Report from the SYSMEX HAEMOSTASIS USER SYMPOSIUM 2001

"The 10th Anniversary Symposium"

Sysmex UK Ltd.

This year's 2001 Sysmex User Symposium marked the 10th anniversary since the formation of Sysmex UK Ltd. This prestigious event was marked in celebratory style with an impressive programme of presentations and entertainment launched within the splendid setting of The Belfrey Hotel, Warwickshire. Over 200 international delegates attended the symposium which this year focused on current issues in haemostasis research and practice. The scientific programme began with a busy Haemostasis Workshop providing an excellent opportunity for the exchange of views and information. Key aspects of research and development in the coagulation field were addressed in the following scientific paper sessions that offered a stimulating forum for lively debate and discussion.

The programme began with an examination of the use of decision analysis based strategies to improve the diagnosis of venous thrombosis and pulmonary embolism. ***Dr. Carl Erik Dempfle*** (Universitatsklinikum, Mannheim) outlined the problems of conventional test procedures and highlighted the clinical usefulness of introducing D-dimer measurements to other assessments within a diagnostic framework. Existing methodologies (contrast venography, duplex sonography, scintiography, spiral CT, MR, angiography) may be invasive, lack sensitivity and specificity or may be expensive to perform. Combining diagnostic tests with clinical probability scores based on clinical symptoms, and patient history and status, improves the diagnostic outcome specificity but is still unsatisfactory. However, the highly sensitive D-dimer assay that uses monoclonal antibodies to detect fibrin formation is a simple, rapid and cost-effective diagnostic test that eliminates some 25-35% of outpatients from further diagnostic procedures.

The risk of venous thromboembolism in passengers on long-haul flights has been a recent controversial topic of media interest. The condition has long been associated with prolonged periods of inactivity in cramped conditions and is not restricted to air travel or even economy class conditions. However, air travel conditions: sitting in confined spaces for prolonged periods, pressure on the back of the calves, dehydration and poor air quality, may all be contributory factors, although previous studies have been inconclusive. ***Dr. Ian Mackie*** and colleagues (University College Hospital, London) conducted a prospective randomised study into the benefits of wearing Class 1 compression stockings for 200 volunteers undertaking long-haul economy class flights. Duplex ultrasonography was used to detect embolism formation and blood samples were collected for analysis. Results showed that a significant percentage of non-stocking wearers developed asymptomatic deep vein thrombosis (DVT) whereas none of the stocking wearers did. Further studies are planned in conjunction with WHO but in the interim, those individuals with a history of venous thromboembolism or with a thrombophilia type defect might benefit from wearing Class 1 compression stockings and treatment with prophylactic subcutaneous low molecular weight heparin.

Dr. Mike Makris (Central Sheffield Haemostasis Centre) reported that the incidence of homocysteinuria, although an extremely rare disorder, has increased in the last few years. The condition, which is characterised by extremely high blood homocysteine levels, arises due to a homozygous defect causing a deficiency of an enzyme in homocysteine

Picture of outside the Woodlands Suite where the User Symposium was held!

metabolism and has long been associated with thrombotic complications. Of increasing interest is the strong correlation between hyperhomocysteinemia and thrombovascular disease. Hyperhomocysteinemia is a more common condition caused by heterozygous gene defects associated with homocysteine metabolism and it is now recognised that this condition is likely to interact with other prothrombotic risk defects to increase thrombotic risk. The mechanism by which homocysteine promotes thrombosis is unclear but the vascular endothelium and metabolism are affected. The condition is confirmed by measurement of fasting levels or by methionine loading tests. It is possible that correction of the abnormality by reducing homocysteine levels might be possible using vitamin supplementation (folic acid, vitamin B_6, vitamin B_{12}) but intervention studies are needed to confirm this.

