

Dear Fellow Clotter

Welcome to the first ASTH newsletter for 2004. Thankyou to all the members who contributed to this issue. As promised the winning abstracts from the HSANZ/ANZBT/ASTH meeting in Christchurch are reproduced in this issue along with a conference report from the ASH meeting held at the San Diego Convention Centre last December. Also in this issue is a brief report on an evaluation recently carried out at The Prince Charles Hospital, Brisbane, of the new INR determination cartridges used on the i-STAT 1 analyser. There are also updates from the Clinical Trials Group and the New and Emerging Technologies Group. The Presidents report gives members more details of the ASM in Melbourne this October.

We are planning another 2 issues of the newsletter in 2004. Any contributions are most welcome.

Emma Perrin

PRESIDENT'S REPORT

Welcome to our first newsletter for 2004. Preparations for the Annual Scientific meeting that will be held in Melbourne are near completion. The Council's scientific sub-committee is currently examining and hopefully approving the program. Murray Adams and Emma Jones-Perrin are planning a workshop for the Saturday before the start of the meeting. This workshop will be modelled

on the one held at Westmead Hospital last year, and will undoubtedly complement the scientific program that will follow.

This year, the society is introducing for the first time a special oration in recognition of Barry George Firkin. This will be an annual event. Barry was an eminent haematologist and has contributed significantly to our current knowledge on von Willebrand factor. Barry's contributions extended beyond the field of hemostasis and thrombosis, and he is well known for his work in many other areas in haematology. It is therefore fitting that the ASTH in conjunction with the HSANZ and the ANZSBT will celebrate Barry's contribution annually though a special oration that will be held on the education day. Michael Berndt has kindly agreed to give the first oration. Michael is well known nationally and internationally for his contributions to hemostasis and thrombosis, and we are lucky that he has accepted the invitation.

The Melbourne meeting will feature a balanced program that is strong in both basic science and the clinical aspects of hemostasis and thrombosis. I am pleased to inform you that Bruce and Barbara Furie, Uri Selighson and Xaverio Ruggeri have all accepted invitations to attend and speak at the conference. All these individuals are well known for their many years of work in the field, and we are very lucky to host them this year. The meeting will feature symposia and state of the art lectures delivered by these eminent individuals. For the first time, we will give our young investigators the opportunity to present their work as part of these symposia. This will

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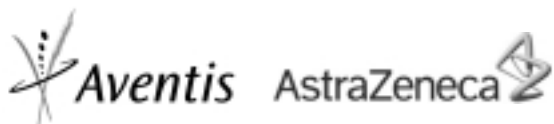
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President's Report *Continued*

give our investigators an excellent exposure and it will be a good recognition of the quality of their contributions. The presidential symposium will continue to feature the best original research that is carried out by members of our societies. This year's session will be held on the last day of the conference and will be followed by the award of prizes. I urge you to make sure that you are around for these very important aspects of our annual meeting.

The societies will this year award three travelling fellowships to help young investigators/clinicians attend the meeting. We are grateful for Kim Gould from AstraZeneca who has agreed to continue to support the best three scientific presentations at the meeting through the award of the AstraZeneca prize.

I look forward to seeing you all in Melbourne, I am confident that this will be a very rewarding experience.

Hatem Salem

SECRETARIAT NEWS

The ASTH welcomes the following new members:

Dr Stephen FLECKNOE-BROWN

Broken Hill Base Hospital, Broken Hill NSW

Ms Prue FREEMAN

Monash University, Box Hill, Victoria

Dr Lay-Cheng LIM

Singapore General Hospital, Singapore

Professor Alan MICHELSON,

Center for Platelet Function Studies, University of Massachusetts Medical School, Massachusetts, USA

Ms Lesley POULTON

Monash University, Box Hill, Victoria

Ms Diane ZEBELJAN

outh Western Area Pathology Service, Liverpool, NSW

Subscription Renewals

I have indulged in a little business process reengineering recently and changed the way I process subscription renewals. I no longer send a terminal voucher and receipt by post (too laborious and time-consuming), but instead email receipts (or fax/post if you do not have email).

Members who have paid their subscriptions should have received a receipt. However, if you would still like to have a terminal voucher, let me know and I can post it to you. Please also contact me if you have paid your subscription but have not received a receipt.

HSANZ/ANZSBT/ASTH Conference

Did you know that this year marks the 10th anniversary of the ASTH? The Council is planning to celebrate this auspicious event at the annual scientific meeting, so we look forward to seeing as many members as possible in Melbourne.

