MONDAY 18 1600 to 1700 Atrium
ASTH Poster Session
Chair: Hatem Salem, Jerry Koutts

P1
Acquired Glanzmann's Thrombasthenia: a Case Report
Claire McLintock, Simon Jones, Christine Algie

Diagnostic Medlab Ltd, Auckland

Acquired Glanzmann's thrombasthenia is a rare haemorrhagic disorder due to the development of an auto-antibody to the platelet receptor GPIIb/IIIa that leads to defective platelet aggregation. We report a case of acquired Glanzmann's thrombasthenia detected in a woman who presented with a history of recent onset of severe menorrhagia that required blood transfusion. Prior to development of severe menorrhagia our patient had no bleeding history and had one child born 4 years previously by caesarean section with no bleeding complications. Investigations Bleeding time >20 minutes. Normal APTT, PR, fibrinogen and von Willebrand Factor Platelet-aggregation studies - classical Glanzmann's phenotype: absent aggregation to ADP, adrenaline and collagen, normal response to ristocetin. Immunoenzyme staining of platelets: positive for GpIIb, GpIIIa and GpIb alpha. Serum platelet antibodies and platelet associated antibodies - demonstrated. MAIPA studies antibodies were directed to anti-GP IIb/IIIa and anti-GP Ia/IIa glycoproteins. The clinical history and laboratory findings support a diagnosis of acquired rather than inherited Glanzmann's thrombasthenia. Currently this woman's menorrhagia is controlled with the oral contraceptive pill and she has no other significant bleeding symptoms. Our patient would like to have another child but with acquired Glanzmann's thrombasthenia would be at risk of severe bleeding in the peri-delivery period. In other cases reports, immunosuppressive therapy has been successful in treating bleeding symptoms and we are considering this as a possible therapeutic option.

P2
Investigation of TFPI Promoter Polymorphisms in Patients with Antiphospholipid Syndrome: Decreased TFPI Activity in a Patient Homozygous for the T-287-C Polymorphism
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Aim: The aim of this study was to investigate whether polymorphisms in the promoter region of the TFPI gene in patients with the antiphospholipid syndrome (aPS) are associated with reduced TFPI activity, which may contribute to the development of thrombosis. Peripheral blood was collected from 28 aPS patients [median age = 47 years, range (26-75 years)] and 58 controls [median age = 63 years, range (22-84 years)]. PCR-RFLP was used to detect TFPI promoter polymorphisms C-399-T and T-287-C. TFPI activity was measured using an amidolytic assay.

Result: Allele frequencies for both polymorphisms were not significantly different between patients and controls [T-287C = 14% vs 17% (p=0.67),C-399T = 19% vs 10% (p=0.47), respectively]. TFPI activity was significantly increased in aPS patients (1.29±0.68 U/mL) compared to controls (0.77±0.19 U/mL; p = 0.0005). However, a subgroup of aPS patients who were homozygous for 287-CC had reduced TFPI activity (n=2, TFPI = 0.36 and 0.89 U/mL) compared to the remainder of the patient cohort and the respective genotype controls (n= 2, TFPI = 0.76 and 0.70 U/mL). Neither of these patients had a history of thrombosis.

Conclusion: This study has reported an aPS individual with a homozygous mutation within the promoter region of the TFPI gene and significantly reduced TFPI activity. Larger studies are required to determine whether polymorphisms within the promoter (and other regions) of the TFPI gene lead to reduced TFPI activity and an increased risk of thrombosis in aPS patients.

P3

D Dimer Request Patterns for Venous Thromboembolism in a Teaching Hospital Emergency Department: Is it Useful?

Cindy Huey-Shin Lee [1], Benedict Carnley [1,2], Jim Thom [1], Ross Baker [1]

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Aim: A negative rapid D dimer assay may be useful in the diagnostic algorithm for excluding venous thromboembolism (VTE) in patients with a low clinical probability of disease.To assess pretest clinical probability of VTE in patients in whom a D-Dimer had been requested in a teaching hospital emergency department.

Method: Randomly selected medical records of 334 patients in whom a D-Dimer concentration had been requested in the Royal Perth Hospital Emergency Department over a 9 month period in 2003, were reviewed. DVT and PE pretest clinical probability scores were calculated using the published Wells score. D Dimer concentration (STA – liatest D-Dimer, STA-R analyser Diagnostica Stago) of greater than 0.4?;g/ml FEU was considered positive.Subsequent clinical management was recorded.

Result: The majority of patients with D dimer requests presented with clinical features of PE (n=291 – 87%). 30 patients had the presentation of DVT (9%). Two cases with snake-bite and 13 cases with insufficient clinical details were excluded from further analysis. Of 233 patients with low pretest probability of PE or DVT, 156 (67%) had a negative D dimer and were discharged with no further VTE related investigations. 88 (27.4%) patients with moderate to high pretest probability of VTE had a D Dimer test when the result should not influence clinical decision making.

Conclusion: In our emergency department, D Dimers are requested to exclude pulmonary embolism in patients with a low clinical probability of disease. However inappropriate ordering is also commonly found suggesting that strategies to reduce inappropriate testing (appropriate diagnostic algorithms and training) are required.

P4

A Review of the Initiation of Anticoagulation: Does Lack of Adherence to Guidelines Result in an Increased Risk of Adverse Outcomes?

Shane Jackson [1], Gregory Peterson [1], Steven Morgan [1], Luke Bereznicki [1], David Jupe [2]

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Aim: Despite the presence of protocols for the initiation of anticoagulation, the actual adherence to recommended doses in initiation protocols in clinical practice has not been often studied. The objective of this study was to assess the adherence to the anticoagulation initiation protocol of the Royal Hobart Hospital (RHH) for inpatients initiated on warfarin.

Method: All patients whom had warfarin initiated as an inpatient of the RHH between the periods February 2002 and June 2003 were eligible for inclusion. Data were assessed on the quality of anticoagulation whilst in hospital compared to the RHH anticoagulation protocol for initiation of warfarin.

Result: A total of 302 initiations were assessed. 36% of anticoagulant initiations followed the RHH anticoagulation protocols. For patents whose initiation did not follow the RHH guidelines, 3% & 8% had INRs greater than 4.0 on days 3 or 4 of their hospital stay, respectively, compared to none on day 3 and 1% on day 4 in the guideline-adherent group. The mean duration of hospitalisation was 1 day longer in patients whose warfarin initiation did not follow the RHH guidelines. Thirty-four per cent of all patients discharged on warfarin were therapeutic at discharge and 60% were sub-therapeutic at discharge.

Conclusion: It is disappointing to find that only 36% of warfarin initiations followed the RHH anticoagulation protocols. This non-adherence to initiation guidelines is potentially putting patients at increased risk of anticoagulant related misadventure and lengthened hospitalisation.

P5

Discrepancies Between the Overall Coagulation Potential (OCP) Assay and International Normalised Ratio (INR) in Warfarin-Treated Patients

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Warfarin therapy requires regular effective monitoring to prevent iatrogenic haemorrhage or recurrent thromboembolism. However unexpected events may occur even when target INR values (2.0<INR<3.0) are maintained. Our aim was to test the effect of oral anticoagulation with warfarin on the OCP, a global coagulation assay of fibrin generation. We hypothesized that patients with INR > 2.0 would have suppressed fibrin generation detectable by a reduced OCP. Over a 6 month period, 105 plasmas from 14 patients attending an INR clinic were assayed. Reference ranges were derived from 60 normal plasmas. Statistical analysis involved calculation of means, standard deviations and Pearson correlations. In patients with an adequate INR we found a wide variation in OCP responses. Only 24.7% of samples showed the expected reduced fibrin generation. 74% of plasmas had a normal OCP despite INR > 2.0. One plasma had an increased OCP consistent with hypercoagulability. Even in plasmas with an elevated INR > 3.0 only 18.9% showed suppression of fibrin generation. We found no correlation between OCP and INR values in the target range (R2 = 0.06). No bleeding or recurrent thrombosis was reported during the study period. There are significant discrepancies between OCP and INR values in patients on long term anticoagulation. This may reflect sensitivity of the OCP to fibrinogen and factor VIII levels which do not influence the INR. We hypothesize that patients with persistence of normal or increased fibrin generation while on warfarin may be at increased risk of recurrent thromboembolism.

P6

Surgical Delay In Acute Admissions On Warfarin: Are We Doing Enough?

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Warfarin anticoagulation is a common cause of surgical delay and thus a concern in any acute surgical admission. We chose to study fracture neck of femur patients on warfarin as they are one of the commonest and any delay does lead to increased distress and morbidity, and potentially, increased mortality.

Materials and Method: Retrospective Analysis: We reviewed 857-fracture neck of femur operations and identified 25 operations while patients were on warfarin. Questionnaire study: This was followed up with a questionnaire study sent to 360 consultant haematologists, designed to assess the current practices. We received 144 responses.

Result: The Problem 1.The average wait to operation was 4.36 days compared to 1.78 days otherwise. 2.Average time for INR to decrease to <2.0 was 3.44 days. 3.The fall of INR was very unpredictable. The Practice 1.73/144 consultant haematologists have responded to be using vitamin K occasionally. 2.130/144 and 63/144 would use vitamin K reversal in patients with atrial fibrillation and mechanical heart valve respectively. 3.80/144 have chosen IV route against 61/144 for oral. 4.Rewarfaranisation and anaphylaxis are a concern for 70/144 and 42/144 haematologists respectively.

Conclusion: Warfarin anticoagulation can cause significant delay in acute surgical admissions. Guidelines are required for vitamin K reversal in semi-urgent situations. Further studies are needed for effective route and dose required. Effect of vitamin K on rewarfaranisation has yet to be validated. We propose a protocol for a multicentric trial to evaluate efficacy of vitamin K reversal.

P7

Development Of A Home Monitoring Program To Optimise Warfarin

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Home monitoring of warfarin therapy is possible with current point of care INR monitors. Home monitoring has many benefits for patients, but INR accuracy and safety are key issues. This study aimed to determine the safety, efficacy and comparative costs of home versus hospital-based monitoring of warfarin therapy in children. A novel education program, based upon an established model of health education, formed the basis for the home-monitoring program (HMP). The CoaguChekTM INR monitor was used for all INR tests. Participating parents (n=14) adhered to a management program that included both home and hospital INR tests. Study duration was 7 months. Data analysis included linear regression, frequency of target range achievement and determination of cross-over significance. Qualitative data and comparative cost analyses were determined. 14 families completed all the study requirements. There was no statistically significant difference between the mean home and hospital INRs. The target range INR was achieved in 65.5% of home INRs compared to 64.4% of hospital INRs. Lin's correlation coefficient between home and hospital INRs was 0.95 ($p<0.0001$), with the mean difference between tests being 0.055 INR units, determined by Bland and Altman analysis. Home monitoring of warfarin therapy was rated as 'highly beneficial' by parents; all participating families wanted to continue home monitoring. Comparative cost analysis determined that hospital-based INRs cost $8.46/test more than home INRs. Parents can safely and reliably monitor their child's warfarin therapy at home if they receive appropriate education and training. Funding models need to be developed to support home monitoring.

P8

Cytochrome p450 CYP2C9 Genotyping and Warfarin Induction Therapy

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Aim: Many factors influence the dose response relationship to commencement of warfarin therapy including age, liver disease and concomitant drug therapy. Recently, identification of a genetic variant of the hepatic enzyme responsible for warfarin metabolism (CYP2C9*3) has been shown to affect response to warfarin therapy and has been correlated with bleeding potential. Our study aimed to determine the correlation between warfarin induction dosage, International Normalised Ratio (INR) and CYP2C9 genotype.

Method: Blood samples from 216 de-identified patients who were referred for warfarin induction dosing were collected. Standard dosing based on clinical criteria was performed. INR was determined by standard procedures. DNA was extracted from blood samples and the CYP2C9 genotype determined by real-time PCR and melt temperature analysis using a Roche Lightcycler.

Result: Of the 216 patients studied, 31 (14%) were found to contain the CYP2C9*3 variant. Using the measure of an elevated INR above the upper limit of the therapeutic range (ie 3.0), we found 45% (14/31) of patients containing the CYP2C9 variant had been over-anticoagulated during the first 12 days of warfarin induction. Using the same criteria for the patients who had the wild-type CYP2C9, only 16% (30/185) had been over-anticoagulated.

Conclusion: Our results suggest that the presence of the abnormal variant of CYP2C9 is associated with an increased risk of over-anticoagulation during warfarin induction dosing. Therefore, detection of variants at the commencement of dosing may allow clinicians to develop improved warfarin dosing protocols to minimise the risk of over-anticoagulation.

P9

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

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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 very large VWF multimers 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 disulphide-bonds that hold VWF multimers together by thrombospondin-1 (TSP-1). 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. TSP-1, like ADAMTS13, bound to the VWF A3 domain. TSP-1 in the wild type mice, therefore, may compete with ADAMTS13 for interaction with the A3 domain and slow the rate of VWF proteolysis. TSP-1, unlike ADAMTS13, is stored in platelet alpha-granules and is released upon platelet activation. Significantly, platelet VWF multimer size was reduced upon lysis or activation of wild type murine platelets but not TSP-1 null platelets. This difference had functional consequences in that there was as an increase in collagen and VWF-mediated aggregation of the TSP-1 null platelets under both static and shear conditions. These findings indicate that TSP-1 influences plasma and platelet VWF multimeric size differently and may be more relevant for control of the VWF released from platelets.

P10

Comparison of Two Point of Care International Normalised Ratio Measurements in Children

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The INR system was developed to enable comparison of prothrombin time results across different analyzer and reagent systems, by relating results to a WHO standardized thromboplastin via the ISI of individual reagents. Point of care INR monitors are more recent advances and studies have validated results of individual monitors against laboratory based INR. Test strips provided with Point of Care monitors have an assigned ISI and it is difficult to validate the ISI. This is the first study to compare two point of care INR monitors in a paediatric setting.

Aim: To compare the Rapidpoint™ Coag to the CoaguCheck™ point of care INR monitor using capillary samples in paediatric outpatients receiving long term Warfarin therapy.

Method: Paediatric patients on long term warfarin were consented to have two capillary samples collected when due for routine INR testing. CoaguCheck™ S results were used for clinical management as this was current standard practice. Results were compared to the INR results from Rapidpoint™ Coag analyser.

Result: 100 separate test results were compared. The overall mean INR from the Rapidpoint™Coag analyser was higher compared to that from CoaguCheck™S, 2.76±0.80 and 2.2±0.6 respectively ($p<0.05$). This represented a 20.3% difference. In 65% of test points, the disagreement between the monitors would have lead to a change in clinical decision.

Conclusion: INR results from different Point of care monitors may not be as comparable as INR results from different laboratory analyser and reagent systems. Further work is required to standarise INRs from point of care monitors.

P11

Reference Ranges for Haemostatic Parameters in Healthy Australian Neonates

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Reference ranges for haemostatic parameters in healthy Neonates were published in a landmark paper by Andrew et al (Blood 1987). Many institutions use these published ranges. However, the results of haemostatic tests are reagent and analyser dependent and have not been reported for Australian neonates.

Aim: To determine Neonatal reference ranges for standard haemostatic assays using an STA-compact analyser in combination with STAGO reagent systems (Diagnostica STAGO, France).

Method: Blood was collected into citrate tubes via clean venipuncture from 101 healthy neonates (day 1 and day 3 post birth). Assays performed included PT, APTT, fibrinogen, coagulation factors (FII, FV, FVII, FVIII, FIX, FX, FXI, FXII), inhibitors of coagulation (Antithrombin, protein C, protein S) and D-dimers. Resuls were compared to paediatric and adult ranges obtained using the same reagent/analyser system, as well as to the previously published results.

Result: This study confirms developmental differences in many coagulation assays. However, the numerical values of the results are significantly different from those previously published. For example, Mean APTT, PT and Fibrinogen results for day 1 neonates were 38.9 sec, 15.6 sec and 2.69 g/l as compared to previously published 42.9 sec, 13 sec and 2.83 g/l, respectively. Data for all parameters tested will be presented. The differences are mainly due to the differences in assay techniques, in particular, method of detection (chromogenic vs clotting), reagents and analysers used to perform the tests.

Conclusions: Analyser and reagent specific age related reference ranges must be developed to enable accurate interpretation of neonatal coagulation tests.

P13
Coagulation Factor Reference Ranges in Adults
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Aim: To establish adult reference ranges for the coagulant activity of factors II, V, VII, VIII, IX, X, XI and XII using modern automated equipment and commercial calibration standards in a large teaching hospital laboratory.

Method: Blood from healthy volunteers was collected by clean venipuncture into 3.2% buffered citrate vacuum tubes, and spun to obtain platelet poor plasma. Samples not tested within 4 hours of collection were stored at -80°C until tested. For FVIII:C 157 samples were tested. For remaining factors between 81 and 87 samples were tested. Thromboplastin and deficient plasma for factors II, V and X were were from Diagnostica Stago. Deficient plasma for factors VII, VIII, IX, XI and XII were from Helena Laboratories. APTT (Platelin-L) reagent was from BioMerieux. Calibration plasma was obtained from Diagnostica Stago. Testing was performed on the STA or STA Compact analyser by PT or APTT-based 1-stage clotting assays using the laboratory's standard protocol, which included testing at two dilutions. Final reference ranges for each coagulant factor were derived from visual inspection of scatterplots.

Result: Coagulant activity reference ranges obtained were: FII:75-135%; FV:70-145%; FVII:70-155%; FVIII:60-200%; FIX: 75-145%; FX:80-150%; FXI:55-120%; FXII:60-150%.

Conclusion: The coagulant factor activity ranges derived using currently available calibrators and testing equipment should be more useful than the previously used ranges of 50-200% for all factors, which were derived from haematology textbooks.

P14
The Tissue Factor Pathway in Ischaemic Stroke Patients
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The aim of this study was to determine whether enhanced activation or impaired control of the tissue factor (TF) pathway may play a role in the pathophysiology of ischaemic stroke. We measured markers of the TF pathway [TF antigen, tissue factor pathway inhibitor activity (TFPIac) and free antigen (TFPIf), and activated factor VII (FVIIa)] within 7 days (acute phase) and at 3-6 months (convalescence) after the acute stroke event in patients with first-ever ischaemic stroke (n=150; age: 65.9±12.5 years) and randomly selected community controls (n=150; 67.1±12.1 years). During the acute phase, TFPIac was increased [mean 1.31 (SD 0.53) vs 1.13 (0.45) U/mL; p=0.0091] and FVIIa was decreased [mean 43.3 (SD 23.8) vs 57.9 (27.2) mU/mL; p=0.0001], but TF and TFPIf were not significantly different compared with controls. During the convalescent phase, a similar pattern remained evident for both TFPIac and FVIIa, but TF was significantly decreased [median 107 (Q1-3: 75±163) pg/mL] compared to both the acute phase [130 (95±235) pg/mL, p=0.0092] and controls [155 (120±250) pg/mL, p=0.0005]. TFPIf was also reduced [mean 15.2 (SD 8.2) ng/mL] compared to both the acute phase [21.2 (12.2) ng/mL; p=0.0006] and controls [23.3 (9.8) ng/mL; p=0.0003]. Activation of the TF pathway during the acute and convalescent phase of stroke is characterised by enhanced TFPI activity. Normal or reduced levels of TFPIf and TF may be due to consumption and could reflect impaired control of the TF pathway by TFPI in stroke patients or a response to antiplatelet and/or statin therapies.

P15

Measurement of Soluble P-selectin and Soluble CD40 Ligand in Serum and Plasma

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Aim: Platelet activation markers soluble P-selectin (sP-selectin) and soluble CD40 ligand (sCD40L) are commonly measured in serum. However these markers are shed from the platelet surface and levels may be artefactually elevated in serum. The objective of our study was to compare levels of sP-selectin and sCD40L in serum and plasma and to explore the effects of delayed sample processing.

Method: We measured sP-selectin and sCD40L levels in serum, citrate and EDTA plasma obtained from 115 patients with cardiovascular disease and 23 healthy controls using commercially available enzyme-linked immunosorbent assay kits.

Result: Mean sP-selectin levels in patients were approximately 2 fold higher in serum compared with citrate (Geometric mean: 239.8 vs. 126.3 ng/mL, $p<0.001$) and EDTA plasma (239.8 vs. 115.5 ng/mL, $P<0.001$). However, serum and plasma levels remained significantly correlated with each other. Mean sCD40L levels in patients were approximately 15 fold higher in serum compared with citrate (5.92 vs. 0.40 ng/mL, $p<0.001$) and 9 fold higher compared with EDTA plasma (5.92 vs. 0.65 ng/mL, $p<0.001$). Serum levels of sCD40L were only weakly correlated with plasma levels. In healthy controls, serum sP-selectin increased by 43% and sCD40L by 210% during the first three hours after blood collection, while citrate plasma sP-selectin increased by 9% and sCD40L by 80%.

Conclusion: Our results suggest that plasma samples, processed promptly after venesection, should be used in preference to serum to measure sP-selectin and sCD40L levels as this is most likely to yield results that reflect physiological levels of these markers in vivo.

P16

Use of Whole Blood Platelet Aggregometry to Assess the Efficacy of Anti-platelet Therapy in Patients with Myeloproliferative Disorders.

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Whole blood platelet aggregation (WBPA) studies allow in-vitro platelet function testing in whole blood, with simultaneous measurement of platelet aggregation by impedence and ATP dense granule release, using luminescence. These studies have been shown to identify patients who are at risk for thrombosis or bleeding, attributable to platelet hyperactivity (spontaneous platelet aggregation, increased impedence and/or increased release) or hypoactivity (decreased impedence and/or decreased release), respectively (Br J Haematol 105:618, 1999; Int J Hematol 76:272, 2002). In this study, we have used WBPA studies to assess the efficacy of anti-platelet therapy in 17 patients with myeloproliferative disorders (MPD) and hyperactive platelet function. The anti-platelet therapy comprised of aspirin (n =12), garlic (n = 6) and/or clopidogrel (n = 2). Clear evidence of anti-platelet drug effect in the WBPA studies were predictive of therapeutic efficacy in preventing future thromboembolic events. One patient (female, 65yrs) had recurrent cerebral transient ischemic attacks (TIA) despite warfarin and anti-platelet therapy. WBPA studies confirmed the persistence of hyperactive platelet function and enabled optimisation of anti-platelet therapy until the drug effect became evident; she has been well and event free for six months. In conclusion, WBPA allows the assessment of platelet function in patients with MPD and to monitor the efficacy of anti-platelet therapy in those at risk for thrombosis due to hyperactive platelet function.

P17

Activated Seven Lupus Anticoagulant (ASLA) Method Development

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The interpretation of assays for the detection of lupus anticoagulant (LA) can be difficult. That no one method is predictably positive for LA, samples reflect the inter-individual heterogeneity of these antiphospholipid antibodies. The combination of DRVVT and KCT methods will detect 80-85% of patients with LA. A supplementary method is required to clarify equivocal results and to increase sensitivity of LA detection. We developed an additional assay for LA using recombinant activated factor VII. The Activated Seven Lupus Anticoagulant (ASLA) method is an extrinsic based assay using a platelet neutralisation step in the confirmatory test. Ratios of patient to normal control clotting times are analysed, with abnormal ratios undergoing assessment of percentage platelet neutralisation. The ASLA was used to evaluate samples previously tested for LA that failed to give clear interpretation for the presence of LA. Results demonstrated a high detection rate with 12 of 18 (67%) KCT positive, and 14 of 15 (93%) known LA, testing positive using the ASLA assay. We propose that the ASLA can be used as a supplementary test for the detection of LA where conventional methods have shown equivocal results.

P18

Choice of Anti-beta 2 Glycoprotein-1 Assay Based on Additional Multiple Cluster Analysis in Preference to Receiver Operator Characteristics Alone

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Aim: Three commercial anti-beta 2 glycoprotein-1 (B2GP) ELISA kits were compared with the intention of adopting the assay that best discriminated between 70 consecutive symptomatic patients harbouring anti-cardiolipin antibodies (ACLA) and/or lupus inhibitor (LAC) and 72 controls, presenting to the Austin Hospital, Melbourne between January 2000 and March 2004, using receiver operator characteristic (ROC) and clustered multiple analysis based on cut-off values recommended by each manufacturer.

Result: Kits 1, 2 and 3 detected anti-B2GP in 29, 46 and 33 (41%, 66%, 47%) of patients with ACLA and/or LAC and none, one and two (0%, 1.4%, 2.8%) of controls. Kit 1 was the most specific (100%) and least sensitive (45%) of the three assays and kit 2, the most sensitive (67%) and slightly less specific (98.6%). Kits 1 to 3 demonstrated areas under respective ROC curves of 0.96, 0.90 and 0.79. Clustered multiple analysis revealed superiority of kit 2 in discriminating between diseased and normal groups over kit 1 ($p=0.0023$) and no statistical difference between performance of kits 1 and 3, or 2 and 3.

Conclusion: Kit 2 demonstrated superior discrimination of patients with ACLA and LAC despite the slightly better receiver operator characteristics of kit 1, and showed clearly better ROC than kit 3. These results therefore support the adoption of kit 2 into the diagnostic armamentarium of anti-phospholipid related disorders at our institution. In addition, this method comparison highlights a general principle of employing more than one analysis tool to facilitate the choice of assay appropriate for an individual laboratory patient population.

