

P01

**Incidence of Bacterial Contamination of aAll Pooled Platelet Units from the Australian Red Cross Blood Bank Service (ARCBS) in the Australian Capital Territory (ACT)**

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**Aim**

To identify the incidence of bacterial contamination of pooled platelet units from the ACT ARCBS pre-release to recipients and the risk of infective or adverse events.

**Methods**

Platelet samples of 0.4-1.2ml came from platelet tails of units at TCH from May to October 2006. 99 episodes were cultured with Paediatric BacT/ALERT bottles and blood, chocolate and anaerobic agar plates. Data were collected on these episodes and on recipients. Neonates, massive transfusion patients and single donor platelets were excluded.

**Results**

3 (3%) positive platelet cultures were obtained but without corresponding positive recipient blood cultures post-transfusion.

Episode	Organism	Paediatric BacT/ALERT	Time to positivity
6	Coagulase Negative Staphylococcus	Positive	1 day 6 hr
79	Coagulase negative Staphylococcus	Positive	1 day 8 hr
93	Candida Albicans	Positive	2 days 21 hr

6 recipients had positive blood cultures up to 118 hours post-transfusion but without positive platelet cultures. However, the low sample volume from the platelet tails may have resulted in colony counts below the detection of the methods used.

**Conclusion**

These pilot study findings have raised suspicions of possible platelet contamination by bacteria and fungi. There is now a need to expand this study to better establish the magnitude of this problem.

**P02****Examination of Laboratory Transfusion Errors: Causes and Remedies**

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Many studies have identified the frequency of incidents at different stages of the transfusion process, from specimen collection through to product administration. The contribution of laboratory error to transfusion incidents, including near misses, is well identified. This review identifies the composition of these errors and possible intervention strategies.

The Alfred Hospital has an active error identification and reporting process that allows analysis of errors as well as corrective procedures. In a 19 month period a total of 26 of all errors documented were internal to the Blood Bank laboratory. These errors consisted of clerical, technical and inventory selection errors. These errors were examined to identify the contributing factors and any intervention strategies required to minimize reoccurrence.

Our laboratory has a diverse patient demographic which is reflected in our inventory. The patients requirement for specialized or modified products is not well supported by our laboratory information system. To overcome this we have tried a number of strategies including; reorganization of the blood product storage, alerts on patient documentation and in electronic files, and the process when releasing products.

This review looks at error identification and corrective actions employed,

**P03**

**Effect of Red Blood Cell (RBC) Age on the RBC Storage Lesion and Erythrophagocytosis**

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RBCs undergo many biochemical and morphological changes during storage, collectively known as the RBC storage lesion. Circulating RBCs have a life span of 120 days, however, the influence of the age of the RBC at the time of blood donation on the RBC storage lesion is not well understood.

**Aim**

In this study the influence of RBC age upon the RBC storage lesion in separated young and old RBCs was investigated. In particular, the expression of the complement regulatory molecules (CRMs) (CD35, CD55 and CD59), cell adhesion molecules (CAMs) (CD44, CD239 and CD242) and the rate of erythrophagocytosis of stored RBCs were determined.

**Methods**

Leukocyte-filtered RBCs were prepared using standard blood bank procedures. RBC units were separated into young and old RBCs by density centrifugation and stored at 2-8°C. Samples were collected from stored RBCs on day 1, 14, 28 and 42. Expression of CRMs and CAMs was determined by flow cytometry. Western blotting was used to detect CD59 in stored supernatants. Erythrophagocytosis assays were performed using the monocytic cell line THP-1.

**Results**

Expression of CRMs and CAMs on young and old RBCs significantly decreased with storage. Old RBCs had significantly lower expression of CRMs and CAMs compared to young RBCs at all storage time-points. The decline in the expression of cell surface CD59 coincided with an increased detection in the RBC supernatant with storage time. Erythrophagocytosis increased with storage for both young and old RBCs. Young RBCs were more actively phagocytosed than old RBCs.

**Conclusion**

Old RBCs have lower expression of CRMs and CAMs compared to young RBCs. However, with storage, young RBCs are more actively phagocytosed compared to old RBCs. The consequences of these multi-faceted effects of RBC age and storage on the efficacy of RBC transfusion require further investigation.

## P06

## Tasmanian State-wide Titration Study

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Since the introduction of column agglutination tests into transfusion the tube skills of workers appears to be decreasing. In an effort to move all tests over to column technology we undertook the study to see if our standard BioVue method could be used for titration purposes

**Aim**

The aim of our study was to compare two methods of titrations.

**Method**

The two methods to compare was the NICE method as stated in ANZSBT guidelines and a standard BioVue method

Methods, cells and plasma were standardised to reduce the potential for variation. The cells used to titre against were designated each month from a pool of three cells from the CSL Phenocell B panel. The plasma to be titred was sourced from Securacell produced by CSL.

The titre of Anti-D as designated by CSL was considered to be the benchmark.

**Result**

A total of 74 titres [41 anti-D titres and 23 non-D titres] were performed by a total of 14 different operators throughout the state. Of the anti-D titres performed 23 were using the BioVue method and the 17 using the NICE method.

The results showed a wider spread in antibody titre when using the NICE method (8 – 256) when compared to the BioVue method (64-256).

	4	8	16	32	64	128	256	512
NICE	0	2	2	2	7	2	2	0
BioVue	0	0	0	0	8	12	3	0

The NICE method on 4 occasions underestimated the titre of anti-D such that at a level <32, further investigation is not required. The BioVue method saw a narrower spread of results suggesting increased precision and on no occasion would significant antibody titre be overlooked.

**Conclusion**

The BioVue method can accurately and precisely determine a titre result. There may be a slight increase in titre with BioVue but there was no estimation that may lead to antenatal investigations not being carried out. Staff were more confident with column agglutination technology.

Overall titre estimation by BioVue is accurate and reproducible and suggests that ANZSBT guidelines may in the future reflect the newer technologies used for such testing.

P07

**Haemovigilance: Seven Years of Blood Transfusion Practice**

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Haemovigilance is the monitoring, reporting and analysis of an adverse event at any stage of the transfusion process. Royal Perth Hospital (RPH) has undertaken a haemovigilance program since 1996. Adverse incidents and adverse transfusion reactions are reported confidentially and collated by the transfusion nurse.

**Aim**

To analyse data collected in the haemovigilance program at RPH from July 2000 to June 2007 and assess incidence of events.

**Results**

Adverse Incidents: 938 incidents, samples received for group and hold 105315.

Sample Collection: 389; Transport of Blood: 72; Laboratory Incidents: 118; Blood Product Handling: 353; Blood Product Collection:

Adverse Transfusion Reactions: 522 reactions, products transfused 129050.

Prods		AHTR	FNHTR	Allerg	Anaphyl	TRALI	Oth	TOT
RBC Tf 80117	# Rxns	1	232	32	0	1	79	345
	Ratio Tf/Rx	1:80117	1:345	1:2503		1:80117		1:232
	Pub Figs <sup>1</sup>	1:38000 1:70000	1:17 1:200	1:33 1:100	1:20000 1:50000	1:5000 1:190000		
Plt doses Tf 15454	# Rxns		25	21	3	0	13	62
	Ratio Tf/Rx		1:618	1:735	1:5151			1:249
	Pub Figs <sup>1</sup>		1:3 1:100	1:33 1:100	1:20000 1:50000	1:5000 1:190000		
FFP Tf 32181	# Rxns		13	49	0	4	29	95
	Ratio Tf/Rx		1:2453	1:321		1:8000		1:338
	Pub Figs <sup>1</sup>			1:33 1:100	1:20000 1:50000	1:5000 1:190000		
WB Tf 1298	# Rxns	0	4	1	0	0	3	8
	Ratio Tf/Rx		1:324	1:1298				1:162
	Pub Figs <sup>1</sup>		Not av.	1:33 1:100	1:20000 1:50000	1:5000 1:190000		
**Oth	# Rxns		3	2		0	7	12
TOT 129050	# Rxns	1	277	105	3	5	131	522 1:253

1. Brecher et al. AABB technical manual, 14<sup>th</sup> ed. Bethesda, M.D.: AABB press, 2002:586-89.

\*\* Other products: Ivlg or combination of products.

**Conclusion**

The haemovigilance program has contributed to policy changes in transfusion practice at RPH and encouraged efforts to reduce adverse transfusion incidents through ongoing education. The incidence of adverse transfusion reactions is lower than published figures for almost all blood products transfused<sup>1</sup>. Changes in products, random donor vs. apheresis platelets and leucoreduction of products, may influence the incidence of reactions. Communication should be encouraged at all aspects of the transfusion chain and information gathered should invite discussion and awareness of the haemovigilance program to maintain integrity of data and patient safety.

**P08**

**Referral of Immunohematology Blood Group Serology in Central Blood Transfusions Service, Indonesian Red Cross**

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**Background**

In Blood Group Serology we have some referral cases with many causes. The targets group is AIHA (Auto Immune Hemolytic Anemia) (56%) with cold type, warm type, warm and cold type or combination. Other cases are rare blood groups (25%), HDN (Hemolytic Disease of the Newborn) (19%), Drug Induce, Paternity, etc. This Referral section has been done since 1953 at that time we test same incompatibility testing on cross matching test and rare blood group, so the point is all about difficulty on blood group interpretation or incompatibility testing.

