Scientific Workshop

Melbourne Convention and Exhibition Centre
Level 2, Meeting Rooms 218 and 219
Melbourne

12\textsuperscript{th} November 2016
Australasian Society of Thrombosis and Haemostasis

The Australasian Society of Thrombosis and Haemostasis (ASTH) was established in 1994. The Society represents approximately 200 clinicians, scientists and other health professionals committed to promoting and fostering the acquisition, exchange and diffusion of knowledge and ideas relating to normal and abnormal haemostasis. The Society serves as a forum for bringing together a broad array of disciplines, which relate to bleeding, thrombosis and cognate fields.

The ASTH Mission Statement

Promote excellence in clinical care for people with clotting and bleeding disorders.
To lead education and training of scientists and clinicians in the field;
To foster innovation through research, discovery and clinical trials;
To advocate and develop policies that improve health outcomes.

Membership Privileges

Newsletters (three per year)
Notices, booklets, flyers or brochures of interest to members
Copies of Media releases and letters to members from Council
Invitations to Society presentations and seminars
Attendance to Annual General Meetings
Nomination for a Council position
ASTH Medal competition eligibility at ASM (45 years of age and under)
Voting rights at Council elections, AGMs and subcommittee meetings
Access to the ASTH Website and Member’s only area, including Discussion group
Discounted registration fees for ASM and Scientific Workshops

Further information
ASTH Secretariat
Email: asth@bigpond.com
8:00 am  Registration and Tea/Coffee

9:00 am  Welcome: Quintin Hughes (Chair)

9:10 am  Liane Khoo (Sydney South West Area Health Service, NSW - Australia)
Episodes FVIII and FIX: A New Era of Haemophilia Treatment

9:40 am  Simon McRae (South Australia Pathology, SA - Australia)
Where do the extended half-life clotting factors fit in clinically?

10:05 am Claire McGregor (Fiona Stanley Hospital, WA - Australia)
Extended half-life products - a patient experience

10:30 am  MORNING TEA and TRADE

11:00 am Justin Hamilton (Monash University, VIC - Australia)
Visualising, measuring, and inhibiting thrombin during human thrombus formation

11:20 am David Rabbolini (University of Sydney, NSW - Australia)
iPS Cell Technology, Disease Modeling and Clinical Applications.

11:40 am Quintin Hughes (WACTH/PBI, WA - Australia)
Development of an anticoagulant with enhanced fibrinolytic activity

12:00 pm  Posters Session

12:20 pm  LUNCH and TRADE

1:30 pm Jeffrey Weitz (McMaster University, Ontario - Canada)
NOAC Reversal Agents: New Developments and Latest Data

2:15 pm Grace Gilmore (PBI/WACTH, WA - Australia)
Andexanet mode-of-action

2:35 pm Chris Ward (University of Sydney, NSW - Australia)
Idarucizumab update

3:00 pm  AFTERNOON TEA and TRADE

3:35 pm Charles Shuttleworth (St. Vincent’s Hospital, NSW - Australia)
Case Study: Drug-induced thrombocytopenia due to aspirin

3:55 pm Yvonne Brennan (Westmead Hospital, NSW – Australia)
Case Study: 2B or not 2B? A prothrombotic tendency masquerading as a bleeding disorder

4:15 pm Closing Remarks

4:30 pm  SUNDOWNDER and TRADE (until 5:30 pm)
Sponsored by ASTH
ASTH would like to thank the following sponsors who have made the 2016 ASTH Workshop possible

Stago
At the Heart of Haemostasis

Werfen
Diagnostic Solutions for Life

Bayer

Helena Laboratories (Australia) Pty Ltd

Sanofi
Notes
Episodes FVIII and FIX: A New Era of Haemophilia Treatment

Liane KHOO
Sydney South West Area Health Service, NSW

The recent development of modified recombinant factor VIII, factor IX and non-factor replacement therapeutic products will create challenges for the haemostasis laboratory in obtaining accurate recovery estimates of these products. The optimum assay method for measuring each new product will need to be assessed for individual products. Challenges include reagent choice, plasma standard, one-stage or chromogenic assays. The aim of this talk is to cover some of these challenges.
Notes
Where do the extended half-life clotting factors fit in clinically?