P19

Intravenous Heparin Monitoring: Should Standardisation And The Therapeutic Range Be Changed?

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Aim: International standardisation of heparin monitoring using the aPTT remains controversial. Each laboratory is required to determine a heparin therapeutic range of 0.2 - 0.4 units/ml (usually between 1.5- to 2.5 control aPTT) using the local reagent and methodology. Recent reports using anti Xa measurement have proposed that a therapeutic range of 2.0 - 3.0 times the normal mean aPTT could be more appropriate. The study aims to determine the heparin therapeutic range of our aPTT reagent (Platelin LS, bioMérieux, Durham, USA).

Method: Anti-Xa levels and aPTT were assessed by a chromogenic technique (Diagnostica Stago, Asnières, France) in 61 patients receiving heparin and compared to the aPTT range obtained from normal plasmas spiked with increasing concentrations of heparin. All tests were performed on the STA-R analyser (Diagnostica Stago).

Result: The mean normal aPTT was 35.0s. For the ex-vivo samples, the reference therapeutic anti FXa range of 0.3 - 0.7 units/ml was equivalent to an aPTT range of 75 - 110 s (ratio 2.1 - 3.1). In vitro spiking was variable between different plasmas with a spiked heparin level of 0.2 - 0.4 units/ml giving aPTTs of 58 - 93s (1.7 - 2.7) in one plasma and 47 - 71s (1.3 - 2.0) in another.

Conclusion: The variability of aPTT to heparin spiked plasma appears to limit the use of this form of therapeutic range determination. Based on anti -Xa levels, our results support the move to the higher therapeutic range of to 2.0 - 3.0 times the normal mean aPTT. Changing the range will necessitate modification of heparin nomograms used for dose adjustment.

P20

Successful Selective B-cell Depletion with Rituximab in the Treatment of a Patient with Acquired Haemophilia A and High Titre of Factor VIII Inhibitor

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Spontaneously acquired factor VIII (FVIII) inhibitors are a rare, life-threatening clinical challenge, as fatal bleeding is a serious complication. Therapeutic options include factor substitution, recombinant FVIIa (rFVIIa), plasmapheresis and immunosuppression. A novel treatment option is rituximab (chimeric monoclonal anti-CD20 antibody, Roche). A 49 year-old female Caucasian patient had a prolonged APTT between 70-99 sec (normal 24-37), which was not corrected by a mixing study with normal plasma. FVIII level was below 1%. There was no evidence of lupus anticoagulant and no family history of haemophilia or bleeding. A Bethesda assay showed a very high FVIII inhibitor titre of 840 BU. She was commenced on immunosuppressive therapy with oral prednisolone and azathiaprine and after 5 months her inhibitor titre dropped to 115 BU. One month later she was readmitted with a spontaneous bleed into her left calf and an inhibitor titre of 148 BU. Haemostasis was achieved with regular doses of rFVIIa (100 mcg/kg/6 hourly for 6 days). A further minor bleed occurred 13 days later, treated with rFVIIa (60 mcg/kg/8 hourly for 5 days). Immunosuppressive therapy was changed to cyclophosphamide and prednisolone orally and adjunctive therapy with rituximab was commenced. Within 3 weeks the inhibitor had dropped to 75 BU and 25 BU by 6 weeks. At four months post therapy, she remained well and has detectable factor VIII activity (16%) with an inhibitor titre of 14 BU. These data support employing of rituximab in the case of acquired FVIII inhibitor. Further properly conducted randomised trials of the efficacy of rituximab are warranted.

P21

Acquired Haemophilia. Local Experience with Acquired Factor VIII Inhibitor

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Monash Medical Centre, Clayton, VIC

Aim: To review the diagnosis and management of acquired haemophilia in a tertiary referral centre.

Introduction: Acquired haemophilia is a rare, often life threatening bleeding disorder, caused in the majority of patients, by antibodies directed against factor VIII (FVIII). It occurs at an annual incidence rate of 1 per million. Most cases of acquired FVIII inhibitor are idiopathic, while 40-50% are associated with other conditions, including pregnancy, malignancy, drugs, diabetes and connective tissue disorders. Unlike congenital haemophilia, haemarthrosis is relatively uncommon, with more frequent manifestations including cutaneous, muscle, gastrointestinal, and genitourinary haemorrhage. Uncontrollable post-operative bleeding or excessive bleeding after venipuncture may also indicate the presence of an underlying inhibitor. The activated partial thromboplastin time (aPTT) is typically prolonged and fails to correct when incubated with normal plasma. The diagnosis is confirmed by assays of specific coagulation factors and the inhibitor activity quantified using the Bethesda assay.

Result and Conclusion: Over the past six months, three patients have been admitted to our institution with acquired haemophilia due to an inhibitor to FVIII. One patient presented with bleeding associated with a ruptured muscle tendon, another with widespread purpura and muscle haemorrhage, and a third with purpura and anaemia. One patient was treated palliatively in the setting of advanced Parkinson's disease, the others received various combinations of corticosteroids, cyclophosphamide, intravenous immunoglobulins, rituximab, factor VIII concentrate and recombinant factor VIIa (Novoseven). The disease was well controlled in the two actively treated patients. The diagnosis, assessment of inhibitor activity and treatment options will be discussed.

P22

Recombinant Activated Factor VII: Treating Post-operative Haemorrhage in Cardiac Surgery

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[1] Department of Haematology, The Royal Melbourne Hospital
[2] Department of Cardiothoracic Surgery, The Royal Melbourne Hospital

Aim: To review the effect of recombinant activated Factor VII (rFVIIa) as rescue therapy in severe post-operative haemorrhage in cardiac patients at our institution.

Method: We present a case series of 11 patients who received rFVIIa on 12 occasions as rescue therapy for post-operative haemorrhage after cardiothoracic operations. We assessed the use of blood products, coagulation parameters (INR, APTT, fibrinogen) and platelet counts in the twenty-four hours before and after the rFVIIa was given, and compared them using the paired t-test.

Result: Bleeding stopped in all cases after the use of rFVIIa. The mean FFP usage was 16Units (range 0-40) pre and 0U (range 0-2) post. The mean red cell usage was 6U (range 0-15) pre and 0U (range 0-1) post. The mean cryoprecipitate usage was 17U (range 0-32) pre and 0 (range 0) post. The mean platelet usage was 20U (range 0-40) pre and 0 (range 0) post; with p-values of <0.001 for all the above. The mean INR was 2.0 (range 1.8-8.5) pre and 0.9 (range 0.7-1.5) post. The mean APTT was 61s (range 30-220) pre and 43.3s (range 30-87) post. The mean fibrinogen level was 3.3g/L (range 1.6-6.4) pre and 3.1g/L (range 1.7-4.5) post. The mean platelet count was 167 x109/L (range 78-257) and 162 x109/L post; these were not statistically significant. There were no thrombotic complications in the observation period.

Conclusion: Our results support the use of rFVIIa as rescue therapy in severe post-operative haemorrhage after cardiac surgery. In our limited cohort rFVIIa was both efficacious and safe.

P23

Recombinant Factor VIIa in the Management of Severe Bleeding in Adult Patients Following Cardiac Surgery: An Audit of Clinical Use

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[2] Georgetown University Medical Center, Washington, USA

Profuse bleeding associated with cardiac surgery is often poorly controlled by conventional pharmacological agents and generally requires the use of blood products. Recombinant Factor VIIa (rFVIIa) may be of value in this group of patients. Data were extracted from the international, internet-based registry haemostasis.com. Search results were manually cross-checked against monthly summary reports and the clinicians were contacted individually to approve the use of their cases, supply any missing information and validate the data that were already held. Automated searches of haemostasis.com identified 14 adult patients undergoing cardiac surgery (five patients had undergone CABG, five had valve replacement, three had aortic root replacement, and one had a thoracic aortic aneurysm repair), who experienced severe post-surgical haemorrhage that was treated with rFVIIa. Doses of rFVIIa ranged from 21-100 µg/kg body weight. Bleeding was reversed completely in five patients, markedly decreased in eight patients and decreased in one patient. Furthermore, the use of all categories of blood products was substantially decreased from a mean 35.5 units (range 12.7-80) within the 24 h before administration of rFVIIa to 3 units (range 0-25) within the 24h after its administration. There was one report of an adverse event (pulmonary embolism) 'probably or possibly' related to rFVIIa.

MONDAY 18 1600 to 1700 Atrium
ANZSBT Poster Session 1 - Hospital Transfusion Practice: Education And Audit
Chair: Peter Flanagan

P24

Effect of a Multifaceted Educational Intervention on Paediatric Nurses- Knowledge of Transfusion Safety and Administration

Janine Furmedge [1,2], Hans Surya [1], Helen Savoia [1], Paul Monagle [1,3]

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[2] Murdoch Children's Research Institute, Melbourne, Australia.
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Aim: Nurses are frequently involved in administering and monitoring transfusions. Nurses are required to accurately identify the patient, administer the blood product and recognise any possible transfusion complications. In order to execute these tasks safely, it is important that nurses have a good understanding of transfusion and its associated risks. We developed a number of strategies to improve paediatric nurses' knowledge regarding blood product administration and transfusion complications in an attempt to enhance transfusion safety and practice.

Method: Nurses in the Haematology/Oncology unit and PICU were targeted because both units are the major blood users in our hospital. This study assessed baseline nursing knowledge of key aspects of transfusion administration and then, via repeat assessment, the effectiveness of a multifaceted educational intervention which comprised of information transfer (posters, in-services, and hospital web-based clinical practice guidelines), and the provision of reminders on the reverse side of the compatibility report issued.

Result: The pre-intervention survey revealed poor knowledge, particularly in such areas as optimal duration of transfusions, recognition of common signs/symptoms of a transfusion complication, and the correct use of blood filters and irradiation. Less than 20% PICU nurses understood the use of leukocyte filters or the role of irradiation. Only approx. 50% nurses in Haem/Onc unit or PICU knew the maximum duration of individual blood unit transfusion. Significant improvements to this knowledge were seen at post-intervention.

Conclusion: The introduction of a multifaceted educational intervention appears to be effective in improving nurses' knowledge with regard to blood product administration and transfusion complications.

P25

A Clinical Audit of the use of Fresh Frozen Plasma (FFP), Platelets (Plts) in a Tertiary Teaching Hospital and the Impact of a New Transfusion Request Form

Chi-Hung Hui, Ian Williams, Ken Davis

Institute of Medical & Veterinary Science

Aim: An audit was carried out to assess (i) the pattern of usage of FFP and Plts in the Royal Adelaide Hospital and (ii) the impact of a self-educating transfusion request form, introduced in Jan 2003, on the appropriate usage of these blood products. Method: The Australian NHMRC/ASBT Clinical Practice Guidelines for the appropriate use of blood components were publicised in Oct 2001. We prospectively audited the indication(s) and laboratory data for 898 FFP and 1014 doses of Plts transfusions issued in the 2-month period of Nov-Dec 2002 and the same period in 2003. Appropriateness was assessed within 48 hours using the Guidelines as a template and, in equivocal or possibly inappropriate cases, assessment by a haematologist.

Results & Conclusion: 1 Reversal of warfarinisation emerged as the commonest contributory indication for FFP (34% compared to 4% previously reported from another tertiary hospital in 1996). Our audit also identifies areas for improvement. 2 In our hospital, 72% FFP and 88% Plts were prescribed in an appropriate fashion in 2002. The use of a self-educating request form had only a modest improvement to the appropriate usage in 2003 (significant for Plts) and met with a fair success in compliance. 3 Designed to incorporate additional prompts in specimen labelling, it also improves transfusion safety and helps reduce the yearly incidence of wrong-blood-in tube since the new form has been in use. 4 Percentage cases of indeterminate appropriateness illustrated the difficulty in this type of audit. There were also difficulties in applying the Guidelines in some scenarios.

P26

Tracking Blood After Release From Hospital Blood Bank

Janine Carnell [1,2], Marilyn Garnham [1], Sukanya Roy [1,2], BB Team [1]

[1] Boxhill Hospital, Box Hill
[2] Blood Matters Breakthrough Collaborative, DHS

Aim: To devise a system of collecting information on blood units after their release by hospital blood banks (BB).Information on the fate of blood is not available to most BBs and patient records are the only way to confirm completion of transfusion. Our method looked at overcoming this issue and aimed to complete the loop from hospital BB, to recipient and back to BB.

Method: A blood tag (BTag) was designed and attached to each released blood unit. Empty bags were returned to BB with the BTag intact. Ward nurses completed tags for each unit. An Access database was used to collate collected data. This included date/ time of transfusion, location, staff name, any identified clerical error, clinical reaction or uneventful transfusion.

Result: This study is ongoing at BoxHill Hospital, Victoria. Data presented is from October 2003 to May 2004. Total number of blood issued was 4128, with 100% empty bags returned. Of all returned BTags, 60% were fully complete, a further 37% captured partial data and 2.5% BTags were returned blank. Of all BTAg reported events (4128), clinical events comprised 0.6%, clerical events comprised 1.9% and 73.3% of all transfusions were uneventful.

Conclusion: Capture of transfusion events has always been a problem. We found high compliance with reporting since the tags provided a prompt. There was real time confirmation of transfusion by BB. All BTag reported events could be followed up promptly by the transfusion nurse.We conclude that all BBs can universally employ BTags for haemovigilance purposes.

P27

Can Transfusion Nurses Make a Difference

Tony Ghent [1,2], Lynelle Foster [2], Bronwyn Williams [1,4], Marianne Wallis [2,3], Sarah Fryer [2]

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[2] Gold Coast Health Service District, Gold Coast Hospital
[3] Griffith University
[4] Royal Brisbane Hospital

In 2003 the Queensland Health Pathology Service and the Gold Coast Health Service District received a grant from Australian Council for Safety and Quality in Healthcare - Safety Innovations in Practice (SIIP) Program Mark II for monitoring appropriateness and safety in clinical transfusion practice. The study looked to improve awareness of and compliance with the NHMRC & ASBT clinical practice guidelines on the use of blood components. Focus was concentrated on 2 of the 16 of the NHMRC recommendations: Clinical Practice - looking at appropriateness of transfusion. Organisational Practice - reviewing informed consent documentation in medical record. Transfusion nurse intervention by awareness sessions to all clinical areas was followed up by mail out newsletters, information on the local intranet, distribution of NHMRC consumer brochures and development of a nursing checklist for administration of blood. Using data from 2001 and current data, the results were analysed using SPSS. Chi square analysis revealed statistically significant improvements in documented evidence of informed consent and appropriate prescription of transfusion. Compliance of documenting informed consent improved from 8% in 2001 to 76% ($p<0.0001$) in 2003. Appropriate prescription of red cell transfusion improved from 74% in 2001 to 86% ($p=0.006$) in 2003.

P28

A Computer Model to Audit Appropriate Blood Usage

Susan Williams [1], John Rowell [1], Peter Russell [1], Peter Hegarty [2], Bruce MacDonald [3]

[1] Qld Health Pathology Service
[2] AUSLAB Support Unit
[3] ARCBS (Q)

Inappropriate blood usage can lead to significant morbidity and mortality. There are significant costs for blood transfusion and blood (and blood donors) are becoming less available. There is a clear need for efficient use of blood and to achieve specific outcomes for each transfusion. Auditing of blood transfusions, using the patient's medical chart and manual review, have proved to be time consuming and cumbersome. There are significant benefits from reporting of audits to medical staff, but unless repeated, improved usage patterns are unsustained. A reliable and repeatable automated method for auditing is required. This would enable regular (monthly) reporting to clients. Most Transfusion laboratories use computerised reporting systems. Incorporation of the auditing practice in existing information systems is beneficial. Queensland Health Pathology Service (QHPS) comprises 33 laboratories that provide a transfusion service to Qld Health facilities. AUSLAB is the statewide laboratory information system which stores most laboratory data. The NHMRC guidelines were used to devise clinical indicators that are added to the computer system when a red cell transfusion is requested either with the request form or when blood is ordered from a 'group and hold'. Data identifiers are then used to extract the information regarding the transfusion episode. Reports based on hospital, consultant and clinical indication are generated to identify which transfusions were considered inappropriate. Reports will be circulated to clients. This method allows repeated audit on a regular basis with an opportunity to assess educational or other strategies to improve blood usage.

P29

The Role and Value of Transfusion Audits in a Major General Hospital

Christine James, John Lown, Ann McNae

Haematology Department, Royal Perth Hospital

The increasing pressures on the blood supply and emphasis on ensuring appropriate use of blood and blood products highlights the need for continuing surveillance of transfusion practice. In addition quality management practices associated with transfusion also require ongoing review. At RPH audits of transfusion practice in all relevant clinical areas have been conducted on a regular basis over the last 8 years with the emphasis on red cells, platelets and FFP. The audit process and associated educational activity was facilitated by the appointment of a Transfusion Nurse in 1996. Parameters recorded for the audits included conformance with hospital transfusion guidelines for usage, documentation of transfusions and the indication in patient's notes by the medical officers, recording of nursing observations, overnight transfusions, C/T ratios, and completion of appropriate reasons for transfusion on request forms. The audits have identified areas of practice that could be improved, especially documentation, resulting in educative strategies which have improved performance. Appropriate clinical indications on request forms, nursing observations in patient's notes, and minimising overnight transfusion, were the areas of practice most vulnerable to fluctuating performance. Rotation of junior clinical staff and nurse staffing issues impacted on compliance with documentation requirements. Regular audits are an essential management tool to continually monitor transfusion related practices and identify and respond to developing problems.

P30

Hospital Transfusion Committees - Monitoring the Health of the System

Erica Wood, Neil Waters, Malcolm Forsyth

Australian Red Cross Blood Service

Aim: Review of hospital transfusion (tx) committee (HTC) activities in Victoria and Tasmania. These groups improve tx practice through policy/protocol development and monitoring use/wastage and adverse events.

Method: Email survey sent to scientist in charge (SIC) of institutional blood banks (BB) in Victoria (82) & Tasmania (6). Review terms of reference, agendas & minutes for HTC meetings attended by ARCBS.

Result: Of 31 replies (35% response), 61% reported that they had any HTC (17 public hospitals, 2 private labs); 4 were considered inactive. All institutions with a dedicated tx nurse had active HTCs. ARCBS participates in 12/15 (80%) active HTCs. 12/31 (39%) responding organisations had no HTC, and few HTCs appear to exist in organisations not responding to the survey. Typical membership included haematologist, SIC of BB and medical/nursing representatives. Some HTCs included institutional & clinical risk management, pharmacy, medical records etc. Activities included protocol development for tx management & product use, product-related issues & tx reaction review. Only 9/19 (47%) institutions regularly reviewed blood use, wastage and/or expiry. Reported difficulties included substantial administrative burden, poor attendance from non-BB members, difficulties implementing HTC recommendations, and lack of institutional management interest in the group's activities.

Conclusion: HTCs are not yet sufficiently widespread or active in all institutions transfusing blood in Victoria and Tasmania. Lack of executive and clinical involvement and resource limitations were often reported as obstacles to a productive HTC. ARCBS can be even more active in supporting HTCs by providing product updates and data for review.

P31

An audit into red cell transfusion.....

Joanna Renee [2], Steven Austin [1], Rosemary Marando [1], Annabel Horne [1], Anthony Dodds [1]

[1] St Vincents Hospital, Sydney
[2] University of Aberdeen, Scotland

An audit into red cell transfusion practice in the Haematology Unit of St Vincent's Hospital was carried out. This was done to assess compliance with the NHMRC/ASBT Clinical Practice Guidelines. Information was sought about the transfusion trigger, number of units received, indication for transfusion, provision of patient information, the obtaining of patient consent and the documentation of the transfusion details in the patient record. Fifty-nine transfusion episodes in 33 patients were studied. These episodes occurred in in-patients (61%) or ambulatory patients (39%) of the Unit. The haemoglobin trigger ranged from 73g/L to 97g/L (mean 85) in 23 transfusion episodes in ambulatory patients and from 79g/L to 96g/L in 36 transfusion episodes in in-patients (mean 87). Eighteen episodes occurred in patients with haemoglobin levels of 90g/L or above. The number of units transfused per episode differed between in-patients and ambulatory patients. In in-patients, 25 episodes involved 1 unit and 1 episode involved 2 units. However in ambulatory patients 18 episodes involved 2 units and 5 episodes involved 3 units with no 1-unit transfusions. Of the 33 patients, 19 had received verbal information about transfusion but only 3 were given written information. 33% could name a side effect or risk and 45% felt they would benefit from more information. No patients in the study had specific documented written consent. Documentation of the transfusion in the patient's record was incomplete in most episodes. In conclusion adherence to the NHMRC/ASBT Clinical Practice Guidelines is problematic in a busy Haematology unit.

P32

Meeting the Ongoing Educational Needs of Hospital Staff in Relation to the NH & MRC/ ASBT Guidelines for Blood Components and Improving Transfusion Practices.

K O'Shea [1], G Aitken [1], J Jupe [1], R deSilva [1], J Bartlett [2]

[1] Royal Hobart Hospital
[2] Baxter Healthcare Australia

Royal Hobart Hospital, Tasmania participated in the Victorian DHHS funded 'Blood Matters' collaborative. The collaborative actively commenced in 2003 with the participation of 14 Victorian hospitals and one Tasmanian hospital. The objective of the collaborative was to implement the NHMRC/ASBT Guidelines for blood components and improve transfusion practice with in the hospitals.

Part of the continuing educational process the Royal Hobart Hospital Blood Matters team requested that Baxter Healthcare contribute to the Blood Matters educational goals in a tangible way. Baxter Healthcare is a worldwide organisation, which provides a diverse range of healthcare solutions to practitioners covering many portfolios. Baxter Transfusion Therapies has long been a member of the medical industry in Australia and provides products that aid in the collection, separation, manufacture and administration of blood and blood products.

In consultation Baxter reproduced RHH Irradiation/ leukodepletion filtration guidelines as a cue card format and a second cue card detailing how to recognise and manage a transfusion reaction. This format is easily accessible at the bedside, without having to log onto a website or access a manual. They were put into bullet points in an easily readable format. The guidelines were then placed in a plastic pocket that could be clipped to the ID badge and worn at all times.

The availability and ease of use of the cue cards have helped to meet the educational goals of the Blood Matters collaborative.

MONDAY 18 1600 to 1700 Atrium
ANZSBT Poster Session 2 - Clinical Transfusion Practice
Chair: Ellen Maxwell

P33
What Blood Users Know About Emergency Transfusion
Shir-Jing Ho, Peter Kyle, Yiu Lam Kwan, Tim Brighton

SEALS Kogarah, St George Hospital, NSW

Background: St George Hospital is a metropolitan teaching hospital providing level 2 trauma facilities. Knowledge regarding transfusion principles and local practice is essential for appropriate resuscitation of patients.

Objective: This study aimed to assess the current state of awareness of emergency blood product use amongst key blood users throughout the hospital.

Method: 445 anonymous questionnaires were sent to medical staff (including JMOs, surgeons, anaesthesists, emergency physicians, intensivists and general physicians) as well as nursing staff in areas of high blood product use (ED, ICU, HDU and Haematology/Oncology units). The survey was based on a clinical scenario of an acute ruptured abdominal aortic aneursym with no previous compatability testing. The patient was shocked with significant anaemia (Hb 60g/dl).

Result: A total of 176 (39.6%)questionnaires were returned. The preferred initial blood product resuscitation fluid was emergency release (O negative) blood in 70% of respondents. 13.7% requested cross-matched blood with 3.5% opting for group specific blood. Median (range) perceived times of blood availability with no pre-transfusion testing performed were 5 (1-1440), 20 (1-720) and 45 (1-1440) minutes for emergency release, group specific and cross-matched blood respectively. The wide range suggests poor understanding of implications of compatibility testing procedures. Results of the survey regarding indications for platelet and other supportive therapy, awareness of massive transfusion protocols and Emergency Release Box strongly suggests further education is required.

Conclusion: This study confirms that appropriate use of blood products requires greater understanding by medical and nursing staff to ensure best practice.

P34
The Trauma Beeper is Going Off
Katrina Sowak, Geoff Magrin, Merrole Cole-Sinclair, Alison Street

Haematology, Alfred hospital

The Alfred Hospital Trauma service has been operational for nearly ten years and requires urgent and ongoing blood product support. In that time the Alfred Hospital Blood bank have made a number of adjustments to improve the service and better manage our resources. Four key areas, inventory management, automation and computerisation, specimen processing and communications have been identified as essential. Automation has assisted handling specimens and electronic transfer of results into the computer system has all but eliminated transcription errors that may occur under time pressure. The inventory has changed dramatically with the advent of new products and the occasional requirement to supply blood in the absence of a reportable blood group. Two emergency reserves are stocked, an O Neg, K negative reserve for patients in whom a group is not available and the other is a group specific K negative reserve for patients with an established blood group. On average we are asked to issue the O Negative K negative units seven times a month. Two doses of group A and O platelets are kept on site at all times. Recently we have been able to offer AB liquid thawed plasma as an immediately available clotting factor product. Strict adherence to ANZSBT guidelines for crossmatch samples is applied to all specimens received. If these guidelines are breached or no sample is received group O negative blood is issued for the next 24 hours The laboratory is included in the Trauma alert loop via a beeper ensuring we receive the estimated time of arrival and any relevant patient information. Blood Bank support for a Trauma Service requires ongoing planning, organisation and communication between the hospital laboratory, the donor service and the trauma unit. We have a system that efficiently manages our inventory and have in place procedures and protocols to handle most situations that arise.

