

P036

Overall Haemostatic Potential (OHP) Assay Results Reflect Changes in Fibrinolytic Factors

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Aim

The Overall Haemostatic Potential (OHP) assay is a global assay of fibrin generation and fibrinolysis. No routine assays are available which quantify abnormalities in the fibrinolytic system. Our aim in this study was to assess changes in fibrinolysis measured by the OHP assay in samples with variable levels of fibrinolytic factors: plasminogen, α 2-antiplasmin, PAI-1 and TAFI.

Methods

Plasmas deficient in plasminogen, α 2-antiplasmin, PAI-1 and TAFI were obtained commercially. Samples were spiked with Lys-plasminogen, Glu-plasminogen, PAI-1, TAFI or pooled normal plasma to produce serial increases in these factors. Potato tuberoses carboxypeptidase inhibitor (PTCI) was also used to inhibit TAFI in PNP and patient samples. OHP assays utilizing either thrombin (0.03 IU/ml) or tissue factor (5 pM) to trigger fibrin generation and rt-PA (350 ng/ml) to trigger fibrinolysis, were performed. Data analysis involved calculation of Spearman correlations for OHP assay parameters and fibrinolytic factor concentrations.

Results

The OHP assay demonstrated markedly reduced overall fibrinolysis potential (OFP) in plasminogen deficient plasmas, which normalised when plasminogen concentration was increased to normal plasma levels. At elevated Lys-plasminogen levels fibrin generation was impaired. OFP was mildly increased in α 2-antiplasmin deficiency. PAI-1 depleted plasma had mildly increased fibrinolysis compared with PNP, but with addition of PAI-1 to concentrations of 300 ng/ml or higher, fibrinolysis was markedly impaired. TAFI deficient plasma had reduced fibrin generation but no change in fibrinolysis was seen, even with elevated TAFI levels, in spiked samples. OFP was not altered by TAFI inhibition with PTCI in PNP and pregnant patients.

Conclusions

The OHP assay shows the predicted changes in OFP, when concentrations of fibrinolysis activators and inhibitors are varied. The assay provides a simple means of quantifying overall fibrinolysis and is sensitive to abnormalities in individual components of the fibrinolytic system. It should be useful for investigating fibrinolytic abnormalities in patients, where no routine assay is currently available.

No conflict of interest to disclose

P037**Pre-analytical Variables in the Calibrated Automated Thrombin Generation Assay**

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Aim

The Calibrated Automated Thrombogram (CAT) is a standardised method for the measurement of thrombin generation. However, there is little data on the influence of pre-analytical variables on this assay, using the commercially available PPP reagent (5pM Tissue factor, 4µM phospholipids).

Method

We measured the endogenous thrombin potential (ETP), peak thrombin concentration, lagtime and time to peak (ttpeak) in blood collected into vacutainers. In ten normal volunteers, we studied five plasma separation methods (single spin 1400g/10min, double spin 1400g/10min, enhanced single spin 2600g/15min, Eppendorf spin 20,000g/ 3min, and 0.2µ membrane filtration). The effect of time (0.5-24 hours) between blood collection and plasma separation was determined in six subjects. The effect of Inhibition of contact activation by corn trypsin inhibitor (CTI, 18.3µg/ml) was investigated in 37 subjects. Results were analysed by two-way ANOVA or paired t-test.

Results

There was no difference in ETP ($P = 0.77$) between the various separation methods. Peak, lagtime and ttpeak all showed significant differences, largely due to the membrane-filtered and 1400g single-spin samples. The latter had a higher mean platelet count ($13 \times 10^3/\mu\text{l}$) than the other centrifugation methods ($1-2 \times 10^3/\mu\text{l}$). Membrane filtration removed all platelets, but also caused a significant ($P < 0.001$) 5% increase in APTT, 28% decrease in factor VIII and 23% decrease in von Willebrand antigen. There was no statistical difference for any of the CAT parameters for samples processed between 0.5 and 6 hours after blood collection, and only peak showed a difference at 24 hours ($P = 0.03$). CTI in the blood collection tube had no significant effect on ETP ($P = 0.63$), peak ($P = 0.13$), lagtime ($P = 0.07$) or ttpeak ($P = 0.29$).

Conclusions

Plasma suitable for CAT analysis can be prepared by an enhanced single spin on the day of collection, without inhibition of contact activation. Membrane filtration is not recommended.

No conflict of interest

A256

P038

Thrombin Generation as a Predictor of Radiotherapy Induced Skin Erythema

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Aim

Biological mechanisms underlying radiation-induced erythema remain largely unknown, with no simple way to accurately predict or prevent extreme cases. Based on recent findings in patients suffering chronic urticaria, we sought to determine if similar mechanisms of hypercoagulation contributed to comparable skin reactions during radiotherapy.

Methods

Plasma levels of prothrombin factor 1+2, D-dimers, and plasminogen activator inhibitor-1 (Pai-1) were tested in 32 women undergoing irradiation following breast conserving surgery for early breast cancer and correlated to peak levels of skin erythema quantitated by reflectance spectrophotometry.

Results

Mean reflectance was found to increase significantly from 16.89 ± 4.61 at baseline to a peak of 27.66 ± 5.14 on day 36 of treatment ($p < 0.001$). Peak reflectance correlated with F1+2 and Pai-1 levels measured both at baseline and day 29 of treatment, and multivariate analysis indicated that these two baseline measurements were the best predictors of peak reflectance, accounting for 59% of the variability in erythema ($p = 0.000004$).

Conclusion

Patients with signs of intravascular thrombin generation are at higher risk of radiotherapy induced skin reactions, providing a new therapeutic avenue for preventing this side effect of cancer treatment.

No conflict of interest to disclose

P039

Overall Haemostatic Potential (OHP) and Thromboelastography (TEG): Novel Assays to Monitor the Anti-Fibrinolytic Effect of Tranexamic Acid (TA) in Treated Patients

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TA is an antifibrinolytic agent used to reduce blood loss and transfusion requirement during surgery and in patients with a bleeding tendency. The therapeutic plasma concentration has been defined as 10 μ g/ml. However there is no routine laboratory test capable of detecting the antifibrinolytic effect of TA *ex vivo*.

The study aims were to determine the sensitivity of the OHP and TEG to TA and their utility for monitoring the antifibrinolytic effect of TA in treated patients. We collected healthy volunteers and patients who undergone cardiac surgery as they represent more than 90% of the requests for TA in our hospital.

The OHP is a simple coagulation assay measuring fibrin generation and lysis in plasma. We use thrombin (0.03U/ml) to trigger fibrin generation and rt-PA (300ng/ml) to initiate fibrinolysis. Serial ODs are measured to generate a fibrin time curve. To optimise the assays, control plasmas were spiked with increasing amount of TA (10ng/ml to 10mg/ml). Using standard assay conditions, the overall fibrinolytic potential (OFP) was above 70% for all controls at baseline. Fibrinolysis was reduced with TA concentrations as low as 0.5 μ g/ml and totally inhibited at 10 μ g/ml. By increasing rtPA concentration we were able to estimate TA concentration up to 30 μ g/ml. All patients tested had a normal BMI and normal renal function. Blood was collected 24h and 48h after surgery and all patients received a standard dose of 5g TA. Patient responses were highly variable with TA activities between 1 and 30 μ g/ml after 48 hours. TEG showed similar results but was less sensitive.

Analysis of patient samples with the OHP or TEG can detect a minimal TA concentration at which fibrinolysis is inhibited, at approximately 10 μ g/mL. We propose that these tests could be used to optimise individual antifibrinolytic therapy during surgery, to achieve haemostasis while minimising the risk of thromboembolism. Both tests are simple and easy to perform and have the potential for routine use in the coagulation laboratory.

No conflict of interest to disclose

A258

P040

An IgM Paraprotein with Unusually Strong Lupus Anticoagulant Activity in a Patient with Splenic Marginal Zone Lymphoma (SMZL)

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Aim

To investigate discrepant PT and APTT clotting times between manual and automated methods in a patient with an IgM paraprotein acting as a strong coagulation factor inhibitor.