We have received same cases from Sumatera (9%), Jawa & Bali (87%), Kalimantan (3%), Sulawesi (1%). In this three years a go, the referral test we have got from BTU (Blood Transfusion Unit) and hospital are decrease. We hope that is because a good care about therapy in clinical used of blood.

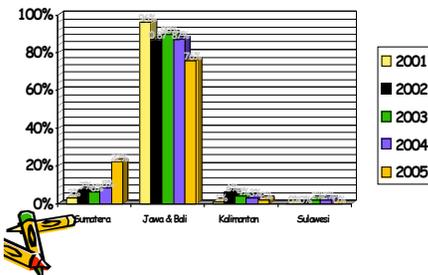
**Description**

The Referral test used conventional method, gel test method and combine that, the combined test is more complete because we got the perfect and accurate result. We used same specific reagent by CBTS (Central Blood Transfusion Service) and other. At AIHA & HDN test we check : 1 blood group ABO & Rhesus, 2 Direct Coomb's Test (Anti Human Globulin Test), 3 screening and identification antigen (the other blood type system : duffy, kidd, MNSs, lewis, etc), 4 Screening and Identification antibody. We used specific technique it depend condition the blood For example ; the cold type AIHA need pre warm test. For referral Blood sample testing we used blood citrate 3 ml and blood non citrate 7 ml in close tube 12 x 75 mm. For referral blood sample, must be completed with the request letter from the doctor who fill the name of patient (with family's / husband's name), age, address, diagnosis of disease, therapy, and signature from the doctor who care the patient. The result of referral test will get in one day. In that result, we give some suggest to the doctor who care the patient.

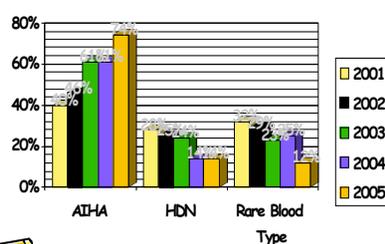
**Conclusion**

We hope can get more referral cases from all BTU and hospital in Indonesia. We want to give more services for increase a quality of health especially about immunohematology blood group serology test.

**Referral Source  
year 2001 - 2005**



**Referral Cases  
year 2001 - 2005**



**P09**

**Queensland Incidents in Transfusion (QiiT) – Developing a Haemovigilance System for Queensland**

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**Aim**

To develop and evaluate a haemovigilance system that will collect data on transfusion related incidents from both public and private health providers in Queensland.

**Method**

The development of QiiT has involved a collaborative approach with involvement of stakeholders from Queensland Health (QH), ARCBS, private health care providers, public hospitals, and both private and public pathology services. The Queensland Blood Board (chaired by the Chief Health Officer) has facilitated this approach. Legal advice confirmed statewide data could be collected from both public and private health care providers. The project involved agreement of a data set, development of methods to validate and allow analysis of reports, a reporting and governance structure, and communication strategy. A major driver has been to minimise the impact of the system on clinical and allied staff.

**Result**

In Queensland approximately 250,000 fresh blood components are issued annually. A data set was agreed that is compatible with the proposed national haemovigilance data set. Based on the UK SHOT system, the QiiT will generate 30-50 reports per year in QLD. The data set was entered into PRIME (QH incident reporting system for public hospitals) in December 2006. During the first 6 months, 31 haemovigilance incidents were reported from all hospitals using PRIME. Two public and two private hospitals have agreed to participate in the pilot; they receive over 30% of red cells issued in QLD. The project commenced in June 2007. To date the reporting rate is in line with that predicted (2 per month). Effective communication remains a key issue in implementing a haemovigilance system in QLD.

**Conclusion**

Improvements in the safety of transfusion practice require methods to monitor relevant incidents. Numerous recommendations for a national haemovigilance system have been made, and the development of QiiT will contribute to the implementation of the proposed national haemovigilance system.

**P10****Transfusion Reaction Reporting to the Australian Red Cross Blood Service**

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**Background**

International experience demonstrates that a formalised system of surveillance encompassing all steps in the transfusion chain can lead to improvements in transfusion practice. The Australian Red Cross Blood Service (ARCBS) investigates serious transfusion reactions with patient, product and donor implications. A national Australian haemovigilance system is in development.

**Aim**

To analyse transfusion reactions reported to ARCBS, creating awareness of the need for and mechanism of reporting such reactions.

**Methods**

Review of transfusion reactions reported to ARCBS during calendar year 2006, with classification according to internationally recognised definitions.

**Results**

A total of 129 reactions were reported nationally:

40 investigations were for suspected transfusion-related acute lung injury (TRALI). In 25 cases donors with granulocyte or HLA antibodies were found.

35 suspected bacterial sepsis episodes, of which 5 were confirmed. Three of these 5 were related to platelet components.

Other reports included allergic reactions (11) ranging from mild to serious; anaphylaxis (2); ABO incompatibility (1); reactions to plasma-derived products (16) and miscellaneous reactions (24).

These cases reported to ARCBS are primarily related to requests for clinical advice, special component support, reference laboratory testing and management of associated components. Timely reporting is essential to recall associated components that may pose a risk to other recipients. ARCBS also manages donor investigations where applicable and any resulting donor management issues.

**Conclusion**

These data identify areas for further patient, donor and product safety developments (e.g. introduction of routine bacterial screening of platelets) and will be important contributions to the national haemovigilance system. Transfusion reaction reporting to ARCBS is important within the broader context of enhancing blood safety in Australia, through provision of clinical advice, specialised testing, and product support for individual cases, as well as analysis of local and national trends and benchmarking with international partners.

## **P11**

### **Evaluation of the iSTAT Buffy Coat Glucose and Lactate Assay as a Device for Quality Assessment of Platelets Stored in Additive Solutions**

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#### **Aim**

Multiple parameters can be used to examine platelet quality including glucose consumption and lactate production. The aim of this study was to determine whether the iSTAT (Abbott) handheld analyser could be used to assess lactate and glucose levels in supernatants from buffy coat pooled platelets stored in T-Sol (PASII) and SSP<sup>+</sup> (PASIIM).

#### **Method**

Pooled buffy coat platelets were split and resuspended in T-Sol or SSP<sup>+</sup> on day 1 and stored at 20-24°C with agitation for up to 7 days. Samples were taken at day 1, 6 and 8, and were tested for glucose and lactate using the iSTAT handheld analyser and the Gluc3 Cobas Glucose method and Lac2 Cobas lactate method.

#### **Results**

Glucose and lactate levels at day 1 were not significantly different in supernatants from platelets stored in T-Sol or SSP<sup>+</sup>. Over the storage period, glucose consumption was accelerated in platelets stored in T-Sol compared to SSP<sup>+</sup>. Lactate production mirrored this finding. The iSTAT yielded lactate results which were, on average, 3.4% lower than the Cobas method. The iSTAT yielded significantly higher glucose results than the Cobas method. Despite large differences in actual value, the correlation between the two methods was 1 and 0.997 for values obtained from SSP<sup>+</sup> and T-Sol respectively. Both glucose test systems demonstrated the expected reduction in glucose over the storage duration.

#### **Conclusion**

The iSTAT handheld analyser is a simple device for measuring glucose and lactate concentration in T-Sol and SSP<sup>+</sup> buffy coat prepared platelet concentrates. Whilst the lactate results were comparable, the levels of glucose measured with the iSTAT are higher than expected for both additive solutions when compared to the Gluc3 method and published data. The iSTAT method did, however, demonstrate an equivalent change in glucose levels over the storage period.

**P12****A Multidisciplinary Approach – The Management of a Pseudotumour in a Mild Haemophiliac**

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**Aim**

Haemophilic pseudotumours, or blood cysts, are expanding destructive lesions of soft tissue or bone in patients with mild haemophilia. Whilst in 1965 17% of patients with severe haemophilia were said to have pseudotumours, the prevalence is now extremely rare in the developed world, especially in patients with mild haemophilia.

**Method**

Case report

**Result**

In this report we present a 48 year old manual labourer in whom mild haemophilia (7iu/dl) was diagnosed aged 24, and who presented with a pseudotumour. Although the retroperitoneal pseudotumour was first detected at the age of 45, the patient was non-compliant with further investigations and treatment. He presented three years later and developed life threatening complications: renal failure, respiratory failure, and bowel obstruction.

**Conclusion**

Clinical strategies were adopted for this patient, highlighting the roles of the multidisciplinary team approach within a comprehensive care centre. The patient was managed conservatively, with an emphasis from the team to develop his coping with chronic disability and promoting a safer more compatible lifestyle.

**P13**

**The Clinical Significance of Anti-M in Pregnancy**

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**Aim**

To determine the clinical significance of anti-M in pregnancy by evaluating maternal and neonatal results in the setting of haemolytic disease of the newborn (HDN).

**Method**

100 women with anti-M from Monash Medical Centre (MMC) and The Royal Women's Hospital (RWH) were included.

Tests reviewed were maternal titration results and neonatal direct antiglobulin test (DAT), M phenotyping and serum bilirubin (SBR). The titration results were divided into two groups, low and high, and then correlated with neonatal results.

The protocols of the clinicians and laboratories were also evaluated to determine treatment standards between the two hospitals.

**Results**

The results indicate no association between high maternal anti-M titre and HDN. The titre level had no influence on neonatal SBR. 17% of neonates had raised SBR with a high titre compared to 38% with a low titre. 41% of neonates SBR were not performed. The DAT results showed no evidence of haemolysis. 79% were negative, 20% not provided and 1% positive.