P35
Recombinant Factor VIIa for Life-threatening Haemorrhage in Surgical and Trauma Patients: a 2 year Single Institution Experience
Anne McNae [1], Julianne LeFante [1], Nicole Staples [1,2]

[1] Royal Perth Hospital
[2] Australian Red Cross Blood Service

Aim: To outline use of Recombinant Factor VIIa (rFVIIa) for life-threatening haemorrhage for non-haemophilia patients in a tertiary referral Teaching Hospital.

Method: A hospital protocol was developed for situations of intractable bleeding in surgical and trauma patients, in which all reversible causes of bleeding had been excluded. The decision to use rFVIIa was made in consultation with the treating Consultants and on-call Haematologist, cost being borne by the requesting Department. All patients were dosed at 100µg/kg rounded to the nearest vial. The Hospital Blood Transfusion Committee audited use.

Result: From July 2002 to June 2004, 31 patients were treated with rFVIIa (4 patients required a second dose of rFVIIa). Median age was 49yrs; 22 (71%) were male, 9 female. Bleeding stopped or decreased in: 13/15 (87%) of cardiac and vascular surgery patients; 5/10 (50%) of trauma; and 5/6 (83%) of other surgical patients. 19 of the 31 patients (61%) survived, and bleeding was the direct cause of death in 6 of the 12 patients who died. Median blood product usage pre and 12 hours post rFVIIa was as follows: red cells pre 11 units (range 4-35), post 2 (range 0-10); FFP pre 12 units (range 4-35), post 2 (range 0-8); Platelets pre 10 single unit equivalents (range 0-31) and post 0 (range 0-16); cryoprecipitate: pre 8 units (range 0-45), post 0 (range 0-16). There were no documented thrombotic complications.

Conclusion: From our experience, there is a role for rFVIIa in managing uncontrolled haemorrhage, particularly in cardiac and vascular surgery patients.

P36

Blood and Components Transfusion in Advanced Liver Disease

Akram Al-Hilali, Khalida Abu Saud, Mohammed Hassan, Rosalia Mago

King Fahad Armed Forces Hospital-Jeddah, Saudi Arabia

Aim: To assess the gravity of need for blood and components by chronic liver disease (CLD) patients in a general hospital in Saudi Arabia, where viral hepatitis and cirrhosis are relatively common.

Result: Over 3 years of retrospective study period total patients transfused were 4,400.They received 10,498 units of packed red cells(PRC),5,875 units of fresh frozen plasma (FFP), 3,013 units of platelets (PC) and 600 units of cryprecipitate (CP). Total number of patients having CLD receiving transfusion during the study period was 63.They were transfused in 109 admission episodes. Age range was 31-94 years (mean 60.6). 39 of them were males and 24 females (M:F ratio 1.63). INR prior to transfusion was 1.2-3.4 (mean 1.91).It was less than 2.0 in 77.8%. GIT was the main bleeding site (60%). 28 patients (44.4%) died during the period and 13(20.6%) had hepatic carcinoma. They required a total of 577 units of FFP (0-41 units per patient-episode). This represents 9.97% of total FFP transfused.They were given 320 units of PRC (0-26 units per episode,3.4% of total). They also needed 88 units of PC (2.92%) and 35 units of CP (5.8% of total).

Conclusion: CLD is a major disease creating need for for all kinds of components in Blood Bank. FFP is the component most widely needed due to coagulopathy. Some patients need enormous quantities of blood and components in an admission, with an unfavourable outcome.

P37

Impact of Oral Anticoagulation on the Blood Bank in a General Hospital

Akram Al-Hilali, Khalida Abu Saud, Mohammed Hassan, Rowena Diamanlatan

King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia

Aim: Evaluating the impact of management of patients by oral anticoagulants (OAC)on the Blood Bank. Such patients may need blood components to correct excessively high INR (GroupI), with or without bleeding, to correct therapeutic INR in situations with bleeding localized lesions (GroupII) or to prepare patients for an invasive procedure (GroupIII).

Result: Over 3 years of the study period, a total of 4,400 patients were transfused with 10,498 units of packed red cells (PRC), 5,785 units of fresh frozed plasma (FFP) and 3013 units of platelet concentrate (PC). Among these patients there were 39 on oral anticoagulants whose files were available for evaluation. In 52 occasions they received 107 units of PRC (1.02% of total), 228 units of FFP (3.94%) and 73 units of PC(2.4%). Another 8 patients were transfused in 13 episodes but their files were not available. They received 36 units of PRC, 48 of FFP and 8 of PC.Total transfusion episodes were 65. GroupI included 28 patients (30 episodes), groupII were 5 (7 episodes) and (groupIII) were 15 patients (18 episodes). Group III received transfusion mainly for the invasive procedures but OAC contributed to the need. GIT was the commonest bleeding site (2.3%). Maximum period for holding OAC was 14 days. Mean admission period was 4.1 days in groupI and 2 days in group II. Group III is actually much bigger than shown in the study, but most did not need transfusion.

Conclusion: OAC creates a group of patients that often need blood components. They represent one of major specific groups with which the Blood Bank has to deal.

P38
Haemoglobin Increments: 2001 vs. 2004
Samantha Brett

Institute of Clinical Pathology and Medical Research

It has been accepted for many years that transfusing one unit of packed red cells will provide a haemoglobin increment of at least 10g/L. I decided it was time to re-look at this value as the volume of red cells in each unit appears to be less than it once was. A retrospective review of stable hospital inpatients was undertaken, which compared pre-transfusion and post-transfusion haemoglobin values from 432 transfusions in 2001 and 420 transfusions in 2004. The post transfusion haemoglobin levels were analysed within 24 hours of transfusion. Actively bleeding and unstable patients were excluded form this review.

Result: In 2001, 74% of transfusions gave a haemoglobin increment of 10g/L or more. The average haemoglobin increment was 13.5g/L per unit and the range was 4.3-35 g/L. In 2004, 62% of transfusions gave a haemoglobin increment of 10g/L or more. The average haemoglobin increment was 12.0g/L per unit and the range was 4-35 g/L.

Conclusion: It appears the average haemoglobin increment per red cell unit transfused in 2004 has slightly declined since 2001 by 12%. This decline corresponds with a change in the blood component preparation to the Optipress - II automated blood component extractor system. This system removes the buffy coat layer from the unit and 13-15% of the red cells (1).

References: 1) Hurtado, C., Bonanad, S., Soler, M., Mirabet, V., Blasco, I., Planelles, M., & de Miguel, A., (2000) Quality analysis of blood components obtained by automated buffy-coat layer removal with a Top & Bottom system (Optipress - II), Haematologica, 85: 390-395

P39
Reduction in Transfusion Labelling Errors
Rosemary McKenna, Jeff Szer, Chris Hogan, Delaine Smith, Michael Haeusler

The Royal Melbourne Hospital

One of the most significant risks to patients in the hospital setting is that of transfusion error. A previous study found 1 in 165 samples were mislabelled and 1 in 1986 samples contained the wrong blood. The Blood Matters Collaborative (BMC) is a Victorian state-funded project involving 13 hospitals. The Royal Melbourne Hospital BMC group trialled a new Transfusion Request Form and Labelling System (TRFLS) with the aim of reducing transfusion request form and sample patient identification (ID) errors and improving the safety of patients requiring blood component therapy. Assessment of the efficacy of the approach was with Rapid Cycle methodology using the Plan, Do, Study, Act (PDSA) quality improvement cycle. A small initial trial in 29 patients (Cycle 1) was powered to show reduction of errors of any kind on request form and sample. Cycles 2 and 3 were conducted in 100 and 240 patients respectively and were powered to show reductions in ID errors (absence or error of key identifiers, unit record number, first or family name, date of birth) on both sample and request form.

Results are tabulated below:

Cycle 1	Reduction in errors of any kind.	N=29	Baseline	Post Trial		68%	21%
Cycle 2	Reduction in serious ID errors	N=100	Baseline	Post Trial	Request Form	8.9%	2%
			Sample			6.1%	2%
Cycle 3	Reduction in serious ID errors	N=240	Baseline	Post Trial	Request Form	8.9%	1.6%
			Sample			6.1%	3.8%

We conclude that the TRFLS significantly reduced errors resulting in the potential to improve patient safety.

P40
Safe Blood Transfusion
Makerita Samau

National Hospital, Samoa

Aim: To determine the rate of appropriateness of blood transfusions usage, within the National hospital and to identify the reactions for recipients in order to target practice improvement intervention.

Method: All test protocols were in accordance with manufacturers recommendations.Our one main laboratory here in samoa, depends on one bloodbank service in times of emergencies,we had supplies of blood from family donors and blood programme with the help of our red cross society.

Result: To approve the how safe of blood that we issued, when in need by clinician, the technician will screen all donors by Hiv, HbsAg, and Syphillis, if its reactive, then investigations is the next step done in confedential.

Conclusion: A significant rate of in appropriate transfusion has been demostrated within regulation of Health. Practice change strategies should be targeted at senior clinicians who prescribe the majority of transfusions and influence the attitudes of junior staff. Our govrnment of Samoa recognises the blood transfusion is an important tool of mordern health care.It saves the lives of many people.

TUESDAY 19 1600 to 1700 Atrium
ANZSBT Poster Session 3 - Transporting Blood Safely
Chair: Neil Waters

P41
Validation of an Innovative New Transport System for Cord Blood Collections
K Bolton [1], G Van der Meer [1], B Pope [2], M Kirkland [1], J Bartlett [3]

[1] The Douglas Hocking Research Institute, Barwon Health, The Geelong Hospital, Geelong
[2] Sydney Adventist Hospital, Wahroonga, NSW
[3] Baxter Healthcare Australia, Brunswick, VIC

Stem Cell Technologies is recently created company dedicated to the safe collection, transport and long term storage of umbilical cord blood. We adhere to strict quality control measures at all stages of our stem cell storage service, which includes Quality Assessment, Maternal Donor Management, Data Collection, Collection Procedures, Transport Procedures, Quarantine and Sample Testing. Baxter Healthcare has recently made available a system for the transport of blood and blood products that utilises a unique Phase Change Material (PCM). The PCM is contained in moulded plastic elements specially shaped for the transport of blood packs. They enable the transport of HSC over distances not normally achievable with conventional systems and as such were deemed ideal for the transport of cord blood back to Stem Cell Technologies two central laboratories, Barwon Health and Sydney Adventist Hospital, for the processing, testing and storage. The Blood-In-Motion system of transport of HSC over long distances at variable temperatures has been a great success for Stem Cell Technologies because it is easy to validate, durable, reusable, easy to clean, does not require complicated storage or packaging instructions, easy for couriers to collect and transport, and is reliable. It has met the standard required for our strict quality control measures and has proved easy to use for all staff concerned.

P42
Validation of the Blood-In-Motion x°Control Bag for the Transport of Vaccines
A Suters, L Parker, P Mealey, G Moore

Baxter Healthcare Australia, Toongabbie, NSW

Aim: The Blood-In-Motion x°Control Bag was validated to obtain data for the transport of vaccines to be used for community vaccination programs in remote sites.

Method: Three sets of conditioned 2-8°C degree Blood-In-Motion extender frames and elements were placed into the x°Control Bag. Into each stack, vaccines were packed and temperature data loggers were placed evenly in the top, middle and bottom positions. The red bag was closed and placed into a constant 30°C degree temperature chamber.

Result: Temperature readings at the top and bottom of the container exceeded 8°C after 3 hours and 50 minutes. The readings in the middle were the best and exceeded 8°C after 6 hours. The vaccines could be stored under 10°C for a minimum of an additional 20 minutes. The advantage of the Blood-In-Motion elements and extender frames are that the elements contain a phase change material that has a melting point of 4°C, which means that the product transported cannot freeze. Ice bricks could potentially damage the product transported.

Conclusion: Up to 500 vaccines (Baxter NeisVac-C) can be carried for approximately 4 hours before exceeding 8°C. Thus the red bag is an ideal transport container for the distribution of vaccines, but could also be extended to other products such as albumin, or Factor VIII products.

P43

New Approach to the Temperature Maintenance of Red Cell Concentrates, while in Transit to Satellite Sites

T Tait [1], T Martinelli [1], J Bartlett [2], A Suters [2]

[1] Austin Hospital, Heidelberg, VIC
[2] Baxter Healthcare Australia, Toongabbie, NSW

Austin Health in Melbourne has adopted an innovative new system of blood transportation to solve the problem of temperature maintenance of Red Cell Concentrates (RCC) once they have left the hospital Blood Bank and are in transit to satellite sites. The Blood Bank at Austin Health has in the past issued blood and blood products on request to the Satellite sites. The RCC have been transported via courier in foam eskies and cold packs. In the past this has led to loss of control of the temperature that the RCC are transported at as there was no way of measuring the temperature. This has led to some product wastage. In an effort to meet the needs of the satellite sites, the patient and the central Blood Bank, the Austin Hospital recently evaluated the new Blood-In-Motion System provided by Baxter Healthcare. Austin Health has found the system easy to use, simple to validate allowing for the safe temperature controlled transport of RCC that meets the needs of all concerned. This solution has enabled the Austin Hospital to conserve a scarce and valuable resource for the community.

P44

Stabilisation of Temperature between 4 and 10°C for Transportation of Haemopoietic Stem Cells Using Blood-In-Motion Systems

C Hutchins, E Varga, S Houston, K James, J Ritchie

Bone Marrow Transplant Unit, Royal Brisbane Hospital, Herston, QLD

In the past, couriers of haemopoietic stem cells (HSC) have used cold packs and insulated coolers to maintain the temperature at between 4 and 10°C during transit. However with the limited number of flights scheduled between Australia and the Northern Hemisphere, transit times in excess of 30h are often experienced. In these instances, couriers of HSC have been required to liase with airlines for the supply of dry ice on board aircraft for re-chilling the cold packs. Even between the East and West coasts of Australia, transit times in excess of 10h have been experienced. Recently a passive transportation system (Blood In Motion, Baxter Healthcare) has been developed for the long distance transportation of blood products either frozen, at 4°C or at 22°C. A system consisting of 4°Control Elements and Extender Frames placed inside a MiniVaq box is recommended for the transportation at 4°C for up to 50h. Whereas for transit times under 12h, the Silver Bag systems may replace the MiniVaq box. The 4°Control Elements and Extender Frames placed inside either the Silver Bag II, Silver Bag III or the MiniVaq box were validated in the laboratory for the transportation of HSC (BM and PBSC). Following validation, the MiniVaq box with a complete complement of 4°Control Elements and Extender Frames has been used for the transportation of HSC (BM and PBSC) between Brisbane, Australia and either Europe or North America on over 12 occasions. The temperature of the HSC during transportation was monitored by the courier using a temperature probe and recorded by data loggers. This system provided a stable temperature environment of between 4 and 8°C with minimal fluctuation in temperature. Although the Blood In Motion MiniVaq box with a complete set of elements and frames is larger and heavier than the coolers usually used for the transportation of HSC, this is outweighed by the ease of temperature maintenance. However, the courier must arrange with the collection centre for 'conditioning' of the phase change media contained within the elements and frames at 2 - 4°C for greater than 12h prior to transportation and for cooling of the HSC to 4°C. Likewise, either the Silver Bag II or Silver Bag III system containing a full complement of 4°Control Elements and Extender Frames has been used for the transportation of HSC within Australasia depending upon the predicted transit time. These systems are easier for the courier to carry due to their smaller dimensions and significantly lower weight. However, the Blood In Motion System consisting of 4°Control Elements and Extender Frames placed inside a MiniVaq box is recommended for the transportation of HSC with transit times greater than 12h. The manufacture of an external cover and the use of a luggage trolley have aided transportation of the MiniVaq box by the courier.

P45

TESTO 174 Data Logger Thermometers - Real Time Monitoring of Temperature Conditions to Aid in Regulatory Compliance

Robyn Nikolai, Robyn Rodwell, Diane Baldry

Queensland Cord Blood Bank, Mater Hospital, South Brisbane, QLD

Aim: To evaluate the role of TESTO 174 Logger Thermometers (TLTs) for monitoring of temperature conditions during the transport of fresh cord blood units (CBUs) and associated blood samples to provide early alert of 'out of range' conditions and objective evidence of compliance to prescribed temperature criteria.

Method: Programmable TLTs provide electronic temperature display, high and low alerts at user defined limits, continuous logging of temperature (range -300C to +700C) at intervals from 1 minute to 4 hours and the option to specify the number of logs of temperature or to employ wrap around memory (continuous for up 2.7 days). A series of calibrated TLTs were allocated identification numbers, programmed and used in a number of validation studies and also placed into routine use with appropriate documentation for the transport of all CBU and associated blood samples. On receipt of transported products the temperature and presence or absence of temperature alerts was recorded from the digital readout. The TLT data was then downloaded and reviewed.

Result: In validation studies, we found the TLTs to reliably log temperature conditions. We identified the potential for a 'false alert' of the true transport conditions if the TLT was hand-held for prolonged periods after initiation of the logger and prior to placement into the transport unit. Over 12 months, data has been downloaded electronically and provided a data log with minimum, maximum and mean temperatures and a graphic format of the temperature conditions during product and sample transport. We found no adverse events with the use of TLTs or inconsistency of the logged data.

Conclusion: TLTs are simple to use, give immediate visual alert to out of limit temperature conditions, provide objective evidence that can be stored electronically or in hard copy to demonstrate compliance to required standards or regulatory codes. TLTs have proven accurate and invaluable for both validation studies and for routine monitoring of a range of temperature environments.

P46

Evaluation of the Blood in Motion 22°C Transportation System.

Robyn Nikolai [1], Robyn Rodwell [1], Dimity Mc Donnell [2]

[1] Queensland Cord Blood Bank, Mater Hospital, South Brisbane, QLD
[2] Baxter Healthcare Pty Ltd Toongabbie, NSW

Aim: To evaluate the Blood in Motion (BIM) 22°C Control Transporter system (Baxter Healthcare) with respect to temperature control for the transport of fresh cord blood units (CBUs) between 8 and 25°C from a regional collection centre to the Cord Blood Bank laboratory by a commercial courier (maximum transport time 4 hours).

Method: The BIM, which consists of a pair of pre-filled elements containing a non-toxic saturated carbohydrate mixture "Phase Change Material" and a series of extender frames is pre-conditioned (minimum refrigeration 1 hour) prior to use and placed within an insulated bag (Silver Bag H). To mimic a normal transport despatch, the BIM was packed with research CBUs. Validation studies were conducted at room (21-24°C) and at elevated temperatures of 34 to 36°C. Two calibrated Testo 174 Logger Thermometers (TLTs) were used to monitor the environmental temperature and the temperature of the product inside the BIM. Following validation studies, the BIM with TLT were placed into routine use.

Result: In two studies conducted over 17 hours at mean temperatures of 23.6°C and 22.9°C respectively, the BIM maintained temperatures of 21.7-23.1°C and 20-20.8°C respectively. In two studies at elevated temperatures (34 - 36°C) with 1 hour pre-conditioning the BIM maintained a temperature of ?25°C for 3 h 8 mins (mean 22.1°C) and for 3 h 15 mins (mean 22.3°C) respectively; with extended 4 hour pre-conditioning in two studies temperatures of 17.5 - 20.3°C (mean 19 °C) and 18.2 - 20°C (mean 18.9°C) respectively were maintained. In routine use, over 8 months (220 transport deliveries) the BIM resulted in 100% compliance to prescribed temperature conditions.

Conclusion: The BIM provides effective control over the temperature conditions of CBUs during transport by commercial couriers even in the extremes of temperature experienced in Queensland. In conjunction with the TLTs, the BIM removes the uncertainty as to the condition or acceptability of the product on receipt at the laboratory, ensures the integrity of the product, its safety for banking and provides regulatory compliance.

P47

Transport of Fresh PBSC and other Blood Products using Blood-In-Motion Transport and Delta T Thermoscan Datalogging System

Noor Parker [1], Dimity McDonell [2]

[1] Wesley Clinic Research Centre Stem Cell Transplant Laboratory, Wesley Hospital
[2] Baxter Healthcare Australia, Toongabbie, NSW

Aim: Due to economic rationalisation, spatial segregation between regional collection centres and central processing centre has become common practice. Transport of fresh blood components including peripheral blood stem cells from the regional centre to the core processing facility has created a quality assurance headache in terms of guaranteeing product quality and integrity. The Blood-In-Motion system was evaluated at the Wesley to determine whether the product met the requirements of the Stem Cell Transplant Laboratory for transport.

Result: The Blood-In-Motion system uses stackable temperature controlled and regulating elements for the transport of multiple bags of fresh blood products. Following conditioning of the elements at 4° C in a standard refrigerator, they interlock with each other allowing the housing of multiple blood product units. Once the interlocked elements are placed in the insulated passive outer container, the internal temperature is accurately maintained between 2 - 8° C for up to 8hrs.

Conclusion: Product temperatures can be maintained for up to 8hrs and are accurately logged using the Delta T Thermoscan data logger allowing controlled transport conditions with a complete data record of the transport process. The Blood-In-Motion System meets the transport requirements of the Stem Cell Transplant Laboratory at the Wesley Hospital.

TUESDAY 19 1600 to 1700 Atrium

ANZSBT Poster Session 4 - Transfusion Testing And Management Issues

Chair: Michael Haeusler

P48

Fate of Transfused Granulocytes Determined by Granulocyte Transfusion Data and Mathematical Modelling

Jeremy Wellwood [1], Lars Nielsen [2], Robyn Minchinton [3], Katrina Williams [1], Kerry Taylor [1], Andrea Rentoul [1]

[1] Mater Health Services Brisbane
[2] University of Queensland
[3] Australian Red Cross Blood Service

Aim: There has been renewed enthusiasm for granulocyte transfusions in patients with severe prolonged neutropenia especially those with active infection. We retrospectively explored neutrophil kinetics after granulocyte transfusions in order to model the kinetics of neutrophil passage in transfusion recipients.

Method: Donor granulocyte yield and host neutrophil increment at 1-4 hour and 8-12 hours were collected from granulocyte transfusions from 1996 to present. Patients had severe prolonged neutropenia (mostly induction for acute leukaemia) and most had significant or life-threatening infection.

Result: 100 granulocyte transfusion episodes had both donor yield and recipient neutrophil increment data available. The median granulocyte yield was 2.1 X 1010 cells (range 0.12 - 9.7 X 1010). The median PMN increment (at 1-4 hour) was 0.2 X 109/ L (range 0 - 4.6 X 109). 38% of episodes had a >= 0.5 X 10 to9 /L increment and the median donor yield to achieve such increment was 2.65 X 1010 (range 0.43 - 9.7 X 1010). Only 10% of infused neutrophils were accounted for in the peripheral blood. This has been attributed to transient sequestration in the lungs. However, the PMN count at 8-12 hours was only 75% (range 0 to 208%) of the count at 1-4 hours indicating that the majority of neutrophils never returned to circulation. There are two possible explanations: 1/ Tissue margination and migration of neutrophils to sites of inflammation. Low blood neutrophil recovery in this scenario may not be a sign of poor efficacy, 2/ Delayed transit through the lungs due to ex-vivo activation of neutrophils or possibly due to donor-recipient granulocyte mismatch. This pulmonary sequestration of neutrophils may be followed by early removal via the reticuloendothelial system. Minimising neutrophil activation may reduce this phenomenon.

Conclusion: Possible strategies to improve functional neutrophil increments after granulocyte transfusion include 1/ increasing granulocyte numbers in the transfusion or 2/reducing neutrophil activation by better donor-recipient matching or with ex-vivo expansion of granulocyte precursors with less susceptibility for such activation.

P49

Red Cell Antibody Detection After Transfusion of FFP

Marian Connolly, Dianne Grey, Wendy Erber

Transfusion Unit, PathCentre, Nedlands, WA

Introduction: On rare occasions fresh frozen plasma (FFP) can generate an immune response to red cell antigens. Five cases of anti-D and two of anti-Fya have been reported in the literature. The Council of Europe guidelines therefore require <6x109/L RBC per unit of FFP. However in Australia the ARCBS does not enumerate the red cell contamination of FFP.

Case Report: We report a case of a 63-year-old B RhD negative male with gastro-oesophageal cancer who received multiple transfusions with RhD negative products over a 4-month period. Seven months later the antibody screen (Diamed-ID Liss/ Coombs) was negative and he was transfused with 7 RhD negative packed red cell units and 6 FFP, 4 of which were RhD positive. Two months later anti-D, -E and -K were detectable in his plasma. The E- and K-antigen status for all red cell units was not known and the anti-E and -K may have resulted from transfusion of antigen-positive packed red cell units.

Conclusion: The anti-D could only have resulted from transfusion of RhD positive FFP, suggesting a primary immune response. The RhD antigen is highly immunogenic and the minimum dose required for immunisation is only 0.03mL or 0.24x109 red cells. Therefore, as the level of red cell contamination of FFP in Australia is not known the RhD status of FFP should be considered in circumstances where multiple units are required. In addition, a history of FFP transfusion should be considered when assessing validity of a patient sample for pre-transfusion testing.