Method

Initial PT and APTT tests were with Recombiplastin and Platelin-L respectively on STA-R and BCS analysers. Lupus anticoagulant (LA) testing was by APTT, DRVVT and KCT methods. 1-stage factor assays were with Platelin-L and Actin FS as APTT reagents on the STAR Evolution. Chromogenic FVIII assays were performed with the Biophen kit.

Results

The APTT was >200 seconds (RR: 25 – 37) and PT was 91.7 seconds (RR: 10.5-13.5), with neither correcting on mixing. The PT showed no correction with added soybean lecithin, and partial correction with washed platelets. LA was detected and remained clearly positive up to 1:100 dilution with normal plasma by DRVVT, APTT and KCT mix tests. Protein electrophoresis required patient plasma to be treated with a reducing agent due to precipitation artefact, and an IgM kappa paraprotein of 13.1 g/L was identified. IgM aCL was >60MPL (RR<20) at 1:32 dilution. FVIII, FIX, FXI and FXII assays gave results <2%. Using LA insensitive APTT reagent and 1/400 dilution, the FVIII:C estimate was 365%. Chromogenic assay for FVIII was 65% at 1/400 dilution. VWF:Ag was 246% by ELISA, but 0% by Stago Liatest, probably due to precipitation of immunoglobulins giving a spurious absorbance change curve. Precipitation was also seen in tilt-tube clotting assays. Two months after treatment of SMZL, paraprotein decreased to 7.6 g/L, but APTT remained >200 seconds.

Conclusion

The patient's LA interfered significantly with clotting-based assays and latex immunoturbidometric assay for VWF:Ag. Precipitation of immunoglobulins caused a falsely short endpoint in the APTT measured on the STA-R analyser, and PT on BCS. It is important the laboratory has alternative methods such as LA-insensitive coagulation factor protocols to allow accurate testing of unusual samples.

No conflict of interest to disclose

P041

Effect of Lipoproteins on the Release of Tissue Factor Pathway Inhibitor and the Expression of Tissue Factor in Endothelial CellsPaul Ellery¹ and Murray Adams^{1,2}¹ School of Biomedical Sciences, Curtin University of Technology, Bentley Campus, Perth, Western Australia, Australia² School of Human Life Sciences, University of Tasmania, Launceston Campus, Launceston, Tasmania, Australia**Aim**

The major lipoproteins LDL, VLDL and HDL have both pro- and anti-coagulant effects. A small number of studies have investigated the effect of these lipoproteins on tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in endothelial cells, though the net effect of each lipoprotein on the TF pathway has not been determined. Furthermore, the effect of HDL on endothelial cell TFPI is unknown. The aim of this study was to systematically determine the effect of HDL, LDL and VLDL on TF expression and TFPI release from human umbilical vein endothelial cells (HUVECs).

Methods

Endothelial cells were isolated from human umbilical veins and grown to confluence in MCDB 131 basal media. HUVECs were treated with HDL, LDL or VLDL for one or 24 hours. Cell surface TF activity and secreted TFPI activity were determined using well defined amidolytic assays.

Results

HDL and VLDL dose dependently increased the secretion of TFPI after one hour, but not 24 hours. LDL increased the secretion of TFPI activity after one hour but decreased it after 24 hours. LDL decreased cell surface TF activity at both timepoints and VLDL decreased TF activity after one hour only, though the effect of both lipoproteins was not dose dependent. HDL had no effect on TF activity.

Conclusion

The data from this study suggests that each of the major lipoproteins may have a short term cardio-protective effect on the endothelium by promoting the release of TFPI, whereas they may have a long term prothrombotic effect by decreasing functional TFPI in the surrounding environment. Further studies are required to define the precise mechanisms by which the major lipoproteins affect components of the TF pathway in endothelial cells.

No conflict of interest to disclose

P042

Establishment of a Reference Range for D-Dimer Levels Using *Innovance* and D-Dimer Plus in Different Stages of Pregnancy for Tasmanian Women

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Study design

We have prospectively measured D-Dimer levels in 500 pregnant women during the period from January 2007 to May 2008 at the Launceston General Hospital (LGH), Tasmania, Australia. The median age was 31 years (range; 18-42) with a median weight of 78 kilograms (range; 46-137). These women have been investigated during each trimester using two different immunoturbidometric techniques with D-D-Plus and *Innovance* (Dade Behring, Marburg, Germany) analysed by Sysmex® CA 1500 (Sysmex, Kobe, Japan).

Results

Interim analysis showed that the mean value in the non-pregnant population for *Innovance* was 0.35 mg/L (mean+2SD = 0.79), while for D-Dimer plus was 135 μ g/L (mean+2SD = 467). The mean value of *Innovance* at different trimesters of pregnancy were respectively 0.5 (M+2SD= 1.1), 0.93 (2.1) and 1.42 (2.81) mg/L, while for D-Dimer plus were 166 (428), 212 (552) and 249 (587) μ g/L in each trimester respectively. About 8% of the pregnant women studied had elevated D-Dimer levels above 2 SD compared to the same women in the trimester group. These patients were monitored regularly every 4 weeks for occurrence of complications during pregnancy. A multivariate analysis showed no correlation between both D-Dimer assays and age, diet, smoking and ethnicity. However, there was an association between both D-Dimer assays and gestational age ($p < 0.001$) as the D-Dimer levels showed a corresponding increase with gestational age. Of interest, we observed that of 5 patients enrolled in the trial with twin pregnancies 4 of these had a high *Innovance* D-Dimer level $>2SD$ compared to 2 out of 5 women who had D-Dimer Plus level $>2SD$, suggesting that the latter might be a more sensitive assay during multiple pregnancy.

Conclusion

Our data suggest that *Innovance* is a reliable and stable assay that directly correlates with D-Dimer Plus in the pregnant population.

This research received a grant from the Clifford Craig Medical Research Trust, Tasmania, Australia.

The authors confirm that there is no conflict of interest in relation to this research.

P043**A Single Institution Audit of the Appropriateness of Utilization of D-dimer Testing****Praveen Kumar Pathak, Simon McRae***Department of Haematology, The Queen Elizabeth Hospital, Adelaide, SA***Aim**

Used appropriately, the D-dimer testing combined with pre-test probability assessment can avoid diagnostic imaging in up to a third of patients with suspected venous thromboembolism (VTE). However, due to poor specificity for VTE (40-50%), inappropriate as a screening test in patients with non-specific symptoms may actually increase the number of imaging tests performed. The aim of the audit was to assess the appropriateness of D-dimer use at a single metropolitan hospital.

Methods

Subjects were identified from consecutive patients referred to the QEH IMVS laboratory for D-dimer testing. Patients with a clear indication for D-dimer testing other than suspected VTE were excluded. The notes of all other patients were obtained and data was extracted on suspected diagnosis prior to D-dimer testing, documentation of pre-test probability (PTP), the performance of diagnostic imaging, and final diagnosis. When not documented, PTP of VTE was determined according to a dichotomised Wells' by the investigator where possible. D-dimer use was determined to be appropriate if test request, interpretation and resulting action were consistent with a locally accepted diagnostic algorithm for VTE.

Results

55 eligible patients were identified. D-dimer use was considered appropriate in 24 (44%) patients, inappropriate in 23 (42%), with no conclusion in 8 patients due to an inability to determine PTP from documented information. Reasons D-dimer use being considered inappropriate included; no clinical suspicion of VTE (11 pts), high clinical PTP of VTE before testing (3 pts), performance of diagnostic imaging with negative D-dimer result (3 pts), and failure to perform imaging when D-dimer was positive (6 pts). Only 11 pts had PTP documented.

Conclusions

D-dimer was inappropriately utilized in over 40% of patients, with frequent use as a screening test to potentially "rule in" VTE. Further education of medical staff on the appropriate utilization of D-dimer testing is required.

*No conflict of interest to disclose***A262**

P044

Evaluation of an ADAMTS13 Activity Elisa Assay for Routine Use

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Aim

Untreated Thrombotic Thrombocytopenic Purpura (TTP) is a life-threatening condition with high mortality rates. Reports in the literature attempt to categorise clinical and/or genetically modifying triggers to predict acute episodes, response to therapy and measurement of ADAMTS13 function with assays developed to assist in this regard. We evaluated the Technozym ADAMTS13 Activity Assay (Technoclone) and assessed its utility as an aid to diagnosis.

