

## **New Approaches in the Measurement of Coagulation**

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### *Dynamic Evaluation of the Clotting Process in the Hemorrhagic Disorders*

Benny Sorensen, Center for Haemophilia and Thrombosis, Denmark

Traditional plasma coagulation assays such as the prothrombin time (PT) and the activated partial thromboplastin time (APTT) usually provide information only on the early start of clot formation. These standard assays diagnose biochemical information such as factor levels, but do not give information on the continuing clot development or on the severity of symptoms. In more recent years, renewed interest has focused on the blood coagulation process and the dynamic characteristics of the hemostatic system, said Dr. Benny Sorensen. He described several new clotting tests that, beyond determining biochemical defect, give continuous plasma coagulation profiles that are useful for monitoring the effects of procoagulant or anticoagulant medication.

Two types of methods – thrombin generation and fibrin generation – record the entire course of clot formation. Thrombin generation methods include in-process subsampling and thermoacoustic tomography (TAT), and continuous recording of thrombin activity using a slow-reacting thrombin reagent. The thrombin generation methods yield more detailed information on the biological differences among patients with bleeding disorders than do standard assays. For example, one of the methods has been used to obtain a dynamic profile of thrombin development during factor VIII (FVIII) substitution in a patient with severe hemophilia A.

Fibrin generation methods include thrombelastometry, thrombelastography, and continuous photometric recording of fibrin formation. Thrombelastography involves continuous whole blood coagulation testing to obtain data on clotting dynamics such as initiation and propagation.

Dr. Sorensen described his laboratory's many years of experience with ROTEM thrombelastometry to monitor continuous whole blood clot formation in patients with hemophilia and other coagulopathies. Using a thrombelastometric model, with minute amounts of tissue factor as activator, the Danish laboratory scientists have been able to demonstrate phenotypic heterogeneity among patients with severe hemophilia and dose titration response to rFVIII, rVIIa, and activated prothrombin complex concentrate (APCC).

The new fibrin generation laboratory techniques have resulted in interesting new information on the biochemical phenotypes of various bleeding disorders, Dr. Sorensen said. The dynamic APTT data better disclose heterogeneity among patients with severe hemophilia A than do standard APTT clotting times, and may serve as supplementary information for selecting appropriate dose regimens. Dr. Sorensen said that thrombelastometric data have provided useful guidance for patient interventions at his centre, while thrombelastography has been used effectively for managing bleeding during knee replacement surgery in a patient with inhibitors.

Future challenges include determining the efficacy of the new instruments for monitoring hemostatic or anticoagulant intervention. Future prospective studies are to investigate the clinical feasibility of the new coagulation tests and parameters.

*Laboratory Measurements for the Choice and Monitoring of Treatment in VWD*

Giancarlo Castaman, Department of Hematology, San Bortolo Hospital, Vicenza, Italy

Dr. Giancarlo Castaman reviewed the pathophysiology of von Willebrand disease (VWD), recent developments, and the best tests used in the diagnosis and subclassification of VWD.

von Willebrand factor (VWF) is a multimeric glycoprotein that plays several functions in the binding activities of hemostasis:

- Carrier of FVIII in plasma, with localization at the site of injury and prevention of inactivation by the protein C system
- Platelet-subendothelium adhesion when elevated shear stress flow occurs
- Platelet-to-platelet cohesion and aggregation, in cooperation with fibrinogen, to promote stable clotting

There are a number of tests for the laboratory diagnosis of VWD, but no single test captures the whole spectrum of VWF's activities and functions, Dr. Castaman said. Basic tests in use for VWD phenotypic diagnosis include:

- Platelet count
- Bleeding time (PFA-100)
- Ristocetin-induced platelet aggregation (RIPA)
- Immunological assay with polyclonal antibody (VWF:Ag)
- Ristocetin cofactor (VWF:RCo)
- Coagulation factor VIII:C (FVIII:C)

Advanced tests include:

- VWF/FVIII binding
- VWF activity based on binding to collagen (VWF:CB)
- Platelet VWF assessment
- Multimer profile

The pathophysiological significance of each test is quite different, Dr. Castaman said. RIPA is a useful diagnostic test for determining the threshold ristocetin concentration that induces a patient's platelet-rich plasma aggregation. It allows discrimination of type 2B VWD, which is characterized by reduced threshold. Desmopressin (DDAVP) may cause thrombocytopenia in type 2B patients, so it is important to identify these patients and provide the appropriate treatment regimen. RIPA is also used to identify type 3 VWD, which is characterized by the absence of ristocetin-induced aggregation.

The evaluation of closure time (CT) with PFA-100 (platelet function analyser) allows rapid and simple determination of VWF-dependent platelet function at high shear stress. This system was

demonstrated to be sensitive and reproducible for the screening of VWD, even though the CT is normal in type 2N VWD and in mild VWD deficiencies.

Assays for VWF:CB are also available and appear to be useful for distinguishing and classifying type 1 VWD (quantitative deficiency) and type 2 VWD (qualitative deficiency or variants). However, the VWF:CB test does not offer substantial advantage compared with VWF:RCo and is not yet well standardized, so it cannot substitute for the ristocetin cofactor activity assay, Dr. Castaman said. FVIII:C and VWF:RCo tests appear crucial in monitoring the safety and efficacy of replacement therapy in VWD.

The goals of treatment in VWD are correction of the clotting defect (particularly important for surgery and soft tissue bleeding) and correction of the hemostatic defect (characterized by mucosal bleeding, bleeding time prolongation, and low VWF).

