Laboratory methods in the haemostatic laboratory

Christine Mannhalter, Peter Quehenberger
Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Vienna, Austria

The current theme issue of Thrombosis and Haemostasis focuses on a panel of important topics regarding laboratory methods in the haemostatic laboratory. There is general agreement that tailoring laboratory assays to correlate with clinical phenotypes is essential for effective coagulation monitoring. Thus, it is not surprising that during the last decade concepts for new assays more closely reflecting the situation in vivo have been developed.

A good example is the development of assays for determination of the time course of thrombin generation. In this theme issue methods for measurement of thrombin generation are presented, and the importance of the determination of thrombin generation to assess disease severity and success of treatment is discussed. In addition, the role of the activated partial thromboplastin time (aPTT) test for the monitoring of unfractionated heparin (UFH) therapy is revisited, and the usefulness of the ProC Global assay in women with a history of venous thromboembolism (VTE) on hormonal therapy is critically evaluated. Furthermore, topics of standardization and quality control, especially the concept of external Quality Assessment Schemes are dealt with.

Thrombin generation

Thrombin is the key enzyme of coagulation. Its activity can be determined indirectly via fibrinogen conversion to fibrin, or by cleavage of a chromogenic substrate. The latter method is more attractive than the first one but is frequently hampered by unspecific cleavage of the chromogenic substrate by thrombin-like enzymes, especially those of the contact phase of coagulation. In spite of the importance of thrombin it is clear that the haemostatic potential is not determined by the thrombin concentration present at the time of analysis. Evidence is growing that the measurement of the endogenous thrombin potential (ETP, the area under the curve), and notably parameters of the thrombogram, are useful in assessing the risk for bleeding or thrombosis and its modification by antithrombotic or haemostatic treatment. ETP may reflect the activity and concentration of the parameters involved in clot formation in addition to thrombin, e.g. platelets, microparticles, or fibrinogen, and could provide a more accurate reflection of the situation in vivo. In a recent review by Sørensen and Ingerslev (1) data were presented showing that the thrombogram seems to be a promising new approach to clinical management of bleeding and thrombotic disease as well as a tool in monitoring treatment with factor VIIa in patients with haemophilia A.

In the articles by Hemker (2) and Dargaud (3) in this theme issue it is shown that it is now possible to obtain a thrombin generation curve (the thrombogram) in plasma, with or without platelets, in an easy routine procedure at high throughput and with an acceptable experimental error (≤ 5%) by the use of a fluorogenic thrombin substrate and continuous calibration of each individual sample.

In-vitro assays and mostly retrospective studies gave good evidence that thrombin generation based upon the individual's blood composition is associated with the risk for thrombosis. Clearly, ETP reflects hypercoagulability and hypocoagulability (4, 5). However, the ex-vivo ETP assay has not been evaluated in prospective studies, and its utility in assessing the severity of thrombosis, or the effectiveness of antithrombotic prophylaxis or treatment, has not been determined. In healthy volunteers it has been shown that prophylactic doses of neither heparin nor low-molecular-weight heparin (LMWH) can inhibit thrombin production (6). This lead to controversial opinions regarding the clinical value of determinations of thrombin production. It is argued that measurement of thrombin production and activity does not provide information useful for clinical decision-making for two reasons: the inability of the measurement to differentiate between physiological (haemostatic) and disease-related (pathological) sources (7) and/or causes of thrombin production in vivo, and the inability of antithrombotic treatment modalities to eliminate the stimuli that cause increased thrombin production evident in venous and arterial thrombosis. These issues will be discussed in the article by Ofoṣu (8).