Method

25 normal samples were assayed with the Technozym ADAMTS13 Activity kit to validate the quoted reference range and confirm cut-off values for the assay. 26 patients presenting with clinical symptoms and laboratory findings of TTP were also tested for ADAMTS13 activity.

Results

We established a normal reference range of 43 to 125%, which correlated with the method's stated reference range. Patients were classified as 'likely idiopathic TTP', 'possible TTP' or 'alternate diagnoses' by the treating clinician. Of the 26 patients tested, 12 were clinically diagnosed as 'likely idiopathic TTP'. 9/12 (75%) yielded baseline pre treatment activity results of <5%, the cut-off considered diagnostic of idiopathic TTP. The remaining 14 patients were categorized as 'possible TTP' or labelled as 'alternate diagnoses' with ADAMTS13 Activity values of >5%.

Conclusion

The Technozym assay has proven to be a reliable method for the determination of ADAMTS13 activity. Reference ranges and reproducibility testing were within acceptable limits and levels of <5% were attained in approximately 75% of likely idiopathic TTP patients, comparing well with literature reports.

This research was supported by Helena Laboratories (Australia) Pty Ltd. The company had no role in analysing the data or preparing the abstract.

P045**A Rapid Assay of ADAMTS13 Activity Predicts Response to Plasma Exchange In Patients With Thrombotic Thrombocytopenia Purpura**

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Thrombotic thrombocytopenic purpura (TTP) is a disease presenting with microangiopathic haemolytic anaemia (MAHA) and thrombocytopenia, with additional variable combinations of neurological involvement, renal impairment, and fever. TTP can be congenital or acquired. The classic immune-based (primary) TTP usually presents as an idiopathic disease, but other cases have common underlying associations. The primary forms are due to formation of antibodies to the metalloproteinase ADAMTS13, with the consequent deficiency resulting in accumulation of unusually large multimers of VWF. These can induce thrombosis by platelet agglutination. Plasma exchange has reduced the mortality rate of primary TTP from 90% to approximately 20%, but is not always effective in other diseases with a TTP-like picture. It has been suggested that severe ADAMTS13 deficiency is a good criterion for diagnosis of primary TTP. In the past, assays of ADAMTS13 activity have been time consuming and labour intensive. However a rapid fluorescent assay method (FRETTS-VWF73) has been developed which enables rapid quantitative measurement of ADAMTS13 activity, with absence of ADAMTS13 activity reported to be 89% sensitive and 100% specific for TTP.

The Canterbury Health Laboratory has recently trialled the FRETTS-VWF73 assay. Six patients with a mixture of primary and secondary TTP are described. Two patients with primary TTP and a patient with HIV-associated TTP had very low levels of ADAMTS13 activity, whilst two patients with secondary TTP and a third patient with possible TTP had normal levels. The treatment and clinical course of these patients is described. This assay, employed for the first time in New Zealand, is promising as a sensitive and specific method for identifying cases of idiopathic TTP and has implications for treatment.

No conflict of interest to disclose

P046

Evaluation of Three Commercially Available Collagen Binding Assays

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Aim

The collagen binding assay is utilised in our laboratory as part of the routine von Willebrands (vWD) screening panel. Recently there have been difficulties in obtaining kits from the supplier, Life Therapeutics. Due to increased regulatory requirements for in house testing we decided to evaluate and compare two other commercially available collagen binding assays with our current assay.

Method

Three commercially available collagen binding assays were selected. Our current assay produced by Life Therapeutics, USA utilises a collagen whose source was not mentioned. The first assay evaluated was Asserachrom VWF:CB, Diagnostica Stago, France which utilises type III collagen from placenta. The second assay evaluated was Collagen binding assay, Vital Diagnostics, Australia which utilises equine collagen (95% type I and 5% type III). Forty four patients were tested in total from samples collected for routine von Willebrand screening. Seven samples were from known vWD patients. Samples were tested according to the provided package inserts for each assay.

Results

The correlation between the Life Therapeutics and the Asserachrom assay yielded an $r^2 = 0.6534$, from all patient samples (n=26). In patient samples with values below 1.0 U/mL (n=19) correlation between these two assays yielded an $r^2 = 0.6377$. The correlation between the Life Therapeutics and Vital Diagnostics assays yielded an $r^2 = 0.4378$ (n=41). Three patients with the Vital Diagnostics assay gave results that were not comparable with the other two assays and the rest of the vWD screen.

Conclusion

All three assays performed well and were easy to use. Although this study was with a small number of patient samples, all samples from known von Willebrand patients were able to be discriminated with two of the kits. Further studies are indicated with a larger cohort of known von Willebrand patients.

No conflict of interest to disclose

P047

ADAMTS13 Activity Testing – Clinically Useful**Sarah Just**, Glen Kennedy, John Rowell*Coagulation and Special Investigations Section, Pathology Queensland - Central, Brisbane, Queensland, Australia***Aim**

To review the utility of an ADAMTS13 activity assay introduced into our laboratory service for diagnosis of thrombotic thrombocytopenic purpura (TTP).

Methods

From December 2006 ADAMTS13 levels were measured using a commercially available fluorescence resonance transfer von Willebrand factor assay (FRETs-vWF; GTI Diagnostics, USA). Requests for ADAMTS13 activity levels were received both directly from within our statewide pathology network, as well as from various pathology laboratories across Australia. Clinical data was obtained from request forms and augmented when possible via phone conversation with the requesting medical practitioner/team.

Results

To date, a total of 82 ADAMTS13 activity assays have been performed in fiftyseven patients, with three patients assayed multiple times. Normal ADAMTS13 activity levels (>70% protease activity) were obtained in 26 patients (46%), mildly reduced activity levels (40-70% protease activity) in six (11%), moderately reduced activity levels (10-40% protease activity) in 16 (28%) and severely reduced activity levels (<10% protease activity) in nine (16%). In total, 8/9 patients with severely reduced ADAMTS13 levels suffered TTP; one patient had no clinical information provided. A further four patients with moderately reduced ADAMTS13 levels and one patient with mildly reduced activity levels had assays initially performed during or after therapy of known TTP. In all three TTP patients with serial levels performed starting at diagnosis and then during/after therapy, ADAMTS13 activity increased from severely reduced to normal (>100%). A significant number of assays (n=21) were performed in patients with normal platelet counts and/or no evidence of microangiopathy.

Conclusion

Our experience suggests that the FRETs-vWF ADAMTS13 activity assay is useful in both the diagnosis of TTP and monitoring efficacy of its treatment. Further clinician education is required with respect to appropriate ordering of this assay, with almost half of requests received in patients without any clinical evidence of TTP.

No conflict of interest to disclose

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P048

The Effect of Capsaicin and Dihydrocapsaicin on Blood Coagulation and Platelet Aggregation *in vitro*

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Aim

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in developed countries. It has been proposed that consumption of capsaicinoids, including capsaicin and dihydrocapsaicin, the 'hot' principles found in chilli, may protect against the development of CVD. Our aim was to determine the effect of capsaicin and dihydrocapsaicin on blood coagulation and platelet aggregation.

Methods

Normal reference plasma (NRP) was spiked with increasing concentrations (3.125-100 $\mu\text{mol/L}$) of capsaicin and dihydrocapsaicin. After incubation for 30 minutes at 37°C, PT, aPTT and coagulation factor activities (FII, FV, FVII, FVIII:C, FIX, FX, FXI and FXII) were measured. ADP-induced (5 $\mu\text{mol/L}$) platelet aggregation was determined in platelet rich plasma (platelet count = $250 \times 10^9/\text{L}$) from six normal donors in the absence and presence of capsaicin and dihydrocapsaicin (concentrations as before).