Have a relaxing Easter break.

Leonie Klomp

Secretariat

CLINICAL TRIALS GROUP REPORT

All has been a bit quiet on the Clinical Trials Group front or has everything been too busy to notice. Following on from the Christchurch meeting momentum continues to build for the ASPIRE study. The current ASPIRE Executive continue to meet by phone every 2 weeks to move the study from the current pilot phase to the main study. In October the CTG unanimously agreed to support new sites with start-up funding to assist in commencing the pilot phase of the ASPIRE study. In December 2003 applications for the start-up funds were reviewed by the Executive and 6 sites were successful. It is anticipated that additional funding maybe advertised in the near future to engage more sites into the pilot phase. Partly as a result of this initiative there are currently 8 sites recruiting patients to the ASPIRE study. As of 22nd March the total recruitment is 31 patients (Auckland 1, Box Hill Hospital 1, POWH Sydney 1, QEII Adelaide 1, Royal Perth Hospital 9, St George Hospital 18). Additional sites will be coming on board in the next few months. The executive are working to establish a Safety and Data Monitoring Committee. Graham Young has agreed to Chair this committee initially and we are very grateful to Graham for his enthusiasm. He will be reviewing SAEs in the near future. New sites to the pilot phase would be welcome and if any investigators are interested please feel free to contact myself or John Eikelboom for more information.

I hope to organise the next CTG meeting via teleconference in late April and will be in touch by email soon.

Comments and suggestions are always welcome.

Tim Brighton

Chairman CTG

ASTH NEW AND EMERGING TECHNOLOGIES GROUP REPORT

As outlined in the previous ASTH newsletter the New and Emerging Technologies (NET) Group aims to:

- Increase the profile of the ASTH for laboratory scientists.
- Increase communication between coagulation laboratories.
- Provide a forum for sharing information e.g. troubleshooting, advice, etc.
- Increase participation of laboratory scientists at ASTH annual scientific meetings.
- Develop collaboration between laboratories e.g. for new method evaluation.

With these aims in consideration, recent activities of the NET have included:

- The NET Group distribution list has been updated to 33

ASTH New and Emerging Technologies Group Report *Continued*

members. The list now includes laboratory scientists from all states in Australia, the ACT and New Zealand. It contains contact details for each member as well as a register of special haemostasis tests. Please contact me if you (or another member of your laboratory) would like to be included, or your details updated.

- Edition #4 of the e-newsletter, distributed in March 2004, included an entertaining article by Liz Duncan and Sue Rodgers on the 8th Scientific Weekend of the Adelaide Blood Club held in the Barossa Valley in 2003.
- The e-newsletter will continue to be distributed in its current electronic form, rather than in the ASTH newsletter, following feedback from NET Group members.
- Three coagulation sessions have been organised for the AIMS National Workshops (30th June - 2nd July 2004). These sessions are titled: "Case studies in coagulation - a potpourri of clinical and laboratory cases," "D-Dimers: Thrombosis answered?" and "Point of care testing and instrumentation - the new revolution?"

The major activity of the NET Group for this year will be a one day workshop to coincide with the 6th Joint Scientific Meeting of the HSANZ, ANZSBT and ASTH in Melbourne (17th - 20th October 2004). The workshop is scheduled for the Saturday the 16th of October, with the proposed venue at the Alfred Hospital.

Further details of the workshop (including preliminary program) should be available by the next ASTH newsletter. Members of the NET Group will be provided with regular updates.

As always, I appreciate any feedback or suggestions.

Murray Adams

Chair NET Group

EVALUATION OF NEW INR CARTRIDGES FOR THE I-STAT POINT OF CARE ANALYSER

Joanne Chudleigh, Jenny Brown, Michelle Williamson, Anne Koerbin, Michael Ray

"Queensland Health Pathology Service, The Prince Charles Hospital/Northside Pathology, Brisbane, Australia"

There is a rapidly increasing use of point-of-care analysers for INR analysis. This is a brief report on an evaluation recently carried out in our laboratory of the new INR determination cartridges used on the i-STAT 1 analyser (i-STAT Corporation).

The i-STAT 1 analyser is capable of capturing the operator identification, patient Hospital Unit Record Number and date of birth. All results are captured on the i-STAT Central Data Station and if patient details have been entered correctly the results will then be sent to the LIS.