P50

Lack of Evidence for Prolonged Rupture of Membranes >=24 Hours without Evidence of Perinatal Infection as an Exclusion Factor for Cord Blood Donors

Robyn Rodwell, Diane Baldry, Jeremy Wellwood, Elizabeth Wolf, Kerry Taylor

Queensland Cord Blood Bank, Mater Hospital, South Brisbane, QLD

Aim: To examine whether prolonged rupture of membranes(PROM >=24 hours (h)without evidence of perinatal infection should preclude the acceptance of cord blood units (CBUs) for processing and banking.

Method: Mothers of cord blood (CB) donors with PROM >= 24h were identified by data extraction of clinical history records collected within seven days of CB donation (n=2750). Clinical and laboratory information was compiled. This included the haematologic score derived from the CB FBC and used routinely in our neonatal unit as a screening test for neonatal sepsis with values of >= 3 considered significant (Rodwell et al. J Pediatr. 1988 112:761-7).Descriptive statistics were performed.

Result: In 27 of 2,750 (0.98%) CBUs accepted for processing PROM >= 24h (mean =< SEM 35.8 =< 4.1h) an arbitrary quality exclusion criterion recently adopted by AUSCORD the Australian Cord Blood Bank Network was identified. Infection was suspected clinically by maternal fever and score of 4 in only one of 27 babies (14 boys, 13 girls, birthweight 3762.4 =< 81.1g, gestational age 39.3 =< 0.2 weeks). The baby was well; CB and subsequent blood cultures were negative and no antibiotics were given. Mean volume and total nucleated cells (TNC) collected in the 27 CB donations was 99.39 =< 5.7ml .and 17.48 =<1.37 x 108 respectively.

Conclusion: There is lack of evidence for PROM >=24h without evidence of perinatal infection as an exclusion factor for CB donations. Some of the 27 CBUs had been validated for release prior to this new quality criterion. Donors with PROM>=24h are no longer collected thereby reducing our accrual rate. The majority of these 27 high quality CBUs with respect to TNC dose, had had 180-day follow-up testing performed on the mothers or the mothers had been advised not to return for testing. These CBUS have been excluded and quarantined. Current cost to bank each CBU is approximately $1,300. This exclusion criterion should be re-examined to avoid the unnecessary loss of high quality CBUs and the associated banking costs.

P51
Is IgG Detection Alone Sufficient to Screen Blood Donors for Anti-CMV?
Lisa Piscitelli, Ken Whitson, Kathleen Doherty, Clive Seed

Australian Red Cross Blood Service

Aim: To determine if the AxSYM CMV IgG assay could replace the CMV Total EIA assay for identifying CMV seropositive and seroconverting blood donors.

Method: 5050 random blood donors were tested prospectively by the Abbott CMV Total EIA assay, AxSYM CMV IgM and IgG assays with discordant samples tested by Immunoblot. Donors displaying possible seroconversion profiles were re-sampled at least two weeks after their index specimen and re-tested by all three screening assays. The ability of each screening assay to detect acute infection was evaluated using 13 seroconversion panels.

Result: In comparison to Total EIA, AxSYM IgG (with a reduced cut-off of 9AU/ml) had a relative sensitivity of 97.68% and a relative specificity of 97.74%. This improved to 99.90% and 97.81% after resolution of discordant results using Immunoblot. In comparison the Total EIA, a strategy of combining the AxSYM IgG and IgM did not increase the sensitivity whereas specificity was negatively impacted. Examination of 13 seroconversion panels demonstrated that the median interval to first sample detected was equivalent for the AxSYM (9AU/ml cut-off) and Total EIA indicating equivalent seroconversion sensitivity.

Conclusion: Using a modified cut-off of 9AU/ml, the sensitivity and specificity of the AxSYM IgG assay in ARCBS donor samples is considered equivalent to the Total EIA. As this equivalence is mirrored in seroconversion samples, the AxSYM assay is considered a suitable alternative assay for anti-CMV donor screening.

P52
The DiaMed ID Syphilis Antibody Test for Blood Donor Screening
Julie-Anne Breese [1], Geoff Nicol [2], Natali Segui [1], Joanne Graetz [1], Ken Whitson [1]

[1] Australian Red Cross Blood Serice
[2] Diamed Australia

Aim: The Diamed ID Syphilis Antibody Test; a particle agglutination immuno-assay for Syphilis antibody screening was evaluated at ARCBS-SA for use on the automated blood grouping system, ID WalkAway System. The assay uses three recombinant antigens bound to a synthetic particle. Random donor samples were assayed initially to evaluate the DiaMed Syphilis Antibody Test protocol and reagents. 1079 syphilis negative samples were subsequently tested to estimate specificity using a modified sample preparation protocol. Sensitivity was estimated from a series of 21 Syphilis positive samples from past (15) and active (6) infections, tested manually and in parallel with the ID WalkAway System.

Result: The initial reactive rate was 0.83% for the 1079 samples using the modified sample preparation protocol. 14 out of 15 past infection samples and 6 out of 6 samples from active Syphilis infections were reactive by the DiaMed ID Syphilis Antibody Test. On the ID WalkAway system, 2 out of the total of 40 positive sample results were initially read as negative during the automated testing but a manual review of the results confirmed the positivity. Replicate testing and subsequent routine operation with operator visual review of all results has shown no further incidents of this type.

Conclusion: In the context of donor screening, where detection of active infections is the primary goal, the performance of the DiaMed ID Syphilis Antibody Test was considered acceptable. Visual confirmation of all results by the operator is recommended.

P53

Intra-uterine Management of Severe Prenatal Anaemia Caused by Antibody to the TSEN Antigen, a Low Incidence Variant in the MNS System

Colin Story [1], Thomas Traino [1], Adam Dichiera [1], Robert Flower [2], Chris Wilkinson [1]

[1] Women's and Children's Hospital, North Adelaide,SA
[2] Pacific Laboratory Medicine Services and University of Sydney, Sydney, NSW

Aim: We present a case history of intra-uterine management of severe prenatal anaemia caused by anti-TSEN. In a previous pregnancy, at 28 weeks gestation, an infant (DR) had been delivered with severe neonatal anaemia requiring postnatal transfusion, as a result of maternal anti-TSEN and possibly anti-MINY haemolytic antibodies. The variant MNS glycophorin was a novel genetic isoform of the low incidence Miltenberger XI phenotype, with different crossover to Glycophorin JL (proposed name GpDR).

Method: The fetus of a woman with multiple recurrent miscarriage (G11 P2) was assessed to be severely anaemic by peak middle cerebral artery blood flow at 20 weeks gestation. Intrahepatic venous blood sampling was followed immediately by intra-uterine transfusion (IUT). Serial IUT followed. Feto-maternal incompatibility was investigated by serology and PCR.

Result: At 17 weeks in this pregnancy, ultrasound surveillance indicated severe fetal anaemia. Maternal IDAT was negative in routine testing but positive with paternal RBCs (anti-TSEN titre 256). Fetal Hb at 20 weeks was 33g/L, DAT was positive. The presumed GpDR incompatibility was confirmed by PCR. Five IUTs of 34-70ml of packed RBCs maintained fetal Hb>100g/L. Delivery at 35 weeks was uneventful: Hb 105g/L, without excessive jaundice or further transfusion requirement (35 week anti-TSEN titre 4000, negative Kleihauer).

Conclusion: Antibodies to low incidence MNS antigens cause neonatal anaemia, possibly from haemolysis of developing RBC in the bone marrow. With intensive transfusion laboratory support, severe fetal haemolytic anaemia caused by these antibodies can be successfully and cost effectively managed by serial IUT, avoiding intensive postnatal management.

P54

Antenatal Management of Fetomaternal Alloimmune Thrombocytopenia (FMAIT) Complicated by Significant HLA Antibodies.ia

Amanda Adams [1] , Peta Dennington [2]

[1] Royal Prince Alfred Hospital, Bronte, NSW
[2] Australian Red Cross Blood Service, NSW

Alloimmunisation against Human Platelet antigen, HPA 1a, is the most common cause of severe neonatal thrombocytopenia. HPA 1a alloimmunisation complicates 1 in 350 unselected pregnancies, leading to severe thrombocytopenia in 1 in 1, 200.1 HLA sensitisation is common occurring in up to one third of pregnancies, however it is only rarely implicated as a cause of FMAIT 2. There are only two previous reports of clinically significant HLA antibodies 3,4 .We report a case of FMAIT secondary to HPA 1a antibodies complicated by platelet refractoriness due to multispecific HLA antibodies. The mother was a 31 year old, G4 P0121. Her previous pregnancy was complicated by a foetal intracranial haemorrhage requiring termination of the pregnancy at 31/40. Genotyping confirmed her to be HPA 1b homozygous and serological testing identified Anti HPA 1a antibodies. At 16/40 an amniocentesis confirmed HPA1a positivity of the foetus. The only established predictor of severity is a history of a sibling with an ICH5, thus this was a very high-risk pregnancy. Maternal treatment with1g / kg weekly IntragamP was initiated at 12/40. FBS confirmed thrombocytopenia (11x109/l) at 25/40 and a HPA 1a matched platelet transfusion was performed. When this produced no increment on repeat FBS 1 week later, maternal HLA antibodies were then sought and detected. The pregnancy was supported with ten, HLA and HPA 1a compatible intrauterine platelet transfusions. A healthy male infant was delivered at 30/40. Thus HLA alloimmunisation can complicate the management of FMAIT.

P55

Antibodies to Low Incidence Antigens with the Potential of Causing HDN

Lanny Ramadi, Janet Wren, Kerman Buhariwala, John Pietryka

Australian Red Cross Blood Service

The identification of antibodies to low incidence antigens does not present a problem in selecting donor units for transfusion. However, since some of these antibodies have the potential of causing HDN, identifying these antibodies is crucial in the management of pregnancy. We have recently investigated three such cases of antibodies to low incidence antigens. Two of these were detected post delivery and the other was referred to us during antenatal testing.

Case 1. A group A RhD positive woman delivered a fullterm O RhD positive infant (baby EA). On day 2 baby EA presented with mild jaundice. Baby's Direct Antiglobulin Test (DAT) was weakly positive. Baby had normal haemoglobin but elevated bilirubin levels and required phototherapy treatment over 24 hours. Elution was not performed due to insufficient sample. Maternal antibody screen was negative, but maternal plasma was strongly reactive with baby's cells. Maternal plasma was found to contain anti-Wra and also reacted with several examples of Miltenberger positive cells (indicating probable anti-Hil). Paternal sample was not available.

Case 2. An O RhD positive infant (baby VC) was found to have a strong positive DAT during routine post natal testing. At birth haemoglobin level was normal but by day 2 bilirubin level was elevated. Baby VC was jaundiced but required phototherapy treatment only. Maternal plasma reacted strongly with paternal cells. It was found to contain anti-Vw and also reacted with several examples of Miltenberger positive cells (indicating probable anti-Mut). Anti-Vw and anti-Mut were eluted from baby's red cells.

Case 3. An antenatal sample of a AB RhD positive woman (JY) and her partner's sample (MP) were referred to us. Plasma of JY was found to contain anti-ce(f) and was also found to be strongly incompatible with MP cells and a single R1R1 (V0012212) panel cell. Upon further investigation, anti-Tar (Rh40) was also found in JY plasma. MP cells and V0012212 were subsequently found to be Tar+ These findings were reported back to the referring laboratory and the patient was subsequently referred to a major obstetric hospital for the management of her pregnancy. All the above examples of antibodies to low incidence antigens have been reported in the literature to have caused HDN. Identification of these antibodies is important to allow proper management of current and subsequent pregnancies.

P56

Disproportionate Number Of Group B Components (Whole Blood/Red Blood Cells) in The Centre for Transfusion Medicine. A Supply or Demand Problem?

Michelle Tzu Fern Peh [1,2], Kwee Kim Leou [1], Hwee Huang Tan [1], Diana Teo [1], Boon Chai Mickey Koh [1]

[1] Centre for Transfusion Medicine, Health Sciences Authority, Singapore
[2] School of Applied Science, Temasek Polytechnic, Singapore

There has been a long-standing observation at the Centre for Transfusion Medicine (CTM) in Singapore that a disproportionate number of Group B units is present in blood stock. The project aims to address this intriguing anomaly of excessive Group B stock and determine if this is due to disproportionate blood donation received or less usage by the blood Group B patients.

The principal methods used:

1)Retrospective Study (sample number: 28430 transfused units) Data were collected on red cell usage from participating hospitals in 2003.

2)Prospective Study This has been set up with all hospitals in Singapore on red cell usage by clinical specialities from January to June 2004.

The statistical analysis employed:

Chi-Square Testing Result: No significant difference was found in the blood group B distribution of the donor population compared to patient population, refuting the hypothesis of excessive blood donation by Group B individuals. Blood usage data revealed a decreased usage of Group B units by the patients, which was statistically significant. The relative usages by clinical specialities were analysed, indicating less Group B patients in most of the diseases categories given.

Conclusion: There is proportionately less usage of red cells by Group B patients, contributing to the excessive Group B blood stock in CTM. The donor and patient population are concordant in the distribution of blood groups. These findings will be of paramount importance in stock management to ensure optimal use of all donations and in policy implementation for continual improvement in the blood bank.

MONDAY 18 1600 to 1700 Atrium

HSANZ Poster Session 1

sponsored by Merck Sharp & Dohme

P57

Bcl-2 Expression Modifies In Vitro Response of Bcr-abl+ K562 Cells to Imatinib Mesylate

Jason Butler, Andrew Boyd, Joanne Cox, Jennifer McCarron, Jason Lickliter

Queensland Institute of Medical Research

Aim: Despite high initial response rates to imatinib mesylate in CML and Ph+ ALL, relapse or disease progression is common. Whilst bcr-abl dependent mechanisms, such as point mutations, account for a number of cases of acquired imatinib resistance, increased expression or up-regulation of other oncogenic pathways, such as bcl-2, may contribute to reduced disease responsiveness. We explored the role of the anti-apoptotic protein, bcl-2, in the development of imatinib resistance.

Method: The bcr-abl+ K562 cell-line demonstrates low bcl-2 expression, and marked in vitro imatinib sensitivity. Utilising electroporation , we successfully transfected the K562 cell line with a bcl-2 expression plasmid. After single-cell sorting, individual high bcl-2 expressing K562 clones were grown, and assessed. Following this SMARTpool bcl-2 siRNA was successfully transfected into the K562 clones utilising lipofectamine. Bcl-2 expression was investigated using flow cytometry. MTS cytotoxicity assays were utilised to assess imatinib sensitivity.

Result: The high bcl-2 expressing (transfected) K562 clones consistently demonstrated resistance to imatinib in comparison to untransfected K562 clones on MTS assays, at concentrations of 1 to 10μM. SMARTpool siRNA substantially reduced bcl-2 expression in these cells and, on MTS assays, demonstrated evidence of imatinib sensitivity similar to untransfected (bcl-2 low) K562 cells.

Conclusion: Bcl-2 up-regulation confers resistance to imatinib in bcr-abl+ K562 cells, and may play a role in the development of acquired imatinib resistance. Down-regulation of bcl-2 restores in vitro sensitivity, and may represent an important therapeutic target in the clinical setting

P58

The Functional Studies of Transcription Factors GATA-1 and Fli-1 in Megakaryopoiesis by Retrovirus-Mediated Gene Transfer

Shu Pan, Feng Yan, Thet Hta Khin, Beng Chong

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Aim: This study aims to investigate the biological roles of transcription factors GATA-1 and Fli-1 during megakaryocyte (Mk) differentiation by over-expressing them in myeloid leukemia cell line M1, mouse bone marrow stem/progenitor cells (BMCs) and embryonic stem cells (ES).

Method: GATA-1 and Fli-1 were separately inserted into the viral vector KMV containing a reporter gene EGFP. The target cells were infected with viruses produced by packaging cells. The infection efficiency was assessed by EGFP positivity. Mk maturation was determined by morphology and Mk specific marker (CD41). Real-time PCR was applied to obtain the expression profiling of megakaryocytic markers and other transcription factors in megakaryopoiesis.

Result: The viral infection efficiency was 23-35% in M1 cells, 10-59% in BMCs and 18-36% in ES cells. M1 cells expressing GATA-1 or Fli-1 showed the megakaryocytic morphology. Enforced expression of GATA-1 or Fli-1 in BMCs and ES cells resulted in an increased percentage of Mks measured by CD41. However, the inhibition of myeloid differentiation by over-expressing GATA-1 was not observed in Fli-1-infected BMCs. Real-time PCR data showed that over-expression of GATA-1 and Fli-1 increased expression levels of Mk specific makers c-Mpl, PF4, AChE and GPIX, as well as the transcription factors FOG-1, NF-E2, PU.1, Ets1, and EKLF.

Conclusion: We demonstrated that over-expression of GATA-1 and Fli-1 indeed enhanced Mk maturation in bone marrow and ES systems, suggesting these two factors play important roles in Mk development. Expression patterns of other essential transcription factors also provided us more insight into transcriptional regulation of mekagaryopoiesis.

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Identification of Immune Evasion Strategies Following Bone Marrow Transplantation in a Patient with Pre-B Acute Lymphoblastic Leukaemia

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Background and Aim: Bone marrow transplantation (BMT) is the only curative option for refractory/relapsed paediatric acute lymphoblastic leukaemia (ALL). However, post-BMT relapse remains an important cause of transplantation failure. Immune mechanisms play a critical role in the anti-leukaemic efficacy of BMT, suggesting that mechanisms enabling leukaemic blasts to evade immune responses are an important mechanism in leukaemia relapse. An improved understanding of the processes underlying post-BMT relapse is required.

Method: We used proliferation assays, cytometric bead arrays, and ELISpot to assess the T-cell responses induced by pre-B ALL cells obtained at various time points from a child with pre-B ALL who relapsed following an allogeneic BMT. Patient samples were also extensively phenotyped in order to determine the degree of co-stimulatory molecule expression on the leukaemic blasts.

Result: A dramatic loss of T-cell stimulatory capacity by later relapse samples was observed. Also, the autologous anti-leukaemic T-cell response demonstrated a Th2 shift compared to the Th1 allogeneic response. These immune stimulatory changes induced by ALL cells at relapse were associated with changes in expression of MHC and costimulatory molecules. HLA-ABC expression was increased on all relapse samples whereas HLA-DR expression was substantially reduced in those relapse samples obtained following immunosuppression cessation. Expression of the costimulatory molecule CD137L was decreased whereas B7-H1 expression was increased on post-BMT relapse samples.

Conclusion: These results reveal mechanisms involved in pre-B ALL relapse, and suggest strategies to prevent evasion of the graft-versus-leukaemia effect by ALL cells.

P60

The Clinical Utility of Trephine Immunohistochemistry in Bone Marrow Diagnosis

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We performed a retrospective analysis of immunohistochemistry (IH) on bone marrow trephine biopsies performed at our institution from January 2002 to May 2004 in order to develop guidelines for its utilization. Immunohistochemistry was performed at the discretion of the reporting doctor in 81 of 794 (10.2%) biopsies. Indications included primary diagnosis (5/81) or staging (15/81) of lymphoma; investigation/monitoring of paraproteinemia (22/81) plasma cell myeloma (6/81) or amyloidosis (4/81); investigation of pancytopenia (11/81), anemia (4/81) or fever of unknown origin (3/81); and other (11/81). Immunohistochemistry was performed on formalin fixed, decalcified, paraffin-embedded tissue sections according to established methods with a streptavidin-biotin detection system and a DAB chromagen. We found IH to be a useful adjunct to diagnosis in the following situations: (i) analysis of plasmacytosis, where IH commonly demonstrated clonality and identified greater plasmacytosis than expected from hematoxylin and eosin stained sections; (ii) characterization of malignant non-haemopoietic tumour infiltration; (iii) leukemia diagnosis and characterisation when bone marrow aspirate was suboptimal. The presence of lymphoid aggregates was a frequent indication for IH (21/81 trephines) and although the distinction between malignant and reactive aggregates could be made in the majority of cases and correlated well with the presence/absence of monoclonal lymphocyte populations on aspirate immunophenotyping, it generally did not alter the diagnosis from that reached by other means. In conclusion, trephine IH was most useful in diagnosis of plasma cell and non-haemopoietic neoplasm. Its utility in lymphoid disorders was more limited, however it provided useful diagnostic information in individual cases.

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The Role of Bone Marrow Examination in Investigating Patients with Suspected ITP

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Aims : (1) To examine the available literature regarding the role of bone marrow examination in patients presenting with isolated thrombocytopenia. (2) To illustrate that each case must be assessed individually as occasionally bone marrow examinations on these patients may yield surprising results. The consensus on the role of bone marrow examination in patients presenting with isolated thrombocytopenia is often debated. We will summarise the available literature and the current recommendations about performing bone marrows examinations on patients with an isolated thrombocytopenia who are suspected of having ITP. A 21 year old male was referred to our service by his general practitioner with a 2-3 week history of bruising. The patient was well and denied any recent history of bleeding, viral illness, transfusion or excessive alcohol consumption. Past medical history was minimal and specific questioning re autoimmune disorders, specific risk factors re HIV infection was negative. Examination revealed a young man who appeared to be in good physical health. The only abnormality found on physical examination was a few small bruises in different stages of development on the legs and torso. FBC revealed a thrombocytopenia (platelet count 29). The other FBC parameters were normal. No blasts were seen on review of the blood film. A bone marrow procedure was performed which revealed 32 % lymphoblasts with high N:C ratio and folded nuclei. Immunophenotyping confirmed the diagnosis of Precursor B-ALL (CD10 and CD19 positive).

Conclusion: this case illustrates that (although infrequent) patients presenting with thrombocytopenia may in fact have alternative diagnoses.

P62

Is Dual Expression of CD9/cytCD68 a Useful Marker of Minimal Residual Disease (MRD) in Acute Promyelocytic Leukaemia (APL)?

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Aim: Aberrant expression of CD9/cytCD68 in bone marrow was evaluated in 16 consecutive patients (103 episodes) referred to the Austin Hospital, Melbourne with APL between 1998 and 2003 at diagnosis, where possible, and follow up in order to investigate the utility of this marker combination for assessment of MRD in APL. MRD was defined as CD9/cytCD68 expression by more than 10% of cells within a CD45+ (dim to moderate) vs log side scatter (moderate to high) region (promyelocyte gate), using multiparameter flow cytometry (FC). A FC episode was then deemed true or false-positive or negative (TP, FP, TN, FN) depending on concordance with both cytogenetic (CG) and molecular (RT-PCR) detection of t(15;17) or PML-RARa fusion transcript respectively.

Results: Only seven episodes were disconcordant. All ten available diagnostic marrows demonstrated the aberrant APL phenotype. One patient showed transient, possibly false, RT-PCR positivity and FC was therefore deemed TN. Of four FN, three were also cytogenetically negative consistent with the comparable detection limits of these two modalities. Further, two of these episodes were from the same patient, on separate occasions, in whom the diagnostic phenotype was not known. Two FP may have represented inadvertent detection of basophilic myeloid recovery following induction as FC was subsequently negative during each patient's maintenance phase. The 96 concordant bone marrow episodes comprised 14 TP and 83 TN resulting in a sensitivity of 78%, specificity 98%, positive predictive value 88% and negative predictive value 95% for FC to predict MRD in APL.

Conclusions: Flow cytometry may provide a useful adjunct in the diagnosis and evaluation of MRD in APL using the unique marker of coexpression of CD9 and cytCD68 particularly if the diagnostic phenotype can be confirmed.

P63

Molecular Characterization of Delta-Beta Thalassemia and HPFH in the Indian Population

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Aim:To study the molecular basis of high HbF levels in adults.

Method: A Gap PCR based strategy was used with three primers that leads to detection of a specific product in the presence of the deletion and a normal band (different size) from the normal allele. The Inversion-deletion rearrangements were detected independently for each break point [Part A -5' deletion and part B -3' deletion]

Result: Molecular analysis was undertaken in 30 adults with increased HbF production from diverse ethnic groups of India[Age 25 -35years]. Their Hb F levels ranged from 10% to 24.8% with variable RBC indices. Three different mutations were encountered. 11 patients showed the presence of the Asian Indian inversion rearrangement. The 48.5 kb Indian deletion (HPFH 3) was seen in 8 cases. The HPFH 6(Vietnamese /Chinese deletion-27 kb) was seen for the firsttime from India in 4 cases. seven cases remained uncharacterized

Conclusion: The Asian Indian inversion and HPFH 3 determinants seem to be the main molecular lesion (63%) leading to increased HbF production in the Indian population. This study has helped us to understand the variable phenotypic expression in double heterozygotes of beta thalassemia with delta beta thalassemia or HPFH and will also be useful for prenatal diagnosis of these disorders.

P64

Haemoglobin J Calabria and Disturbed HbA1c Measurement

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Glycosylated haemoglobin, HbA1c, is used in patients with diabetes to evaluate long term control of the disease. HbA1c is the result of non-enzymatic addition of a glucose residue to one or both N-terminal valines of the haemoglobin B chains. This is commonly measured by ion exchange HPLC and expressed as a percentage of total haemoglobin. In this report we describe how the presence of abnormal haemoglobin variants (haemoglobinopathies) can produce erroneous results. A 30 year old female with a history of diabetes presented for routine testing. HbA1c assay with the Bio-Rad Variant II HPLC method revealed an unknown peak in the pre-A area. Review of the patient's results showed a consistently high haemoglobin, MCV and PCV, suggesting the possibility of a high affinity haemoglobin variant. Further HPLC analysis showed a peak poorly resolved from A but not identifiable. Electrophoresis suggested Hb J Calabria. Sequencing of the beta globin gene revealed a single base change at nucleotide 324 (g324G>A) that results in a Glycine to Aspartic acid substitution at codon 64. This result confirmed the abnormal haemoglobin to be Hb J-Calabria. The increased use of HbA1c measurement has resulted in the identification of an increasing number of individuals with haemoglobinopathies, many of which are haematologically silent. The principle clinical problem in these cases is the invalidation of the HbA1c result (if recognised) and difficulty in managing the patient using the DCCT guidelines.