Results

Both capsaicinoids decreased FVIII (capsaicin by 36% and dihydrocapsaicin by 41% at 100 $\mu\text{mol/L}$) and FIX (capsaicin by 34% and dihydrocapsaicin by 20% at 100 $\mu\text{mol/L}$) activity in NRP, in a concentration-dependent manner, but did not affect the activity of factors II, V, VII, X, XI and XII. Concentration-dependent decreases in ADP-induced platelet aggregation were observed using capsaicin (0 vs 100 $\mu\text{mol/L}$; mean \pm SEM); max % aggregation (79.8 \pm 4.0% vs 70.0 \pm 4.6%), % area under curve (65.1 \pm 3.5% vs 55.0 \pm 4.5%) and dihydrocapsaicin (0 vs 100 $\mu\text{mol/L}$; mean \pm SEM); max % aggregation (79.9 \pm 3.4% vs 70.5 \pm 4.7%), % area under curve (65.6 \pm 2.9% vs 56.6 \pm 4.4%). Capsaicinoids had no effect on platelet count, morphology, PT or aPTT.

Conclusion

This study suggests that capsaicinoids may provide a cardio-protective effect by inhibiting platelet aggregation and reducing the activity of coagulation factors VIII and IX. Further studies are required to determine whether these effects are consistent using dietary trials and to evaluate their potential role in the prevention of CVD, either alone or in combination with other prevention strategies.

No conflict of interest to disclose

P049**Reference Range Interpretation and Reporting for Platelet Aggregation – Is It Better?**

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Background

New guidelines for platelet rich plasma aggregation (PRPA) will demand interpretation and reporting of results with regard to an established reference range (RR). While intuitively appealing we question whether this approach will improve our laboratory's evaluation of PRPA.

Methods

We are currently establishing a new RR for our PRPA method on a new AggRAM aggregometer (Helena Laboratories). So far 32 healthy controls have been tested over a 12 month period. The parameters evaluated were lag time, rate initial aggregation, and maximum aggregation, and pattern of response. The RR was initially constructed by parametric (log transformed) and non parametric statistical approaches. Comparison was also made with our previous RR. So far 20 patients with bleeding and possible platelet dysfunction have been evaluated on the AggRAM aggregometer. The PRPA results were reported at time of testing based a previous RR and "experience". Re-reporting of these results with new RR was performed and conclusions compared.

Results

Updated RR data will be presented. Derivation of RR for most agonists proved challenging given the heterogeneity of platelet response. In our view statistical approaches incompletely capture important aspects of the individual aggregation response including effect of agonist dose response and overall pattern of response. The comparison of re-reporting of patient evaluations will be presented.

Conclusion

In our experience deriving a RR for platelet aggregation has proved to be a challenging exercise. Evaluation of platelet aggregation according to RR incompletely captures information relevant to the final interpretation of platelet evaluation.

No conflicts of interest perceived or declared

P050
Platelet Aggregation for the Paediatric Setting – Comparison of Platelet-Rich Plasma Lumi-aggregometry and Whole Blood Flow Cytometric Aggregation

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Background

Assessment of Paediatric patients with possible platelet dysfunction can be challenging due to the sample volume requirements for full platelet rich plasma aggregation (PRPA) studies. In this context we have performed a preliminary evaluation of a whole blood flow cytometric aggregation (WBPA) method.

Methods

A comparison of aggregation patterns of PRPA and WBPA was performed in 21 healthy controls. The pattern of aggregation in PRPA is measured continuously over 5-8 minutes. In WBPA analysis is performed at 3, 8 and 15 minutes. Two patients with bleeding and possible platelet dysfunction were also evaluated. Reduction in percentage maximum aggregation (%MA) was determined for a range of agonist concentrations.

Results

We found comparable patterns of %MA in healthy controls between PRPA and WBA (Table 1). Similar patterns of reduction in %MA was also seen in the two potential cases.

Agonist	N	%MA (+/-95%CI)		Case 1 (EB)		Case 2 (MM)	
		PRPA	WBA*	PRPA	WBA	PRPA	WBA
ADP (μ M)							
1.25	21	33 \pm 14	55 \pm 8	30	35	0	32
2.5	21	60 \pm 13	65 \pm 7	85	56	30	41
5.0	21	77 \pm 8	82 \pm 5	85	65	30	41
PAF (μ M)							
5.0	20	61 \pm 12	60 \pm 10	0	13	0	31
Collagen (μ g/ml)							
0.5	21	54 \pm 14	82 \pm 8	5	24	0	43
1.0	21	71 \pm 10	89 \pm 6	90	58	0	53
Adrenaline (μ M)							
5.0	22	66 \pm 11	90 \pm 5	70	94	10	51
TRAP (μ □)#							
5	21		88 \pm 6		38		80
20	16	81 \pm 8		93		12	

* WBA at 8 minutes, # different concentrations TRAP in PRPA & WBA

Conclusion

WBPA has the potential to perform platelet aggregation on a small sample volume and looks promising for the evaluation of paediatric patients with possible platelet dysfunction.

No conflict of interest

P051**Warfarin and Risk of Bleeding: Root Causes and System Inadequacies**

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Aim

Warfarin is the mainstay of prophylaxis against stroke in atrial fibrillation and valve replacement. Its low safety margin requires that an effective system of care underpins its use. This research aimed to identify common predisposing factors amongst patients with significantly elevated INR levels (≥ 6.0), through the use of root cause analysis methodology.

Methods

Patients and doctors were recruited with assistance from a large metropolitan pathology provider. Patients were eligible if following initial titration they had a single INR level ≥ 6.0 and provided informed consent. Structured interviews were conducted in patients' homes and at their doctor's practice. Patient interviews investigated potential predisposing factors including demographic characteristics, comorbidities, diet, current medication, warfarin knowledge and health service utilisation. Standardised measures evaluated cognition, mood, social support, functional independence, medication complexity and adherence. The pathology service provided monitoring and dosing histories.

Results

40 patients (mean age 75.5 years) were studied. Cognitive dysfunction was present in 65%; possible depression in 40%, with 78% reporting difficulty adhering to medication schedules. Patient knowledge of potential interactions with concomitant medications was minimal. Only 57% recalled receiving any warfarin education.

Conclusions

Our findings suggest a need for more comprehensive assessment and support for patients when prescribed warfarin, including psycho-social factors, particularly in older adults. When significant deficits are identified, patients should be provided with a more cohesive management structure, incorporating additional assistance with medication management.

No conflict of interest to disclose

A270

P052

Bedside INR Testing and Warfarin Dose Adjustment by Nurses from a Hospital @ Home Clinical Outreach Service When Starting Warfarin Therapy

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Many patients now start warfarin at home or with early discharge. Nurses from outreach services like 'hospital – at – home' (H@H) have traditionally been limited to giving low molecular weight heparin injections or supervise self-injection, collecting blood for INR testing in the laboratory, and checking for recurrence or bleeding. Portable point of care (POC) machines now permit bedside measurement of INR and immediate face-to-face dose-adjustment in the patient's home.

Aim

A pilot study of INR testing and warfarin dose adjustment by trained nurses.

Methods

Trained nurses used a CoaguChek XS POC INR machine and age-sensitive dosing algorithms for testing and dose-adjustment, with haematologists available for backup in 224 consecutive patients referred to H@H when starting or restarting warfarin therapy. Major diagnostic categories were AF (106), DVT (55), PE (28), previous heart valve replacement (13), and embolic stroke or acute coronary syndrome (12). Patients with a recent valve replacement were excluded. Age was 23 – 89 years; 40% were women; 143 also received enoxaparin. Patients were discharged from H@H when INR was >2.0 and stable for several days.

Results

Pre-determined criteria for assessing the quality of warfarin dose-adjustment included: (1) days to achieve ≥ 2 INR > 2.0 (mean \pm SD and range): 3.4 ± 2.0 (3 – 10) (2) INR < 2.0 when stop enoxaparin 3/224 (1.3%) (3) 1 or more days INR > 4.5: 11/224 (4.9%) (4) readmission within 1 month of discharge from H@H: 14/224 (6.3%; 2 = INR <2.0, 1 = recurrent VTE, 11 disease related).

Conclusion

Bedside POC INR testing and warfarin dosing by trained nurses from a H@H outreach program achieves a good quality of treatment with few complications.