25% of neonates with a high maternal titre at MMC and 83% at RWH were followed up for possible HDN. Of these, 33% at MMC and 100% at RWH were antigen M phenotyped.

At MMC a high titre is  $\geq 32$ , at RWH it is  $\geq 16$ . At MMC the reference range of SBR varies depending on hours post birth but at the RWH the range is 0-125 $\mu$ mol/L.

**Conclusion**

This small study found no evidence that anti-M is clinically significant in pregnancy as neonates with a high maternal titre had no laboratory findings consistent with HDN. Until further evidence can be evaluated the current protocol of titration of anti-M should continue.

It has also been determined that there is no standard protocol for testing maternal and neonatal samples at MMC and RWH.

**P14****CellPrep Whole Blood Washing by Gentle Dialysis Provides Improved Specificity in a New Flow Cytometric Washed Whole Blood Microassay for Platelet Associated IgG (PAIgG)**

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Detection of platelet associated IgG by immunofluorescence methods has suffered from a lack of standardisation and of poor specificity due to non-specific binding of proteins such as antiphospholipid inhibitor and anticardiolipin antibody which are difficult to remove efficiently using routine manual washing.

**Aim**

Our aim was to develop a reliable PAIgG assay that also discriminated between normal and large platelets using refined flow cytometric data collection and analysis.

**Methods**

We optimised and evaluated semi-automated CellPrep (Beckman Coulter) washing compared with manual washing using 50 $\mu$ l of EDTA whole blood. Patient groups were adults with thrombocytopenia including SLE patients with reduced platelet counts often with detectable circulating inhibitors and neonates investigated for thrombocytopenia. Reference ranges were established for adults and neonates for both manual and CellPrep washed platelets. We used a platelet activation assay to examine platelet surface membrane after washing and carryover was determined for CellPrep washed whole blood using anti Rh (D) to detect Rh (D) Pos erythrocytes.

**Results**

PAIgG results were expressed as % platelets positive for IgG. Ranges for patient samples with non-immune thrombocytopenia, manual wash (M) PAIgG mean = 10.2%, CellPrep wash (CP) 5.5% (n=80), neonates M mean = 6.6%, CP mean = 3.7% (n=54), SLE/antiphospholipid patients without significant "thrombocytopenia", M mean = 24.8%, CP mean = 4.4% (n=13), SLE patients with ITP, M mean = 30%, CP mean = 19.3% (n =6).

Increased platelet antibody binding was found after CellPrep washing of neonatal cells with anti HPA1a binding, M mean = 67%, CP mean = 86%, (N=35) allowing more efficient typing. Platelets were activated after both washing procedures - CD62P, M mean = 27%, CP mean = 26% (N=5). Carryover of Rh (D) Pos cells was 0.1%.

**Conclusion**

A preliminary washing step is required for all platelet antibody assays. CellPrep washing increases specificity for the PAIgG assay by removal of non specifically bound plasma products and should contribute to standardisation of all immunofluorescent platelet antibody detection and platelet cross-matching techniques.

**P15**

**Autologous Stem Cell Transplantation using Planned Platelet Support in a Patient with Platelet Refractoriness due to HPA-1b and HLA Antibodies**

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**Method**

A 60 year old woman undergoing re-induction therapy for AML in 1<sup>st</sup> relapse experienced life threatening intestinal haemorrhage associated with refractoriness to random donor platelet transfusions. Multispecific HLA Class I antibodies and anti-HPA-1b were identified by the solid phase and MAIPA platelet antibody assays. A decision was taken to abandon attempts at consolidation chemotherapy and proceed directly to autologous stem cell transplantation. Planned support included stem cell collections targeted to achieve an adequate number of CD34<sup>+</sup>c-mpl<sup>+</sup> megakaryocyte precursors as well as HLA and HPA (Human Platelet Antigen) matched voluntary donations and frozen storage of autologous platelet collections. Searching of the Australian Bone Marrow Donor Registry was conducted for HLA matched donors with subsequent HPA-1b genotyping of the available HLA matched donors. Three compatible donors were identified from over 18 000 HLA typed donors in the Sydney CBD. Autologous platelets were collected and frozen. G-CSF primed stem cell collections were performed until a CD34<sup>+</sup>c-mpl<sup>+</sup> dose >6 x 10<sup>4</sup>/kg was achieved.

**Results**

An autologous stem cell transplant was performed following Bu/Cy conditioning with a CD34<sup>+</sup> cell dose of 3.8 x 10<sup>6</sup>/kg and a CD34<sup>+</sup>c-mpl<sup>+</sup> cell dose of 14.4 x 10<sup>4</sup>/kg. The first HLA and HPA-1b matched platelet donation was given at day 5 when the platelet count had fallen to 40 x 10<sup>9</sup>/L. A one hour increment to 70 x 10<sup>9</sup>/L was documented. An autologous platelet transfusion given on day 7 produced a significant increment in the platelet count from 24 to 61 x 10<sup>9</sup>/L. The third platelet transfusion donor platelets given on day 9 achieved another good increment from 34 to 87 x 10<sup>9</sup>/L. No bleeding complications were observed and the patient was discharged on day 13. The nadir in the platelet count post-transplant was 24 x 10<sup>9</sup>/L.

**Conclusion**

Planned platelet support can enable delivery of (high dose) chemotherapy despite multispecific HLA and HPA-1b antibodies.

Acknowledgement: Platelet studies were also done on the frozen platelets courtesy of E. Favaloro ICPMR Westmead Hospital.

**P16**

**An Audit of Laboratory Turnaround Times (TAT) in Massive Transfusion (MT) events and the Implementation of Laboratory Strategies with a Hospital MT Protocol**

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**Aim**

The management of MT demands timely availability of test results to guide blood component replacement. We describe the lab strategies put in place in a referral tertiary hospital as well as an audit of the lab TATs.

**Methods**

A multi-disciplinary MT protocol was implemented in June 2005, taking into account best practices and local scenarios. We prospectively monitored all MT events (129 test episodes; 84 patients in 25 months) and collected the TATs for coagulation (PT, aPTT +/- Fibrinogen) and CBE (Hb/Platelet) .

**Results**

The lab strategies are summarised. Following an initial phase of familiarisation, we found a shortening of median TATs (from collection-to-phoned results) from 45 to 30 min for coagulation, and from 32 to 20 min for CBE, with a trend of improvement, tighter TATs with less outliers. When we began to monitor receipt of specimens-to-phoned results, we noted that the interval between specimen collections to their dispatch became a limiting factor. The median TAT for coagulation/CBE in the lab were 17/7 min (receipt-to-phone) respectively in the last 11 months.

**Conclusions**

Auditing of lab TATs allows identification of areas of improvement in the management of MT patients. A multi-modality approach is helpful.

**Table1:** New services and strategies

Main Lab	Blood bank
“Red bag” alert system, with pre-printed tests + sample tubes	Massive Transfusion Pack consisting 4u FFP, 1 adult dose Platelets, 5u Cryoprecipitates with each 6 unit of RBCs issued – initiated acc to agreed algorithm.
Clinical team/ blood bank alerting lab of imminent cases	“Red bag” attached with each MTP issued
Hospital shute system prioritised for specific locations	Thawed group O/A FFP, stored at 4°C
Stat-spin centrifuge for plasma separation	Involvement of transfusion senior staff / haematologist
One staff to follow-through tests; no repeat for “aberrant” results	
Regular reinforcements of staff and of performance targets	

P17

**Timing of Sensitization to Fetal RBC Antigens in Pregnant Women**

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**Aim**

Haemolytic disease of the newborn (HDN) still causes significant problems. We examined the trimester and pregnancy during which allo-immunization to D and other antigens was first detected.

**Method**

An analysis was performed of the 39 women who had records available and allo-antibodies detected between 2000-2006 in Christchurch DHB. Antenatal screening was by an IAT-based technique.

**Results**

Antibody (n)	Pregnancy (trimester)											
	1 (1)	1 (2)	1 (3)	2 (1)	2 (2)	2 (3)	3 (1)	3 (2)	3 (3)	4 (1)	4 (2)	4 (3)
D (21)	1		4			9	2		4			1
C (12)	1		2			5	1		3			
E (10)				1	1	4			3			1
c (10)			2	3	1	3			1			
K (3)				3								
Jka (1)									1			
Fyb (1)									1			
Kpa (1)						1						
Hi(1)									1			
Cw (2)			1			1						

39 women developed 62 antibodies. 18/39 (46%) developed only one whilst 21/39 (54%) developed more than one. Anti-D (21/39 or 54%) and anti-C (12/39 or 31%) were the commonest. In 9/39(23%) anti-C and anti-D were found together. 4/39 (10 %) of women had received a transfusion. All these women were RhD-negative and had received RhD-negative components.

48/62 (77%) of all allo-antibodies were detected during the 3<sup>rd</sup> and 23/62 (33%) during the 1<sup>st</sup>/2<sup>nd</sup> trimesters. This raises the possibility of sensitisation late in the third trimester or at delivery.

All 3 women with Kell antibodies became positive in the first trimester of the second pregnancy . This suggests that the Kell antigen, which is expressed early on RBC precursors, may also be expressed early in gestation.