P65

Metastatic Merkel Cell Carcinoma Presenting as Primary Marrow Failure with Circulating Tumour Cells

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Aim: Merkel cell carcinoma (MCC) is a neuroendocrine skin tumour involving locoregional lymph nodes that may metastasize to skin, lung, liver or brain. Bone marrow (BM) involvement is rarely described, and to our knowledge no cases of circulating MCC have been previously reported.

Method: We report two patients with haematogenous involvement by MCC.

Result: Patient one, a 55yo man, presented with a rapidly enlarging axillary mass. Subsequent biopsy diagnosed MCC, with a small thumb lesion identified as the primary site. Ten days later, isolated thrombocytopenia of 35 x 109/L was found. Circulating medium to large cells (0.13 x 109/L) with a round nucleus, multiple indistinct nucleoli, scanty basophilic cytoplasm and CD56+ CD45- immunophenotype were identified in peripheral blood (PB); BM biopsy confirmed extensive marrow infiltration. Immunohistochemistry of BM trephine and PB cell block showed characteristic perinuclear 'dot' positivity for AE1/3 and cytokeratin 5.2. The patient received palliative chemotherapy with a partial tumour response, and remains alive six weeks post admission. Patient two, a 66 yo man with a history of cardiac transplantation, presented with acute liver and renal failure of uncertain aetiology. Six months previously a MCC was excised from his forearm; four months later he received radiotherapy for axillary nodal recurrence. On admission isolated thrombocytopenia of 11 x 109/L was present; BM examination confirmed involvement by MCC. The patient deteriorated rapidly and succumbed five days later.

Conclusion: MCC involvement of PB and BM is rare but can be identified by characteristic morphological, immunophenotypic and immunohistochemical features.

P66

Plasma Cell Quantification by Flow Cytometry Significantly Underestimates Bone Marrow Involvement in Multiple Myeloma as Compared to Immunohistochemistry Staining for CD138 on Trephine Biopsies

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Aim: To compare the percentage of plasma cells identified by flow cytometry and morphological assessment in bone marrow samples in patients with multiple myeloma (MM).

Method: The percentage of plasma cells present in bone marrow samples of 10 consecutive MM patients was determined by flow cytometry (FC) and immunohistochemistry, using CD138 expression as the basis for plasma cell identification in both methods. Flow cytometry was performed on aspirate samples, counting a minimum of 50,000 events per analysis. Percentage of plasma cells was determined by the number of bright CD38-FITC (BD HB7) / CD138-PE (BD M:15) events recorded. Immunohistochemistry for CD138 (Dako MI15) was performed on trephine samples, with quantification of plasma cells performed by calculating the mean percentage of CD138 positive cells present in 20 consecutive high power fields (x100). Results between the 2 methods were compared using the Student's t-test and Pearson correlation coefficient analysis.

Result: The percentage of plasma cells determined by FC was significantly less than that calculated by morphological assessment ($p=0.02$). In every case, results obtained by FC were less than that calculated from trephine samples, varying between 3% and 73% of plasma cell percentages counted on morphological assessment. There was no relationship between differences in results obtained between the 2 methods and degree of plasmacytosis present ($r=0.4$; $p=0.25$).

Conclusion: Our results show that FC estimation of plasma cell numbers in bone marrow samples significantly underestimates the actual degree of plasmacytosis present, as assessed by immunohistochemistry on trephine biopsies. This information should be taken into account when interpreting FC analysis in MM.

P67

Molecular Investigation of a Rare Translocation t(11;17)(q23;q12 or 21) in a Child with AML M5b

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The t(11;17)(q23;q12or21)in acute myeloid leukaemia(AML) is found as a rare variant translocation in acute promyelocytic leukaemia but its appearance in cases of AML M4 or M5 is especially rare. We describe a case of a 12 year old girl with AML M5b and t(11;17)in all bone marrow cells analysed at diagnosis. The breakpoint at 11q23 is the location of the MLL gene whose involvement in childhood leukaemia and in AML is well known and carries a poor prognostic outcome. The breakpoint on 17q is difficult to define by conventional cytogenetics but includes 3 genes of possible significance MLLT6(AF17), LASP1, and RARA. In most of the previously described cases of t(11;17) in AML M4 or M5 it appears that involvement of MLL and a gene proximal to RARA is the distinguishing feature of this translocation. The MLLT6 gene proximal to RARA at 17q21 was identified several years ago as a new fusion partner to MLL in t(11;17) but more recently the LASP1 gene has also been identified at this chromosomal location and found to partner MLL in a single case of an infant with AML M4. LASP1 is approximately 250kb distal to MLLT6 making it difficult to distinguish the involvement of these 2 genes in this translocation. We applied conventional FISH using commercially available probes to determine the involvement of MLL and RARA. We found a rearrangement of MLL but that RARA was translocated not rearranged suggesting a gene proximal to RARA is MLL's fusion partner. To distinguish the involvement of MLLT6 or LASP1 we used BAC derived probes and FISH to provide information which may lead to a better understanding of this translocation and it's role in AML M4 or M5.

P68

Expression of Chromatin Remodelling Enzymes In Chlorambucil Treated Lymphoid Cell Lines

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Therapy of lymphoid malignancies (eg CLL) has included alkylating agents, such chlorambucil (CLB) and corticosteroids. The basis of drug resistance observed in many patients treated with CLB is complex and may be related to defective chromatin remodelling mechanisms that may include abnormal methylation and expression of apoptotic genes (eg TRAIL receptors, caspase-8, Bcl-2) and histone acetylation/deacetylation derangements. These processes are regarded as possible therapeutic targets.

Aim: To explore an association between the apoptotic response of lymphoid cells to CLB and expression of two chromatin remodelling enzymes, DNA methyltransferase 1 (DNMT1) and histone acetylase 1 (HAT1).

Method: Lymphoid cell lines, LP1 and NCI-H929, cultured in RPMI1640/10% FBS, were treated with 20 μ M CLB, 10 μM dexamethasone (Dex) or vehicle alone (0.1% ethanol) for up to 72 hours. Induction of apoptosis was assessed by flow cytometry at 0, 48 and 72 hrs using Annexin V-propidium iodide method. Expression of DNMT1 and HAT1 relative to PGK1 was measured using real time PCR. Results were evaluated using unpaired t-test.

Result: CLB induced apoptosis in both cell lines. Necrotic (propidium iodide positive) cells comprised 73.7% ± 6.47 SEM, n=5) of CLB-treated LP-1 cells at 72 hrs (vehicle alone 22.0% ± 5.62, n=5, p=0.0007) and 80.8% ±9.92, n=5 in NCI-H929 cells (Vehicle alone 23.4&±4.75, n=5, p=0.0008). DNMT1 expression was elevated 194 fold ±0.246, p=0.0245) at 48 hrs in LP-1 cells (relative to control) but not at 72 hrs (p=0.3975). DNMT1 expression in NCI-H929 cells did not change significantly at any time. HAT1 mRNA was elevated in LP-1 cells 1.525 fold (±0.157, p=0.0165) at 48 hrs but not at 72 hrs (p=0.206). Similar changes in HAT1 expression profile were observed in NCI-H929 cells (1.636 ± 0.136, p=0.0087, n=5 at 48 hours and non-significant afterwards). Dex induced significant apoptosis in NCI-H929 cells only. No changes in DNMT1 and HAT1 expression could be observed following Dex treatment.

Conclusion: Changes in expression of chromatin remodelling enzymes, DNMT1 and HAT1, may be associated with induction of apoptosis by CLB but are unlikely to be significantly involved in Dex-induced cell death in these two lymphoid derived cell lines. Similar experiments using fresh B-lymphocytes from CLL are in progress.

P69
Variant Deletions of 20q in MDS and AML: Defining the Deleted and Retained Regions
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Aim: The del(20q) is a well-recognised chromosome abnormality in MDS and AML. The smallest known consistently deleted region is 2.6Mb within 20q12. However, not all chromosome 20 rearrangements appear to be straightforward deletions. Using mBAND fluorescence in situ hybridisation (FISH) we proposed to determine whether 20q12 was deleted in all variant chromosome 20 abnormalities.

Method: Metaphase spreads from seven cases with chromosome 20 abnormalities were mBANDed with XCyte 20 (Metasystems) which identifies specific regions of chromosome 20. Metaphases were captured and analysed using a Zeiss Axioplan 2 microscope with Isis image analysis software (Metasystems).

Result: The two isochromosomes were duplicated around different points, one on the proximal long arm resulting in deletion of most of the long arm and duplication of the short arm and proximal long arm, and the other at the centromere of a del(20q) resulting in duplication of the deleted long arm. Thus, different areas were retained but both had lost 20q12. Three cases of simple dic(17;20) were formed via two chromosome 20 breakpoints, with resultant deletion of 20q12 material and retention of part of 20q13. Two complex dic(17;20) cases had lost all or most of 20q, including 20q12.

Conclusion: All seven variant abnormalities had deletion of 20q12. Monosomy 20 was described in three of the original karyotypes. Deletion of the critical sequence at 20q12 occured by means other than straightforward deletion. We suggest that any chromosome 20 abnormality in MDS and AML should be considered a potential del(20q).

P70
An Unexpectedly High Rate of X Chromosome Abnormalities in Haematological Malignancies Revealed by mFISH
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Aim: Small unbalanced chromosome translocations may go unidentified or unnoticed with standard G banding analysis. These difficulties can be overcome with multicolour fluorescence in situ hybridisation (mFISH), which allows the identity of even small chromosome segments to be determined. During mFISH analysis of fifteen haematological malignancies, we found four unbalanced translocations involving gain of small parts of the X chromosome. Recurrent X chromosome translocations are not commonly identified in malignancies using G-banding. We sought to determine whether the same chromosome region was involved each time, indicating possible clinical significance.

Method: Metaphases were probed with an mBAND probe (XCyte X, Metasystems) which identifies specific regions of the X chromosome, then captured and analysed using a Zeiss Axioplan 2 microscope with Isis image analysis software (Metasystems).

Result: Three of the four abnormal chromosomes (from cases of plasma cell leukaemia, Philadelphia negative CML and relapsed AML) contained Xq26-Xqter or Xq27-Xqter. The fourth abnormal chromosome (CLL) contained Xp21.3-pter.

Conclusion: It is noteworthy that all four cases were male (40% of male patients studied), and that all cases involved non-pseudoautosomal regions of the X chromosome. A gain of Xq27-28 material was found in three cases (30% of males tested). MTCP1 (Mature T-Cell Proliferation 1) is among several known oncogenes in this region. Although this study comprises a small number of cases, further use of mFISH in cytogenetic analysis will reveal whether this is a recurring abnormality, as suggested by the early trend. It appears possible that gain of an oncogene(s) in the region of Xq26-qter contributes to the disease phenotype in these and other cases.

P71

Minimal Residual Disease in Childhood Acute Lymphoblastic Leukaemia; Concordance between Flow Cytometry and PCR

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Approximately 25 % of children with acute lymphoblastic leukemia (ALL) relapse after frontline therapy.Current stratification methods using clinical/biological criteria fail to identify a significant proportion of these children. More patients (pts) classified as standard/medium risk, relapse than those with high-risk ALL. Early identification of this group with modifications of therapy may prevent this. We compare two techniques, multiparameter flow cytometry (FCM) and polymerase chain reaction (PCR) to monitor minimal residual disease (MRD) after initial therapy in newly diagnosed pts. We report our initial experience in pts with B-lineage ALL from a single institution (CHW) treated on the ANZ Children's Haematology-Oncology Group Study VIII protocol for ALL. Multiparameter FCM was performed using two different four colour combinations of antibodies: CD19 APC/ CD45 PerCP/ CD10 FITC/ CD20 PE and CD19 APC/ CD45PerCP/ CD34 FITC/ CD9 PE. A series of dual parameter displays were generated to define normal B cell differentiation pathways which allowed the discrimination of malignant precursor B cells. Real time PCR used clone specific primers after sequencing immunoglobulin heavy chain and/or T-cell receptor gene rearrangements from the diagnostic leukemic clones. Critical time points for analysis were Day (D) 33 and 79 during induction/consolidation. Of 31 pts, 30 had informative PCR markers: 7 with high MRD levels on D33 (> 10-3), which persisted in 2 on D79. By FCM 29 of 31 pts had a diagnostic template. In contrast to PCR, 3 had positive MRD (>0.1%) on D33 compared with 6 on D79.The 2 with high MRD by PCR were also identified by FCM. Both pts had been classified as standard/medium risk by conventional criteria. The techniques may be complementary in identifying pts with significant MRD. Only a proportion of pts are identified by both methods. Longer follow up and a larger cohort will allow determination of optimal surveillance/stratification strategy.

P72

Chemokine Receptor Expression on B-cell Malignancies by Flow Cytometry

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Chemokine receptors are expressed by B lymphocytes and function to mediate cell trafficking and migration. Normal B-cells are known to express CCR6, CCR7, CXCR3, CXCR4 and CXCR5 receptors but not CCR5. To assess Chemokine receptor expression profiles of B-cell malignancies and to determine if chemokine receptor profiles may allow detailed subclassification of these disorders, whether differences in receptor expression may correlate with tissue localisation, and whether such expression profiles may provide a mechanism to further our understanding of normal B-cell ontogeny. We studied chemokine receptor expression in normal peripheral blood lymphocytes (n=20), and various B-cell malignancies including CD10-positive follicular centre lymphoma (n=16), precursor B-acute lymphoblastic leukaemia (n=16), chronic lymphocytic lymphoma (n=21), small cell lymphocytic lymphoma (n=8), hairy cell leukaemia (n=9) and other lymphomas (n=9). CXCR3 expression was highly variable on normal B-cells as well as other B-cell malignancies. Normal B-cells were typically positive for CXCR4, CXCR5 and CCR6, negative for CCR5 and variable for CCR7. B-ALL cells expressed CXCR3 and CXCR4, but were negative for other chemokine receptors. B-CLL cells lost expression of CCR6 but up-regulated CCR7. Cells from tissue-restrict B-CLL (SLL) showed a similar profile to those of B-CLL, but interestingly lost CCR7 expression. HCL cells lacked CXCR5 and CCR7 expression, but were the only B-cell malignancy that expressed CCR5; interestingly, variant HCL (n=3), defined by lack of CD25 expression, showed absence of CCR5 expression. FCCL cells showed down-regulation of CXCR4 and CXCR5, and obvious loss of CCR6 and CCR7 expression. Our study demonstrated that CCR5 was a useful marker for HCL diagnosis, and especially for its differentiation from variant HCL. Loss of CCR6 and CCR7 expression was a feature of most tissue lymphomas. CCR7 expression was strong in B-CLL but absent in SLL.

P73

Disruption of a Lymphocyte Development Gene Cluster at 10q24 by a Novel t(5;10) Translocation in Acute Lymphoblastic Leukaemia (ALL)

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Disruption of a lymphocyte development gene cluster at 10q24 by a novel t(5;10) translocation in acute lymphoblastic leukaemia (ALL) Sheryl M. Gough1, Suzanne M. Benjes1, Ruth Spearing2, Peter Ganly1,2 and Christine M. Morris1,2. 1 Cancer Genetics Research Group, Department of Pathology, Christchurch School of Medicine & Health Sciences, Christchurch, New Zealand 2 Haematology Unit, Christchurch Hospital, Christchurch, New Zealand. The molecular dissection of structural chromosome alterations has identified many genes important to the cause or progression of leukaemia. Aside from their diagnostic and prognostic value, the identification of these genes is critical to our current understanding of the molecular pathways of leukaemogenesis, and a necessary precedent to the design of targeted, less toxic treatments. However, the genetic basis of up to 48% of acute lymphoblastic leukaemia (ALL) cases remains unknown. We hypothesise that novel chromosome translocations may mark the location of genes potentially rearranged more frequently by cryptic submicroscopic mechanisms in leukaemia patients. To this end, we have targeted the breakpoints of a novel t(5;10)(q22;q24) translocation found as the sole abnormality in the leukaemic cells of a 59 year old male patient with ALL. Mapping efforts using a combination of fluorescent in situ hybridisation (FISH), targeted large-insert bacterial-, yeast- and P1-artificial chromosome (BAC, YAC, PAC) clones, genomic database analyses, and hybridisation to leukaemic t(5;10) chromosomes, has facilitated the identification of a YAC and subsequently a PAC clone containing the 10q24 breakpoint site. Genomic probes from within this PAC clone and Southern hybridisation studies of patient DNA resolved a model of the 10q24 breakpoint site in a genomic region not previously associated with leukaemia. The breakpoint region harbours a small cluster of lymphocyte development genes, one of which we have found, unexpectedly, to be flanked at its 5' end by novel RNA transcripts. We now have confirmed evidence of unique transcriptional activity spanning the 10q24 breakpoint and have identified novel transcripts potentially implicated in this leukaemia.

P74

Allogeneic Bone Marrow Transplantation (BMT) for T-cell Prolymphocytic Leukaemia (T-PLL)

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T-PLL is a rare condition, comprising approximately 2% of cases of small lymphocytic leukaemia in adults over the age of 30 years. Response rates to non-transplant therapies are generally poor and although responses are seen to treatment with CAMPATH-1H and purine analogues, relapse is inevitable. A 45 year old man presented with lymphocytosis, lymphadenopathy and pulmonary infiltrates and a diagnosis of T-PLL was made based on morphology of lymph node and marrow, immunophenotype and the detection of T-cell receptor gamma gene rearrangement. The white cell count (WCC) at diagnosis was 76.7x10^9/L (lymphocytes 37.9x10^9/L) and platelets 91x10^9/L. He was initially treated with 3 cycles of CVP and pentostatin with a partial response (WCC 19.7, lymphocytes 12, platelets 175) and then underwent a planned HLA-identical sibling myeloablative allogeneic BMT, 5 months from diagnosis. The post transplant course was complicated by severe graft-vs-host disease of the gastrointestinal tract and skin requiring significant immunosuppression. As a consequence, he developed cerebral nocardiosis and life-threatening gram negative sepsis on two occasions. Corticosteroid use also resulted in myopathy and osteoporosis. At 12 months after transplant he was in complete remission (CR) as determined by bone marrow and radiological examination. Six months later, he presented with abdominal pain, fever, rapidly increasing hepatosplenomegaly, hepatic and renal failure. Lymphocyte count increased from 0.9 x 10^9/L to >200x 10^9/L over the course of 7 days. Examination of the bone marrow confirmed relapse with 55% lymphocytes and sheets of CD3+ T lymphocytes identified on the trephine, but with an immunophenotype (CD3+/CD5+/CD7+/CD8+/CD4-) somewhat different to that seen at diagnosis (CD4+/CD8+). He died of progressive disease, 533 days after the BMT. There is a paucity of literature regarding allogeneic BMT for T-PLL but those experiences described so far include a relapse at day 84 and ongoing CR at 2, 11, 24 and 36 months post BMT. Although most patients present with a typical aggressive, progressive disease course, a subgroup has been described with an initially indolent course followed by subsequent secondary progression. Our patient may fit into this group. We conclude that the role of myeloablative and non-myeloablative BMT in T-PLL is still to be defined.

P76

Characterisation of Endothelial Progenitor Cells

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[1] St George Hospital
[2] Prince of Wales Hospital

Introduction: Endothelial progenitor cells (EPC) are bone marrow (BM)-derived cells that have been identified in peripheral and umbilical cord blood (CB). They have the potential to differentiate into mature endothelial cells (EC) and to improve the oxygenation of ischaemic tissues through new vessel formation. Whilst the properties of EPC are only beginning to be fully characterised, AC133, CD34 and Flk-1 are three markers that identify early EPC. Determining the best source of EPC is difficult, as various studies have not been consistent in their definition of EPC, their method of isolation and culture conditions required for differentiation and expansion. We sought to fully characterise EPC derived from mobilised peripheral blood (PB) and identify the optimal culture conditions for their differentiation and expansion.

Methods:. Mobilised PB samples were collected with compliance of SESAHS Human Ethics Committee. Mononuclear cells were firstly isolated using Ficoll Paque, then monocytes allowed to adhere. AC133+ cells were selected from non-adherent population using magnetic bead separation (Miltenyi Biotec, Sydney). Enriched cells were analysed using PCR and flow cytometry for phenotypic markers and were also grown under various culture conditions with either fibronectin or collagen coated plates.

Results: Preliminary results demonstrated that AC133+ selected cells express AC133 (76.6 ±14.2%), CD34 (97.9 ±1.4%) and CD45 (99.4%) by flow cytometry. PCR confirmed the expression of Flk-1 and demonstrated the presence of Tie-2 receptor and Ang-1. Cells were negative for CD14, CD31, Flt-1, Ang-2 and VEGF-A. Enriched cells adhered to both collagen and fibronectin but only proliferated on fibronectin. Optimal culture conditions were M199 with 10%FCS, 50ng/ml VEGF, 1ng/ml bFGF and 2ng/ml IGF; medium changed once weekly.

Discussion: Phenotypic analysis confirms that we have isolated EPC from mobilised peripheral blood. These cells are presently being assessed for their ability to differentiate into functional mature EC and will be compared with the ability of EPC from BM and CB to proliferate and differentiate in culture.

P77

Prolonged Remission of Refractory Lymphomatoid Granulomatosis after Autologous Haemopoietic Stem Cell Transplantation

Anna Johnston, Luke Coyle

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Aim: Lymphomatoid granulomatosis is a rare EBV-associated lymphoproliferative disease. Even with combination chemotherapy mortality is high. There is no standard therapy for relapsed or refractory disease. There is only one report of a complete remission with high-dose chemotherapy and autologous stem cell transplantation in the literature.

Method: We present the case of a patient with progressive lymphomatoid granulomatosis who was successfully treated with autologous stem cell transplantation after conditioning with high dose chemotherapy and total body irradiation.

Result: A 46 year old man presented with lymphomatoid granulomatosis (angiocentric immunoproliferative lesion grade II) with initial lung, skin and peripheral nerve involvement. In spite of combination chemotherapy he manifested progressive disease and underwent autologous stem cell transplantation with peripheral blood progenitor cells after conditioning with total body irradiation and high dose cyclophosphamide. Post-transplantation he received maintenance therapy with interferon alpha 2A for 45 months. The patient remains well and in remission 7 years post transplantation.

Conclusion: This is the first report of a durable (>1 year) complete remission after high dose chemotherapy and autologous stem cell transplantation in lymphomatoid granulomatosis. The role of high dose chemotherapy and autologous stem cell transplantation in relapsed or refractory cases merits further evaluation. The exact place of interferon in treatment of lymphomatoid granulomatosis remains to be clarified but appears to be promising.

P78

Early Followup of Non-Myeloablative Allogeneic Stem Cell Transplantation in Multiple Myeloma

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Multiple Myeloma is refractory to chemotherapy alone with relapse common after autologous PBSCTx. Allogeneic stem cell transplantation can result in a durable graft-versus-myeloma effect but with 40-50% TRM in the first 100 days.

Aim/Method: Retrospective assessment of 8 patients undergoing non-myeloablative allogeneic transplantation at the RAH using Badros/Barlogie or Seattle protocol. Median age was 54 years. Six underwent sibling allogeneic transplants and 2 mini MUDs. Each patient had poor prognosis cytogenetics; B2M >4 or relapse after autograft.

Result: Three were in CR at transplantation and 5 PR. Six patients had undergone 1 autologous stem cell transplant and 2 patients 2 previous transplants. No TRM occurred in the first 100 days. Seven of 8 patients engrafted. By D+100 engrafted patients had 90-100% donor chimerism. Autologous recovery occurred in the non-engrafted patient. GVHD developed in 7/8 patients, 3 acute GVHD, 2 progressing to chronic GVHD. Four patients developed chronic GVHD only. CMV antigenaemia occurred in 3/4 donor positive to recipient positive transplants. Three of 8 patients developed peri-transplant septicaemia. No VOD occurred. The 3 patients transplanted in CR have remained in CR. Two patients transplanted in PR are currently in CR, one having responded after relapse to decreased immunosuppression. Both relapsed patients and the patient who failed to engraft were transplanted in PR. One relapsed patient died 17 months post mini MUD in full donor chimerism. Median time since transplant is 13 months.

Conclusion: Non-myeloablative allogeneic bone marrow transplantation is well-tolerated and exerts graft-versus-myeloma effect in high risk patients transplanted both in CR and PR.