Monitoring of unfractionated heparin with aPTT

Immediate anticoagulant treatment is essential to reduce morbidity and mortality in patients with acute VTE. Although UFH has been replaced by LMWH for many indications it is still widely used. UFH is still recommended for patients with renal impairment and for those at high risk of bleeding. As UFH exhibits more variability in anticoagulant response among individ-
uals laboratory monitoring is recommended. The most widely used laboratory assay is the aPTT. Multiple devices are available for point-of-care aPTT and heparin concentration testing. For each test, there is some variability in results between devices and between reagents used in the same device. The recommended therapeutic range of an aPTT of 1.5 to 2.5 times of the control value is only supported by weak evidence. Although recommended by the American College of Chest Physicians’ Seventh Conference on Antithrombotic and Thrombolytic Therapy, establishing a heparin concentration-derived therapeutic range for UFH is rarely performed. Despite these limitations, anticoagulation monitoring of UFH using aPTT is common in clinical practice, particularly when evaluating anticoagulation associated with interventional cardiology procedures and cardiopulmonary bypass surgery. Even though standardisation of aPTT methods for monitoring of UFH is recommended it is not widely used. Would a surrogate such as the International Normalised Ratio (INR) contribute to standardization of aPTT? What are the ideal characteristics of a laboratory assay used for monitoring the anticoagulant effect of anticoagulant drugs? These important questions are reviewed by Eickelboom and Hirsh (9).

ProC Global assay

Clinical laboratories are confronted with increasing demands for thrombophilia work-up. Recently, methods able to investigate the protein C anticoagulant pathway globally have been proposed. ProC Global is a coagulation assay that measures the aPTT in a plasma sample before and after activation of endogenous protein C by Protease, a snake venom. It reflects abnormalities in the whole protein C pathway which are found in the majority of patients with thrombophilia. Previous studies have suggested that abnormalities in this test are associated with an increased risk of VTE. The ProC Global assay has a high sensitivity for detection of protein C deficiency, APC-resistance, and the factor V Leiden mutation while the sensitivity for detection of protein S deficiency is controversial (10, 11). Improvement of sensitivity toward this component of the protein C anticoagulant pathway would enroll the global test as a suitable candidate for screening. However, since antithrombin, which also remains undetected by this test, is an additional important risk factor for venous thrombosis, efforts to develop global tests able to detect defects in both the antithrombin and protein C pathways are warranted. In patients with confirmed VTE an association between abnormal ProC Global results and recurrent VTE showed a strong trend (odds ratio, OR 3.6). Patients with a first episode of idiopathic VTE expressed significantly lower ProC Global results than those with secondary VTE. These results suggested that ProC Global could represent a predictor of recurrent VTE (12). Oral anticoagulants as well as hormone replacement therapy have been shown to increase the risk for venous thrombosis, even current low-dose oral anticoagulants carry a certain risk. As over 100 million healthy women use hormonal therapy, the identification of women at high risk of VTE when taking oral contraceptives is important. Screening for APC-resistance using CoaTest APC, ProC Global, or ProC APC-FV-Leiden clearly identified homozygous mutant carriers. However, with regard to heterozygous mutant carriers, the sensitivity and specificity of these tests, especially during oral contraceptive intake, was limited (13). In a meta-analysis on the risk of clinical complications in women taking oral contraceptives nine studies for oral oestrogen preparations were included. Significant associations of the risk of VTE were found in women with factor V Leiden; deficiencies of antithrombin, protein C, or protein S, elevated levels of factor VIII; and factor V Leiden and prothrombin G20210A. For hormone replacement therapy, a significant association was found in women with factor V Leiden (14). A cost-effectiveness analysis showed that universal screening of women prior to prescribing hormone replacement therapy was cost-effective. In contrast, this procedure prior to prescribing combined oral contraceptives was not a cost-effective strategy. There is considerable difference in the magnitude of the risks among different patient groups with different thrombophilic defects. However, the absolute risk remains low. Large prospective studies should be undertaken to refine the risks and establish the associations of thrombophilias with VTE among hormone users. To do this, a reliable economic screening test that is suitable to detect the majority of persons at risk would be very useful. In this theme issue, data on the use of ProC Global assay in women with a history of VTE under hormonal therapy are presented by Galit et al. (15).

References