**Conclusion**

Most sensitisation to the D antigen occurred late in pregnancy - therefore we support anti-D prophylaxis in the last trimester. Some sensitisation to the D antigen may also occur early. The subset of pregnant women (without obvious risk factors) in whom this may happen needs to be defined.

**P18****Determining the Indications, Urgency and Deferability of Red Cell Transfusion: The Bloodhound 2007 Audit of Red Cell Usage in Victoria**

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**Background**

Careful planning is critical to ensure that blood products are available for urgent indications during times of reduced supply or increased demand, such as an influenza pandemic or major disaster. Few data concerning usage, including urgency, are available in Australia or internationally to inform planning.

**Aims**

To develop methods to assess the potential utility of triaging blood components in an emergency by restricting use only to urgent indications.

**Methods**

Several approaches were considered in consultation with internal and external stakeholders, including transfusion laboratories, and experts in statistics and public health. Data requirements and optimal method of collection were determined.

**Results**

Approaches including 'census' of all issued red blood cells (RBCs), user surveys, and longitudinal audit of a random subset of issued RBCs were each considered. A longitudinal audit was considered statistically valid and logistically viable.

In the final design, random RBCs are tagged with a case report form at production, distributed and used as usual. A total of 5000 units will be tagged in Victoria between June and December 2007.

At issue, the blood bank completes the form. This outlines recipient demographics, clinical indication, urgency of supply, and, where issued to support surgery, urgency of surgery.

Forms are machine readable. A custom-built database will reconcile and assist analysis of forms.

Forms, instructions, and data collection were refined following feedback from units (n=30) distributed in a pilot study in May 2007, involving three laboratories.

**Conclusion**

Auditing red cell usage using randomly tagged units over 6 months should accurately determine the proportion of RBCs used for urgent and elective indications, and the proportion of use which could potentially be deferred. These data will inform local and national planning with respect to triaging blood components in times of shortage. The methods are potentially applicable to other blood components.

**A242**

**P19**

**Audit of FVIIa and Blood Product Use in a Single Centre Cardiothoracic Unit over Five Years**

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**Aims**

To assess the impact of the availability of Coagulation Factor VIIa (FVIIa) on blood product use and clinical outcomes in a public hospital cardiothoracic unit.

**Methods**

Cases were identified via searching and cross-referencing the cardiothoracic database, pharmacy dispensing records and cases submitted to the haemostasis registry. This was done to ensure that all patients who received FVIIa were reviewed. Patients under the age of eighteen, and cardiothoracic surgical cases performed in the private hospital were excluded. Parameters assessed included amount of FVIIa administered, surgical variables, blood product use, clinical outcomes and costs.

**Results**

Between Jan 2003 and July 2007 a total of 1989 cardiothoracic cases were performed, of these 65 (3.3%) received FVIIa. The percentage per year of patients receiving FVIIa increased from 1% in 2003 to 6.6% in 2007. Blood product use was consistently higher in the FVIIa population compared to the general CTS group (red blood cells 7.27 vs 4.18 units, fresh frozen plasma 9.37 vs 5.68 units and pooled platelets 3.71 vs 2.58 units). High blood product users (predefined as receiving >15 units of any blood product) constituted 61.4% of the FVIIa group compared to 7.92 % of the general CTS group. Overall re-operation rates for bleeding were 3.10% in 2003. In 2007 they are 2.10% to the current time. Two thrombotic events occurred in the FVIIa group (3.1%). Total cost of FVIIa for the entire period is estimated at \$567,937.65. The average cost per patient was \$7569.93 (range \$6231.55 in 2003 to \$10038.64 in 2006).

**Conclusions**

This audit has allowed the profiling of FVIIa and blood product use in a public hospital cardiothoracic unit over the past five years. Overall blood product use has remained stable and there may be a trend towards reduced re-operation rates for bleeding. Of note the use of FVIIa has increased, a trend which requires careful scrutiny with regard to clinical benefit and cost implications.

**P20****Buffy Coat Platelets Stored in Platelet Additive Solutions T-Sol and SSP<sup>+</sup>: The Effects on Soluble P-Selectin, Annexin V and CD40L**

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**Aim**

Platelet quality can be assessed by multiple parameters including the expression of surface activation markers such as P-Selectin, secretion of soluble P-Selectin and CD40L or release of Annexin V as a consequence of cellular injury. The aim of this study was to determine whether third generation platelet additive solutions (PAS) affected the levels of platelet derived soluble products P-Selectin, Annexin V and CD40L produced by buffy coat pooled platelets over an extended storage period when compared to the current additive solution T-Sol.

**Method**

Buffy coat pooled platelets (n=14) were split and resuspended in either T-Sol or SSP<sup>+</sup> and stored at 20-24°C with agitation for up to 7 days. Samples were taken at day 1, 6 and 8 and supernatant was tested for the concentration of soluble P-Selectin, Annexin V and CD40L using ELISA assay kits.

**Results**

Supernatant levels of each marker were significantly higher in T-Sol suspended platelets compared to SSP<sup>+</sup> platelets at both day 6 and 8 of storage. At day 6 the concentrations of P Selectin, Annexin V and CD40L were 36%, 18% and 19% respectively (p<0.01) higher in platelets stored in T-Sol compared to SSP<sup>+</sup>. Between days 6 and 8, P-Selectin levels increased by 33% in T-Sol compared to an 11% increase in SSP<sup>+</sup> (p<0.001) which correlated with the increased surface expression of CD62P. However a similar magnitude of change between day 6 and day 8 was not observed for CD40L levels or Annexin V.

**Conclusion**

Reduced expression and secretion of CD62P and CD40L into the supernatant of buffy coat platelets stored in SSP<sup>+</sup> in comparison to T-Sol suggests reduced storage induced activation, coupled with reduced cellular damage as determined by soluble Annexin V levels.

**P21**

**Platelet Management Quality Improvement Project: The Effect of Audit Feedback and Process Change on Platelet Wastage**

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**Background**

SEALS Central Blood Bank provides platelet cover for emergency use, planned surgery and patients with haematological malignancies. St George Hospital is a major trauma centre located 60-90 minutes by taxi from the ARCBS. The hospital encourages early use of platelets in massive transfusion, and the Blood Bank keeps spare platelet pools (platelets) on site. Routine usage audit showed median monthly platelet wastage of 41% (average 36%, n=950) over 7 months. Factors thought to govern wastage included the distance from ARCBS, over-ordering pre-operatively, and the age of platelets when received.

**Aim**

To investigate factors governing platelet wastage, and provide solutions that maintain wastage below 30% without compromising patient safety.

**Method**

Strategies aimed at reducing the number of platelets kept on-site were introduced, including a 3-month retrospective audit of requesting and usage patterns. Audit results were fed back to identified problem areas, while the routine usage audit was extended to include age of discarded platelets when received. Requesting and usage patterns were reaudited after 6 months.

**Results**

Following initial intervention, wastage was maintained at  $\leq 30\%$  per month for 11 months (n=1175) and monthly average of 19%. No complaints related to availability were recorded. Average % wasted reduced from 36% to 22% in the 2 months immediately following intervention, prior to feedback of retrospective audit results, with downward trend continuing after feedback. The retrospective audit identified that pre-operative requests from two surgical areas, A&B, represented 50% (n=104) of all platelets requested, not used. After feedback, area A showed a drop in platelets requested, not used from 70% (n=43) to 40% (n=55), while area B showed no significant improvement in ordering pattern. In this study, no direct link between age of platelets when received and wastage was established.

**Conclusions**

Our study shows that sustained wastage reduction can be achieved by process management within the Blood Bank, without compromising patient safety. The results suggest that audit and feedback raise awareness of factors governing wastage and increase confidence in change management strategies, and that further small savings may be made by feedback to other areas.

## P22

**Comprehensive Hospital Haemovigilance Data Are Important and Have Practical Consequences for Patient Care**

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<sup>2</sup>*Transfusion Medicine Services, Australian Red Cross Blood Service*

**Background**

Haemovigilance programs can improve transfusion safety however the direct impact of transfusion reaction reporting on clinical care remains poorly defined.

**Aim**

To describe a metropolitan teaching hospital comprehensive voluntary transfusion reaction reporting system, including reaction type, cause and resulting clinical interventions.

**Methods**

Review of all reactions reported between January 2005 and June 2007. Bayside Health requires all suspected transfusion reactions be reported to the transfusion team. Results are captured prospectively and reported to clinicians after evaluation.

**Results**

191 reactions were reported in 171 patients (male 65%, age 17-95 years, median 54); 16 had more than one. Clinical areas included haematology in 85 (44.5%), gastroenterology 16 (8.4%), trauma 15 (7.9%), oncology 14 (7.3%), others 61 (32%).

Implicated products included red cells 140 (73.3%), platelets 25 (13.1%), fresh frozen plasma 13 (6.8%), intravenous immunoglobulin (IVIg) 8 (4.2%), others 5 (2.6%). Presenting symptom was fever in 135 (70.7%), urticaria 17 (8.9%), dyspnoea 11 (5.8%), rigors 10 (5.2%), asymptomatic 4 (2.1%), others 16 (8.4%). Reaction type was febrile non haemolytic transfusion reaction (FNHTR) in 70 (36.6%), possible FNHTR 35 (18.3%), underlying sepsis 27 (14.1%), allergy 15 (7.8%), anaphylaxis 3 (1.6%), delayed haemolytic reaction 2 (1%), circulatory overload 3 (1.6%), bacterial contamination (proven) 2 (1%) and transfusion-related acute lung injury 1 (0.5%). Five patients (2.6%) experienced severe morbidity; no deaths or acute haemolytic reactions were recorded in this period.