The INR cartridges will be available in Australia from March 2004. They accept non-anticoagulated capillary or venous samples. The cartridge is loaded out of the instrument, facilitating capillary collection. The cartridge contains a recombinant thromboplastin with an ISI close to 1.0 which has been calibrated by WHO methods against International Reference Preparation rTF/95. Thrombin generated during the test splits a substrate releasing positive ions to produce an electrogenic curve, the mid-point being the clotting end-point.

A total of 55 patients on whom venous blood was being collected for INR determinations were also tested on the i-STAT and the INR result compared to that from the laboratory. The latter was performed on an ACL Futura using Thromborel S, the ISI of which had been calibrated

UPCOMING MEETINGS

MEETING	WHERE/DATES	CONTACT
Thrombophilia Testing Seminar	Auckland 23 April 2004	paulh@adhb.govt.nz
44th British Society of Haematology ASM	Cardiff 19-21 April 2004	www.b-s-h.org.uk
XVIIth International Symposium on Technological Innovations in Laboratory Haematology	Barcelona, Spain 13-15 May 2004	www.islh.org
50th Annual Scientific and Standardization Committee Meeting	Venice, Italy 17-19 June 2004.	www.sscvenice.it
18th International Congress on Thrombosis	Ljubljana, Slovenia 20-24 June 2004	www.thrombosis2004.org
AIMS National Workshops	Perth 30 June – 2 July 2004	www.aims.org.au
33rd Annual Scientific Meeting of the International Society for Experimental Hematology	New Orleans, USA, 17-20 July 2004	www.iseh.org
6th HSANZ/ANZBT/ASTH ASM	Melbourne 17-20 October 2004	www.asth.org.au
XIth International Congress on Antiphospholipid Antibodies	Sydney 14-18 November 2004	http://www.xith-icaa2004.unsw.edu.au/sydney/index.html
The American Society of Haematology 46th Annual Meeting	San Diego 4-7 December 2004	www.hematology.org

Evaluation of new INR cartridges for the i-STAT Point of Care Analyser *Continued*

with 20 reference plasma (Helena) and the mean normal PT calculated on the plasma from 20 healthy volunteers.

Quality control was performed daily, a bottle of reconstituting fluid simply being tipped into a second bottle containing the lyophilised control plasma.

The i-STAT results agreed closely with those from the laboratory, the Passing Bablok agreement test producing a slope of 1.000 and an intercept of 0.000 (Fig.1). Linear regression showed r2 to be 0.93. The mean difference was 0.004 (Fig.2). The QC over 29 days showed acceptable precision (Table 1).

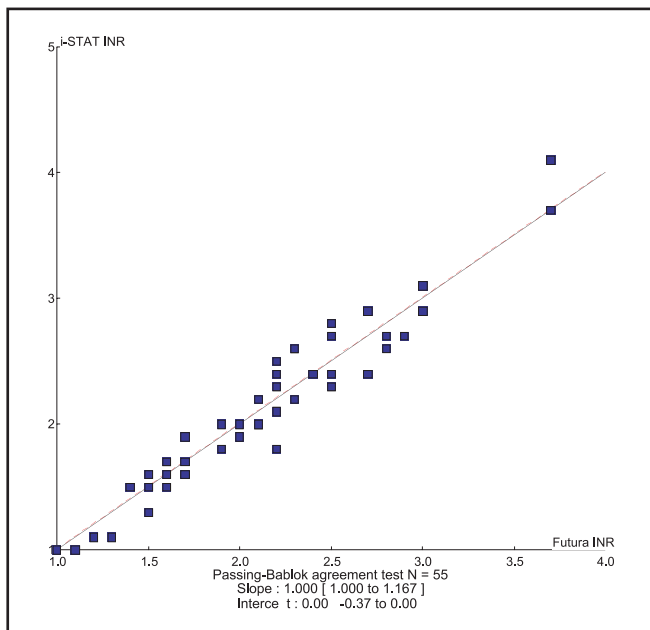


Fig. 1. Passing Bablock agreement test between INR estimations by ACL Futura and I-STAT