No conflict of interest

P053**An Evaluation of Thrombotic Changes in Bereavement**

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Bereavement has been associated with increased cardiovascular risk. Prior studies have reported increased morbidity and mortality in bereavement, especially in the early weeks. However, the physiological responses to bereavement over time are not well understood. This prospective study evaluated thrombotic parameters in spouses and parents, within 2 weeks of bereavement and again at 6 months.

Sixty five subjects were studied and compared to a control group of sixty six non-bereaved relatives of discharged patients. All participants had haemostatic measures obtained between 8 and 11am and blood samples were processed within one hour following collection. White cell subpopulations and activation marker levels were obtained using flow cytometry. Von Willebrand factor (vWF) antigen, factor VIII and fibrinogen plasma levels were assessed and calibrated automated thrombogram (CAT) and overall haemostatic potential (OHP) were also performed. Group results were analysed using SPSS.

Acutely bereaved subjects had higher number of platelet-granulocyte aggregates (427 vs 394 events/ul p= 0.025), evidence of increased in vivo platelet activation and CD62p exposure. That was lower at 6 months (398/ul p=0.016)

Bereaved also had higher levels of vWF-Ag than non-bereaved(129% versus 118% p=0.04) that was lower at 6 months (124%; p=0.046) and higher factor VIII acutely (1.38 versus 1.23 p=0.045). There were no significant changes in fibrinogen from acute to 6 months however fibrinogen was still significantly higher than the control group at 6 months (3.59 mg/ml versus 3.34, p=0.043). Global coagulation assay showed reduced fibrinolytic potential in bereaved subjects (p=0.033).

Acute bereavement is associated with increased in vivo platelet activation and prothrombotic changes which could be linked to hypercoagulability. These findings provide insight into potential mechanisms for the increased cardiovascular risk associated with bereavement.

No conflict of interest to disclose

A272

P054

Peri-Procedural Manipulation of Anticoagulation in Patients with Mechanical Heart Valves

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Background

Significant uncertainty exists relating to the optimal regimen for peri-operative anticoagulation in patients with mechanical heart valves due to the lack of conclusive evidence to support any one of a number of proposed regimens.

Aim

To evaluate outcomes in patients with prosthetic heart valves referred for peri-procedural anticoagulation management at a large teaching hospital.

Methods

Between January 2005 and July 2008, 735 patients have been assessed for peri-operative anticoagulation manipulation at Royal Perth Hospital and assigned a thromboembolic (TE) and bleeding risk, as determined by pre-defined criteria. Patients were given intravenous heparin (IVH) or low molecular-weight heparin (LMWH) or a combination as determined by TE and bleeding risks. For each case, data relating to the form of anticoagulation, the achievement of required INR in the peri-procedural phase, time to therapeutic INR post-procedure, the occurrence of TE, major and minor bleeding episodes (within peri- procedure and 30 day post-procedure period) was obtained.

Results

Patients with prosthetic valves accounted for 147 (20%) of all cases. Eighty-one of 147 cases (55%) had an AVR; 59 of 147 (40%) had an MVR; and 7 cases (5%) had dual prosthetic valves. Forty-four (30%) of cases were admitted for IVH with the remainder of the cases managed as outpatients with LMWH. All patients achieved an INR of less than 1.5 on the day prior to the procedure. Median time to therapeutic INR was seven days. There were no episodes of thromboembolism during the follow-up period. Seven patients experienced a bleeding complication (4.8%) of which two patients had a major bleed (1.36% of all cases).

Conclusion

The methods of anticoagulation management employed in this cohort are comparable in terms of TE outcomes, to that of published data. The bleeding rate of 1.36% is at the lower end of the range stated in published data (0% - 5%). LMWH can be utilised in the majority of mechanical heart valve cases but UFH is still required in the high risk bleeding and/or thrombosis patients because of ease of laboratory monitoring and rapid reversal.

No conflict of interest to disclose

P055

Circulating Plasma Procoagulant Microparticles Are Increased After Acute Stroke

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Background

Patients with acute ischaemic stroke have a high risk of early recurrent events that may be related to elevated blood concentrations of procoagulant microparticles produced from various cellular sources but predominantly from activated platelets.

Aim

To investigate whether there is evidence of ongoing platelet activation, as measured by elevated blood concentrations of circulating microparticles (MP), during the first week after acute ischaemic stroke, using two recently developed techniques.

Methods

Tests were performed on double centrifuged citrated plasma collected from patients within 7 days of their first acute ischaemic stroke and from an identical number of age and sex matched healthy controls. The XACT test (Haematex, Australia) measures procoagulant phospholipid by a clotting technique in which the activity of FXa is enhanced by phospholipid present in plasma. The Zymuphen-MP Activity kit (Hyphen BioMed, France) is an ELISA technique estimating the activity of circulating MP expressing negatively charged phosphatidylserine (PS) on the surface membrane. Circulating MP that bind AnnexinV provide a matrix for the production of thrombin that in turn cleaves a synthetic chromogen.

Results

The geometric mean procoagulant phospholipid for 193 patient samples (XACT test) was significantly elevated in stroke patients (0.95 µg/ml, [95% CI 0.89 – 1.02]) when compared to controls (0.83 µg/ml [95% CI 0.77 – 0.88], $p=0.02$). The PS microparticle assay for 149 patient samples was also significantly higher (9.93 nM [95% CI 8.80 – 11.22]) when compared to controls (6.74 nM [95% CI 6.12 – 7.43], $p = <0.00001$). There was no correlation between the techniques ($r = 0.19$). There was no age or gender significance with either test.

Conclusion

Both techniques show significantly increased circulating MP phospholipid activity in the patient group after ischaemic stroke. The poor correlation between the two tests might be explained by lack of surface expression of PS in a proportion of MP, or that a share of circulating PS activity is derived from plasma micelles and/or liposomes.

No conflict of interest to disclose

A274

P057

A Case Illustrating Severe Acquired Antithrombin III (AT III) Deficiency

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Introduction

Antithrombin III is a potent inhibitor of coagulation. Congenital AT III deficiency is associated with a high risk of VTE. Severe AT III deficiency can occur when there is excess consumption and/or decreased production in sick patients causing VTE.

Case history

A 19 year old male with a 4 year history of severe Crohn's disease poorly controlled by medical measures, developed extensive venous thromboses involving the inferior vena cava, the right iliac and femoral veins and multiple pulmonary emboli. At presentation, the protein S 43% (50-130) and antithrombin III 46% (70-130). He was treated with IV heparin and long term warfarin.

One year later his colitis worsened requiring a sub-total colectomy and ileostomy. Postoperatively, despite enoxaparin prophylaxis, he developed a further right leg DVT and PE.

The development of peritonitis required a laparotomy which showed a leaking rectal stump. Postoperatively he was hypoalbuminaemic and thrombocytopenic due to a combination of sepsis, disseminated intravascular coagulation, possible heparin induced thrombocytopenia and malnutrition. Enoxaparin was discontinued and anticoagulation continued with lepirudin.

AT III levels indicated a severe deficiency of 14% (82-125). Congenital deficiency of AT III was excluded by a family study. (The prior AT III results from interstate were not available). With intensive support the AT III level increased to 87% over 11 days, with albumin rising to normal from 10g/L. AT III concentrates were not used.

Conclusions

Severe AT III deficiency can occur in sick patients in the ICU setting and maybe associated with venous thromboses despite LMW heparin prophylaxis. The distinction from hereditary AT III deficiency may be difficult. The role of AT III concentrates in acquired AT III deficiency and sepsis is uncertain.

No conflict of interest to disclose

P058**Implementation of Subcutaneous Administration of DDAVP for Children With Mild Bleeding Disorders**

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Aim

To assess the tolerability and effectiveness of administering DDAVP by subcutaneous injection to children with mild haemophilia A and von Willebrand disease.