Recommendations were made in 65 (34%) cases: for example: "issue modified blood products" in 6 (3.1%), "modify blood products if reaction recurs" in 31 (16.2%) and "premedicate prior to transfusions" in 18 (9.4%).

**Conclusion**

Implementation of a comprehensive institutional haemovigilance system can improve clinical care significantly, and provides a model for clinical governance. Ongoing efforts to improve recognition, appropriate management and reporting of transfusion reactions, and to provide a framework for national haemovigilance, are required.

**P23**

## **Ceftriaxone Induced Intravascular Haemolysis**

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Drug induced haemolytic anaemia (DIHA) can be life-threatening. Several mechanisms for DIHA have been described: drug adsorption e.g. penicillin, drug-dependent immune complex type e.g. ceftriaxone, and drug independent e.g. methyldopa. Most DIHAs are associated with a positive direct antiglobulin test (DAT) due to IgG alone or a combination of IgG and complement (C3). This report describes a previously well 68 year old woman with acute intravascular haemolysis during ceftriaxone therapy for endocarditis. Initial investigations revealed a negative red cell antibody screen and strongly positive DAT (IgG negative, C3d 4+). The differential diagnosis is discussed and further investigations demonstrated ceftriaxone-specific antibodies. This is the first time that serological investigation of drug immune complex formation has been performed in our Blood Bank and is the first reported case of haemolytic anaemia due to ceftriaxone notified to the Centre of Adverse Reactions Monitoring (CARM) at the New Zealand Pharmacovigilance Centre.

The patient recovered after cessation of the drug.

There have been a number of published case reports of haemolytic anaemia due to cefotetan and ceftriaxone. Cephalosporins are used relatively frequently to treat a variety of infections and as surgical prophylaxis. It is important for clinicians to understand the interactions between red blood cells and cephalosporins and recognize signs and symptoms of haemolysis. This complication may occur more frequently with less severity and thus be under-recognized.

**P24****A Simple Method for Validation of Assessment of Fetomaternal Haemorrhage by Flow Cytometry**Trish Walker*Austin Health, Heidelberg, Victoria***Aim**

In Australia, successful prevention of Rhesus D alloimmunisation due to fetomaternal haemorrhage involves routine prophylaxis with anti-D in addition to extra doses when a significant fetomaternal haemorrhage occurs. Thus both reliable detection and accurate quantification of fetomaternal haemorrhage are important. Flow cytometry is suggested as more accurate and precise alternative to the traditional acid elution Kleihauer Betke technique, yet many laboratories are still not offering this test in routine practice.

This study aims to demonstrate a simple method for validation of a flow cytometry technique utilising anti-F and anti-D.

**Method**

Adult Rhesus D positive blood was “spiked” with a known amount of cord Rhesus D negative blood and the two methods: flow cytometry and manual Kleihauer Betke were performed. Accuracy was assessed by agreement between expected and observed (measured) volumes of fetal cells. Precision and lower limit of quantification were determined by replicate testing.

**Results**

Both methods showed a high degree of agreement between expected and observed percentage of fetal cells ( $R = 0.96$  for manual Kleihauer Betke and  $R = 0.99$  for flow cytometry). Flow cytometry however was more precise than the manual Kleihauer Betke test ( $CV\% = 9.7\%$  vs  $27.1\%$ ) and had a lower limit of quantification of less than 3ml. Samples for flow cytometry were able to produce reliable results even when stored for several days prior to testing.

**Conclusion**

Flow cytometry is an accurate alternative to the manual Kleihauer Betke test and shows superior test characteristics in terms of precision and lower limit of quantification. The method of validation used in this study is simple and able to be implemented in laboratories with flow cytometry capability.

**P25**

**Role of Indonesian Red Cross (IRC) in Reducing HIV Transmission Through Linked Confidential Policy in Blood Transfusion Service (BTS): Yogyakarta Case**

Robby Nur Aditya

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**Background**

In Indonesia, HIV is spreading rapidly in many areas, including Yogyakarta, central part of Java. As HIV can be transmitted by blood transfusion, implementing HIV screening and linked confidential policy in BTS could reduce HIV transmission.

**Problem**

Government has released two policies in HIV screening of blood transfusion, those are Unlinked Anonymous and Linked Confidential. Most of BTS in Indonesia including Yogyakarta use Unlinked Anonymous regulation. According to BTS, if blood contaminated by HIV was detected, the blood should not be used and BTS may not inform the donor as stigma about person infected HIV still strong. On the other hand, other diseases such as Hepatitis B, C and Syphilis, BTS may inform the donor to have further management. Recently, there is program from some Non Governmental Organization ( NGOs ), coordinated by the government, to establish a Voluntary Counselling and Testing ( VCT ) in centre/province hospital. Yogyakarta BTS cooperate with VCT clinic in Dr.Sardjito Hospital to give further management for persons whose blood contaminated by HIV by implementing Linked Confidential policy. When program is running, there is a lack of coordination between the stakeholders in BTS. Many of stakeholders are still not understand and familiar with Linked Confidential policy in blood donors and its role to reduce the HIV transmission. The government also seems not encourage BTS to implement linked confidential policy in their practice. So in the reality, many BTS in Yogyakarta still implement unlinked anonymous policy than linked confidential policy.

**Solution**

To solve this problem the Government should have agreement with BTS about what the best policy and efforts that could be implemented by BTS in reducing HIV transmission by blood transfusion. It is necessary by the government to encourage the BTS to implement linked confidential policy on blood contaminated with HIV. The networking between BTS and VCT service in hospital should be improved so the HIV prevention could be increased and people whose blood contaminated by HIV can have management sooner.

**P26****Upper Limb Venous Thrombosis: A Retrospective Audit of Medical Management**Ninfa Rojas, Nada Hamad, Jennifer Curnow, Christopher Ward*Northern Blood Research Centre, University of Sydney**Dept of Haematology and Transfusion Medicine, Royal North Shore Hospital, St Leonards, NSW, Australia*

Venous thrombosis of the upper limb differs from the more common lower limb thrombosis, in its association with strenuous exercise, intravenous catheters, oestrogen therapy and the role of extrinsic vascular compression. The optimal management of upper limb venous thrombosis remains controversial, and it has been suggested that early surgical intervention improves functional recovery. We have conducted a retrospective audit of cases, seen in our thrombosis clinic, who had not undergone surgical treatment (thrombolysis, venous stent placement or rib resection). Seven cases of deep vein thrombosis (UDVT) and 5 of superficial vein thrombosis (USTP) were identified. The seven UDVT cases were all female with an average age of 41. Three cases were idiopathic, with the others associated with central intravenous lines or ovarian hyperstimulation for IVF. In contrast, the 5 USTP patients (3 female and 2 male) were older (mean age of 57), and all associated with intravenous catheters for chemotherapy. All UDVT and 80% of USTP patients received therapeutic doses of anticoagulation. Four cases of UDVT showed complete sonographic resolution of thrombus, at an average time of 4.3 months. Three others showed partial resolution at a mean follow-up of 9.3 months. 5/7 of UDVT cases had residual signs at follow-up, including mild arm swelling, erythema and prominent superficial veins. However, all UDVT patients reported good functional recovery and had returned to normal activities. This preliminary analysis suggests that medical management (anticoagulation) of UDVT can produce good functional results, but that a significant subset will have residual thrombus and mild post-thrombotic changes. A similar review of surgically-treated UDVT patients will be conducted to assess rates of functional recovery and thrombus resolution.

**P27**

## **Management of Pulmonary Embolism in Pregnancy of a Patient with Known Antithrombin III Deficiency**

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### **Introduction**

Antithrombin III deficiency is the most thrombogenic of the hereditary thrombophilias. It is a rare, autosomal dominant disorder, with a prevalence of 0.02%. Untreated, it is associated with a very high (50%) risk of venous thromboembolism (VTE) in pregnancy.

### **Case Report**

We report the case of a 26 year old Gravida 1 Para 0 with congenital antithrombin III deficiency (baseline activity 40%), who presented at 24 weeks with an extensive left leg DVT. She had a strong family history, but no personal history, of thromboembolic disease. She had been commenced at 14 weeks on prophylactic LMW heparin, at a dose of 40mg/day. Despite treatment with LMW heparin at 1mg/kg bd, she was readmitted 3 days later with bilateral pulmonary emboli.

Following international discussion, she was treated with human derived ATIII factor replacement, in conjunction with therapeutic LMW heparin. ATIII boluses were administered daily until a steady state was reached, then on a 3 times a week basis for a further 8 weeks. ATIII levels were maintained between 80-120%. Anti Xa levels were maintained at 0.6-1.0 iu/ml at 4 hours post dose.

The patient was readmitted at 38+5/40 weeks for induction of labour. A continuous IV heparin infusion was commenced, and ATIII boluses were given to maintain therapeutic levels for 1 week post-partum.

An emergency Caesarian section was performed due to failure to progress at 18 hours. Post-operatively, IV heparin was run for 48 hours, before conversion to LMW heparin. Due to patient preference, she has continued on heparin during the period of breast feeding. She and her baby continue to do well.