Simon MCRAE

South Australia Pathology, SA

Recombinant clotting factor products that have been modified with the intention of producing an extended half-life have now been approved by regulatory bodies and are available for the management of haemophilia in some jurisdictions. There remains ongoing debate about patient selection and eligibility, and the use clinical versus laboratory methods to guide dose selection and adjustment. This talk will present available data on the effectiveness and safety of the extended half-life clotting factors and discuss some of the contentious issues regarding their use.
Extended half-life products - a patient experience

Claire MCGREGOR

Fiona Stanley Hospital, WA

**Aims:** Within Australia, extended half-life products are currently only available for use in patients on clinical trial. The Haemophilia Treatment Centre at Fiona Stanley Hospital has been involved in a number of clinical trials involving extended half-life products. This case presentation aims to examine the use of extended half-life products and the impact the products can have on an individual.

**Method:** A review of the current patients on clinical trial was undertaken to identify a suitable patient.

**Outcome:** A patient was identified who had benefited from undertaking prophylaxis with the extended half-life product as well as undergoing procedures. The patient had previously declined prophylaxis due to the frequency of injections but also could not appreciate the difference prophylaxis could make to someone who already had significant joint disease. He commenced prophylaxis twice a week but was then moved to every 5 days. After a number of years on clinical trial he underwent coronary angiogram and stenting for cardiovascular disease and following this had a cardiac arrest when a stent became blocked. As a result, his dosing regimen was re-evaluated to reduce the peak FVIII level whilst maintaining a baseline level to continue the ongoing benefits to joint health. He remains on prophylaxis every third day.

**Discussion:** Whilst the obvious patients to place on extended half-life products may be those on prophylaxis, it is those individuals that will only use on-demand due to frequency of traditional prophylaxis that may benefit from these products. However, the question of prophylaxis regimens in an ageing population given the possibility of cardiovascular disease is one that needs careful consideration.
Visualising, measuring, and inhibiting thrombin during human thrombus formation

Justin HAMILTON

Australian Centre for Blood Diseases, Monash University, VIC

Thrombin is the most important enzyme in coagulation. Thrombin is also the most potent endogenous activator of platelets, which it achieves in human platelets via N-terminal cleavage of the protease-activated receptors (PARs), PAR1 and PAR4. There has been much recent interest in targeting platelet PARs for antithrombotic therapy: the first PAR1 antagonist (vorapaxar) was recently approved for clinical use and the first PAR4 antagonists are currently in early stage clinical trial. We have examined the relative roles of PAR1 and PAR4 in the setting of human thrombus formation in order to rationalise the utility of agents targeting these receptors for novel antithrombotic therapy.

In the setting of thrombosis, a subpopulation of the platelets become procoagulant as a result of extended activation, and bind coagulation factor complexes on their surface. These procoagulant platelets thereby facilitate the generation of further thrombin and the consequent amplification of the overall thrombotic response. We have previously shown that PAR4 drives this procoagulant response in human platelets. However, visualising and measuring thrombin generation in the setting of thrombus formation in flowing blood has proven difficult. We have overcome this by using a fluorescence resonance energy transfer (FRET)-based probe for thrombin activity/cleavage, linked to an anti-platelet antibody to prevent the signal from being washed away in flowing blood. This approach has enabled us to sensitively visualise and accurately measure thrombin activity in an ex vivo human whole blood thrombosis assay, and to examine the impact of inhibiting PARs on this response.