Method

DDAVP remains the therapy of choice for mild haemophilia A and von Willebrand disease. The administration of DDAVP by intravenous infusion is a challenge in young children. We have implemented administration of DDAVP by subcutaneous (s.c.) injection to assess its tolerability and effectiveness. The dose of DDAVP administered continues to be 0.3 µg/kg, and we have adapted our existing protocol of monitoring and 24 hour fluid restriction post-DDAVP. Blood sampling for post-DDAVP factor levels is performed at 90 minutes when the peak levels are expected. Multiple doses of DDAVP s.c. can be given through an insulafon to cover surgical procedures.

Result

To date, six DDAVP trials via the s.c. route have been performed. These procedures have been well tolerated, and the administration of DDAVP is easier than had been previously experienced with intravenous infusion of DDAVP. As insertion of an i.v. cannula is no longer required, there has been a significant saving in time spent by staff on this procedure. No significant side effects were experienced. In addition, five invasive procedures have been successfully managed with s.c. DDAVP. One patient with von Willebrand disease (baseline VWFRCo 0.17 U/mL) underwent an uncomplicated tonsillectomy and adenoidectomy, for which he received 7 days treatment with s.c. DDAVP.

Conclusion

The administration of DDAVP by subcutaneous injection for children with mild bleeding disorders has resulted in a practice that is both more convenient and better tolerated by patients, and has led to significant reductions in staff time spent administering DDAVP. Trial doses of DDAVP and surgical procedures have been successfully undertaken using this mode of delivery of DDAVP.

No conflict of interest to disclose

A276

P059

Acquired von Willebrand Disease Presenting with Myocardial Infarction

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Clinical Presentation

56-year-old female, evaluated initially in 1999 for bruising with history of uncomplicated hysterectomy and spontaneous deliveries presented with acute myocardial infarction. No family history of bleeding diathesis. Initial evaluation showed an IgG paraprotein; markedly low Factor VIIIc, von Willebrand antigen (vWF: Ag) and activity levels –von Willebrand ristocetin activity (vWF: RCo), von Willebrand collagen binding (vWF: CB), poor response to DDAVP. A provisional diagnosis of acquired von Willebrand (AvWS) disease was made. During the current presentation, initial treatment included Aspirin, Clopidogrel, heparin covered by a Biostate continuous infusion, which was complicated by epistaxis.

Laboratory Tests and Management

Markedly reduced vWF: Ag, vWF: RCo, vWF: CB and FVIIIc. Poor increment in vWF: RCo and vWF: CB to continuous infusion and bolus doses of Biostate was noted. Sustained increases in levels were noted following intravenous immunoglobulin (Ivlg). Coronary artery stenting was avoided given the difficulty in using antiplatelet agents. The patient successfully underwent coronary artery bypass graft after Ivlg infusion under cover of bolus doses/infusion of biostate and novo-seven.

Discussion

AvWS is rare - this case suggests an unusual antibody mediated mechanism with qualitative and quantitative reduction in von Willebrand activity. As illustrated by this case and other reports in literature, Ivlg can be effective in restoring vW Ag and VIII levels to therapeutic levels for a safe surgical intervention in AvWS.

No conflict of interest to disclose

P060**A Case Study of Pseudo von Willebrand Disease**

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A pregnant woman at 34 weeks gestation, with a maternal history of abnormal bleeding, was referred for investigation. Initial findings revealed prolonged PFA-100 results along with abnormal von Willebrand studies. Tests were repeated post-partum which confirmed earlier abnormal results with von Willebrand studies showing a functional discordance indicating possible type 2 von Willebrand Disease (vWD).

Further testing raised doubts that this case was a 'typical' case of type 2B vWD and in fact may be pseudo (platelet type) vWD. Pseudo vWD was diagnosed following detailed platelet aggregation testing and confirmed at the molecular level. This case demonstrates that this rare sub-type of vWD may be under diagnosed due to its phenotypic similarities to type 2B vWD.

No conflict of interest to disclose

P061

Microangiopathy, Thrombocytopenia and Renal Failure After Tiger Snake Envenomation

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We report a case of a 55 year old lady who was transferred to our facility from a regional hospital after being bitten by a tiger snake. Her only significant past medical history was hypertension and smoking. She was bitten on her left 5th toe by an unidentifiable snake and driven to her local hospital. She developed a headache and nausea so was transferred to a larger hospital where she arrived 3 hours after the bite.

Her initial bloods revealed a coagulopathy, with normal renal function and full blood count. A swab of the bite site was positive for Tiger snake venom and she received Tiger snake antivenom. Her coagulopathy resolved. However she developed microscopic haematuria and proteinuria with worsening renal function. By day 3 post-bite she was in florid oliguric acute renal failure and her LDH was rising.

On day 4 post-bite she was noted to have thrombocytopenia and microangiopathic haemolytic anaemia (MHA), along with worsening renal failure. At this point she was transferred to the Austin hospital where she commenced haemodialysis, plasma exchange and prednisolone 1mg/kg daily. She went on to receive a total of 12 days of plasma exchange and 8 days of haemodialysis with complete resolution of renal failure, microangiopathy, and thrombocytopenia.

Venom-induced coagulopathy is the main manifestation of snake bites in Australia. The triad of delayed thrombocytopenia, MHA and acute renal failure after resolution of the coagulopathy has frequently been described in cases of Brown snake envenomation. However we are unaware of this being reported after Tiger snake envenomation.

No conflict of interest to disclose

P062

Factor Deficiencies Associated with Venom Induced Consumption Coagulopathy in Australian Snakebite

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Objective

There is limited information on individual factor concentrations and their recovery in patients with venom induced consumption coagulopathy (VICC). We measured factor concentrations in patients with VICC to better understand the time course of the coagulopathy, the effect of antivenom and factor replacement.

Methods

This study used samples from 524 patients recruited to the Australian Snakebite Project (ASP) with VICC including 55 cases of brown snake envenoming, 54 cases of tiger snake group envenoming and 3 taipan envenoming. Severe VICC was defined as patients with an international normalised ratio [INR] > 3. Citrated plasma samples were collected from each patient at various time points over 0.5 to 6 days after the bite. All samples were processed and stored according to a national standardized protocol. Prothrombin time (PT), activated partial thromboplastin time (aPTT), factor assays for II, V, VII, X, vWF antigen and D-dimer concentrations were measured on a Behring Coagulation System (BCS; Dade Behring, Marburg, Germany). Fibrinogen, and factor assays for VIII and IX were performed on a Sysmex CA1500 (Sysmex Corporation, Japan).

Results

Patients who were admitted within 1 to 2 hours of snakebite demonstrated almost complete absence of detectable fibrinogen, factor V and factor VIII. Furthermore, there were reduced concentrations of factor II, but little change in factors VII, IX, X and vWF antigen. Fibrinogen recovered the slowest returning to concentrations >1g/L only after 20 to 40 hours, while factor V and VIII reached >50% within 5 to 15 hours in the majority of cases. Improvement in the INR (to less than 2.0) and the aPTT appeared to correlate best with the recovery of factors V and VIII. There was no major difference between brown snake, tiger snake group and taipans in the severity and reconstitution of the factor deficiencies. There did not appear to be an association between the time of antivenom administration and the timing of recovery of the factors. The D-Dimers were exceedingly high and in many cases above the limit of quantification of 900mg/L and returned to normal levels over 2 to 4 days.

Conclusion

VICC in Australian elapid envenoming is characterized by rapidly developing almost complete deficiencies of fibrinogen, factor V and factor VIII, and a partial deficiency of prothrombin; similar for all snake types. There is no delay in recovery and the time course appears consistent with re-synthesis of the factors.

No conflict of interest to disclose

A280

P063

Clinical Features and Therapy for Patients with Immune Thrombocytopenic Purpura (ITP) at an Australian Hospital

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Aim

ITP is a common cause of thrombocytopenia in our community and is an important cause of morbidity. There is a paucity of data regarding clinical features and treatment of ITP patients in Australia. The aim of our study was to gather information regarding presenting features and management of ITP patients at our institution, St George Hospital, NSW. We sought to determine efficacy of standard treatments, either as first line therapy or therapy in relapsed/refractory cases, including splenectomy.

Method

The medical records of 56 patients diagnosed with ITP at our institution, from 1993 to 2007, were analysed.