P79

Validation of a System for the Temperature Stabilisation, Monitoring and Transport of Human Stem Cell Products

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[3] Baxter Healthcare Pty Ltd, Sydney, New South Wales

To ensure safety and efficacy the Australian Bone Marrow Donor Registry (ABMDR) specifies that Human Stem Cells (HSC) cells must be transported at stable temperatures between 4° C and 10° C. Ice packs and coolers have been used to maintain HSC temperature during transport. A supply of dry ice was required to re-chill cold packs when transit time exceeded 30 hours. Using this method the product temperature can fall outside the required range for a considerable time during transport >= a median of 35% of temperature readings were out of range for seven HSC transported for periods from 2.5 hours to 47 hours. The Blood in Motion (BIM) system from Baxter Healthcare is a passive transport system developed for transportation of blood products consisting of 4° C control elements and extender frames. These are filled with a Phase Change Material (PCM) and used in conjunction with a vacuum-insulated container (MiniVaq). Once solidified at 4° C the PCM is placed in the MiniVaq where it can maintain stable temperatures between 4° C and 10° C for the extended periods required for international transport. Using this system the product reached the appropriate temperature range within 2 hours of packing, temperature could be maintained between 4°C and 10°C for up to 50 hours and was minimally affected by handling during customs inspection. For shorter interstate transport of HSC the elements and frames may be used inside a silver-lined rucksack. The BIM system provides a reliable, safe and thermally-stable environment for the transport of HSC contributing to product safety and efficacy.

P80

Use of Pegylated G-CSF in Stem Cell Mobilisation for Autologous Peripheral Blood Stem Cell Transplantation - the Fremantle Hospital Experience.

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[2] Fremantle Hospital, Pharmacy Department

Aim: Autologous peripheral blood stem cell transplantation (ABSCT) is an important means to allow delivery of high-dose cytotoxic therapy in the treatment of various haematologic neoplasms. Traditionally, daily administration of Granulocyte Colony Stimulating Factor (G-CSF) is used to mobilise haemopoietic stem cells (HSC). Daily administration may have a negative impact on patient compliance, cost and resource utilisation. The development of polyethyleneglycol(PEG) conjugated G-CSF (PEG-GCSF) has allowed single administration regimens following cytotoxic therapy. Preliminary results comparing single-dose PEG-GCSF with daily-dose G-CSF regimens have shown that it is able to mobilise sufficient HSC's for ABSCT. As a result, 5 patients at Fremantle Hospital have had a trial of HSC mobilisation using PEG-GCSF.

Method: Five patients (3 Multiple Myeloma, 1 Acute Lymphoblastic Leukaemia and 1 Non-Hodgkin's Lymphoma) were given a single 6mg dose of PEG-GCSF, following priming chemotherapy. Daily full blood counts (FBC) were performed from day +8 and a CD34 count was performed when the total white cell count (WCC) was >2.0 x 109/L. HSC's were collected using the Cobe Spectra Apheresis Machine when the CD34 count was >10x106/L.

Result: All patients had sufficient HSC's mobilised and harvested. The mean time from chemotherapy to HSC collection was 10.6 (8 - 13) days and from administration of PEG-GCSF to collection was 8.4 (7 - 9) days. Three patients received PEG-GCSF on Day +2 following Cyclophosphamide priming and 2 patients on day +5 of HyperCVAD. Three patients were collected over 2 days and 2 patients over 1 day. The mean collection was 12.28 x 106/kg (3.0 - 29.1). The mean peripheral total WCC at collection was 5.44 x 109/L (2.02 - 6.9) with a mean CD34 count of 108.43 x 106/L (16.93 - 314).

Conclusion: Single dose PEG-GCSF is effective at mobilising HSC's for ABSCT.

P81

In vivo Dendritic Cell Depletion, in a Murine Transplantation Model, to Attenuate Graft versus Host Disease.

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Patient outcome after haemopoietic stem cell transplantation (HSCT) is governed by time to engraftment, tumour relapse and development of graft versus host disease (GvHD). Current immunosuppressive strategies for GvHD patients are centred mainly on manipulation of the effector T-cell; however, new interest in the possibility of DC depletion is emerging. Our intention is to shift the focus from elimination of activated T-cells, to elimination of DCs that activate these T-cells. We have previously shown that immunoregulatory DC subsets exist in mice that make good targets for manipulation in a transplant setting. In addition, we have also shown one DC subset in particular, mature plasmacytoid DCs, to be increased in mouse spleen after conditioning by radiation. The purpose of this study is to develop novel strategies to deplete candidate DC subsets in order to control GvHD and improve outcomes for HSCT recipients. Intra-peritoneal (ip) injection of N418, a monoclonal antibody against mouse leukocyte integrin CD11c, is expected to deplete murine DC in vivo. Dendritic cell depletion by ip injection of N418 cannot be monitored using a commercial anti-CD11c-APC conjugate due to epitope blocking. This problem was overcome using a complex staining matrix of DC markers which include MHCII, 33D1, DEC205, CD4, CD8a, CD11b, CD45R (B220) and Gr-1 to identify sub-populations of CD11c. Recent results show the depletion of murine DC using this method. It is our intention through the use of DC depleting antibodies to provide an effective alternative to current T-cell based immunosuppressive prophylaxis for GvHD.

P82

Significance of Abnormal Protein Bands (APB) in Patients with Multiple Myeloma Following Autologous Stem Cell Transplantation (ASCT)

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Aim: We studied the characteristics of small APB (oligoclonal bands (OB) and new monoclonal bands with isotype switch (IS)) that are frequently detected by serum protein electrophoresis in the post transplant setting.

Method: A retrospective analysis of patients with myeloma undergoing ASCT was performed. Paraprotein identity and quantification was performed using standard immunofixation electrophoresis. The nature of any new bands was determined by isoelectric focussing which distinguished between oligoclonal banding and distinctly new monoclonal bands.

Result: 24 cases were reviewed: median transplant age 61yrs(range,32-75yrs); female 50%; all stage III disease. 50% of patients achieved CR, 46% PR and 4% <PR. Median follow-up was 42 months(range,5-95months) and 13 patients have relapsed at a median of 11 months (range,3-65months). Twenty (83%) developed APB. Isoelectric focussing demonstrated four to have both OB and IS, and 16 having OB only. The median time to development of APB was 1.3 months(range,0-27months). Bands of up to 5g/l were observed. Eight patients had more than one episode of OB. Of 30 episodes of OB, 25 resolved after a median duration of 3.4 months(range,0.2-34+ months). Of four episodes of IS, two resolved after two and 18 months respectively, one still persists after two months, and one represented true disease progression. There was no correlation between the presence of APB and relapse or survival.

Conclusion: APB are very frequent post ASCT and probably represent normal immune reconstitution. IS may represent relapsed disease with an alternate malignant clone, but more likely is a transient phenomenon representing regeneration of a limited immune response.

P83

Non Myeloablative Stem Cell Transplantation in CLL

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We present six cases of heavily pretreated and/or refractory Chronic Lymphocytic Leukemia (CLL) with multiple comorbidities (including AMI), who underwent non-myeloablative stem cell transplantation (NMSCT) with Melphalan,Fludarabin and ATGAM conditioning. Follow up varied from 4.5 years to 3 months, and four out of six remain in morphological/molecular CR. One patient demonstrates persistent disease is on weaning immunosuppresion. One patient has early relapse over 12 months post transplantation. We have proven clinically useful graft vs CLL effect. To date there have been no transplant related deaths. NMSCT is well tolerated and potentially curative therapy for refractory CLL.

P84

Does Donor Collection Centre Affect Outcome in Unrelated Donor Stem Cell Transplantation (UD-SCT) ?

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The Leukemia/BMT Program of British Columbia and The Division of Hematology, Vancouver General Hospital, British Columbia Cancer Agency and the University of British Columbia

Aim: Solid organ transplant data suggests inferior allograft outcomes when donors are not harvested at the transplant center. Although general guidelines for the collection and transport of non-cryopreserved BM and PBSC exist, no studies have compared outcomes of patients transplanted using locally versus distantly collected BM and PBSC's. We sought to determine the effect of donor collection centre on transplant related outcomes in UD-SCT.

Method: All UD-SCT's performed between 6/88 and 12/01 by the Leukemia/BMT Program of B.C were reviewed. The 'local' patient cohort comprised patients whose donor collection centre was in Vancouver, B.C. The 'distant' cohort comprised patients whose donor collection centre was outside B.C. Variables analysed were age, sex, diagnosis, cell dose, T-cell depletion (TCD), HLA match, and ABO compatibility. OS at 30 days, EFS, time to neutrophil and platelet engraftment, graft failure rate, relapse rate, and rates of GVHD were evaluated.

Result: 240 patients, 193 'distant' and 47 'local', were identified. There was no significant difference in OS at day 30, cumulative EFS, graft failure, relapse rate, or GVHD between the two cohorts. Patients receiving a TCD-graft, regardless of cohort, had significantly poorer EFS (p=0.0007). In those receiving a non-TCD-graft, there was no significant difference in neutrophil (p=0.84) or platelet (p=0.72) engraftment time regardless of cohort.

Conclusion: The use of distantly collected and transported non-cryopreserved stem cell product does not adversely affect outcome in UD-SCT. Larger collaborative studies will be required if significant qualitative defects in distantly collected and transported stem cell products are to be detected.

P85

Design and Implementation of a Comprehensive Computer Management System for Haemopoietic Stem Cell Collection, Processing and Storage Facilities.

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Aim: Currently most, if not all, haemopoietic stem cell transplant centres in Australia rely heavily on paper based record systems. This is largely due to the unavailability of systems specifically designed to meet the needs and practice models of Australian laboratories. This lack of World's Best Practice data management systems will severely hamper the efforts of Australian institutions to partner with international collaborative groups and funding sources in pursuit of stem cell research and clinical trials. Thus there remains a critical need for a comprehensive computerised management system, which encompasses the collection, processing, storage and release of products.

Method: The Diagnostic Haematology Department at the Royal Melbourne Hospital has commenced the development of a comprehensive software suite for data capture and management, labelling requirements and report generation. The goal is to develop a system that covers all aspects of the process from the collection couch through to the storage, release and infusion of products in a manner consistent with code of Good Manufacturing Practice, the National Pathology Accreditation Advisory Committee Guidelines, and the standards published by The Foundation for the Accreditation of Cellular Therapy.

Result: The resulting database, hosted on a centralised server running the Microsoft SQL server architecture, consists of over 90 individual data fields, with a user Interface through a custom developed client program written in Microsoft Visual .NET. The security and data integrity requirements, as set down in the prevailing US standard 21 CFR part 11, have been fulfilled by careful selection of the database architecture and computing infrastructure employed. The labelling requirements of 21 CFR 175 have also been incorporated.

Conclusion: The project is currently in the testing and validation stages of development and a live demonstration of the software is included in the presentation.

P86

Expansion of the Peripheral Blood Stem Cell Collection Service at Peter Mac to Enable TGA licensed Leucopheresis Collections at an Offsite Location.

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[1] Peter MacCallum Cancer Centre, Division of Haematology & Medical Oncology
[2] Cabrini Hospital Apheresis Service
[3] Australian Red Cross Blood Service

Aim: The Therapeutic Goods Administration (TGA) Licensed Centre for Blood Cell Therapies (CBCT) at Peter MacCallum Cancer Centre (PMCC) provides various cell therapy activities including Peripheral Blood Stem Cell (PBSC) collection, cryopreservation and autologous transplantation under Good Manufacturing Practice (GMP) conditions. Previously, all PBSC collections were performed by the Apheresis Unit onsite at PMCC. An agreement between CBCT & Cabrini Hospital Malvern (CHM) was developed to investigate the expansion of PMCC PBSC apheresis service to include PBSC collection at the CHM Apheresis Unit.

Method: To facilitate the extension of our existing TGA license providing collection of PBSC's at CHM for subsequent cryopreservation at PMCC, various quality assurance activities were undertaken.

These included:

1. Identification of a concurrent/retrospective validation process for the Cobe Spectra Cytapheresis machine at CHM;

2. A validation/implementation was performed of the Blood in Motion Transportation System for delivery of PBSC's from CHM to PMCC;

3. Patient identification issues were identified and addressed allowing formulation of unique identifiers recognised by both hospitals;

4. Staff training including development of appropriately controlled standard operating procedures common between PMCC and CHM apheresis units;

5. Logistical problems relating to appropriate and timely pre-collection testing were identified and overcome;

6. Appropriate environmental & critical material monitoring systems were developed at CHM in line with the systems already in place at PMCC.

Results & Conclusion: The various validations and processes will be presented in more detail. Application to extend our TGA license to allow collection of PBSC's at CBH was granted in April 2004. Continual review and monitoring of the PBSC collection services will be undertaken within existing CBCT Quality Systems.

P87

Recovery or Viable CD34+ cells from Cryopreserved Haemopoietic Stem Cell Products

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The recovery of viable CD34+ cells reinfused into patients at the time of autologous or allogeneic transplantation is clinically an important variable, which can determine graft success or failure. In this study we analyse the recovery of viable CD34+ cells /kg pre and post cryopreservation on a total of 73 autologous stem cell products as well as 4 cryopreserved stem cell products from allogeneic donors. CD34 enumeration was performed on all samples pre and post cryopreservation using a novel in-house no-lyse CD34 assay (previously described ASH 2003 abstract no.1685). Cells were labelled with CD45, CD34 and 7AAD in TRUCOUNT tubes using a modified single platform ISHAGE protocol. The absolute number of viable CD34 + cells per Kg was determined. For the 73 samples before freezing the median viable CD34+ cell count was 6.0 x 10^6 /Kg (range 0.3-25.2 x 10^6/Kg). For post thaw samples the median viable CD34+ cell count was 5.0 x 10^6/Kg (range 0.2-24.6 x 10^6/Kg). The median recovery was 83.3% (range 48-100%). This represents an average loss post freeze/thaw of 16.7%. Further analysis showed a median loss of 10% for NHL (range 0-52%, n=34), 17.5% for MM (range 0-44%, n=12), 8.5% for acute leukaemia (range 0-29% n=8) and 7% for non-haematological malignancies (range 0-50% n=21). Interestingly the greatest loss occurred in allogeneic donors, where viable CD34+ counts on fresh samples averaged 5.7 x 10^6/Kg (range 3.1-11.8 x 10^6/Kg, n=4), whereas post freeze/thaw averaged 2.2 x 10^6/Kg (range 1.2-3.3 x 10^6/Kg). Representing a mean loss of 58% of CD34+ cells. Assaying the viability of CD34+ cells post cryopreservation may identify patients at risk of poor haematological recovery that could benefit from further stem cell collections.

P88

Iron Overload After Allogeneic Bone Marrow Transplant

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Aim: To analyse incidence of iron overload after BMT and assess the role of venesection in preventing complications.

Method: Retrospective analysis of 189 consecutive patients undergoing allogeneic BMT at RMH between 1998-2003 surviving >d180. Data was collected from notes, computer records and blood bank. Iron studies were routinely done pre-BMT, at D100, 1 & 2 years post BMT.

Result: Data was available in 168 patients. Raised ferritin post BMT (>1000mcg/L at any time) occurred in 115 patients, 57 of whom (34% of total) had transferrin saturation >70% on at least one occasion. 23/57 underwent liver biopsy, 17 of whom demonstrated tissue iron overload as per consensus criteria for haemochromatosis (iron concentration > 80umol/g dry weight, hepatic iron index >1.9, hepatic iron grade 3-4). 7/57 with no biopsy met criteria for iron overload on CT (CT liver iron >1.0 mg/ml). 25/57 did not undergo liver biopsy or CT, predominantly due to disease relapse (n= 12), or alternative reason for raised ferritin-severe liver GVHD (n=4). Compared to patients with lower values, those with a raised ferritin and saturation were more likely to have been transplanted for acute leukaemia (36/57 (63%), vs. 27/110 (25%) $p<0.001$), to have been transfused with a higher number of units of red cells (mean 44 vs.17, $p<0.001$) and to be C282Y heterozygotes (10/37 with available data (27%) vs. 4/42 (10%) $p=0.07$). There was no difference in the two groups with respect to acute or chronic GVHD, age or sex. A mean of 12.3 units were venesected in 22 patients (Range 2-46), all of whom had received >25 units of red cells. ALT fell significantly (mean ALT pre venesection 189 IU/ml, post 36 IU/ml, $p<0.05$), as did transferrin saturation (mean pre venesection 68%, post 29%, $p<0.05$).

Conclusion: 1. Tissue iron overload is common after BMT. 2. Biochemical measures of iron stores (ferritin and transferrin saturation) may be unreliable in this context, radiological or histological assessment to distinguish hyperferritinaemia due to inflammation from true tissue iron overload may be required. 3. Patients at risk of iron overload (Transfusions >25 units, C282Y heterozygotes) should be closely monitored and early venesection therapy instituted to prevent organ damage.

P89

Towards Safer Transportation of Haemopoietic Stem Cells

Deborah Hayes [1], Pamela Dyson [2], Karen Olson [1], Debra Page [1], Jane Bartlett [3]

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The Australian Bone Marrow Donor Registry (ABMDR) specifies that Human Stem Cell (HSC) must be transported at temperatures between 4° C and 10°C. The temperature must remain stable during transport times from 2 >= 50 hours. Traditionally couriers of HSC have used ice packs and coolers to ensure maintenance of temperature during transport and have liaised with airlines to ensure a supply of dry ice to re chill cold packs for extended transit times. The courier is responsible for all aspects of product safety including the reading and recording of package temperature at frequent intervals in transit. Products are frequently transported over long distances, often necessitating flights in excess of 30 hours. This can result in a very tiring and stressful procedure for the courier. So that courier fatigue does not compromise product safety it is essential to adopt any technologies that may improve the process. Cold packs may now be replaced by a passive transport system for the transportation of blood products that is available from Baxter Healthcare. The Blood in Motion (BIM) system consists of 4° C control elements and extender frames filled with a patented Phase Change Material (PCM). Once solidified at 4° C the PCM is placed in a transport container where it can maintain stable temperatures between 4° C and 10° C for the periods required for transport. For extended transit times the control elements may be used in conjunction with a vacuum-insulated container (MiniVaq) and for shorter transit times a silver-lined rucksack or bag may be used. The choice of transport container depends on the anticipated transit time and should not compromise the occupational safety of the courier.

P90

Bilateral Breast Lumps in Sex Mismatched Allogenic Transplant for Aplastic Anaemia

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[2] Department of Anatomic Pathology, Newcastle Mater Misericordiae Hospital

An unusual diagnostic problem in a 35 year old female with bilateral breast lumps, axillary lymph node swellings, and subcutaneous mass in the right infra mammary region is being presented. The patient was diagnosed as aplastic anemia in August 1998. She had no response to standard doses of antithymocyte globulin, methylprednisone and cyclosporin. She then underwent a sex mismatched sibling allogenic transplant. She had essentially no Graft-vs-Host disease and, after 5 months post transplant, did not return for follow up when all drugs were ceased. Her presentation now was to the breast clinic where the above findings were documented. An Fine needle aspiration (FNA) was performed which suggested a haematological tumour and she was subsequently referred to haematology for diagnosis. Diagnostic investigations carried out included: 1) FNA cytology of the breast lesion and biopsy of the subcutaneous lesion. Flow cytometry and immunohistochemistry on these samples was performed. 2) Tissue biopsy of the infra mammary lesion and immunohistochemistry on the paraffin sections was performed. 3) A simultaneous analysis of peripheral blood and bone marrow was also undertaken. The tests performed on the latter were morphology, cytochemistry, fluorescent in situ hybridization (FISH) and cytogenetics. FNA of the breast lesion showed a monomorphic population of neoplastic cells suspicious of a haemopoietic malignancy. Tissue biopsy showed a poorly differentiated malignancy which expressed CD45, CD34, CD117 and CD4. Blasts (85%) in the bone marrow could not be characterized by morphology or standard cytochemistry. Flow cytometry on the bone marrow sample confirmed a myeloid leukemia with expression of CD34, CD33, CD117 and aberrant expression of CD4. FISH analysis showed the tumour cells to be of female origin, but with 15% residual male cells. The classical cytogenetics revealed 46 XX, add(5)(p15) karyotype. A WHO (world health organization) classification of acute myeloid leukemia, minimally differentiated was ascribed to this tumour. Being a sex mismatched transplant it also provided an opportunity to determine the origin of the malignant cell (patient or donor). The malignancy was of patient origin thus providing an opportunity for a second transplant and/or donor lymphocyte infusions. In summary, a rare and peculiar presentation of an acute myeloid leukemia in a post bone marrow transplant patient, which was both educative and challenging, is being described.

P91

Development of a Policy for the Storage and Disposal of Cryopreserved Haematopoietic Progenitor Cells for the NSW BMT Network Laboratories

Vicki Antonenas [1], Ian Kerridge [1], Ken Bradstock [1], Peter Shaw [1], Annette Trickett [2]

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[2] NSW BMT NETWORK

Although cryopreserved haematopoietic progenitor cells (HPC) can be stored indefinitely, there are questions about long-term viability in vapour phase of liquid nitrogen. As indications for transplants expand and the number of patients undergoing second or tandem transplants increase, there are also increasing pressure on available storage space. The NPAAC guidelines do not provide sufficient direction resolving policies for the storage or retention or disposal of cryopreserved autologous or allogeneic HPC for adults and pediatrics. The NSW BMT Network have developed a policy and procedure on storage and disposal of HPC for BMT laboratories in NSW. The policy developed aims to provide clinical flexibility for patients and physicians while at the same time recognising space limitations, evidence based practice and the current limitations of ex-vivo expansion. The process for storage and disposal of HPC is indeed based around the following:

* Obligatory recording of patient preference for disposal of HPC
* Immediate notification of haematologists receiving stem cell harvest yield.
* Mandatory clinical justification for storage beyond 7 years or in situations where there is an inadequate yield.

Details of the policy for the NSW BMT Network will be discussed.

TUESDAY 19 1600 to 1700 Atrium
HSANZ Poster Session 2

P92

Pegylated G-CSF (Neulasta-Amgen) In The Management of Severe Chronic Neutropenia.

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Aim: To investigate the use of Pegylated G-CSF in the management of severe chronic neutropenia, particularly in patients with poor compliance to daily G-CSF and recurrent sepsis. including- (a) optimal dose and frequency of administration (b) neutrophil response including peak counts and days of neutropenia (c) episodes of infection and inpatient admission (d) side effects of therapy. Two sisters (19 & 23 years old) with severe congenital neutropenia with life-long recurrent sepsis, admissions and suboptimal compliance were studied. Cost analysis was performed.

Result: Neutropenia resolved with pegulated G-CSF administration. There was moderate to marked neutrophilia with 6mg dosage, moderate bone pains for 3 days and one patient developed minor thrombocytopenia. Neutrophilia and pains were less marked elevation when the 3mg dosage was used. The number of days of resultant neutrophil counts greater than 1.0 x109/l varied between subjects, and required adjustment in the treatment schedule. One patient had no significant infective episodes and no admissions since changing from standard to Pegylated G-CSF, and was stabilised on 3mg every 10 days. This resulted in predictable control of the neutrophil count. The other patient was stabilised on 3mg every 7 days, again with satisfactory neutrophil counts. The latter patient had ongoing compliance problems and had febrile neutropenia requiring hospital admission after failing to administer a planned injection.

Conclusion: Pegylated G-CSF appears safe and effective in the management of severe congenital neutropenia. The optimal treatment schedule is individualised, and provides potential advantages of improved compliance, reduced injections to patients and cost effectiveness.

P93

The Baseline BCR-ABL Transcript Level in Imatinib-Treated Newly Diagnosed Patients with Chronic Myeloid Leukaemia (CML) Does Not Impact on the Timing and Extent of the Molecular Response

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The selective inhibitor of the BCR-ABL tyrosine kinase, imatinib mesylate has demonstrated significant therapeutic benefit for patients with CML. Molecular monitoring of residual BCR-ABL transcripts in patients achieving a complete cytogenetic response (CCR) reveals a stratification of values that have prognostic significance. It has previously been established that a 3-log reduction (major molecular response) by 12 months of imatinib therapy predicts for both a long duration of CCR and a high probability of progression-free survival. The analysis used a standardised protocol, which measured BCR-ABL values of individual patients relative to a median baseline value for untreated patients. It is not established whether the individual patient baseline BCR-ABL value influences the timing of the molecular response or whether the actual log reduction is a better predictor of treatment response than the standardised log reduction. We used real-time quantitative PCR to measure BCR-ABL transcript levels in 123 newly diagnosed patients in chronic phase (median months of imatinib therapy was 15). The median BCR-ABL/BCR% value at baseline was 113% (range 9% to 405%). Patients were divided into 2 groups according to the baseline value; those with a low baseline value (median BCR-ABL/BCR=71%) and those with a high baseline value (median BCR-ABL/BCR=180%), $P<0.001$. From 3 months of imatinib therapy there was no significant difference between the median BCR-ABL values for each group, indicating that the timing and extent of the molecular response is independent of the baseline value. Similarly, there was no significant difference in the probability of achieving a major molecular response by 12 months when the actual log reduction values were compared to the standardised log reduction, $P<0.0001$. The data suggest that the actual baseline value can be used to assess the molecular response to imatinib.

P94

The Australian Cancer Anaemia Survey: A Snapshot of Anaemia in Adult Cancer Patients in Australia

Tara Seshadri [1], Miles Prince [1], David Bell [2], Paul Coughlin [3], Philip James [4]

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Aim: To evaluate the prevalence and management of anaemia in Australian adults with solid and haematological malignancies.

Method: Six month, prospective, multi-centre study included 694 patients from 24 oncology centres. Data included tumour type, disease status, cancer (ca) and anaemia treatment (Rx), performance status (PS), baseline and trigger haemoglobin (Hb).