### **Discussion**

Review of the literature reveals that evidence-based guidance on management of these women is very limited. Guidelines suggest that all women with ATIII deficiency, irrespective of their personal history, should be considered at high risk for VTE during pregnancy, and thus should receive therapeutic dose heparin from early pregnancy. Treatment of pregnancy-associated VTE in patients with ATIII deficiency is controversial. It has been suggested that plasma derived ATIII concentrate may be useful in those patients who have unusually severe thrombosis, have difficulty in achieving adequate anticoagulation or develop recurrent thrombosis despite adequate anticoagulation.

However, there remain many unanswered questions at present, regarding the use of ATIII concentrates (both plasma derived, and the newer recombinant preparation), in this setting. There are no trials comparing the efficacy of ATIII as a single agent in VTE treatment, versus a potentially synergistic effect when combined with heparin. This case report documents the successful use of plasma-derived ATIII in a patient with congenital antithrombin deficiency complicated by pregnancy associated VTE.

**P28****Pulmonary Embolism Complicating Sequential Therapy with Novoseven and FEIBA for a High Titre FVIII inhibitor in a 6 Year Old with Severe Haemophilia A**

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**Aim**

Inhibitor formation in children with severe haemophilia A remains the most significant complication of FVIII therapy. Bleeding episodes in individuals with a FVIII inhibitor involves the use of haemostatic agents such as recombinant activated Factor VII (rFVIIa; Novoseven) and activated prothrombin complex concentrates such as FEIBA. The response to these agents is variable between patients, and sequential use of rFVIIa and FEIBA in patients poorly responsive to both agents used alone has been studied by one US centre. This centre has not reported any significant thrombotic complications. We report a case in which a pulmonary embolus (PE) developed in a child treated with both FEIBA and rFVIIa.

**Method**

Case report.

**Result**

A 6 year old child undergoing immune tolerisation with high dose FVIII and Rituximab presented with a left knee haemarthrosis not responding to rFVIIa therapy (200µg/kg 2 hourly). Switching to FEIBA (50 U/kg b.d.) led to resolution of the haemarthrosis, but he represented with dental bleeding 3 days later. Six loose teeth were extracted. However, despite increasing doses of FEIBA (50 U/kg 8 hourly) he continued to bleed from a dental socket. Topical antifibrinolytics, fibrin glue and re-suturing had no effect. At this point the fibrinogen and platelet counts decreased to 1.8 g/L and  $171 \times 10^9/L$ , respectively. The dental bleeding finally responded to rFVIIa and oral antifibrinolytics. Twenty one days after initial presentation the patient was admitted with pleuritic chest pain. A pulmonary embolus was confirmed with a CT angiogram.

**Conclusion**

The prothrombotic potential of both FEIBA and rFVIIa should not be underestimated. This is the second case that we are aware of, in which a PE has complicated sequential use of these haemostatic agents in patients with inhibitors. This case highlights the potential risks and the difficulty of obtaining adequate haemostasis during bleeding episodes in individuals with FVIII inhibitors.

**P29**

## **Successful Augmentation Mammoplasty in Women with Bleeding Disorders**

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### **Aim**

We report our single centre experience of augmentation mammoplasty in women with bleeding disorders. An underlying bleeding disorder has been considered an absolute or relative contraindication to this procedure. A search of Medline failed to find any reports on the outcome of this surgical procedure in women with a bleeding disorder. The aim was to describe the outcome of this procedure in a cohort of patients.

### **Method**

Retrospective cohort study of cases who had undergone augmentation mammoplasty at one centre.

### **Result**

To date 6 women have undergone 6 primary augmentation mammoplasties, and 2 further augmentations (1 unilateral and 1 bilateral). The two women who underwent revisions of their mammoplasties did so for cosmetic reasons. The underlying bleeding disorders in these 6 women was symptomatic carrier of haemophilia B (n=2), von Willebrand disease (n=2), FXI deficiency (n=1), and platelet function disorder (n=1). Haemostatic prophylaxis was provided with FIX concentrate (n=2), recombinant FVIIa (n=1), tranexamic acid alone (n=1) and DDAVP (n=4). Of the 4 procedures utilising DDAVP, asymptomatic hyponatraemia was observed on 2 days out of a total of 14 treatment days. The median length of inpatient stay was 7 days (range 5-14 days). The median length of time to removal of the drains from the operative site and total fluid drained were 4.5 days (range 3-7 days) and 162 mL (range 90-860 mL). Only one patient had a post-operative haemorrhage, which required a re-operation, despite normal FIX levels.

### **Conclusion**

In conclusion 7 bilateral and one unilateral augmentation mammoplasties have been performed successfully and without excess bleeding complications in women with an underlying bleeding disorder. A bleeding disorder should not be considered an absolute contraindication to this surgical procedure.

P30

## Extradural Haematoma with Spinal Cord Compression in a Neonate Following Lumbar Puncture as the Presentation of Severe Haemophilia A

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### Introduction

We report a case of an extensive extradural haematoma with spinal cord compression following a lumbar puncture in a neonate with previously undiagnosed severe haemophilia A. Bacterial meningitis is a significant cause of morbidity and mortality in the neonatal population. Consequently, lumbar puncture is considered an important part of a neonatal sepsis work-up and this is routinely performed without coagulation screening. Spinal haematoma is an uncommon complication of lumbar puncture. However, the risk is increased in patients with acquired or congenital coagulopathy.

### Case report

A 3920g baby boy was delivered at 41 weeks gestation by Caesarean section without labour following failed induction. At delivery there were signs of chorioamnionitis. Within 24 hours the baby experienced fever and respiratory distress. A septic work-up was performed including lumbar puncture, and antibiotics were commenced. Subsequently, the baby experienced apnoeic episodes and later developed neurologic signs. MRI revealed an old, small subependymal haemorrhage and an extensive acute extradural bleed extending from the base of the brain to the level of L2 with two focal areas of spinal cord compression. At that time a coagulation profile revealed normal PT and fibrinogen, and a prolonged APTT at 100s that corrected when mixed with normal plasma. FFP was administered to the neonate at 15mL/kg while awaiting further test results. Factor VIIIc level was 0.01, with normal FIX and vWF levels consistent with the diagnosis of severe haemophilia A. Treatment with recombinant factor VIII was initiated at 50IU/Kg bd achieving adequate FVIIIc levels. Urgent surgical intervention was performed with laminectomy at multiple levels resulting in successful decompression of the haematoma within 6 hours of the MRI scan and 24 hours of the LP. Recombinant FVIII was continued at full treatment dose for 14 days followed by three times per week prophylaxis. An infusaport was inserted at age 11 days under rFVIII cover. Despite rapid identification of the haematoma and management with factor replacement and surgical decompression, the infant has been left with some neurologic deficit in the lower limbs requiring physiotherapy, and a neurogenic bladder requiring intermittent catheterization, and thereby requiring ongoing prophylactic rFVIII. Genetic testing has revealed a previously undescribed splicing mutation c.144-1G>C in the factor VIII gene, with confirmation of maternal carriage.

### Conclusion

Spinal haematoma may complicate lumbar puncture in neonates with undiagnosed haemophilia. This is one of the youngest cases reported with this rare but devastating complication.

**P31**

**A Bloody Good High**

Irene Goodhew<sup>1</sup>, Kent Chapman<sup>1</sup>, AnoopEnjeti<sup>1</sup>, Ian White<sup>2</sup> and Michael Seldon<sup>1</sup>  
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*2 Department of Toxicology and Pharmacology, Calvary Mater Hospital, Newcastle, NSW, Australia*

**Case Report**

We report an unusual acquired bleeding problem in a 21 year old female who presented to the hospital for sudden onset of abdominal pain, distension and extensive bruising. She was markedly pale with an Hb of 75G/L with a coagulation profile showing both the PT and APTT in an unrecordable range.

A CT scan showed extensive intra-abdominal bleeding and a possible ruptured ovarian cyst. She was resuscitated with blood products, including fresh frozen plasma. Detailed coagulation testing showed her to be deficient in Factor II, VII, IX and X. These are Vitamin K dependent factors produced in the liver and the scenario was consistent with Vitamin K antagonism. There were no inhibitors detected. With extensive blood product support and intravenous Vitamin K for the next 48-72 hours her coagulation profile returned back to normal. She had a subsequent laparoscopy, which showed extensive intra-abdominal haematoma but no other pathology.

She denied any access or abuse of warfarin. She had a reasonably good diet and had no family history of bleeding. On further enquiry reported having smoked marijuana at varying intervals in the recent past. She was reviewed by the toxicologist who raised the possibility of her marijuana being laced with a 'superwarfarin', bradifercoum, which has been reported to cause bleeding. As the recovery time in this patient was too short, unlike with bradifercoum, this was more likely to be warfarin toxicity.

A warfarin level on the post transfusion sample was 3.5mg/L which confirmed toxicity by vitamin K antagonist although the exact source for this was not clear. The possibilities are surreptitious ingestion or smoking marijuana contaminated with sweet clover although no other cases of such bleeding have been reported in this area. In conclusion, this represents an unusual and severe bleeding problem which is consistent with vitamin K antagonism.