The Platelet Function Analyser (PFA-100) is a compact benchtop analyser that offers a new approach to platelet function diagnostics and is attracting a great deal of interest for its utility in a range of clinical applications. ***Dr. John Francis*** (Florida Hospital Center for Hemostasis and Thrombosis) outlined the deficiencies of the standard bleeding time (BT) test for diagnostic purposes and described the advantages of the PFA-100 based on a prospective comparison of the two methodologies for screening platelet dysfunction (vWD and platelet function defects) in hospital inpatients. vWD appears to be common in women with menorrhagia and the PFA-100 may be a useful tool for screening such patients. In Jersey, vWF is thought to affect 1 in 300 of the 85,000 islanders and therefore a convenient method for testing suspected cases is of great importance. According to Lynne Davis (Jersey General Hospital) the only screening method available prior to the PFA-100 was the BT test that was inconvenient to perform and distressing for children. The PFA-100 offers a rapid, less invasive test and now provides an in-island method of distinguishing between patients likely to have vWD and those bleeding for other reasons.

Dr. John Francis, Florida Hospital Centre

Fibrin formation is the end product of the clotting cascade and an established marker that shows coagulation has occurred. However, according to ***Dr. John Biggerstaff*** (Biomolecular Research Institute, University of Florida), fibrin may have another role in the detection of cancer. It is present in the stroma of many cancers and this is confirmed by confocal laser scanning microscopy (CLSM). When metastasis occurs, tumour cells can bind soluble fibrin (SFn) and this augments platelet-tumour cell adherence and may be partly responsible for many of the coagulation defects observed. In addition, in vitro studies have indicated that SFn also influences the immune response to cancer by binding to leukocyte and tumour cell adhesion receptors, resulting in inhibition of adherence and tumour cell destruction. The results implicate fibrin as an important mediator of tumour cell progression and cancer metastasis. This raises the possibility of therapy using anticoagulants and antithrombin and suggests that antifibrin antibodies may well be an important area for investigation.

The clinical utility of screening for disorders of haemostasis and the significance of risk analysis for such disorders has to be assessed within a balanced framework of risks and benefits. ***Dr. David Keeling*** (Oxford Radcliffe Hospitals) examined the genetic basis for a range of clotting disorders and questioned the relevance of screening and risk assessment with particular reference to contraceptive pill (CP) and HRT use. Relative and absolute risk values provide very different types of risk assessment and care must be taken with the presentation and interpretation of results.

Dr. John Biggerstaff, University of Florida

The Scene of the User Symposium

Nevertheless, the increased risk of thrombosis for women taking the pill and HRT (CP: × 4.0 for normal women; × 35 for Factor V Leiden women; HRT: × 3.5 for normal women; × 25 for Factor V Leiden women) suggests that it may be of benefit to offer screening programmes to those individuals with a history of thrombophilia who are contemplating pill or HRT use.

Antiphospholipid syndrome (APS) is an important thrombophilic condition because it is common and has a high morbidity and mortality. ***Prof. Mike Greaves*** (Aberdeen Royal Infirmary) explained that diagnosis relied on the occurrence of a thrombotic event (deep vein thrombosis, pulmonary embolism, ischaemic stroke or recurrent miscarriage) in combination with a positive laboratory test for the antiphospholipid antibodies (APabs). However, assays have suffered from variable sensitivity, lack of specificity and lack of standards, etc., and there are clinical difficulties because the exact mechanism of action is uncertain and antibodies can be transient (e.g. after infections) or drug-induced, or may be incidental with no signs of disease. Prof. Greaves summarised the latest developments in current testing and the important considerations to be made for the interpretation of such tests. He stressed that it was important for clinicians to recognise the limitations of test systems and to interpret the results carefully alongside the clinical evidence.