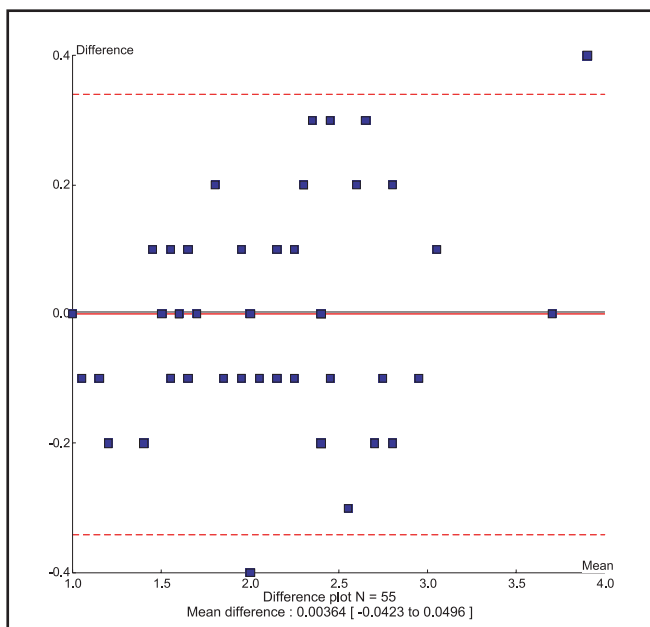


Fig. 2. Bland Altman difference test between INR estimations by ACL Futura and i-STAT

Table 1. QC results over 29 days of INR estimations on i-STAT.

Control	Target	Mean	SD	CV (%)
i-STAT PT Level 1	0.9-1.3	1.2	0.04	3.46
i-STAT PT Level 2	2.0-3.2	2.98	0.15	5.17

In conclusion, this point-of-care analyser was able to provide INR results of a similar accuracy and precision to the laboratory. Its ability to specify the patient with two points of identification, to download results to a LIS, and to be monitored from the laboratory makes this system, when run under the supervision of a laboratory, a suitable way of providing INR results at a location remote from that laboratory.

Editors note. This article was published for scientific content and the interest of the readership. The above evaluation was carried out independent of the ASTH and the conclusions reached are not necessarily those of the Editor of this newsletter or the ASTH council. No conflict of interest is perceived or declared.

ASTH WEBSITE

Thrombophilia Testing

Thrombophilia testing remains a contentious issue. Why test? Who should we test? Which tests should we do? When should we test? These are all questions that still tax us when managing patients with venous thrombosis. This month a seminar will be held in Auckland to look at some of these questions. To help get some background information about your views on thrombophilia testing, I have put a simple questionnaire on the web-site. Please visit and share you views. I have also put together some thrombophilia case studies. These are fictitious cases but are based on problems that I have been faced with over the last few months. How would you manage these cases? Please have a go - remember there are no right answers. I will give some feed-back on the web-site next month.

Haemophilia Guidelines

The haemophilia treaters in New Zealand have drawn up some revised treatment guidelines. These will be available on-line very soon -

www.asth.org.nz/guidelines.htm. All comments welcome.

Do Trials Change Practice?

The PREVENT study and Clot in Cancer study were published in the last 12 months. Have they changed your practice? From the comments so far, PREVENT seems to have had little impact on practice. Why not add your comments?

For those of you who feel that our web-site is in need of a facelift, please keep watching as we hope to revamp soon with a more professional design.

CONFERENCE REPORTS

San Diego Convention Centre, December 2003.

Good weather and excellent congress facilities made for an enjoyable meeting in San Diego. The following are selected coagulation-oriented presentations that I found particularly interesting.

The Vascular Biology session on the Endothelium was good. Robert Kerbel, from Sunnybrook and Women's College Health Sciences Centre, Toronto, summarised the challenges for clinical translation of angiogenesis inhibitors. He contrasted the recent success of a phase III clinical trial testing bevacizumab, the humanized anti-VEGF antibody, in combination with standard chemotherapy for the first line treatment of metastatic colorectal cancer to the failure of previous phase III clinical trials involving angiogenesis inhibitors. Key challenges include definition of the optimal biologic dose [OBD], monitoring anti-tumor activity, how to best combine an antiangiogenic drug with chemotherapy agents, where standard MTD chemotherapy regimens may be inappropriate; low-dose "metronomic" regimens may be superior – while being less toxic.

Biemond et al from Amsterdam gave a related abstract [2036] entitled "Strong Thrombogenic Activity of the Combined Administration of Thalidomide and Doxorubicin in Experimental Thrombosis in the Rabbit". They analyzed the effect of thalidomide, doxorubicin and the combined administration of thalidomide and dexamethasone with or without doxorubicin in a New Zealand rabbit thrombosis model. The combined administration of thalidomide, doxorubicin and dexamethasone induced a strong increase in thrombus growth, while the drugs separately and the thalidomide and dexamethasone combination did not. Potential risk factors for enhanced thrombus growth were analysed. No significant differences in factor VIII activity, von Willebrand factor activity, protein C activity, PAI-1 activity, euglobulin clot lysis or thrombin generation were demonstrated between the study groups. The study therefore did not clarify the underlying prothrombotic mechanism.