**P32****Thrombophilia Related Factors and their Response to Thromboprophylaxis:  
Case Study of Pregnancy Loss Women**Catharina Suharti Suyono*Dept of Medicine, Dr Kariadi Hospital/School of Medicine, Diponegoro University,  
Semarang, Indonesia***Background**

Abortion or fetal loss is serious problem in a pregnant woman. Some causes of pregnancy loss are known, but another cases did not clearly yet. We identified thrombophilia related factors in unknown causes. The nadroparine calcium was used to thromboprophylaxis throughout their pregnancies.

**Methods**

We evaluated medical records of 7 pregnant women who had pregnancy loss history. Four factors had been identified: Protein C, Protein S, ACA IgG and ACA IgM. In several cases also identified Fibrinogen, Platelet Agregation Test, and AT-III. All cases received nadroparine calcium during pregnancies.

**Result**

Among 7 cases of pregnancies loss women, 3 cases revealed Protein S deficiencies, 2 cases revealed both ACA IgG and ACA IgM positive, 1 case ACA IgG positive and 1 case ACA IgM positive. After nadroparine calcium thromboprophylaxis, 6 cases reach mature gestation and 1 case got spontaneous abortion.

**Conclusions**

This study did not completely identified thrombophilia related factors of pregnancy loss women. Most cases (6 cases ) reach mature gestation preventing by nadroparine calcium. Cause of spontaneous abortion in 1 case did not clear.

**Keywords**

Thrombophilia related factors, thromboprophylaxis, pregnancy loss women

**P33**

**Case Report: Pseudohomozygosity for Activated Protein C Resistance**

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2. *Liverpool Hospital, Liverpool NSW Australia*
3. *Institute of Medical and Veterinary Research, Adelaide SA Australia*

**Aim**

To investigate a discrepancy between a heterozygous Factor V Leiden genotype and a phenotype of APC resistance in the homozygous range, in an individual with DVT and PE.

**Method**

Blood samples were collected from an individual with DVT/PE to re-confirm a previous finding of FVL genotype/phenotype discrepancy. Additional blood from 5 siblings was similarly tested. APC-Ratios, FV:C, INR, APTT and liver function tests were determined in each family member. APC-Ratios were determined by each of two venom-based kits: the Gradileiden V test and the Pentapharm Pefakit test.

**Result**

The DNA tests showed five of six siblings to be heterozygous for the FVL (G1691A), and the sixth negative. Of the 5 FVL heterozygotes, three had APC ratios in the range normally associated with heterozygosity, and two had APC ratios in the range normally associated with homozygosity for FVL. This finding was consistent in both clotting tests. The latter two individuals had mildly reduced levels of FV:C (56% and 66%, Ref. Range 70-120%), while the other three had clearly normal FV:C levels. INR, APTT and liver function tests were normal in all siblings.

**Conclusions**

The most likely explanation for the FVL genotype/ APCR discrepancy in two siblings is the presence of compound heterozygosity for FVL and FV deficiency. The presence of a FV null mutation in trans with FVL results in all the circulating FV protein being FVL, hence the homozygous APCR phenotype. This has been described in the literature as FVL pseudohomozygosity. Thrombosis-free survival curves in family studies suggest that clinically these individuals behave like true homozygotes rather than heterozygotes [1]. Though rare, such cases would not be detected by DNA testing alone.

1. Paolo Simioni et al. Blood 2005, 106 (7): 2363-2365.

**P34****Trial of the CoagUChek XS in Monitoring INR in a Clinical Environment**

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*Haematology Dept, Sullivan Nicolaides Pathology, Brisbane, Australia*

The CoagUChek XS INR Point of Care testing system is marketed as a simple, fast and convenient method for monitoring patients on oral anticoagulation therapy. The test uses capillary blood obtained from a finger prick to provide an INR result within a few minutes. This allows monitoring of therapy to be done by the general practitioner or the patient and avoids the time delay in obtaining a result. The patient is then able to receive follow up instructions at the time of consultation.

Sullivan Nicolaides Pathology has performed a trial involving patients referred by clinicians from a local general practice clinic. Patients were tested using the CoagUChek XS instrument and the results obtained from this technique compared with standard laboratory INR measurements, performed on a venous collection. Over 160 comparisons have been made, with results demonstrating good correlation between the different methodologies across a large range of INR values. This comparison acknowledges the value of point of care testing and has proven its utility in providing consistent INR results in clinical practice.

**P35**

## **Accuracy and Clinical Usefulness of CoaguChek XS Point of Care Device in Patients Commencing Warfarin Therapy in a Community Setting**

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*Flinders Medical Centre, South Australia*

### **Aim**

To assess the accuracy and clinical usefulness of the CoaguChek XS portable international normalised ratio (INR) device during warfarin initiation in patients receiving concomitant enoxaparin therapy in a community setting.

### **Method**

337 paired laboratory (ACL Futura, Thromborel S) and point of care (POC) INR readings (CoaguChek XS, Roche) were collected from 99 consecutive patients commencing warfarin at home through hospital at home program (H@H) between January and August 2006. 16 trained nurses performed POC INR measurements using 4 CoaguChek XS analysers. All patients also received therapeutic dose enoxaparin. Microsoft Excel was used to plot laboratory INR against POC INR, linear regression and derive Bland-Altman plots. The percentages of paired INR measurements within 0.5 INR units of each other and within 0.8 INR units of each other were calculated for laboratory INR values of < 2, 2-3.5 and >3.5 INR units. The clinical usefulness of CoaguChek XS was assessed using published criteria for clinical agreement.

### **Result**

Using linear regression analysis there was an excellent correlation between the two measures ( $r^2=0.9514$ ). There was no evidence of a trend in difference with increasing magnitude of the measurement on Bland-Altman plot. Overall, 93.5% of POC INR readings were within 0.5 of laboratory INR values and 95.7% of POC INR readings were within 0.8 of laboratory INR values. Clinical agreement as assessed by expanded criteria occurred in 99.7% of cases and by narrow criteria in 99.4% of cases.

### **Conclusion**

CoaguChek XS is suitable for INR monitoring in patients commencing warfarin and receiving concomitant enoxaparin therapy. Accuracy of the device is excellent across INR values, which is in contrast to our earlier experience with CoaguChek S device.

**P36****Evaluation of IL D-Dimer HS on the TOP CTS and the D-Dimer Method Measured by the Miniquant**

Nick Michalopoulos, Kevin Tan, Jill Finlayson, Brad Augustson  
*PathWest, Perth, Australia*

We have previously evaluated the mini-Vidas D-dimer method and it is now the established assay in our laboratory. However there is a need to find a suitable replacement to achieve a better turn around time (TAT) while not losing any sensitivity and if possible improving specificity. We have recently purchased two ACL TOP CTS analysers and have evaluated the IL D-Dimer HS method with the aim to improve TAT. At the same time, we are also in a position to evaluate the D-Dimer latex method measured on the MinQuant-1.

Consecutive outpatients (from the Emergency Department) with clinically suspected DVT or PE had a D-Dimer measured at presentation. Clinical assessment was based on a three month follow up review for each and the sensitivity, specificity and negative predictive value (NPV) of the assay was compared to the mini-Vidas. The mini-Vidas has been assessed previously and presented in poster format in 2001 at the ISTH.

A total of 200 patients were included and results will be presented for both the IL D-Dimer HS and MinQuant-1 methods. Data will be presented to highlight the usefulness and application of each method.

**P37**

## **Measurement of Tissue Factor Procoagulant Activity and the Effect of Plasma-derived Microparticles**

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### **Aim**

Tissue factor (TF) microparticles are submicron sized, membrane-derived vesicles released by cells in response to activation or apoptosis. Although they are likely to have a significant role in thrombogenesis and cardiovascular diseases, a lack of sensitivity and correlation between methods for their measurement has limited the understanding of their physiological role. The aims of this study were to 1) modify and validate a method to measure TF procoagulant activity (TFPA), and 2) determine the TFPA in plasma derived microparticles from normal donors.

### **Methods**

A clotting assay to measure TFPA was modified from a previously published prothrombin time based method using pooled normal plasma (PNP). Assay specificity was assessed using a monoclonal anti-TF antibody and factor VII and X deficient plasmas. Microparticles were isolated from the plasma of normal donors (n=24) by centrifugation and assessed for TFPA.

### **Results**

Serial dilutions of rabbit brain thromboplastin generated a dose-dependent increase in the clotting time of PNP (range: 28.7 – 249.0 seconds). TFPA was generated in a factor VII and X dependent manner and was reduced by a monoclonal anti-TF antibody. The intra- and inter-assay coefficients of variation were 3% and 18%, respectively. Microparticle fractions isolated from normal donors demonstrated mean +/- SD clotting times of 205.9 +/- 26.2 seconds (range: 142.6 – 246.4 seconds; buffer control > 300 seconds) in PNP.

### **Conclusion**

The TFPA assay was modified and validated using an anti-TF antibody and factor VII and X deficient plasmas. Microparticle fractions collected from normal donors generated variable clotting times in the TFPA assay. Further studies are required to evaluate methods for the measurement of microparticle associated TF, which will allow improved determination of its significance in disease states.

**P38****Platelet Function Testing Using a Modified Cone and Plate Assay on the DiaMed Impact-R**

Beatrice Muj, Alessandra Bianchi

*Haematology, Diagnostic Pathology Unit, Concord Hospital, Concord West, NSW***Aim**

The Impact-R is a cone and plate analyzer (CPA) for assessment of platelet adhesion and aggregation under physiological flow conditions. This study is to demonstrate platelet aggregation induced by different agonists using a modified CPA assay on the Impact-R as compared to traditional platelet aggregometry.