Using this approach, we have demonstrated that selective inhibition of PAR4, but not of PAR1, produces an ~50% reduction in thrombin activity and consequent fibrin formation in thrombi formed in human whole blood. These new approaches have therefore uncovered a novel function for PAR4 in the setting of human thrombus formation and have provided rationale for the further investigation of PAR4 antagonists as novel antithrombotics.
iPS Cell Technology, Disease Modeling and Clinical Applications

David ROBBOLINI

Northern Blood Research Centre, Kolling Institute of Medical Research
University of Sydney, NSW

Mechanisms of inherited thrombocytopenia include defects of megakaryocytic differentiation and maturation, as well as, defects of proplatelet formation and/or platelet release. Only platelets are found in large quantities in the peripheral blood making the study of megakaryopoiesis difficult without invasive procedures such as bone marrow biopsies or mobilization of CD34+ cells from the peripheral blood with colony stimulating factors. Moreover, not all human phenotypes caused by mutations affecting megakaryopoiesis are expressed in animal models. These limitations have contributed to the development of patient specific approaches using induced pluripotent stem cells (iPS cells) for the study of inherited diseases, including inherited thrombocytopenia.

Moreover, the logistical and biosafety limitations associated with donor derived platelet transfusions have stimulated research into the use of platelets generated in vitro from IPSCs. Our ability to harness this technology offers an exciting opportunity in transfusion medicine.

This talk will introduce the reprogramming process and will cover important definitions and milestones in the history of IPSC development. I will discuss important steps in characterizing IPSCs and then describe our local experience and rationale for using IPSCs to model an inherited thrombocytopenia caused by mutations in the transcription factor, GFI1B. I will end by discussing the use of IPSC derived platelets in transfusion medicine and highlight some of the recent advances made in this field.
Notes
Development of an anticoagulant with enhanced fibrinolytic activity

Quintin HUGHES

Western Australian Centre for Thrombosis and Haemostasis (WACTH) and the Perth Blood Institute (PBI), WA

Aptamers are short, synthetic DNA (single-stranded) or RNA oligonucleotides that undergo unique, sequence-dependent conformations resulting from Watson-Crick base-pair induced secondary and tertiary folding. Through a rigorous selection process, aptamers can be targeted against a wide range of molecules (proteins, drugs, chemical compounds etc) with a high degree of specificity and affinity. Anti-thrombin aptamers that bind to exosite I (Bock et al. 1992) and exosite II (Tasset et al. 1997), have been previously published, but these studies have generally just assessed the effect of these aptamers in standard clotting assays such as the Thrombin Clotting Time (TCT). In addition to the TCT, our laboratory has further evaluated their effects using the Thromboelastograph (TEG) and Thrombin Generation Assay (TGA). Thrombin exosite I is required for binding to fibrinogen. Therefore, addition of the exosite I-targeting aptamer exhibited TCT, TEG and TGA profiles in healthy control, very similar to those observed in blood samples derived from patients taking the direct thrombin inhibitor, Dabigatran. Although previously reported to have minimal effect on the TCT, we identified an interesting fibrinolytic effect when assessing the exosite II aptamer using the TEG with normal blood.

We are currently collaborating with an expert in nucleic acid modification to create derivatives of the exosite I and II aptamers that are more stable and resistant to degradation in whole blood.
NOAC Reversal Agents: New Developments and Latest Data