Mr. Chris Gardiner (University College Hospital, London) continued the APS story by proposing a specific role for autoantibodies in clotting mechanisms. It is known that APabs interfere with the protein C pathway and that this frequently manifests in thrombophilia screening as acquired activated protein C resistance (APCR) in the absence of factor V Leiden. The laboratory has developed a highly sensitive test for APab-associated APCR that is essentially a modified Russell's viper venom time, performed with and without endogenous protein C activation. Studies utilising the test concluded that APCR may be more common than previously thought and it is potentially a useful thrombogenic marker in patients with antibodies associated with thrombosis.

Heparin-induced thrombocytopenia (HIT) is a challenging disorder to diagnose and study and has a complex physiopathology. It is characterised by a fall in the platelet count (usually between days 5 to 10) after exposure to heparin and surgery may be the initiator of this reaction. A significant percentage of patients will develop HIT antibodies and a proportion of these patients will go on to develop thrombotic complications. ***Mr. Simon Davidson*** (Coagulation Department, Royal Brompton Hospital) explained that the diagnosis should be based on two criteria: clinically evident abnormalities such as thrombocytopenia with or without thrombosis; and the detection of HIT antibodies either by functional or antigenic methods. Mr Davidson outlined the range of laboratory diagnoses available for HIT antibody measurement (functional platelet isotope assays, platelet activation/aggregation assays and enzyme immunoassay) and suggested that a combination of assays (e.g. heparin/PF4 used in conjunction with a functional method of platelet activation) can increase the sensitivity significantly. Endothelial markers can also assist in disease diagnosis since there is some evidence that endothelial disturbance occurs in this disorder.

Dr. Steve Kitchen (Royal Hallamshire Hospital, Sheffield) reported on the implementation of the CA-7000 coagulometer into a busy haemostasis reference laboratory. The CA-7000 is a highly advanced random access instrument with 20 test protocols and operates using barcode reading with a bidirectional interface and cap-piercing of primary tubes. Clotting, chromogenic and immuno assay methods can be selected. The analyser offers a multidilution analysis programme for clotting factor assays that allows use of a stored calibration curve, with automated linearity and parallelism assessment are available on a graphic display. Throughput, accuracy and precision were excellent for a number of techniques and the assay software was found to be particularly useful in the construction of valid assays of coagulation factors.

Mr. Chris Gardiner, UCLH London

Some of Sysmex Staff at User Symposium

Sysmex Haemostasis User Symposium 2001

Date and Place: 1st - 2nd November, 2001 The Belfry Hotel, Warwickshire, UK

Speakers:

1st November

1. Diagnosis of Venous Thrombosis and Pulmonary Embolism by Decision Analysis Based Strategies including Clinical Probability and Laboratory Assays

Dr. Carl Erik Dempfle, Universitatsklinikum, Germany

2. Thrombosis and Air Travel

Dr. Ian Mackie, University College Hospital, UK

3. HIT: Laboratory and Clinical Analysis to Improve Diagnosis

Mr. Simon Davidson, Royal Brompton Hospital, UK

4. New Clinical Application of the PFA-100

Dr. John Francis, Florida Hospital Center for Hemostasis and Thrombosis, USA

5. PFA-100 Application in a Routine General Hospital

Ms. Lynne Davis, Jersey General Hospital, USA

6. Fibrin and Cancer

Dr. John Biggerstaff, Biomolecular Research Institute, University of Florida, USA

2nd November

1. Thrombophilia: The Contraceptive Pill and HRT

Dr. David Keeling, Oxford Radcliffe Hospitals, UK

2. Lupus Screening Tests Overview

Prof. Mike Greaves, Aberdeen Royal Infirmary, UK

3. APC Resistance Associated with Anti-phospholipid Antibodies

Mr. Chris Gardiner, University College Hospital, UK

4. Homocysteine & Thrombosis

Dr. Mike Makris, Central Sheffield Haemostasis Centre, Royal Hallamshire Hospital, UK

5. CA-7000 – Implementation into a Haemostasis Reference Laboratory

Dr. Steve Kitchen, Central Sheffield Haemostasis Centre, Royal Hallamshire Hospital, UK