Result: Median age of patients (61% female) was 61 years. At enrolment: 35% were anaemic (Hb <120g/L); 65% received chemotherapy (CRx), 4% chemo-radiotherapy (CXRT), 1% radiotherapy (XRT) and 30% no Rx; 27% had breast ca, 24% gastrointestinal (GIT) ca, 22% lymphoma/myeloma, 7% lung ca, 6% gynaecological ca, 6% leukaemia, 3% urogenital ca, 1% head/neck, 5% other. Frequency of anaemia at enrolment or during the study ranged from 49% for lymphoma/myeloma to 85% for urogenital ca (51% GIT, 55% breast, 56% leukaemia, 71% head/neck, 76% lung and 81% gynaecological). Patients receiving CXRT at enrolment had the highest frequency of anaemia during the survey (86%) compared with XRT (71%), CRx (57%) and no Rx (55%). Of CRx patients not anaemic at enrolment 25% became anaemic after 2 further cycles of CRx, (25% breast, 32% lymphoma/myeloma, 17% GIT, 55% lung). Anaemia was treated in 32% of patients with solid tumours and 45% with haematological ca. Anaemia Rx was either none (77%), transfusion (19%), iron (3%) and erythropoietic agents (1%). Median trigger Hb for initiating anaemia Rx ranged from 105g/L (GIT ca) to 85g/L myeloma/lymphoma ($p<.05$)

Conclusion: Anaemia is widely prevalent amongst Australian cancer patients. Transfusion is the most common management for anaemia.

P95

Hyperphenylalanaemia (Acute Phenylketonuria) Following L-Asparaginase Therapy

Steven Stylian, Jeremy Wellwood, Cameron Turtle, Subrat Mishra, Anne Haughton, Elizabeth Gibson, Frank Bowling, Kerry Taylor

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Aim: To increase awareness of the metabolic side effects of Asparaginase therapy and their management and in particular stress the rare effect of hyperphenylalanaemia.

Method: A 22 year old patient with ALL failed induction following one cycle of the HyperCVAD regimen. Second line chemotherapy was then commenced, consisting of the High risk ALL Hoelzer protocol. A component of this included L-Asparaginase IV D15-28. The following disturbances were subsequently encountered; On D25 it was noted that the bilirubin level was 92 micromol/L (conjugated 5). Despite cessation, the level continued to rise, peaking on D31 at 296. AST, ALT, GT, ALP were also significantly raised but without any clinical evidence of acute liver failure. Severe hyperlipidaemia was also documented, cholesterol 9.2 mmol/L and triglyceride 17 mmol/L. There was no clinical or biochemical evidence of pancreatitis. Hypofibrinogenaemia was treated with cryoprecipitate. Other causes of acute hepatitis were excluded. An ammonium level of 245 was measured (5-35 micromol/L). Partial measures included a low protein diet, Lactulose and a single dose of Benzoate 200mg/kg IV over 24 hours. Dystonia, ataxia, loss of speech and confusion was noted. The symptoms demonstrated diurnal variation, worse at night and did not correlate with the ammonium levels. Metabolic investigations showed severe hyperphenylalanaemia 1175 micromol/L (210-660) and phenylketonuria. The mechanism likely relates to inhibition of pterin and folate cofactors by Asparaginase resulting in phenylalanine hydroxylase inhibition. Depletion of pterin cofactors as well as high phenylalanine levels may be associated with acute and chronic neurologic dysfunction. Emergent treatment consisted of oral Folinic Acid, oral Tetrahydrobioterin (100mg/kg) and a low phenylalanine diet given via naso-gastric due to its unpalatable nature. Rapid resolution of the phenylalanine level but also bilirubin was subsequently noted.

Result: The patient made a good recovery without neurologic deficit. This case demonstrates the metabolic side effects of Asparaginase which include: hepatitic dysfunction, hyperammoniaemia, hyperphenylalanaemia, hyperlipidaemia and hypofibrinogenaemia. The hyperphenylalanaemia is a rare side effect previously only noted in children treated with Asparaginase. Asparaginase also inhibits the urea cycle, but the ammonium level in this patient was not sufficiently elevated to explain the neurological dysfunction. Pterin deficiency may also occur with Methotrexate, Vincristine and Cytosine Arabinonside.

Conclusion: Asparaginase, a very useful agent in management of ALL, has a pleiotropic side effect profile including potential for significant disturbance of amino acid and neuro-transmitter metabolism and attendant neurologic effects.

P96

In-vivo Response to Imatinib Measured by Inhibition of Crkl Phosphorylation Identifies Good Responders in the TIDEL Trial

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[3] Australasian Leukaemia Lymphoma Study Group

Imatinib mesylate (Glivec, Novartis) reverses the clinical and haematological abnormalities of CML by blocking the binding of ATP to the kinase domain of the BCR-ABL fusion protein. Whilst remission rates are high with imatinib therapy, not all patients achieve cytogenetic and molecular responses. Differences in the level of BCR-ABL kinase inhibition achieved in CML patients on imatinib may explain this variability. Assays predicting response facilitate early dose escalation or combination therapy. We used western blotting to assess phosphorylation of Crkl (p-Crkl), a major substrate for BCR-ABL in blood samples from 45 CML patients in the TIDEL trial. Samples were used to measure the in-vivo level of p-Crkl 7-14 days and again 21-28 days after imatinib start. The % decrease of p-Crkl was calculated relative to the level of p-Crkl measured pre imatinib, and patients were grouped into high and low % p-Crkl response relative to the median. These groups were then compared to molecular responses at 3 and 12 months. In the first 14 days of therapy the median % decrease in p-Crkl was 27% (R: 14-78%). Log Rank survival analysis showed no significant difference in achieving a 2 log depletion at 3 months (high 47%; low 33%: p=0.225). Similarly there was no difference in the probability of achieving a 3 log depletion by 12 months (high 62%; low 42%: p=0.253). However, analysis of % decrease p-Crkl performed day 21 - 28 (median 41% R: 14-80%), revealed a significant difference in the probability of achieving a 3 log depletion by 12 months (high 72%; low 32% with p=0.004). In conclusion a surrogate measure of in-vivo BCR-ABL kinase inhibition by imatinib was strongly linked to the subsequent achievement of major molecular response.

P97

DNA Sequencing of ABL Subclones Detects Mutations in Imatinib Treated CML Patients at an Earlier Timepoint than Direct Sequencing.

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Mutations in the ABL kinase region of BCR-ABL are commonly associated with CML patients who develop resistance to imatinib. Some patients may harbour these resistant mutations in a small number of cells, which rapidly expand under the selective pressure of imatinib therapy. The current mutation detection method, direct sequencing, reveals mutations present in approximately 20% of cells and therefore may not be sensitive enough to detect the mutations in minor clones. We have developed a more sensitive method involving the subcloning of the ABL kinase domain. This requires PCR amplification of the ABL kinase domain of the BCR-ABL allele, cloning it into pGEM-Teasy vector, and sequencing individual clones. We have analysed 2 newly diagnosed patients who developed mutations, detected by direct sequencing within 12 months of commencing imatinib treatment, to investigate whether these mutations could be detected earlier and whether they were pre-existing. In both patients the cloning method detected mutations at time points prior to their detection by direct sequencing. In the first patient where direct sequencing detected the mutation after 6 months of treatment, the clonal approach demonstrated the mutation at 3 months in 20/51 clones (39%) and at 1 month in 1/107 clones (0.9%). Similarly in the second patient where the mutation was detected by direct sequencing at 3 months, clonal sequence analysis demonstrated the mutation at 2 months in 12/106 clones (11.3%). Analysis of these two patients demonstrates that sequencing of individual clones provides a more sensitive approach for mutation detection. The baseline samples of the patients who developed mutations are currently undergoing analysis to determine whether the mutations were present at diagnosis and if so, at what level.

P98

The Response of the Myelodysplastic Patient to Erythropoietin Therapy.

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Introduction: A retrospective audit of erythropoietin use showed that myelodysplastic syndrome (MDS) was most common condition treated with erythropoietin by our department. This report follows the progress of 15 MDS patients.

Method & Result: Pharmacy, patient & transfusion records were examined to identify 17 patients with MDS, including one with chronic monomyelocytic leukemia, who received erythropoietin between October 2002 & March 2003. All but one had a diagnostic bone marrow biopsy with most patients having refractory cytopenias with multi-lineage dysplasia. Treatment response could not be assessed in 2 patients who ceased erythropoietin within 4 weeks of commencement. Thus 15 patients were evaluated. Overall, 12 of 15 MDS patients (80%) responded to therapy; haemoglobin response in 11, transfusion response in 9 with a mean duration of response of 86 weeks. The mean weekly erythropoietin dose used in responsive patients was 25000 units. Seven of 15 patients remain alive with 3 remaining erythropoietin-responsive & 4 now erythropoietin non-responsive. Seven of 8 patients are dead secondary to progressive MDS, which was usually preceded by loss of response to erythropoietin. Eight of 15 patents had pre-treatment erythropoietin levels performed, mean 51.1U/L, range 6.8U/L to 194U/L. All of these patients responded to therapy. None of the 12 patients with iron studies performed pre-treatment were iron deficient but 2 became iron deficient on therapy.

Conclusion: Erythropoietin is a valuable treatment for the anaemia of MDS with sustained responses observed (mean 86 weeks). This translates to reduced blood transfusion requirements & reduced hospital contact. Patients should be monitored for secondary iron deficiency. Response should be optimised through dose adjustment, iron supplementation & regular clinic review.

P99

Glivec Induced Aplasia

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Aim: To describe complications of Glivec induced aplasia in two patients with late chronic phase chronic myeloid leukaemia (CML).

Method: Two cases resulting in fatal aplasia following the use of Glivec are outlined. Case 1: A 77 year old patient with Ph positive CML of 17 years duration had been previously treated with multiple intermittent courses of Busulphan. Increasingly difficult haematologic control was encountered. BMAT showed CML in CP with Ph chromosome in 100% of metaphases. Glivec 400 mg daily was commenced. Eight weeks later, severe BMAT showed aplasia and the agent was ceased. Further complications ensued including basal pneumonia, ischaemic coronary syndrome, epistaxis, recurrent malaena and fulminant neutropenic sepsis. Progressive decline in neutrophil count to zero was seen, unresponsive to G-CSF. Specific treatment of the aplasia with IV Methylprednisone, Cyclosporin and Oxymethalone was unhelpful and the patient died of sepsis after 8 weeks. Case 2: A 35 year old patient had Ph positive CML diagnosed 16 years previously. Past treatment included Busulphan and Interferon. Haematologic control was achieved but marrow remained 100% Ph chromosome positive. Glivec 400mg daily was commenced and approximately 9 weeks later there was severe neutropenia; N < 0.2 and Platelets < 20. A BMAT showed aplasia and the agent was ceased. The patient subsequently developed LUL pneumonia. Despite broad spectrum antibiotics, G-CSF and Stem Cell Factor the patient developed respiratory failure necessitating ventilatory and inotropic support and died three weeks later.

Result: Both patients developed early onset aplasia following commencement of Glivec. There was a relentless progression of the cytopenias despite cessation of the agent. These were unresponsive to G-CSF, Stem Cell Factor and immunosuppressive therapy and death ensued due to infection. Neither patient had chronic phase stem cells stored at time of diagnosis.

Conclusion: In CML patient with long standing disease there is potential Glivec induced aplasia presumably due to paucity of normal residual stem cells. We recommend that extreme caution should be exercised when commencing Glivec in such patients. Collection of CP stem cells should be routine in all CML patients and perhaps long term marrow cultures to identify normal residual stem cells should be undertaken prior to institution of Glivec in such patients.

P100

Oligospermia in a Patient Receiving Imatinib Therapy for Hyper-Eosinophilic Syndrome (HES)

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Imatinib mesylate (Glivec®, Novartis) is an inhibitor of the Bcr-Abl tyrosine kinase of CML, but also inhibits c-abl, c-arg, and the receptors for platelet-derived growth-factor (PDGF) and stem-cell factor (c- kit). Imatinib has been effectively utilised therapeutically against these kinases in other malignancies. The physiological functions of cellular targets of Imatinib are still being elucidated and their inhibition may contribute to unwanted effects. Gene-targeted animals lacking c-kit and PDGF-receptor have impaired testosterone production and male infertility, and gynaecomastia has been associated with Imatinib therapy. We report a case of oligospermia in an 18 year-old man treated with Imatinib for HES (diagnosed 10/02). Initial treatment was hydroxyurea (3g/day), prednisolone (60mg/day) and interferon-alpha (3 million units). Semen was cryopreserved pre-imatinib in 12/02 (sperm count 20x106/mL, 40% motility). Imatinib was then commenced at 400mg/day for 1Mo, then escalated due to poor response to 600mg/day for 5Mo, and is ongoing at 800mg/day at 18+Mo. Repeat semen analysis in 12/03 showed oligospermia (< 1x106/mL, 25% motility). Testosterone levels were normal. His HES remains in remission, all haematological parameters are normal, and no other medications had been given between the two semen analyses. This oligospermia may be mediated via inhibition of c-kit. The role of the Imatinib dose used is unclear.

Conclusion: The risk of impaired fertility should be addressed when counselling patients prior to initiating Imatinib therapy, and pre-treatment semen storage considered. More studies of the fertility effects of imatinib are required to define the true incidence of this phenomenon.

P101

Myelodysplastic Syndrome with t(6;21): A Report on Clinical and Pathological Features

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Aim: To describe the clinical and pathological features associated with a novel t(6;21) translocation.

Method: The clinical progress of a patient with a myelodysplastic syndrome and a novel translocation between chromosomes 6 and 21 was reviewed and pathological findings described.

Result: A 65 year old male with a history of mitral incompetence, resected colon cancer and resected prostate cancer presented with dyspnoea. He was clinically in cardiac failure. Blood examination demonstrated normocytic anaemia (Hb 102g/L, MCV 91fL) with leukoerythroblastic changes, target cells, basophilic stippling and hypogranular neutrophils. Bone marrow aspirate confirmed erythroid and granulocytic dysplasia with ringed sideroblasts, blasts <1% and t(6;21)(q15;q22) in all metaphases. Cardiac failure treatment was commenced, however within four months developed progressive dyspnoea and cough. Clinical and radiological findings indicated pulmonary infiltration. Blood examination revealed Hb 74g/L and WCC 186x109/L, with numerous pleomorphic blasts. Bone marrow examination confirmed Acute Myeloid Leukaemia (WHO classification: AML with multilineage dysplasia following a myelodysplastic syndrome.), with marked granulocytic dysplasia and prominent ringed sideroblasts. After initial cytoreduction, the patient was treated with FLAG (Fludarabine, Cytarabine, G-CSF) with resolution of the pulmonary infiltrate. Post induction marrow examination demonstrated only 2.0% myeloblasts, however there were persistent dysplastic changes and 5/20 metaphases remained positive for t(6;21). A further modest improvement was achieved with consolidation therapy.

Conclusion: The novel t(6;21) was associated with myelodysplastic syndrome undergoing rapid transformation to acute myeloid leukaemia, responsive to a cytarabine based regimen. However, there remain persisting cytogenetic and morphological changes of the primary myelodysplastic syndrome.

P102
Linezolid Induced Red Cell Hypoplasia
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Linezolid is a fluorinated oxazolidinone, the first in a new class of antimicrobials designed to increase our repertoire against an increasing number of multi-drug resistant gram-positive cocci. Experience with these agents is however limited and despite extensive post marketing surveillance there continues to be a need for vigilance. We present a case of linezolid induced, 'chloramphenicol like', red cell hypoplasia. A 66-year-old man with multiple co-morbidities including type 2 diabetes, hepatitis C and renal impairment, was admitted following coronary artery bypass graft surgery with a sternal wound infection. Methicillin Resistant Staphylococcus Aureus was cultured from the wound and blood cultures. Intravenous vancomycin was initially commenced, however, after worsening renal impairment was replaced by linezolid 600mg twice daily. Four weeks following commencement of linezolid he was admitted with cardiac failure and anaemia. FBE revealed Hb of 73 g/L, WBC of 7.37 X 109 cells/L and platelet count of 130 X 109 cells/L his reticulocyte count was 8.0 X 109 cells/L. The blood film demonstrated a small number of 'helmet' cells. A haematinic screen was unremarkable. The bone marrow aspirate was hypocellular with moderately reduced, dysplastic and sideroblastic (>15% ringed sideroblasts) erythropoiesis. Prominent cytoplasmic vacuolation of early erythroid precusors and large dysplastic megakaryocytes were also noted. Two weeks following the cessation of linezolid there was notable recovery of erythropoiesis with a Hb of 100 g/L and reticulocytes of 134 X 109 cells/L. The similar morphological appearance of vacuolated pronormoblasts and ringed sideroblasts suggest linezolid, like chloramphenicol, causes suppression of protein synthesis in the mitochondria leading to myelosuppression. Whilst chloramphenicol induced myelotoxicity may sometimes be irreversible, thus far it appears that linezolid induced red cell hypoplasia may be reversed with the cessation of therapy. However if not recognised early linezolid induced red cell hypoplasia may be overlooked leading to potentially life threatening consequences. Baseline and regular monitoring of haemoglobin and reticulocyte counts should be the standard of care in patients on linezolid therapy.

P103
CNS involvement with Chronic Myeloid Leukaemia on Imatinib: A Case Report and Review
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Aim: To review a the role of imatinib in central nervous system (CNS) myeloid progression of Chronic Myeloid Leukaemia (CML).

Method: Retrospective review of the clinical and pathological features of a case of accelerated phase CML with myeloid blast crisis involving the CNS, and review of the literature.

Result: A 40 year old male with accelerated phase CML [t(9;22)(q34;q11) and t(15;21)(q22;q22)] at diagnosis was treated with imatinib after initial cytoreduction. Five months after diagnosis he developed intermittent diplopia and hip pain with normal neurological examination. Bone marrow examination confirmed continuing haematological remission, with persistent cytogenetic changes. Within three weeks there was evidence of raised intracranial pressure and an absent ankle jerk. Bone marrow aspirate revealed 20% myeloblasts, and progression of cytogenetic changes. CSF examination showed numerous blasts. A 5x2mm hyperdense lesion was noted in the right frontal lobe on CT imaging, consistent with a chloroma. Treatment with intrathecal and systemic chemotherapy lead to marrow and cytogenetic remission. Persisting CSF involvement after first consolidation, responded to further intrathecal chemotherapy. He underwent a 5/6 matched unrelated donor transplant and succumbed shortly afterwards to transplant related complications. Discussion CNS involvement with lymphoid blast crisis is a recognised complication of CML, however myeloid involvement is less common. There have been recent reports of lymphoid blast crisis in the CNS whilst the marrow remains in remission. The CNS may be a sanctuary site for CML in patients on imatinib, due to its low penetration, and may favour CNS progression.

P104

The Use of the PETHEMA Protocol in Acute Promyelocytic Leukaemia: A Single Institution's Four Year Experience

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Aim: The Spanish PETHEMA protocol for newly diagnosed PML-RARA positive APML was first published in 1998 and demonstrated a high molecular remission rate as well as substantially lower therapy related toxicity compared to previous regimes. This study reviews the results of this protocol in a single institution over a four year period as first line therapy in patients with APML.

Method: A retrospective chart review of patients diagnosed with acute promyelocytic leukaemia and treated with the PETHEMA protocol was carried out. nineteen consecutive patients between January 2000 and June 2004 were identified. Clinical and laboratory data was analysed.

Result: 19 patients were treated in our institution, with all in haematologic remission and 10 (53%) in molecular remission following initial induction therapy. Four (21%) obtained molecular remission at the end of the first consolidation cycle, one (5%) at the end of the second cycle and a further two (11%) after the third cycle of consolidation. There have been three treatment related deaths, one cardiac while on protocol a secondary MDS following completion of maintenance therapy, and a cardiac death following second relapse post bone marrow transplant. Fourteen patients remain in molecular remission with a median follow up of 22 months (1 40), with three haematologic relapses. The overall survival is 74%

Conclusion: The haematologic and molecular remission rate in this single institution review is similar to that reported by the Pethema group. Toxicity has been acceptable although the case of MDS following maintenance therapy is of concern. Further follow up and analysis of larger patient cohorts following maintenance therapy is required.

P108

The Early Use of Daspone in Refractory Immune Thrombocytopenia

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Aim: The use of Dapsone in immune thrombocytopenia (ITP) has often been restricted to chronic patients refractory to prednisone. In this setting it has comparable efficacy to other agents including danazol and immunosuppressive drugs. We aimed to analyse the efficacy and toxicity of Dapsone when introduced early as the principal second-line agent, the common recent practice at our institution.

Method: In a retrospective case series, the records of 24 consecutive patients receiving Dapsone for ITP between 1999 and 2004 were analysed for efficacy and toxicity data. ITP was diagnosed according to standard criteria.

Result: The median disease duration before Dapsone was 1.25 months (range 0.25 to 228). The mean platelet count at therapy onset was 21. All patients received prior treatment with prednisone and 18 (75%) remained on weaning prednisone while receiving Dapsone 100mg daily. A complete response (platelet count sustained >150 x 109L for 6 weeks) was seen in 6 patients (25%), and a partial response (platelets 50-150 x 109) in 9 (37.5%). There were 9 nonresponders / lost to follow-up. Neither age, baseline platelet count, prior treatments or degree of hemolysis correlated with positive response. Relapse post Dapsone withdrawal was common (75%). Six ceased therapy due to toxicity / hemolysis.

Conclusion: This study confirms the efficacy of Dapsone in immune thrombocytopenia, when used earlier in the disease course than previously described. Our higher rate of response may relate to selection bias and intercurrent corticosteroids. Hemolysis lead to therapy cessation in several patients.

P109

Using Mouse Models of Thrombocytosis to Identify Genes Involved in the Pathogenesis of Essential Thrombocythemia

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Classical Essential Thrombocythemia (ET) is an acquired clonal stem cell disorder characterised by an increased platelet count associated with a proliferation of megakaryocytes in the bone marrow. In contrast to some other myeloproliferative diseases, no specific cytogenetic or molecular abnormality has been identified in this disorder. A genome-wide mutagenesis screen has been conducted in mice to identify genes that are critical in platelet production. This breeding program has yielded several mutant mouse lines (designated Plt2, Plt3 and Plt4) that display prominent platelet over-production associated with a proliferative bone marrow. Plt3 and 4 mice exhibit thrombocytosis (Plt4 platelet count 3936±618x109/L compared to wild type 1479±128) associated with hyperplasia of mature megakaryocytes and megakaryocyte progenitors. The point mutations responsible for the Plt3 and 4 phenotypes map to different domains of the haemopoietic transcription factor, c-myb. To determine whether similar mutations are present in ET we have recruited 27 patients with this disorder (M:F=12:15, age range 33 to 90) to study the human c-myb gene. Genomic DNA was isolated from peripheral blood granulocytes and individual exons and the exon-intron boundaries from the c-myb gene were sequenced from PCR products. Multiple single nucleotide polymorphisms have been identified from these individuals but no pathogenic mutation has been identified in the exons studied. Random mutagenesis provides a novel approach to examine genes that may be involved in the pathogenesis of ET. Further genes identified to cause thrombocytosis in this mutation screen will be correlated with the samples from individuals with ET.

P111

A Case of Quinine Induced Thrombocytopenia Purpura Induced by the Ingestion of Bitters

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Aim: We present a case highlighting the role of bitters in the aetiology of quinine induced thrombocytopenia.

Method: Case report

Result: A 34 year old female initially presented with severe thrombocytopenia (platelet count <10x109/L) and bleeding following the ingestion of quinine tablets. The FBC was otherwise normal and other causes of thrombocytopenia were excluded. Treatment with intragam and high dose prednisolone resulted in recovery of her platelet count to normal levels. Subsequently, testing for quinine associated platelet antibodies (PIFT method) was positive. Whilst on a weaning dose of prednisolone (75mg) she ingested a lemon, lime and bitters softdrink and she represented within 24 hours with bleeding and a platelet count of <10x109/L. She was given a further three doses of intragam and her prednisone dose was increased to 100mg/day. The platelet count rapidly improved and six months after cessation of steroids her platelet count remains normal. Further investigation suggested that the lemon, lime and bitters beverage contains quinine, not because of any tonic water component, but because quinine is present in bitters. Whilst gentian bitters are more common, quinine bitters are also used in a variety of cocktails as well as the lemon,lime and bitters drink.

Conclusion: Patients with quinine induced thrombocytopenia should be counselled that bitters, along with the more commonly known quinine containing agents, should be avoided.

P116

A Unique Case of Histiocytic Sarcoma

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Aim: To define the unique clinico-pathological features of a histiocytic sarcoma: a rare cause of refractory peripheral thrombocytopenia, hypogammaglobulinemia and hypoalbuminemia

Method: The clinical course of the patient, pathology and autopsy findings were reviewed and compared with reports in the medical literature

Result: A seventy-four year old gentleman presented with persistent bleeding following excision of a skin lesion. Investigation revealed severe thrombocytopenia, with plentiful megakaryocytes on bone marrow biopsy, suggesting peripheral platelet destruction. Associated findings included hypogammaglobulinemia and hypoalbuminemia. An abdominal ultrasound revealed a mass lesion consistent with a haemangioma. The patient's thrombocytopenia remained refractory to standard therapies for presumed immune thrombocytopenia, before proceeding with splenectomy, which failed to achieve an increased platelet count. Splenic pathology demonstrated a littoral cell angioma. In the following months the patient remained severely thrombocytopenic and became anemic, requiring red cell transfusion support, and progressively hypoalbuminemic. Repeated bone marrow biopsies, liver biopsy and extensive imaging failed to achieve a definitive diagnosis although underlying malignancy, complicated by haemophagocytosis, was suspected. The patient received further empiric therapy with Mabthera, Etoposide, pulse Dexamethasone but died from hepatic failure ten months after presentation. Autopsy revealed disseminated histiocytic sarcoma involving liver, lymph nodes and bone marrow.