Jeffrey WEITZ

McMaster University, Ontario - Canada

Non-vitamin K antagonist oral anticoagulants (NOACs) are at least as effective as vitamin K antagonists (VKAs), but are safer and are more convenient to administer. Because of these features, current guidelines now give preference to the NOACs over VKAs for stroke prevention in atrial fibrillation and for treatment of venous thromboembolism. Consequently, there are more prescriptions for NOACs than for VKAs in many countries. Although life-threatening bleeding is less frequent with the NOACs than with VKAs, serious bleeding can still occur and patients taking NOACs may require urgent surgery or interventions. Therefore, specific reversal agents are desirable to streamline patient care in such circumstances. Idarucizumab is licensed for reversal of dabigatran. A monoclonal antibody fragment, idarucizumab binds dabigatran with high affinity to form a 1:1 stoichiometric complex that is then cleared by the kidneys. Andexanet alfa and ciraparantag are in development for reversal of rivaroxaban, apixaban and edoxaban. Andexanet, which is undergoing phase III evaluation, is an inactive factor Xa variant that binds the drugs and sequesters them until they can be cleared. Initial data indicates that andexanet reverses the anti-factor Xa activity of apixaban and, to a lesser extent, rivaroxaban and improves hemostasis in patients taking these drugs who present with serious bleeding. More data are needed about the safety of andexanet and its prothrombotic potential. Ciraparantag is a synthetic cationic small molecule that binds the NOACs. Phase II data in volunteers given a single dose of edoxaban indicates that ciraparantag reverses the prolongation of the whole blood clot time induced by edoxaban, but data in patients are lacking. The availability of a reversal agent for dabigatran simplifies management of patients with serious bleeding or requiring surgery. Approval of reversal agents for rivaroxaban, apixaban and edoxaban will eliminate another perceived barrier to widespread uptake of the NOACs.
Andexanet mode-of-action

Grace GILMORE

Perth Blood Institute (PBI) and Western Australian Centre for Thrombosis and Haemostasis (WACTH), WA

Andexanet alfa is a recombinant modified human FXa decoy protein that has been specifically designed as an antidote to neutralise the effect of the direct and indirect FXa inhibitors.

Aim: To determine whether the addition of andexanet alfa can overcome the interference of rivaroxaban and apixaban on LA testing in plasma samples from both LA positive and LA negative patients.

Methods: Rivaroxaban (Bayer) and apixaban (Bristol-Myers Squibb) tablets were crushed and dissolved in DMSO. Andexanet alfa (Portola Pharmaceuticals, Inc.) was dissolved in distilled water and aliquots were frozen at -40°C until used. Plasma samples were spiked with varying concentrations of FXa inhibitors and andexanet alfa. LA-sensitive aPTT, PT, TCT, dRVVT (Siemens Healthcare Diagnostics) and anti-FXa level (Diagnostica Stago) were performed with and without andexanet alfa on the Sysmex CS 5100 analyser. Andexanet alfa (at a final concentration of 250µg/mL) was added to LA positive and LA negative plasma samples to assess the impact on the dRVVT normalised ratio.

Results: Andexanet alfa demonstrated a concentration-dependent increase in LA sensitive aPTT and dRVVT clotting times, with negligible impact on PT or TCT. Andexanet alfa was capable of reversing FXa inhibition in a concentration-dependent manner in plasma samples containing rivaroxaban or apixaban. When rivaroxaban is added to normal plasma it significantly prolongs the dRVVT without phospholipid more than with excess phospholipid leading to a false-positive LA ratio at final concentrations >100ng/mL. This level of rivaroxaban is in the therapeutic range. The addition of andexanet alfa reduced the measurable anti-FXa level and returned the normalised dRVVT ratio to LA negative result (<1.25). In contrast, apixaban and andexanet alfa proportionately prolongs the dRVVT with or without excess phospholipid and even at high concentrations, the normalised LA ratio remains negative (<1.25).

Conclusion: Andexanet alfa corrects the false positive LA ratio in patients on rivaroxaban without affecting the true diagnosis in LA positive patients. Apixaban and andexanet alfa proportionately prolong the dRVVT with and without phospholipid, so the LA ratio remains negative.
Notes
Idarucizumab update

Chris WARD

Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, NSW; Sydney Clinical School, University of Sydney, NSW