Results

Prednisolone (~1mg/kg) and intravenous immunoglobulin (IVIG) was the most commonly used first-line treatment. Steroids had a lower response rate when used as second line treatment. Danazol was the most commonly used second-line treatment, and was well tolerated. IVIG and vincristine had high response rates but these were not sustained in most cases. Splenectomy was effective, with 83% of patients achieving a sustained response while being free of medications. Patients were enrolled in clinical trials for thrombopoietin receptor agonists and Staphylococcal protein A. Both agents showed good response.

Conclusion

First line treatments (steroids, IVIG) yielded sustained response in 40% of patients. It is worthwhile using steroids as second-line treatment in ITP. There are a large number of options for second-line treatment, most having reasonable response rates while being well tolerated. Splenectomy results in high rates of long term efficacy, and should be considered in any patient with relapsed/refractory disease. Patients who have not responded to initial treatment should be enrolled in clinical trials to receive new agents, such as rituximab and thrombopoietin receptor agonists, as these drugs have shown efficacy with minimal adverse effects.

No conflict of interest to disclose

P064

Eltrombopag in the Treatment of Chronic Refractory Immune Thrombocytopenic Purpura (ITP)

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Introduction

ITP is an autoimmune disease in which anti-platelet antibodies accelerate the destruction of platelets. Megakaryocyte production can also be impaired in chronic ITP.

Case-report

We report on a 41 year old Caucasian male who presented 10 months previously with very severe thrombocytopenia (platelets <1/nl) and significant bleeding (nose, GIT) with normal WCC and Hb. Initial treatment with steroids (1g IV methyl prednisolone) did not improve his platelet count; therefore, intravenous immunoglobulin (IVIG) was added at a dose of 2g/kg BW with normalisation of his platelet count thereafter.

However, upon reduction of his corticosteroid intake after 4 weeks, his platelets again dropped to a critical level of <10/nl. Use of dexamethasone did not alter the platelet count. He ultimately responded again to IVIG.

After several months of relapses and treatment with IVIG, he was commenced on IV Rituximab in an attempt to control antibody-production. A bone marrow biopsy showed adequate megakaryopoiesis without evidence of other haematological disorders. Haemolysis, immune disorders, viral and Helicobacter infection were excluded.

The patient presented after 9 months of ongoing immunosuppressive treatment with a platelet count of 15/nl and epistaxis, therefore Eltrombopag was commenced (50mg orally daily) with good toleration. His platelets increased significantly to 45/nl within a week of treatment. The dose of Eltrombopag was increased to 75mg to achieve maximum benefit with a further increase of his platelet count to 96/nl. Once Eltrombopag was commenced, IVIG was discontinued with a simultaneous reduction in his prednisolone-dose. A planned splenectomy was cancelled due to the positive effect of Eltrombopag.

Conclusion

Eltrombopag is an orally bioavailable, small molecule, thrombopoietin receptor (TPO-R) agonist currently being developed for the treatment of a variety of medical disorders associated with thrombocytopenia including chronic ITP This report highlights the value of increasing platelet production, especially in the treatment of chronic refractory ITP.

The authors confirm that there is no conflict of interest in relation to this research.

A282

P065

Pancreatitis Preceding Acute Episodes of Thrombotic Thrombocytopenic purpura: Report of Three Patients and Possible Mechanism of Action

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Background/Aim

Thrombotic thrombocytopenic purpura (TTP) is a rare disorder manifested by thrombosis in the microvasculature of several organs. Pancreatitis following TTP has been noted in 1-2% of cases, however the incidence of TTP preceding pancreatitis is unknown.

Method

We report three patients diagnosed with TTP following pancreatitis. A PUBMED search with key words “pancreatitis” and “TTP/Thrombotic microangiopathy” was performed to identify additional patients with similar presentation.

Result

All of our patients presented with short period of abdominal pain (2-5 days) with features of acute pancreatitis, renal impairment and microangiopathic haemolytic anaemia. The cause of pancreatitis was variable, one due to alcohol, a second due to cholelithiasis and a third idiopathic. Two patients tested had normal functional ADAMTS13 level by ELISA at presentation. The PUBMED review identified another 14 patients who had TTP following pancreatitis, three of which had recurrent TTP following a subsequent episode of pancreatitis.

Conclusion

In the majority of reported cases, pancreatitis preceded the diagnosis of TTP, suggesting that inflammation of pancreas is a triggering event. While the pathophysiologic mechanism remains unclear, the endothelial injury is thought to play an important role. It has been postulated that release of pancreatic enzymes or production of cytokines (IL1, IL6, TNF α) may be potential causes of endothelial injury. Other possible mechanisms include: modification of circulating von Willebrand Factor (vWF) by pancreatic proteases, leading to platelet aggregation; damage to endothelium by circulating trypsin; release of platelet activating factor and other chemoattractants such as nitric oxide; and release of high molecular weight vWF multimers from damaged endothelium which is thought to be predictive of recurrence.

No conflict of interest to disclose

P066**A Diagnostic Dilemma: PET or Pregnancy Associated TTP - A Case Presentation****Cameron Curley**, Glen Kennedy*Department of Haematology, Royal Brisbane and Women's Hospital, Brisbane QLD***Introduction**

Thrombotic microangiopathy in pregnancy can present a difficult diagnostic challenge to the clinician with both TTP and PET/HELLP often presenting with similar clinical and laboratory pictures. Outcomes are frequently poor when there are delays in initiation of specific therapy for both conditions.

Case Report

We present the case of a 32 year old woman who presented at 39 weeks gestation, G1P0, with a short history of abdominal pain, headaches, blurred vision and severe thrombocytopenia, platelet count of $6 \times 10^9/L$. Initial evaluation revealed BP of 130/80 with normal neurological examination and live intrauterine foetus. Microangiopathic haemolytic anaemia with moderate red cell fragmentation on blood film examination was accompanied by marked proteinuria, acute kidney injury, mildly deranged liver function tests and normal coagulation profile. The patient, felt to have indistinguishable features PET/HELLP and TTP was managed with high dose corticosteroids, intravenous immunoglobulin, fresh frozen plasma and platelet transfusion prior to proceeding to emergency C-section resulting in a live baby. Post operatively the patient received daily plasma exchange for 4 days with improvement of platelet count within 24 hours and normalisation within 48 hours. ADAMTS13 activity was determined on stored samples subsequently at our laboratory and found to be <5% acutely, returning to 100% during her ongoing remission. Whilst moderate reductions in ADAMTS13 activity are a feature of late pregnancy and HELLP, severely decreased ADAMTS13 activity is highly specific to TTP and confirms a diagnosis of pregnancy associated TTP in our case.

Conclusion

Whilst pregnancy associated TTP and PET may be difficult to distinguish acutely, the above case demonstrates ADAMTS13 activity testing acutely and during remission may provide useful diagnostic and therapeutic information.

No conflict of interest to disclose

P067

Single Centre Experience with the Use of PaGIA for Screening of HIT

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Aim

The aim of this study was to investigate the role of a rapid immunological assay, the particle gel immunoassay (PaGIA) heparin-PF4 antibody test (DiaMed Israel Ltd) as a screening test for patients with a clinical probability of heparin-induced thrombocytopenia (HIT).

Methods

Between July 2003 and July 2008, 151 patients' sera with intermediate and high risk pretest probability were screened with PaGIA for diagnosis or exclusion of HIT. Retrospective chart review was performed. To ascertain the sensitivity of PaGIA as a screening test, thirty four blood samples were also sent for heparin/platelet-factor-4 enzyme immunoassay (HP4-ELISA).

Results

Antibodies against HPF4 were detected in 19/151 (12.5%) by PaGIA. Of the 34 blood samples that were sent for HP4-ELISA, 17 were positive (50%). Nine patients positive in HP4-ELISA were not detected by PaGIA. In a considerable number of samples the results of PaGIA were equivocal.

Conclusion

PaGIA is a rapid immunoassay that is marketed as a screening test for diagnosis of HIT; however in our hands it was not as sensitive as previously reported. Hence, at present it cannot substitute established immunologic HIT assays. Compared to ELISA the PaGIA had reduced sensitivity. Interpretation of PaGIA results is difficult in a considerable number of samples.