A related abstract [2040] by Woodley-Cook et al from McMaster University "Mechanisms by Which the Chemotherapeutic Agent Doxorubicin Induces a Procoagulant State in Vascular Endothelial Cells" used HUVECs to study the effects of doxorubicin on the protein C anticoagulant pathway, the extrinsic tissue factor (TF) procoagulant pathway, and apoptosis. They observed a dose-dependent down-regulation of cell surface endothelial protein C receptor (EPCR), a necessary receptor in the protein C anticoagulant pathway. The decrease in EPCR expression resulted in a decrease in the ability of HUVECs to convert protein C zymogen to APC. Doxorubicin treatment of HUVECs also resulted in a dose-dependent up-regulation of cell surface TF and endothelial apoptosis. Their results highlight a chemotherapy-induced shift of the hemostatic equilibrium by down-regulating the protein C anticoagulant pathway, upregulating the TF procoagulant pathway, and inducing cellular apoptosis.

John Griffin, from The Scripps Research Institute, summarised "Endothelial Protective Effects of Activated Protein C", in relation to clinical trials of recombinant activated protein C (APC) that showed reduced 28-day mortality in severe sepsis patients, in contrast to a score of anti-inflammatory agents as well as two potent anticoagulants (antithrombin and recombinant TFPI) which all failed. The presentation focused on the cytoprotective effects of APC on endothelial cells. He showed that APC can directly modulate gene expression profiles, resulting in up regulation of some cell survival genes, including protease activated receptor-1 (PAR-1) and EPCR. APC-induced neuroprotection in a murine model was observed at APC doses that had no effect on fibrin deposition, indicating that the anticoagulant activity of APC was probably not responsible for at least some of APC's in vivo neuroprotective effects. EPCR appears to be an "APCmodulin" that switches APC activity from anticoagulant to cytoprotective, through switching APC's proteolytic substrate target from factors Va and VIIIa to PAR-1 on endothelial cells.

For those interested in Pre-Implantation Genetics, the Scientific Committee session on Transplantation Biology was instructive. John Wagner from the University of Minnesota spoke on "Embryo Selection to "Create" a Genotypic Identical Hematopoietic Stem Cell (HSC) Donor". He described experience with the Fanconi anemia (FA) gene as well as HLA typing of each day 3 embryo generated by IVF prior to intrauterine transfer, resulting in a successful pregnancy and delivery of a healthy HLA identical infant. Umbilical cord blood HSCs were used for allogeneic transplantation for a 6.5 year old female sibling with FA. He highlighted a number of practical limitations, including the potential requirement for multiple IVF/PGD cycles and consequent delays in HSC transplant therapy as well as high cost of repeated IVF procedures.

Jeffrey Kahn, also from the University of Minnesota, spoke on the "Ethics of Creating a Stem Cell Donor". He emphasised the importance of ethical, legal and policy oversight, as the use of PGD to select embryos as potential stem cell donors becomes more widely adopted. He questioned whether there should be limits on what characteristics parents may choose when using PGD. The classic uses of PGD are to help parents avoid bearing children affected by genetic disease (severe haemophilia is a potential model here). Selecting for HLA compatibility crosses an implicit barrier between using PGD to avoid disease and using it to select for non-disease traits, chosen less for the child who will be born than to help save the life of a sick existing child. However assessing parents' motives in pursuing reproductive options is nearly an unprecedented measure in US social policy.

Moving on to the Plenary Session, abstract [5], by Landolfi et al from Italy spoke on "Efficacy and Safety of Low Dose Aspirin In Polycythemia Vera (ECLAP Study)". This was a double-blind, randomised trial involving 518 PV patients with no indication for- or contraindication to- aspirin, randomised to aspirin (100 mg daily) or placebo. At three year follow up, aspirin significantly lowered the risk of

cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke. The risk of both major and minor thrombotic events was also significantly decreased. No excess of major bleeding was observed in patients on aspirin. These results indicate a high benefit/risk ratio of aspirin in a low risk polycythemic population.

