperative period[8].

4. Blood coagulation assessments in acquired hemophilia A

Acquired hemophilia A is a disease characterized by highly serious bleeding caused by an anti-FVIII autoantibody that develops suddenly in healthy individuals, and the patient's FVIII activity levels or inhibitor titers reportedly do not reflect the clinical severity of the condition at all[9]. Despite having the same level of activity, acquired hemophilia A involves markedly disordered coagulation function compared with congenital hemophilia A[10]. Its mechanism of action was found to involve the presence of FVIII/IgG complex, which inhibits the formation of FXa complex and hence severely disorders thrombin production[10]. As a differential diagnosis of acquired hemophilia A, antiphospholipid antibody syndrome is found in some cases, and it is often difficult to diagnose using the one-step coagulation technique. One study reported on the feasibility of their differential diagnosis with the use of CWA[11].

5. Coagulation monitoring of bispecific antibody (emicizumab)

Emicizumab is a bispecific antibody that binds an FIXa molecule to one of its antigen-binding sites, and an FX molecule to the other antigen-binding site to replace the FVIIIa cofactor function and promote blood coagulation reactions and is administered by subcutaneous injections to prevent bleeding in patients with hemophilia A[12,13]. The aPTT cannot accurately be evaluated because it shortens markedly in periodic administration of the product. The potency of emicizumab was calculated to be 2.5–5 μg/mL/IU/dL on the basis of FVIII activity from the hemolytic effect of emicizumab administered in a monkey model of acquired hemophilia A[14]. This is considered to be equivalent to approximately 10–20 IU/dL based on FVIII activity, because the blood concentration of emicizumab expected from its likely clinical dose is approximately 50 μg/mL. It is not well known, however, whether this range of converted values is valid for patients with hemophilia A because no appropriate method of hemostatic monitoring is available. In the case of periodic administration of emicizumab, hemolytic monitoring is required for comprehensive coagulation functional assessments in patients on emicizumab treatment, hemostatic management for serious bleeding (in combination with FVIII product or BPA), perioperative hemostatic management, and coagulation functional assays upon the development of antiemicizumab antibody[15].

We previously reported on the clinical utility of blood coagulation assessments in the presence of emicizumab using CWA[16]. Two measures are taken to facilitate reliable assays of samples in the presence of emicizumab. First, since fibrinogen concentrations essentially influence the percent transmission of clot waveform, the transmission of the postcoagulation phase is adjusted to 0% to minimize the influence of fibrinogen concentrations. Second, coagulation is induced with an exogenous reaction (PT reagent) and enhanced with an endogenous reaction (aPTT reagent) to produce fibrin, with the endogenous and exogenous systems being combined in the appropriate proportion. The resulting mixed reagent is used as a trigger reagent for CWA. As a parameter of the waveform thus obtained, adjusted |min1| well reflects the functional activity of emicizumab. It is considered highly useful for general purposes because it can be measured using a fully automated coagulation analyzer in ordinary laboratory testing. It also became possible to assess the coagulation function of emicizumab alone or in combination with FVIII product or bypass hemostatic agent[16].

6. Clot-fibrinolysis waveform analysis[17] (Fig 2)

We have recently established a measuring method that enables us to concurrently assess clot-fibrinolysis dynamics using clot-fibrinolysis waveform analysis (CFWA) by adding tissue plasminogen activator (tPA) at the same time as with the aPTT reagent $CaCl_2$. In CFWA, quantitative analysis is possible as follows: for the coagulation phase, the CT and clot waveform are differentiated in the first order to calculate the coagulation velocity, and its maximum |min1| is used for the analysis; for the fibrinolytic phase, the time from the CT to the start of lysis (FLT; fibrinolysis lag time) and fibrinolysis waveform are differentiated in first order to calculate the fibrinolysis velocity, and its maximum (FL-|min1|) and endogenous fibrinolysis potential