The two main therapeutic approaches to VWD are (1) DDAVP, which is safe (with no risks of infections), cheap, and effective in 60%–70% of patients (mainly with type 1 VWD); and (2) FVIII/VWF concentrates, which are used for patients with type 2B VWD (because DDAVP may induce thrombocytopenia), type 3 VWD, and type 1 and 2 VWD patients who do not respond to DDAVP.

Significant limitations on the use of DDAVP in surgery include the following:

- Non-responders – patients who do not respond to DDAVP
- Short half-life of released factors – some subtypes are characterized by increased clearance
- In responders, prolonged DDAVP treatment may be difficult and accompanied by risks of tachyphylaxis, and antidiuretic and other side effects
- Contraindications include patients with cardiovascular disease and type 2B VWD

Issues to consider regarding the use of FVIII/VWF concentrate for treating VWD include:

- Content of VWF and FVIII – (1) VWF:RCo/VWF:Ag ratio and multimeric VWF composition (owing to variations in the amount of functional VWF among the different concentrates); and (2) FVIII:C/VWF:RCo ratio
- Virus inactivation
- Pharmacokinetics and clinical efficacy from retrospective and prospective studies

Some considerations for dosing of a FVIII/VWF concentrate are:

- Concentrates should be labelled with both VWF:RCo and FVIII:C content
- Dosing using FVIII:C can lead to unduly low levels of VWF
- Dosing using VWF:RCo has been advocated

It is feasible to dose FVIII/VWF concentrate (Haemate P®) for surgery in VWD based on a pre-operative pharmacokinetic analysis, said Dr. Castaman. Reports on the use of FVIII and VWF concentrates in surgery reflect good hemostasis achieved by using concentrates in a median dose range of 51–82 IU VWF:RCo/kg. During major surgery, FVIII/VWF concentrate

substitution must be monitored every 12 hours on the day of surgery, and every 24 hours thereafter, and should preferably be within 50–150IU/dL. Patients should be checked for unexpected bleeding resulting from low VWF:RCo.

A practical approach to VWD treatment is to administer a test infusion of DDAVP to measure FVIII and VWF activity, and base dosing for major surgery on the response, Dr. Castaman said.

*Mild Hemophilia with Normal Results:*

*Is it a Lab Phenomenon or a Local Issue?*

John Lloyd, Division of Haematology, Institute of Medical and Veterinary Science, Adelaide, Australia

Dr. John Lloyd gave an overview of discrepant mild hemophilia A, a variant of hemophilia A documented by a number of centres. Patients with this variant have levels of FVIII that are much higher when measured by a FVIII one-stage assay than by a two-stage assay. This finding has implications for the diagnosis of patients with mild hemophilia A.

From 1976 to 1980, manual methods of clot detection started being replaced with automated methods. As the two-stage FVIII assay was difficult to automate, one-stage assays came to displace the two-stage assay in the majority of centres. At the South Australia Haemophilia Treatment Centre, the one-stage assay proved unsuitable for a significant group of patients with mild hemophilia. The centre has retained the two-stage assay for diagnostic purposes, and uses the one-stage assay to monitor treatment.

In patients with discrepant mild hemophilia A, the baseline plasma levels of FVIII obtained by a one-stage assay are about one-and-a-half- to two times higher than those from a two-stage FVIII assay, Dr. Lloyd said. A study of 140 adult patients in hemophilia A in South Australia found 97 to have mild hemophilia – of these, 37 individuals (38%) have the discrepant phenotype.

Many families in other countries have now been described with this variant. At least 17 missense mutations have been identified in families with discrepant hemophilia, occurring in the A1, A2, and A3 domains, and one reportedly in the C2 domain. These mutations render the FVIII less stable, so that activated FVIII is inactivated more quickly in these patients.

The one-stage assay is standard for FVIII in most diagnostic laboratories. However, since baseline plasma levels fall only slightly behind or within the normal reference range in many patients with discrepant hemophilia, use of this assay leads to diagnostic uncertainty, Dr. Lloyd said. On the other hand, the more complex two-stage coagulation assay is available only in a few centres, is difficult to automate, and is time-consuming when carried out manually.

Chromogenic FVIII assays, based on a two-stage technique, are available in some diagnostic laboratories. Results using this technique have been variable, but they usually result in levels that are lower than a one-stage assay but higher than a two-stage assay.

Discrepant mild hemophilia A is relatively common, occurring in 40% of mild hemophilia A. In patients with discrepant hemophilia, FVIII levels are often only borderline low and may be

normal. Therefore, said Dr. Lloyd, all hemophilia treatment centres must have access to properly optimized chromogenic assay for diagnosis of this variant.

*The Impact of the Cone and Plate(let) Analyzer*

David Varon, Institute of Thrombosis and Hemostasis, Sheba Medical Center, Tel Hashomer, Israel

The Cone and Plate(let) Analyzer (CPA) is one of the few technologies that measures platelet function in whole blood samples under flow conditions, said Dr. David Varon. The cone and plate apparatus was developed for testing platelet adhesion and aggregation on a polystyrene surface, and is suitable for both basic studies and clinical application. The automated device has undergone intensive development by DiaMed/Bio-Rad Laboratories and is expected to be available soon for clinical use.

Plasma proteins, in particular fibrinogen and VWF, are instantly immobilized on the polystyrene surface and form a thrombogenic surface to which platelets adhere. The CPA is useful for a complete screening of patients with bleeding disorders and confirmation of hemostasis abnormalities, including Glanzmann thrombasthenia, afibrinogenemia, and VWD. The test is also useful for detecting bleeding risk and platelet function in thrombocytopenia patients and is a sensitive predictor of bleeding, surface coverage, and blood loss for cardiac surgery patients.

The CPA can also be modified for testing platelet aggregation, and used as a “reverse aggregometer” for the diagnosis of thrombotic thrombocytopenic purpura (TTP).