In abstract [7], Francis et al presented on "Efficacy and Safety of the Oral Direct Thrombin Inhibitor Ximelagatran Compared with Current Standard Therapy for Acute, Symptomatic Deep Vein Thrombosis, with or without Pulmonary Embolism: The THRIVE Treatment Study". Ximelagatran, an oral direct thrombin inhibitor, has a rapid onset of action and its active form, melagatran, has predictable pharmacokinetics without known CYP450 drug or food interactions. This was a randomized, double-blind, trial involving 2489 patients with DVT, (37% with PE), randomly assigned to either oral ximelagatran 36 mg bid for 6 months without coagulation monitoring, or standard LMWH followed by warfarin for 6 months. The aim was to determine whether ximelagatran is noninferior to enoxaparin/warfarin in the prevention of recurrent VTE at 6 months. There was a 9.6% incidence of serum ALT elevations (> 3 times the upper limit of normal) in patients receiving ximelagatran versus 2.0% for patients receiving enoxaparin/warfarin. They concluded that ximelagatran was noninferior to standard treatment. Ximelagatran was associated with a favourable outcome with respect to major bleeding.

Abstracts of related interest included the following: Brenner et al from Israel, abstract [43], presented "Enoxaparin Treatment Improves the Gestational Outcome of Pregnant Women with Thrombophilia and Recurrent Pregnancy Loss". This was a prospective randomized study of women with thrombophilia and RPL (3 losses in first trimester, 2 losses in second trimester or 1 loss in third trimester). Compared to the patients' historical rates of live birth and pregnancy complications, enoxaparin treatment significantly increased the rate of live birth, decreased the rate of pre-eclampsia and decreased the rate of placental abruption.

Finazzi et al from Italy [44] conducted a randomized clinical trial of two intensities of oral anticoagulant therapy in patients with the Antiphospholipid Syndrome. They found that high-dose warfarin (INR 3.0-4.0) was not more effective than conventional treatment (INR 2.0-3.0) at preventing recurrent thrombosis. This study gives useful evidence on which to base anticoagulant strategy for APS patients, minimising the risk of hemorrhagic complications.

The laboratory diagnosis of TTP attracted interest at the meeting. Two different and new ELISA methods were presented. A medical student by name of Wenhua Zhou from the Bronx, NY, gave an elegant presentation [433] entitled "Enzyme Immunoassay of ADAMTS13 Activity Distinguishes Patients of Thrombotic Thrombocytopenic Purpura from Normal Individuals and Carriers of ADAMTS13 Mutations". ADAMTS13, a recently cloned zinc metalloprotease, cleaves von Willebrand factor (VWF) at a site within the VWF A2 domain. By cleaving conformationally unfolded VWF multimers, ADAMTS13 prevents the accumulation of super-active forms of VWF in

the circulation. Technical complexity of current assays of ADAMTS13 limits their application in many laboratories. Zhou demonstrated that recombinant VWF A2 fragments may be used in a format of enzyme immunoassay (EIA) for determining ADAMTS13 activity. His results suggest that this EIA of ADAMTS13 activity identifies patients with acquired or hereditary TTP and has the advantage that the procedure is technically simple and can be completed within hours instead of days.

A similarly interesting assay was presented by Manfred Rieger from Baxter BioScience [434] "Detection of Anti-ADAMTS-13 Autoantibodies by a Newly Developed Highly Sensitive Enzyme-Linked Immunosorbent Assay (ELISA) System". Anti-ADAMTS-13 antibodies in acquired TTP have not previously been measured directly. However, in a clinical setting, it might be of considerable interest to quickly know if the patient is anti-ADAMTS-13 antibody positive or not. Using recombinant ADAMTS-13 (rADAMTS-13) he established an ELISA system capable of detecting IgG and IgM antibodies to ADAMTS-13. Briefly, C-terminal His-tagged rADAMTS-13 was immobilized on an ELISA plate via an anti-his-tag antibody. Plasma was added in dilutions and anti-ADAMTS-13 antibodies were detected using alkaline phosphatase labeled anti-IgG or anti-IgM specific antibodies. In summary, this new assay allows a rapid and sensitive determination of anti-ADAMTS-13 antibodies in human plasma.

It appears that rapid laboratory characterisation of TTP cases may soon be a realistic possibility for haematology diagnostic laboratories.