Conclusion: This case of disseminated histiocytic sarcoma represents a rare cause of severe refractory peripheral thrombocytopenia. Diagnosis is difficult and the triad of thrombocytopenia, hypogammaglobulinemia and hypoalbuminemia should prompt investigation for this malignancy. Histological appearances may be difficult to distinguish from a littoral cell angioma.

P117

Anaplastic Large Cell Lymphoma Presenting as Paraneoplastic Pemphigus

Amanda Davis, Prudence Russell, Merrole Cole-Sinclair

The Alfred Hospital, Toorak, VIC

Aim: We report a case of fatal anaplastic large cell lymphoma presenting with paraneoplastic pemphigus, in order to raise awareness of this uncommon association.

Method: A 23 year old female presented to hospital (day 1) with 1 week of rash, fever, myalgia and arthralgia treated with ciprofloxacin and prednisolone by her local doctor. Recent medical history included urinary infection treated with amoxicillin (5 weeks prior), surgical termination of pregnancy (3 weeks prior), and sexual intercourse with a tampon in situ (10 days prior). The rash was disseminated and erythrodermic with desquamation of her hands and feet and mild erosions in the mouth and perineum. No organomegaly or lymphadenopathy was palpable. On day 4 she developed respiratory failure requiring ventilation and intensive care support. A provisional diagnosis of toxic epidermal necrolysis secondary to antibiotics was made and IV gammaglobulin given. A skin biopsy performed at this time however, favoured staphylococcal toxic shock syndrome and meropenem and vancomycin were commenced. By day 9 she had developed progressive multiorgan failure with abdominal distension, ARDS, lactic acidosis and DIC. Laparotomy demonstrated hepatomegaly, splenomegaly and retroperitoneal lymphadenopathy. Splenic, lymph node and marrow biopsies showed involvement with anaplastic large cell lymphoma, small cell variant, ALK-protein positive. Skin blistering was noted and repeat skin biopsy and review of the original skin biopsy was consistent with paraneoplastic pemphigus. Despite advanced severe multiorgan failure and an apparently grave prognosis, dose reduced and modified CHOP chemotherapy was administered along with G-CSF and IV gammaglobulin. After initial clinical improvement, on day 11 post chemotherapy she died of presumed sepsis. Interpretation of autopsy findings was somewhat limited due to autolysis but, these included: extensive lymph node involvement with almost complete necrosis of tumour secondary to chemotherapy, multiple pulmonary findings, the main features being bronchiolitis obliterans and tumour involvement, and acute cholestasis and DIC.

Result: Paraneoplastic pemphigus, an autoimmune mucocutaneous disorder with an associated neoplasm and unique IgG autoantibody, was first described in 19901. The majority of reported cases are linked to non-Hodgkin lymphoma, chronic lymphocytic leukaemia or Castleman's disease. and characteristically antibodies against the epidermal proteins desmoglein 1 and 3, and proteins of the plakin family are seen2. Pulmonary epithelial damage and progressive respiratory failure (including bronchiolitis obliterans) are features of paraneoplastic pemphigus3, as seen in this case.

Conclusion: This case highlights the need for increased awareness and early recognition of this uncommon erythrodermic condition, paraneoplastic pemphigus, which should prompt timely investigation for an associated, often haematological, malignancy.

1. Anhalt GJ, Kim SC, Stanley JR et. al. Paraneoplastic pemphigus: an autoimmune mucocutaneous disease associated with neoplasia. N Eng J Med 1990;323:1729-35.
2. Anhalt GJ. Paraneoplastic pemphigus: the role of tumours and drugs. Br J Dermatol 2001;144:1101-1104 3. Nousari HC et.al. The mechanism of respiratory failure in paraneoplastic pemphigus. N Eng J Meg 1999;340:1406-1410.

P118

Fludarabine Combination Therapy is Highly Effective in Frontline and Salvage Treatment of Patients with Waldenstrom's Macroglobulinaemia (WM)

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Aim: Alkylating agents or single-agent purine analogues are modestly effective as front-line therapy of WM, but responses of <50% are achieved in the salvage setting. Fludarabine combination therapy may be more effective, but no large studies exploring these regimens specifically in WM are available.

Method: 19 epsiodes of fludarabine-based combination therapy were administered to 15 patients from 12/94-8/03 at PMCC: fludarabine (F 25mg/m2x3) & cyclophosphamide (C 250mg/m2x3; n=9), & mitoxantrone (M 10mg/m2x1; n=3), FC-R (FC & rituximab 375mg/m2x1; n=6) or FR (n=1). Pt characteristics: median age 58 yrs (range 44 °C 89), male 89%, previously untreated 21%, #prior therapies 2 (0-7), prior single-agent F 26%, alkylator refractory 47%, time from diagnosis 30 months (0-130), baseline paraprotein 25g/L (5-56).

Result: Patients received a median of 4 cycles (range 1-6), with grade 3+ neutropenia and infection complicating 27% and 3% cycles, respectively. 16 treatments were evaluable: objective responses, all partial, were observed in 13 (81%). Response rates did not differ sigificantly by any of regimen (FC 7/7. FM 2/3, FCR 4/5, FR 0/1), disease status (untreated 3/4, relapsed 4/4, refractory 6/8), previous F exposure (5/5 vs 8/11 no previous exposure), alkylator refractoriness (6/8 vs 7/8 not refractory) or time from diagnosis (6/8 <30 months vs 7/8 >=30 months). 5-year actuarial remission duration and survival were 25=<15% and 57=<15% respectively. There have been no cases of secondary AML/MDS seen.

Conclusion: Fludarabine combination therapy is highly effective in the treatment of patients with WM, achieving response rates of >75% in patients with refractory or relapsed disease, with often durable remissions.

P119

Interim Results of a Phase II Study of Ancestim (SCF) and Twice-Daily High-Dose Filgrastim for Mobilization of Peripheral Blood Stem Cells (PBSC) in Patients with Indolent Lymphoproliferative Disorders Previously Treated With Fludarabine

Kirsten Herbert, Susan Morgan, John Reynolds, Henry Januszewicz, Miles Prince, David Westerman, Max Wolf, John Seymour

Peter MacCallum Cancer Centre, East Melbourne VIC

Indolent histology and prior fludarabine exposure are risk factors for impaired PBSC mobilization. In our previous analysis of patients with prior fludarabine treatment (Leukemia May 2004) only 14 of 41 (35%) achieved PBSC yields of >= 2x106/kg using growth factor +- chemotherapy.

Aim: To assess mobilization efficacy of an alternative approach using ancestim plus high-dose filgrastim without chemotherapy, given the known synergy between G-CSF and SCF.

Method: Study drugs: Ancestim (SCF) 20 µg/kg/day sc from day 1; Filgrastim 12µg/kg/BD sc from day 4. Apheresis scheduled to commence day 6, provided PB $CD34^+$ count > ~3 x 10^6/L. Patients from the previous study served as historical controls.

Result: Thus far, of 16 patients recruited, 14 are evaluable; median age 51 years (range 31-63), median cumulative fludarabine dose 663 (480-900) µg. Nine of 14 (64%) of patients collected >= 2 x 10^6/kg $CD34^+$ cells, compared to 35% with the previous approach (p=0.06). The median $CD34^+$ yield was 2.21 (lower and upper quartiles 1.61-3.26) x 10^6/kg compared to 1.93 (0.2-2.64) x10^6/kg in the previous study. Means were not significantly different. Mobilization scheduling was predictable: all patients commenced apheresis on day 6 (n=9) or 7(n=5). The regimen was well tolerated, with injection site reactions [grade 1-2(n=8)], bone pain [grade 1-2 (n=8)], lethargy (grade 1-2 (n=3)], and no serious adverse events or infectious complications.

Conclusion: The regimen of ancestim and BD high-dose filgrastim shows promising efficacy in this interim analysis of patients previously treated with fludarabine with good tolerance, predictable kinetics and no infectious morbidity.

P120

Infectious Complications of Fludarabine Therapy in Low-Grade Lymphoproliferative Disorders

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Department of Haematology and Transfusion Medicine, Royal North Shore Hospital, St Leonards, NSW

Fludarabine is a potent nucleoside analogue with activity against lymphoid and myeloid malignancies. Therapy with fludarabine-containing regimens can be associated with major infectious complications, due to prolonged immunosuppression. The optimal regimen for prophylaxis of infections has not been established.

Aim: A retrospective review of 16 patients with low-grade lymphoproliferative disorders (CLL or NHL) treated with fludarabine was performed in our institution to assess the safety of this agent and the efficacy of infection prophylaxis.

Result: Twelve of 16 patients (75%) were admitted on at least one occasion for probable infection following therapy. Increased age was associated with an increased number of infections. All of the patients admitted were neutropenic and the respiratory tract was the most common site of infection. Fifty percent of these infections were attributed to bacterial causes (E.coli in 3 cases). Viral infections were predominantly due to reactivation of herpes viruses and 75% of fungal infections were due to Candida spp. Eighty-three percent of the patients developing infections after fludarabine had also been admitted after previous therapies. All patients received some form of infection prophylaxis; 15/16 patients were on cotrimoxazole and no cases of Pneumocystis carinii pneumonia (PCP) were seen. 50 percent of patients who developed viral infections were receiving valacyclovir prophylaxis. All 4 patients who experienced fungal infections had been treated with fluconazole.

Conclusion: Infectious complications are common after fludarabine therapy in this group of patients; PCP prophylaxis is effective, but improved strategies are needed to prevent bacterial, viral and fungal infections.

P121

Six Cases of Primary CD56+CD4+ LIN- Plasmacytoid Dendritic Cell Leukaemia/Lymphoma (BLASTIC NK-CELL LYMPHOMA/LEUKAEMIA)

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[2] Department of Dermatology, St Vincent's Hospital, Melbourne

CD56+CD4+blastic NK-Cell-lymphoma (WHO classification) is a rare, aggressive, skin-tropic lymphoma, previously postulated to arise from NK-cells. More recent work suggests early-plasmacytoid dendritic cell-of-origin with CD123 and tcl-1 expression. Differentiation from T or B-lineage cutaneous lymphoma is made on histological appearance, absence of lineage-specific immunophenotypic markers and clonality on TCR molecular testing. The absence of EBV-genome helps differentiate CD56+CD4+lin-plasmacytoid dendritic cell leukaemia/lymphoma from NK-nasal-type-lymphoma. We present 6 cases to highlight diagnostic and treatment difficulties with this disorder. The mean age of our cases was 73.8 years (range 63-90yo), 5/6 were male. 5 presented with skin lesions (3 localised, 2 multicentric) and 1 with acute leukaemia. All 5 skin-biopsied lesions had epidermal-sparing skin infiltrates which were CD56+CD4+lin- and EBV-ISH negative. The original diagnosis was amended on 5 occasions when pathology was reviewed at our institution and determined prospectively in 1 case (acute leukaemia). Treatment was varied: 2/6 received CHOP and CNS prophylaxis with infusional methotrexate, 1/6 HyperCVAD, 1/6 CVP, 1/6 AML induction(7+3 then HiDAC). Intrathecal prophylaxis was given in 1/5 with skin-disease. One patient was treated palliatively with skin radiotherapy and oral chlorambucil/prednisolone. The clinical course showed 2/6 developed progressive lymphadenopathy and bone marrow involvement within 4 months of treatment, and 1/6 CNS involvement. 2/6 patients died during follow up within 2 years from disease progression or treatment complications. In our series, the original diagnosis was amended on review in 5 cases highlighting the need for clinicopathological interaction and consideration of the diagnosis. Aggressive treatment where feasible seems justified given the relapse rate.

P122

Use of Arsenic Trioxide in the Treatment of Refractory Multiple Myeloma

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[2] Department of Pharmacy, Fremantle Hospital, Fremantle, WA

Aim: Multiple myeloma (MM) is characterised by infiltration of bone marrow by slow growing plasma cells which remains incurable. Most patients respond to initial chemotherapy but invariably progress to chemoresistance. Arsenic Trioxide (AT) has been used in the treatment of refractory Acute Promyelocytic Leukaemia (APML), inducing partial differentiation and promoting apoptosis. The exact mechanism is not known but AT affects signal transduction pathways and causes alterations leading to apoptosis and inhibition of cell growth. AT may have properties that overcome chemoresistance. Studies of non-APML myeloid leukaemias and MM, have shown activity in these neoplasms. Glutathione may inhibit AT-induced cell death, through conjugation of AT or by sequestering reactive oxygen induced by AT. Ascorbic Acid (AA) reduces glutathione levels and is shown to potentiate the effects of AT on MM cells. Curiously the combination relatively spares normal haemopoietic cells.

Method: We report on 4 cases of refractory MM at Fremantle Hospital. Patients were given AT 0.25 mg/m2, AA 1000mg and Dexamethasone 40mg orally daily for 4 days in week 1 as induction and then twice a week as maintenance for weeks 2-12.

Result: Four patients were identified, 1 was deceased. The mean paraprotein at commencement was 19 g/L (9-31). Responses varied with a mean maximum reduction in paraprotein of 6.25 g/L (4-13). Two patients reported side effects, one with worsening heart failure (but with worsening anaemia at the time) and another with diarrhoea (which stopped with cessation of therapy). Another patient had problems with hyperglycaemia on Dexamethasone.

Conclusion: AT with AA and Dexamethasone appears to have activity against refractory MM with moderate side effects. There is considerable variation in response and further study is required.

MONDAY 18 1600 to 1700 Atrium
Nurses Poster Session

P130
Case Study: Red Cell Exchange
Peter Casey, Beverley Wake, Anne Canty, Allan Hayward, Andrew Atkins

Australian and New Zealand Society of Blood Transfusion

The Haematology Day Centre (HDC), Royal Adelaide Hospital (RAH),provides an Apheresis service to the RAH. There are 400 - 500 Apheresis procedures performed annually in our centre consisting of Peripheral Blood Stem cell Collection, Therapeutic Plasma Exchange and Therapeutic Reductions. Red Cell Exchange (RCE)is not a procedure that we commonly perform. The case study is about a patient with Sickle Cell Disease in crisis who had RCE. The decision to perform RCE was complicated by the patients pregnancy and the presence of multiple Red Cell Antibodies. The Cobe Spectra was used to perform this procedure. We present our experience in planning and performing this procedure. RCE proved to be unproblematic with acceptable patient outcomes.

P131
Extension to the Centre for Blood Cell Therapies' Therapeutic Goods Administration licence to collect Peripheral Blood Stem Cells in a Mobile Collection Site within the Peter MacCallum Cancer Centre
Melisa Darby [1], Dominic Wall [1], Maria Maroulis [2], Kerrie Stokes [1], Miles Prince [1]

[1] Peter MacCallum Cancer Centre
[2] Australian Red Cross Blood Service

Aim: In June 2003 the Centre for Blood Cell Therapies (CBCT) at Peter MacCallum Cancer Centre (Peter Mac) was successful in obtaining a Therapeutic Goods Administration (TGA) licence for the collection, processing and storage of Peripheral Blood Stem Cells (PBSC) for autologous transplantation. This licence included the collection of PBSC's in the Apheresis Unit which operates Monday - Friday. In the event that a patient required collection of PBSC's out of operational hours two nurses were required to be present in the Apheresis Unit to perform the procedure which proved extremely demanding on the limited human resources available. An application to extend the current TGA license was submitted to the TGA and subsequently approved to include a mobile collection site within Peter Mac. This mobile site therefore ensured that patients who had complex or unpredictable outcomes were also offered a high quality Apheresis Service.

Method: To prepare for the application to extend the licence conditions, various activities were conducted which included the following:

1. Identification of an inpatient area for the procedures to be performed,
2. Internal audit conducted to ensure selected area met requirements of Code of Good Manufacturing Practice (cGMP),
3. Development of Standard Operating Procedures to perform the collection procedure,
4. Apheresis Nursing Staff training and development,
5. Pre-procedure inspection checklist to ensure that the area meets collection requirements,
6. Communication with Inpatient Area Nurse Unit Manager to assist with bed availability,
7. Identification and control of Critical Materiel and equipment for the collection procedure.

Conclusion: The Application to TGA to extend the current licence to include the mobile collection venue was successful. This demonstrated that the code of GMP can be applied to the clinical setting to facilitate the collection of PBSC's out of operational hours to meet the unpredictable requirements of patients. The successful application also demonstrated that GMP can be implemented without negatively impacting current human resources, and ultimately increasing staff satisfaction as out of hours work was consequently reduced.

P132
Improving Transfusion Practice with the Implementation of Mandatory Nursing Competencies for the 'Administration of Red Cells'.
Nadine Gilby, Illona Sharp, Sanchia Aranda, David Westerman, Denise Spencer

Peter MacCallum Cancer Centre, East Melbourne, VIC

Aim: To ensure 100% of 269 Division 1 Registered Nurses in the relevant clinical areas at Peter Mac, successfully complete the competency on Red Cell transfusion. This requires understanding procedural and product requirements around red cell transfusion, attaining knowledge regarding the NHMRC/ ASBT guidelines and hospital requirements relating to documentation, and reporting of adverse transfusion events. Patient education was also emphasised within the competency.

Method: The competency package, based on the Hospital Red Cell policy & procedure was initiated and mandated by the hospital transfusion committee and developed by members of the Practice Development Committee. The package compromises a knowledge and a practical component. Nurse representatives from each clinical area participated in a training day, preparing participants to train and assess their colleagues. The goal was to achieve 100% completion of competency assessment, by the identified clinical nursing staff, within 7 months.

Result: At 2 months following the Assessor's Training Day 14% of clinical staff had completed the package. At 5 months the completion rate was 60% and at 6 months the completion rate stands at 75%. The process of rolling out this competency package has contributed to raising the profile of transfusion practice and provided a framework for intensive transfusion medicine education to nursing staff by the transfusion nurse and the 'assessors', across the organisation. Improvements in compliance to hospital policy regarding completion of the 'preadmission checklist', has improved from 61% to 95%. Similar improvements have been documented with compliance to recording patients' vital observations throughout the transfusion episode from 63% to 96%. Monitoring the completion of the competency package by new staff to the hospital will be maintained through the hospital transfusion committee in the form of a Key Performance Indicator.

P133
A Retrospective Audit of Oral Mucositis in Patients Undergoing Autologous Stem Cell Transplantation
Adel Gray, Nichola Naidoo, Bartrum Baker

Palmerston North Hospital, Palmerston North, New Zealand

We wished to investigate oral mucositis in patients undergoing autologous stem cell transplantation (ASCT) to determine whether patients and clinical staff have different perceptions of the severity of this complication. Of 24 consecutive patients undergoing ASCT at Palmerston North Hospital between May 2002 and April 2004, all 19 surviving patients were sent a brief questionnaire, asking them to rank seven common side effects of chemotherapy and to grade the severity of their oral mucositis using the WHO scale. A member of the medical staff, blinded to the results of the patient survey, independently assigned each patient a WHO grading based on retrospective review of their hospital records. Fifteen patients (79% of those eligible) responded to the questionnaire. Two respondents (13%) rated oral mucositis as their worst symptom and a further five (33%) rated it as one of their worst three symptoms. Eight patients (53%) assigned a lower severity score, six patients (40%) the same score and only one (7%) a higher score than that assigned independently from review of the medical records. This small, retrospective audit confirms that oral mucositis is one of the most significant problems facing patients undergoing ASCT, although a significant proportion of patients assign lower severity scores for this symptom than those suggested from review of medical and nursing records. This difference in perception will need to be taken into account when designing future prospective intervention studies.

P134
Methylene Blue in the Reversal of Ifosfamide-Induced Neurotoxicity
Natalie Maier, Kerrie-Ann, Murphy, Christine Pecenka, Elizabeth Browne

Prince of Wales Hospital, NSW

Ifosfamide is a cytotoxic drug commonly administered in the haematology/oncology setting, which is used in the treatment of diseases such as Non Hodgkins Lymphoma and gynocological tumours. One of the side effects includes ifosfamide-induced encephalopathy(IIE), which can range from mild confusion to a comatose state and death. Current data suggests that Methylene Blue is the best treatment for IIE, although there are no uniformly accepted administration guidelines. Risk factors for IIE include hypoalbuminaemia and renal and hepatic impairment. Prior to the development of IIE in a patient at Prince of Wales Hospital in 2001, no guidelines for the prevention and monitoring of this complication were in place. As a consequence of this incident, we have devised a protocol for the early detection of IIE . Predisposing factors are recognised and treated prior to the administration of ifosfamide. A nursing neurological assessment chart has been instituted as a diagnostic tool to identify early cerebellar dysfunction caused by IIE. The administration of Methylene Blue as a reversal agent for IIE has been included in the protocol. Two case studies illustrate the role of the POWH protocol in minimising the severity of IIE by early detection triggering the administration of Methylene Blue.

P135
Royal Hobart Hospital Blood Matters
Karen O'Shea, Gina Aitken, Rae Desilva, Shelby Jarrell, Trish Beck

Royal Hobart Hospital, TAS

Safe blood transfusion practice is a high priority of the Australian Council for Safety and Quality. A Tasmanian clinical team spearheaded by a Blood Transfusion Nurse employed on a fixed term basis has participated in the Victorian Government Blood Matters Collaborative. The National Health Development Fund, the Royal Hobart Hospital -Hobart Private Hospital Franchise funds and more recently department of Health and Human Services have provided one-off funding to enable this participation. The resultant activities have been like a game with some losses along the way and importantly some significant wins demonstrated by improvement in clinical practice and reducing usage of red cell units.
The activities in focus areas have demonstrated that:

* Improvements in clinical transfusion practice can be achieved but ongoing expert time, effort and skill are required to sustain and extend these improvements and ensure quality practice and adequate patient information.
* Unsafe practices in transfusion at the RHH and other hospitals are currently exposing patients to avoidable risks and exposing the organization to:
* Unnecessary risks of error at a time when the community and statutory authorities have an increased interest in quality and safety in hospitals
* Preventable waste in time and money with inappropriate use of resources State-wide reduction of wastage of outdated blood products has been initiated through state-wide interlaboratory stock inventory management. This is an ongoing initiative being undertaken by the RHH blood matters team with ongoing consultation with the Australian Red Cross through a statewide forum.

P136
Meeting the Australian Red Cross Blood Services Training and Educational requirements: A National Perspective
L Steel [1], M Ford [1], S Shanahan [1], J Bartlett [2], G Moore [2]

[1] Australian Red Cross Blood Service - Bass
[2] Baxter Healthcare Australia

The Australian Red Cross Blood Service (ARCBS) is a national organisation, which is responsible for the supply and manufacture of blood products as well as the plasma necessary for fractionation into many other blood plasma products. It has long been the practice, and is indeed the expectation, that Healthcare companies that provide the ARCBS with medical equipment or devices will also provide accompanying training and on going service. Many of the Healthcare companies provide education to staff using this equipment on an on going basis or when new software versions or hardware modifications become available. To date this training has been provided on a state-by-state basis. To meet the changing needs of the ARCBS national structure, Baxter Healthcare has recently instigated a new innovative approach to providing staff education. In response to the ARCBS need to have a national approach to all procedures and in consultation with key opinion leaders among the staff, Baxter proposed a National Based Training session to introduce Apheresis nurses from each state to a new software version, S.M.A.R.T. V6, for the Autopheresis - C plasma collection device. By centralising the training and education of nursing staff, it has allowed for a consistency of procedure continuity that has been perhaps been lacking in the past. It also allowed for the nursing staff from each state to interact and network which also promoted the consistency of policy and procedure. The ARCBS and Baxter Healthcare worked together on this innovative approach to provide standardised training and education. Based on the feedback received by both Baxter and the ARCBS this approach will be used again in the future.

P137
Blood and Blood Product - Quick Reference Chart
Tamla Tait, Tony Martinelli, Carole Smith

Austin Health, Heidelberg, VIC

The employment of a Transfusion Nurse at Austin Health as part of the Department of Human Services (DHS) funded 'Blood Matters Collaborative' has led to improved staff knowledge and understanding of blood and blood products. The Transfusion Nurse through discussion and education sessions with both nursing and medical staff has identified deficits in knowledge relating to blood and blood products. Liaison with Austin Health Blood Bank staff has further highlighted the need for information about blood & blood products to be available to staff. To assist staff a blood and blood product quick reference chart has been developed. The chart is A3 in size and includes information relating to fresh blood products including:-

* components, administration, storage and handling,
* an ABO compatibility chart for red cells, platelets and plasma products,
* adverse event recognition and treatment,
* correct specimen and pathology request form labelling criteria
* processing times for group and screen, crossmatch
* products available form Austin Health Blood Bank

The chart has been distributed throughout the hospital and staff have found it to be an invaluable reference tool.

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