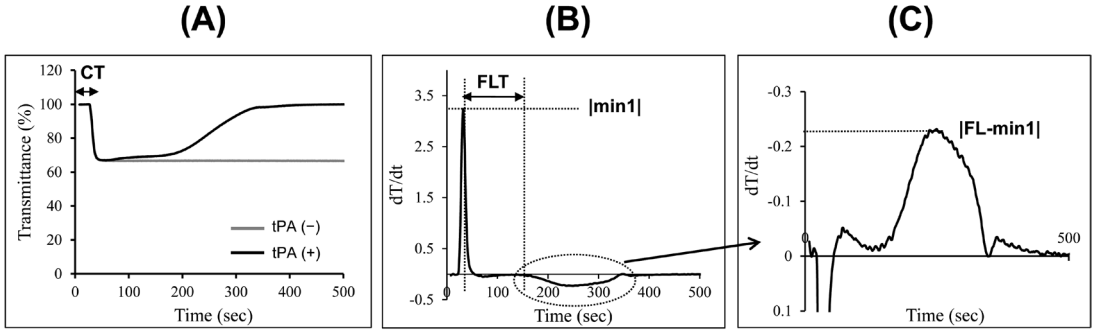

Fig 2. CFWA in normal plasma

Panel (A) shows clot-fibrinolysis waveforms from normal plasma in the absence and presence of tPA and their parameters.
Panel (B) shows a first-order differential waveform from panel (A).
Panel (C) shows a reverse waveform of the fibrinolytic portion of panel (B).

tPA: tissue-type plasminogen activator, CT: clot time, FLT: fibrinolysis lag time
|min1|: maximum coagulation velocity, FL−|min1|: maximum fibrinolysis velocity

(EFP) are used for the analysis.

This method was used to analyze the coagulation fibrinolysis dynamics in normal plasma in the presence of argatroban (thrombin inhibitor), tranexamic acid (TXA), and thrombomodulin added thereto. When argatroban was added, the CT prolonged, and the |min1| decreased, in a concentration-dependent manner. Furthermore, the FLT prolonged, and the start of fibrinolysis was delayed with increasing CT. In the presence of TXA, the coagulation phase was not influenced, but the FLT prolonged and the FL-|min1| decreased, in a concentration-dependent manner. In the plasma with the addition of low-concentration thrombomodulin, the coagulation phase was not influenced, but the FL-|min1| decreased. High-concentration thrombomodulin inhibited both the coagulation phase and the fibrinolytic phase. These results showed that this method reflected the interplay between the coagulation potential and fibrinolytic potential[17].

CFWA analysis of FVIII-deficient plasma found that the coagulation phase prolonged when compared with normal plasma and that the fibrinolytic phase began before fibrin formation[17]. Although the FL-|min1| was nearly the same as with normal plasma, a biphasic fibrinolysis waveform was evident, and the EFP increased markedly. This result also showed that there was an imbalance between the coagulation system and fibrinolytic system in hemophilia A. When FVIII- or FIX-deficient plasma was analyzed using CFWA in the presence of FVIII or FIX product added at various concentrations, both products improved the CT and |min1| in the coagulation phase in a concentration-dependent manner. The fibrinolytic phase did not improve in the presence of low-concentration FVIII (< 1 IU/dL), whereas in the presence of FIX, the FLT and EFP improved in a concentration-dependent manner even at low concentrations (< 1%). When added to FVIII-deficient plasma and FIX-deficient plasma, TXA suppressed the fibrinolytic phases of both forms of factor-deficient plasma even at low concentrations. When added at high concentrations, TXA nearly completely suppressed fibrinolytic reactions. It was considered possible to comprehensively assess coagulation fibrinolytic dynamics in the pathologic analysis of coagulation factor deficiencies, and the therapeutic effects of coagulation factor products and antifibrinolytic drugs, using CFWA. We have recently reported that CWA/CFWA is also clinically applicable to nonhemophilia diseases[18,19].

Conclusion

It seems possible to understand the essential hemostatic potential in terms of blood coagulation in patients with hemophilia by combining a factor activity determination and a comprehensive coagulation functional assay. Furthermore, it is likely possible to predict the patient's severity of bleeding and to draw up a strategy for future hemostatic management, and even to draw up a long-term strategy for hemostasis, by using these various combinations of assessments. We will proceed to increase the cumulative number of cases and demonstrate that comprehensive coagulation functional assessments are more useful in clinical settings.

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