Mark Smith

ABSTRACT WINNERS FROM 2003 CONFERENCE

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The Rate of Thrombus Resolution following Treatment of DVT is related to the Site and the Precipitating Cause of Thrombosis

Young L. McKelvie S, Rolfe-Vyson V, Shirley K. Ockelford PA, Harper PL

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The success of treatment for DVT has been measured using mortality, incidence of PE and rate of recurrence as end points, but few studies have examined the incidence of vessel recanalisation. Incomplete restoration of vessel patency may be an important predictor of DVT recurrence. We report the incidence of vessel patency following anticoagulant treatment in above and below knee DVT and correlate this with known risk factors for thrombosis. A total of 688 cases of lower limb DVT were reviewed. 344 patients had an ultrasound scan after completing 3 to 6 months of anticoagulant therapy. The scan results were categorised into 3 groups; those with complete resolution of thrombus, those with partial

improvement and those with no improvement or progression. The patients were categorised into five risk groups; (1) with a malignancy; (2) with a recent history of surgery or trauma; (3) pregnant/post partum, or on oestrogen therapy; (4) immobility or recent air travel; and

(5) with no identified precipitating factor. In above knee DVT, 39% of cases had complete resolution of thrombosis and 48% partial improvement. In below knee DVT resolution was significantly better with 72% with clear vessels and 24% partial improvement.

Follow up Scan	Malignancy	No cause	HRT/OC/ Pregnancy	Surgery or trauma	Immobility/ air travel
No change or worse	14(23%)	28(14%)	2(8%)	4(4%)	1(3%)
Partial improvement	26(43%)	87(45%)	11(42%)	30(33%)	13(42%)
Vessels clear	20(33%)	80(51%)	13(50%)	56(62%)	17(55%)
Totals	60	95	26	90	31

Patients with a transient risk factor, such as surgery, trauma, immobility or air travel are most likely to have complete resolution of the thrombosis with only 4% showing no improvement after treatment. In contrast patients with malignancy have a low incidence of complete resolution (33%) and in 23% of cases there was no improvement. Although the majority of patients with a malignancy had above knee DVT, this difference was not entirely due to the site of thrombosis. In a subgroup

analysis of above knee DVT the rate of thrombus resolution was still significantly lower in the malignancy group, than the surgery/trauma group (30% v 55%). We conclude that the rate of vessel resolution is related to the precipitating cause of DVT and in all groups with above knee DVT more than 50% of patients have some residual vessel changes. The significance of these changes in relation to recurrent DVT is being examined.

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Role of Thrombospondin-1 in the Control of von Willebrand Factor Multimer Size in Mice

Pimanda JE¹, Ganderton T¹, Lawler J², Kershaw G³, Maekawa A¹, Chesterman C¹ and Hogg P¹

¹Centre for Vascular Research, University of New South Wales and the Department of Haematology, The Prince of Wales Hospital, Sydney; ²Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston; ³Department of Haematology, Royal Prince Alfred Hospital, Sydney

Plasma von Willebrand factor (VWF) is a multimeric glycoprotein from endothelial cells and platelets that mediates adhesion of platelets to sites of vascular injury. In the shear force of flowing blood, however, only the ultra large VWF multimers (ULVW) are effective in capturing platelets. The multimeric size of VWF can be controlled by proteolysis at the Tyr⁸⁴²-Met⁸⁴³ peptide bond by ADAMTS13 or cleavage of the disulfide bonds that hold VWF multimers together by thrombospondin-1 (TSP-1). A severe deficiency of ADAMTS13 is associated with thrombotic thrombocytopenic purpura, a disorder characterized by the persistence of ULVWF multimers in plasma and the thrombotic occlusion of arterioles. To clarify the role of TSP-1 in the control of VWF multimer size in vivo, we have studied the vWF multimer pattern in TSP-1 null mice. Surprisingly, the average multimer size of plasma VWF in TSP-1 null mice was significantly smaller than in wild type mice. In addition, the multimer size of VWF released from endothelium in vivo was

reduced more rapidly in TSP-1 null mice than in wild type mice. These findings indicate that TSP-1 inhibits the activity of ADAMTS13 in vivo. TSP-1, unlike ADAMTS13, is stored in platelet α -granules and is released upon platelet activation. Accordingly, platelet VWF multimer size was reduced upon lysis or activation of human and wild type murine platelets but not TSP-1 null murine platelets. This difference was reflected in a significantly faster rate of shear-induced aggregation of the TSP-1 null platelets. These findings indicate that TSP-1 influences plasma and platelet VWF multimeric size differently and may be more relevant for control of the platelet VWF pool.