Non-Vitamin K dependent, or direct-acting oral anticoagulants have significantly changed clinical practice in both stroke prevention for atrial fibrillation and the treatment and secondary prevention of venous thromboembolism. The lack of a "reversal agent" for these drugs has been a barrier to uptake amongst both prescribers and patients, despite acceptable bleeding rates in trials and registries and major bleeding outcomes that are not inferior to warfarin. Idarucizumab, a Fab’ molecule that binds dabigatran with 350-fold higher affinity than thrombin, has recently been registered for use in Australia. Prior to this, clinical experience with idarucizumab was limited to trial patients in the REVERSE-AD open-label trial, where it was administered to patients on dabigatran with life-threatening bleeding or requiring emergency surgery. Small numbers of Australian patients were recruited to this trial, in contrast to the New Zealand experience where dabigatran is the sole NOAC with public subsidy. Clinicians are now gaining experience with dabigatran reversal, and the trial entry criteria serve as a reasonable starting point for patient selection. Several recent cases from Australian hospitals will be presented to demonstrate the use of this agent, and some of the practical challenges that we need to address, including providing rapid access to distant hospitals. The role of laboratory testing is evolving; as dabigatran levels were not required for enrolment in the REVERSE-AD trial, a sizeable proportion of subjects did not have detectable drug at the time of idarucizumab treatment. Waiting for dabigatran levels using the dilute thrombin time or similar assay will not always be possible, particularly in time-critical situations such as intracranial haemorrhage. In other patients, it may be possible to select candidates for reversal based on their laboratory profile, and avoid using idarucizumab on patients with no residual dabigatran activity. Although the agent is very effective at reversing in vitro clotting times, the clinical impact of reversal remains to be established. Clinicians should work together to monitor outcomes and develop a consistent approach to the reversal of dabigatran.
CASE STUDY: Drug-induced thrombocytopenia due to aspirin

Charles SHUTTLEWORTH

St. Vincent’s Hospital, NSW

Aspirin is one of the most ubiquitous medications in modern medicine. Similarly, thrombocytopaenia is a common problem, particularly in hospitalized patients. Whilst aspirin is often ceased in cases of thrombocytopaenia due to concerns about haemostasis, less than a handful of cases have described a direct association between aspirin and thrombocytopaenia itself. Here we present a case of thrombocytopaenia in a hospitalized patient post cardiothoracic surgery which was initially treated as Heparin Induced Thrombocytopaenia (HIT) with minimal response. Following this, a diagnosis of aspirin induced thrombocytopaenia was made on clinical grounds, and this was then confirmed using platelet immunofluorescence via flow cytometry. This case highlights both the key steps in making a diagnosis of drug induced thrombocytopaenia, as well as some of the diagnostic pitfalls associated with HIT.
Notes
CASE STUDY: 2B or not 2B? A prothrombotic tendency masquerading as a bleeding disorder

Yvonne BRENNAN
Westmead Hospital, NSW

Type 2B von Willebrand Disease (VWD) and platelet type (PT)-VWD are rare bleeding disorders in which increased affinity of von Willebrand factor (VWF) for platelets, or increased affinity of platelets for VWF, results in clearance of large VWF multimers and platelets. Both are characterised by a positive low dose ristocetin-induced platelet agglutination assay (LD-RIPA). Our patient presented with an abnormal coagulation profile performed as a pre-operative screening test, as he required biopsy of a spinal mass. A series of investigations eventually identified metastatic renal cell carcinoma and a positive LD-RIPA assay. We believe this positive LD-RIPA was a false positive result from non-specific hyperaggregable platelets rather than true type 2B or PT-VWD. The patient later developed a deep vein thrombosis (DVT) post-surgery. Genetic investigation failed to confirm either 2B or PT-VWD, but instead uncovered a prothrombotic polymorphism in the GPIBA gene. If the patient had been inappropriately diagnosed as having 2B or PT-VWD based on laboratory testing, prohemostatic treatment may have promoted further adverse thrombotic events.
Notes
WE LOOK FORWARD TO WELCOMING YOU TO SYDNEY IN 2017

The Annual Scientific Meeting of HAA (Haematology Society of Australia and New Zealand, the Australian & New Zealand Society of Blood Transfusion, and the Australasian Society of Thrombosis and Haemostasis) is the societies’ major forum to exchange ideas and discuss clinical and research issues, including haematological malignancies, stem cell transplantation, transfusion medicine, haemostasis and thrombosis and other non-malignant haematological disorders.