No conflict of interest to disclose

P068**Bilateral Adrenal Haemorrhage Due to Heparin-Induced Thrombotic Thrombocytopenia Syndrome (HITTS) Following Prophylactic Low Molecular Weight Heparin****Nicholas E Murphy**¹, Alvin Chung², Adrienne Anderson², John C Duggan¹¹ *Department of Haematology, Austin Health, Melbourne, Victoria, Australia*² *Department of General Medicine, Austin Health, Melbourne, Victoria, Australia*

We report a case of a previously well 69 year old woman admitted to our facility 10 days after a total hip replacement with fever and hypotension. The hip replacement was the patient's first hospitalisation and she was on no regular medications. The patient received Low Molecular Weight Heparin (LMWH) as DVT prophylaxis and there was no intra-operative exposure to Heparin.

The patient was transferred from a rehabilitation ward with vague flank pain, fever (38.5), confusion, tachycardia (HR 100 bpm) and hypotension (systolic BP 90 mmHg). There was no clinically obvious source of sepsis. E.Coli was cultured in urine and treated. The patient's haemoglobin subsequently fell, requiring a total of 4 units of packed red blood cells. Gastroscopy was normal. The patient failed to improve with ongoing flank pain and hypotension after the haemoglobin had stabilised and was found to have adrenal failure {Serum Cortisol < 11 nmol/L (N = 275-555), Short Synacthen Test} with contrast-enhancing adrenal lesions. Throughout, the platelet count was stable at 70-90 x10⁹/L.

Adrenal replacement therapy was commenced with a rapid improvement in blood pressure. On day 20 post-hip replacement, the patient developed extensive bilateral pulmonary emboli, demonstrated on VQ scan. At this stage, a clinical diagnosis of HITTS was made, and confirmed on both HITTS screen immunoassay and serotonin release study.

Danaparoid was commenced (target anti-Xa 0.5 – 0.8) with a rapid platelet count recovery, and subsequent warfarin for a duration of 6 months undertaken without incident.

Adrenal vein thrombosis and adrenal haemorrhage is a rare manifestation of HITTS. HITTS is a highly thrombotic disorder caused by antibodies to heparin-platelet-factor 4 complexes. HITTS is more common in surgical than medical population. The rate of HITTS has been estimated at 0.2% in patients receiving prophylactic LMWH.

No conflict of interest to disclose

A286

P069

Management of Life-Threatening Complications of Bronchiectasis in a Patient with Congenital Factor XIII Deficiency

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Background

Congenital factor XIII deficiency is a very rare autosomal recessive bleeding disorder, with a number of causative mutations identified.

Case Report

We report the challenging case of a 35-year old Polynesian male, with congenital FXIII deficiency and severe bronchiectasis, who was retrieved with life-threatening haemoptysis and hydropneumothorax secondary to *Streptococcus milleri* empyema. Additionally, factors II, V, VII, and XI were low due to the hepatic synthetic dysfunction associated with his severe infection. He underwent two attempted bronchial artery embolisations, an unsuccessful decortication procedure which was complicated by significant bleeding, and ultimately a thoracotomy with rib resection to excise necrotic lung. He was supported with plasma derived FXIII concentrate (*Fibrogammin P*), cryoprecipitate and fresh frozen plasma throughout this period, and he achieved good haemostasis with his last surgery.

Results

There is little consensus regarding the FXIII target level, and it is reported that a relatively low level is sufficient to prevent bleeding in deficient patients. We attempted to maintain a trough level of at least 0.4U/ml when he was actively bleeding with intermittent dosing as well as additional dosing pre-operatively. Dosing was guided by a quantitative FXIII assay (commercial trans-glutaminase assay) and his levels were within the measurement range for FXIII assay (0.10 – 1.0 U/ml).

FXIII is characterised by a long half-life of 150 – 300 hours. However, in this case the FXIII half-life during the immediate post-operative period ($t_{1/2}$ = 36 - 62 hours) was much shorter than the half-life pre-operatively ($t_{1/2}$ = 120 – 320 hours) and later in the admission ($t_{1/2}$ = 130 – 190 hours).

Additionally, we determined that this patient and his affected sister appeared to be homozygous for a novel c.1982T>G Leu661Arg missense mutation of the FXIII gene.

Conclusion

We found the FXIII activity assay to be an extremely useful tool to guide appropriate FXIII treatment in the management of his bleeding and surgeries.

No conflict of interest to disclose

P071

DIC Associated with Hepatocellular Carcinoma and Alpha-1 Antitrypsin (AAT) Deficiency and a Question of Fibrinogenolysis

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Aim

To report a case of DIC associated with hepatocellular carcinoma and apha-1 Antitrypsin (AAT) Deficiency.

Case Report

A previously well 80 year old widower presented with jaundice, bruises and haematuria. Over 12 days he had 69 donor units of cryoprecipitate, 14g fibrinogen concentrate, 21 units FFP, 2 pooled platelets and 3 packed red cells. Heparin therapy was ruled out due to high risk of bleeding. On day 11, he had a massive intra-abdominal bleed, and he died a day later despite rFVIIa treatment. Post-mortem revealed cirrhosis and a solitary hepatoma with PAS positive eosinophilic globules characteristic of ATT deficiency, Pi phenotype of MZ was confirmed.

Discussion

Day	1	2	3	4	5	6	7	8	9	10	11	12
Hb, g/L	124	115	101	90	88	83	73	97	94	75	52	66
Platelet,x10 ⁹ /L	10	9	14	12	35	39	48	56	70	93	69	99
PT, s	25	27	45	48	37	25	19	25	20	25	26	34
APTT, s	46	46	69	59	62	44	34	43	32	36	41	34
Fibrinogen,g/L		0.3	0.3	0.3	0.3	0.4	1.3	0.4	1.3	0.4	1	1.5
D-dimer,mg/L		20	80	80	80	80	80	80	66	32	29	11
Plasminogen,%	47		42		37							

Snake bite or toxic exposure was suspected initially due to the acute onset of DIC. When his liver impairment became more evident, its etiology remained unclear until after post-mortem. The rapid consumption of fibrinogen pointed towards overt fibrinolysis, but it also raised the suspicion of concurrent primary fibrinogenolysis which we could not exclude. The reduced plasminogen level may reflect impaired liver synthesis.

Conclusion

This demonstrated a fatal case of extreme DIC associated with cirrhosis and hepatocellular carcinoma from undiagnosed hereditary AAT deficiency. His management was difficult because the underlying cause was not apparent as well as the intensity of DIC.

No conflict of interest to disclose

P072

Development and Implementation of an Electronic Antithrombotic Management System for Clinicians (eATMS)

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Aim

Antithrombotic medications are widely used across acute health services for a variety of indications and have the potential to result in significant bleeding complications resulting in substantial morbidity and mortality. Ready access to clear and concise antithrombotic management guidelines are essential to minimise prescription error and adverse outcomes. The Antithrombotic Management Committee at Barwon Health aimed to reduce antithrombotic-related adverse events through implementation of an electronic antithrombotic clinical guideline management system that was easily accessible by clinicians.

Method

The primary focus was to identify, review and rationalise all antithrombotic clinical guidelines and to develop a process to facilitate easy electronic access to all antithrombotic guidelines in clinical use across Barwon Health. All existing antithrombotic guidelines and protocols were initially identified and reviewed. Duplication of guidelines was eliminated and new guidelines were developed for those clinical areas devoid of existing protocols. Using the hospital-wide electronic clinical information system (BOSS) accessed by all clinicians, an interface that employed hyperlinks to antithrombotic guidelines was developed and implemented. Monitoring of adverse events occurring in relation to protocol use enabled identification of those guidelines requiring review.

Results

The project enabled rationalisation of antithrombotic guidelines and removed duplication. Existing guidelines have been streamlined and new guidelines developed where needed. No adverse events have been recorded where eATMS guidelines have been used.

Conclusion

eATMS has the potential to improve anticoagulation safety through improved access to standardised hospital antithrombotic protocols and ongoing monitoring of protocol safety.

No conflict of interest to disclose