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IgG Fractions from Subjects with the Antiphospholipid Syndrome Demonstrate Inhibitory Activity Against TFPI and Increased Thrombin Generation

Adams M¹, Breckler L¹, Thom J², Baker R², Oostrick R¹

¹School of Biomedical Sciences, Curtin University, Perth, ²Department of Haematology, Royal Perth Hospital, Perth

Aim: We have previously demonstrated that plasma from some subjects with the antiphospholipid syndrome (aPS) has inhibitory activity against tissue factor pathway inhibitor (TFPI). Impaired ability of TFPI to regulate the tissue factor (TF) pathway of coagulation may be an important contributor to a prothrombotic state in these subjects. The aim of this study was to investigate the effect that IgG fractions from aPS subjects containing anti-TFPI activity have on TF induced thrombin

generation. Methods: TFPI and anti-TFPI activities were determined in aPS subjects (n=23) and normal controls (n= 18). TFPI activity was determined using an amidolytic assay based on the generation of factor Xa. Anti-TFPI activity was determined after incubation of IgG isolated from subject or control plasma with normal pooled plasma, using the TFPI amidolytic assay. The influence of IgG fractions on TF induced thrombin generation was determined using a chromogenic assay of thrombin activity, in the aPS subjects and 10 normal controls. Results: Anti-TFPI activity was demonstrated in 11 of 23 (48%) subjects (mean = 0.024 U/mL; range = 0 - 0.095 U/mL) and 0 of 18 (0%) controls. There was no significant difference in TFPI levels demonstrated between subjects (1.23 +/- 0.33 U/mL) and controls (1.17 +/- 0.27 U/mL). The effect of IgG fractions on TF induced thrombin generation was not significantly different between aPS subjects (95 +/- 19%) and the 10 normal controls (100 +/- 5%) tested. Anti-TFPI activity was weakly correlated with the effect of IgG fractions on thrombin generation in

subjects (r = 0.33). IgG from four aPS subjects demonstrated TF induced thrombin generation higher than 10%, of which two (13.1% and 12.1%), also demonstrated anti-TFPI activity (0.080 and 0.095 U/mL, respectively). Conclusions: Anti-TFPI activity was confirmed in aPS subjects. IgG fractions from aPS subjects demonstrate variable interference to TFPI function and thrombin generation compared to controls. IgG fractions from two subjects demonstrated both anti-TFPI activity and increased thrombin generation. This may represent a subset of aPS subjects in which high anti-TFPI activity leads to up-regulation of the TF pathway. Although autoantibodies to TFPI have recently been demonstrated, cross-reacting antiphospholipid antibodies and/or other entities may also interfere with TFPI function, resulting in a net increase in thrombin generation and an increased thrombotic risk. The overall inhibitory contribution of anti-TFPI activity toward TFPI *in vivo* therefore remains to be elucidated.

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A prospective study assessing the clinical utility of D-dimer levels and a clinical risk assessment model for the exclusion of pulmonary embolism

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The clinical diagnosis of pulmonary embolism is difficult. Objective testing using ventilation perfusion scanning (VQ) is expensive and not readily available in many institutions. There have been several studies utilising clinical risk assessment models in combination with Ddimer (DD) assays to assist with the diagnosis of PE, negating the need for VQ in patients with a low clinical risk and a normal DD. Monash Medical Centre has

conducted a prospective study on 704 patients who were admitted to our Emergency Department during the period November 2002 - July 2003. We assessed the success of a protocol utilising the clinical risk assessment model of Kline et al and an Instrumentation Laboratory D-dimer test for the exclusion of PE, the results are displayed in table 1 below. We also performed four D-dimer methods including the IL Ddimer (Automated immunoturbidometric assay), Agen dimertest gold (ELISA assay), Vidas D-dimer (Rapid ELISA), and Agen Simplify (Bedside qualitative test) on a subset of these patients, the results are displayed in table 2. We have noted that the reproducibility of some assays at the cutoff level have high coefficient of variation. However, our results showed that the combined clinical risk assessment and Ddimer protocol is a safe strategy for excluding patients who are at a low risk of PE and withholding anticoagulation therapy.

	n	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
Risk assessment (RA)	235	45 (39-51)	84 (80-88)	41 (35-47)	86 (82-90)
IL- DD	249	92 (89-95)	43 (37-49)	30 (24-36)	96 (94-98)
Risk assessment and DD	230	98 (96-100)	41 (35-47)	26 (20-32)	99 (98-100)

Table 1

	n	Sensitivity (CI)	Specificity (CI)	PPV (CI)	NPV (CI)
IL dimer	73	95 (90-100)	10 (3-17)	31 (20-42)	83 (74-92)
ELISA GOLD	73	73 (63-83)	57 (46-68)	42 (31-53)	83 (74-92)
VIDAS	73	86 (78-94)	55 (44-66)	45 (34-56)	90 (82-98)
Simplify	73	77 (67-87)	59 (48-70)	45 (34-56)	86 (78-94)

Table 2