**Method**

Blood was collected with a loose tourniquet into 3.2% citrate tubes from 12 normal subjects, 5 males and 7 females, and 5 patients on antiplatelet therapy.

Platelet function testing was performed on each sample by three methods:

1. Chronolog Whole Blood Aggregometer (WBA) using ADP (5uM/ml), Collagen (2ug/ml), Arachidonic Acid (0.5mM) and Ristocetin (1.0mg/ml). Impedance aggregation results are expressed in ohms.
2. CPA assay on the Impact-R measuring the % surface coverage (SC) and the platelet aggregates size (AS).
3. A modified CPA method, each sample was pre-incubated for 2 mins with the same agonists as in method 1. The %SC was measured on the Impact-R as in method 2.

All testing on each sample were performed within 3 hours from collection.

Mean and SD were calculated on results from normal subjects and were used to compare with each patient's results.

**Results**

The modified CPA assay pre-incubation of platelets with all agonists in normal subjects showed markedly reduced %SC compared to the Basic CPA %SC, demonstrating that pre-incubation of blood with the agonists results in a transient micro-aggregates formation, resulting in a significant reduction of %SC in the modified CPA assay.

Patients on antiplatelet drugs showed a decreased aggregation response to some of the agonists tested in the WBA. This pattern of reduced aggregation corresponded in the modified CPA assay to an increase %SC when the patient result is compared to the normal range.

**Conclusion**

The modified CPA assay on the Impact-R may be useful for assessing the effect of different agonists on platelet aggregation and for monitoring antiplatelet therapy.

P39

**Comparison of the PFA-100 Assay with von Willebrand Testing in the Laboratory Assessment of Response to DDAVP in Coagulation and Platelet Disorders**

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**Aim**

DDAVP is widely used in bleeding disorders prior to invasive procedures, however a therapeutic trial is required in advance to assess response to this agent. This is often utilizes von Willebrand factor (VWF) assays, however in many laboratories, this has a relatively slow turnaround time and limited availability, limiting its role in the acute setting. We aim to compare the performance of the more rapid and simpler PFA-100 assay, to the VWF assay in the assessment of response to DDAVP in a variety of bleeding disorders.

**Methods**

Patients with bleeding disorders (both paediatric and adult) undergoing a DDAVP trial in our institution were retrospectively identified from a laboratory database. Diagnosis and clinical details were confirmed via chart review. DDAVP was administered at 0.3 µg/kg (maximum 20µg) via intravenous infusion over 30 minutes. VWF\* and PFA-100‡ assays were performed at baseline, then 60 minutes following DDAVP infusion, and recorded as abnormal if either component of the PFA-100 was prolonged or any part of the VWF assay was below the lower limit of the reference range.

**Results**

Sixty two patients were identified between May 2000 and January 2006, with a median age of 22 years (range 1.4 to 72), females 55%. Represented in this group were: VWD type 1 63%, VWD type 2 11%, platelet dysfunction 3%, unclassified 8% and coagulation disorders 3%. Results are presented in the table below:

Disease	DDAVP	All patients	VWD-type1	VWD-type 2	Platelet dysfunction
PFA abnormal (%)	Pre	50/61 (82%)	32/39 (82%)	5/6(83%)	7/8 (88%)
	Post	15/62 (24%)	7/39 (18%)	7/7(100%)	2/8 (25%)
VWF abnormal (%)	Pre	41/62 (66%)	29/39 (74%)	5/7 (71%)	1/8(13%)
	Post	8/62 (13%)	4/39 (10%)	3/7 (43%)	0/8 (0%)

**Conclusions**

These results show the PFA-100 is at least comparable to formal vonWillebrand factor testing in patients with type 1 VWD in assessing responsiveness to DDAVP. Further study is required to assess whether correction of the PFA-100 time correlates in a reduction of clinically significant bleeding.

‡ Collagen/ epinephrine and collagen/ ADP cartridges

\*vWF antigen and activity, vWF collagen binding activity and factor 8 levels

## P40

**PFA-100® Closure Times and Blood Loss During Total Knee Arthroplasty in Patients Taking NSAIDs**

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**Aim**

PFA-100® is useful for monitoring various congenital and acquired platelet dysfunctions, and the effect of aspirin. However the lack of clinical outcome correlation remains the weakness of this monitor. We previously showed arthroplasty patients taking NSAIDs preoperatively have a wide range of PFA-100® closure times. This study aims to evaluate whether different pre-operative PFA-100® closure times in these patients may predict intraoperative bleeding differently.

**Method**

With IRB approval, we recruited patients taking NSAID and undergoing total knee arthroplasty. All patients were allowed to continue the NSAID up to the time of surgery. No LMWH was used. Preoperative blood was taken for PFA-100® ADP and epinephrine closure times (ADPCT and EPICT) measurement immediately before surgery. Non-closures were assigned ADPCT or EPICT values of 300 s. Surgery was performed by the same group of surgeons in a standardised manner under general anaesthesia. No deliberate hypotension was performed and body temperature was maintained. Intraoperative blood loss, duration of operation, fluid requirements and surgeon assessment of ease of haemostasis were the outcome measures. Linear correlation was sought between ADPCT and EPICT and these outcome measures.  $P < 0.05$  was considered statistically significant.

**Results**

30 patients were recruited (age =  $66.3 \pm 8.6$ ; BMI =  $27.4 \pm 5.3$ ; M/F = 7/23), representing 51 knee arthroplasties. Mean ADPCT was  $120 \pm 60$  s and EPICT was  $199 \pm 79.6$  s. There was no significant correlation between ADPCT or EPICT and any of the outcome measures. However, intraoperative blood loss was significantly correlated with duration of operation ( $r = 0.43$ ,  $P < 0.01$ ), surgeon assessment of haemostasis ( $r = 0.55$ ,  $P < 0.01$ ) and transfusion of red cells ( $r = 0.59$ ,  $P < 0.01$ ) and colloids ( $r = 0.35$ ,  $P < 0.05$ ).

**Conclusion**

Both ADPCT and EPICT of the PFA-100® fail to predict intraoperative blood loss, surgeon assessment of bleeding or intraoperative fluid requirement during total knee arthroplasty.

**P41**

**Restructuring the Haematology Coagulation Laboratory Practical for Year 2 Students at the Australian National University Medical School to Make it Clinically Relevant**

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**Aims**

Medical education in Pathology remains challenged to find teaching strategies that actively engage students in the learning process, especially in the laboratory. Laboratories that accompany medical courses are often non-inquiry oriented, with activities that promote learning of technical skills but not skills of clinical integration [1]. The coagulation laboratory practical for year 2 students of the Australian National University Medical School was first held in 2005. This was formatted along traditional lines, but student feedback indicated that it was not perceived as relevant to their needs. This project made an attempt to restructure the practical to make it more interesting and clinically relevant for students.

**Methods**

An e-survey with open-ended questions was posted to 80 medical students who participated in the practical. Survey responses were used to frame in-depth interviews for consenting students. Teacher interviews were also conducted to obtain additional perspectives. Qualitative analysis was performed on all responses and common themes identified.

**Results**

Greater clinical orientation was the predominant additional objective identified by students and teachers to improve the practical. Strategies recommended for achieving this included better time management in the practical, provision of a greater theoretical base and incorporation of case-based sessions in small group settings. Most respondents voted in favour of preserving bench work even while acknowledging that such work was deprioritised in the overall curriculum by not forming part of their assessment.

**Conclusions**

Based on the responses, a new format for the practical was set up. This involved use of a mini-lecture covering theoretical aspects, followed by a case-based session incorporating bench-work. A wind-up session with case discussion was used to tie laboratory results in with clinical management.

**References**

1 David B. Rivers. Using a course-long theme for inquiry-based laboratories in a comparative physiology course. *Adv Physiol Educ* 26: 317-326, 2002

**P42****Management and Outcome of Patients on Warfarin Presenting with PT-INR >9**

Zoe McQuilten and Merrole Cole-Sinclair

*Haematology Unit, Alfred Pathology Service, The Alfred Hospital***Aim**

The PT-INR is one of the major determinants of the risk of serious bleeding in patients on oral anticoagulant therapy. We reviewed management, risk factors and outcome in patients in whom a PT-INR >9 had been recorded.

**Method**

A retrospective single-institution review of patients on warfarin in whom a PT-INR result >9 was recorded during the period 2005- mid 2007 inclusive.

**Results**

Fifty-one patients were identified. Indications for warfarin included: atrial fibrillation in 30 (58.8%), mechanical heart valve 9 (17.7%), venous thromboembolic disease 7 (13.7%) and other 5 (9.8%). The most recent PT-INR had been performed in the preceding 2 weeks in 20 (36%) and had been within the target range (2-3 or 2.5-3.5) in 33 (65%).

Causes identified for an elevated PT-INR were: addition of a new drug 16 (31.4%), development of inter-current illness 15 (29.4%) and non-compliance or patient error 9 (17.6%).

Ten patients (19.6%) had symptoms due to bleeding at presentation. Three had an intracranial haemorrhage (two were fatal), three had gastrointestinal bleeding (two required transfusion) and four were minor (e.g. epistaxis). There were three deaths directly attributable to excessive anticoagulation.

Forty-nine patients received vitamin K (oral or parenteral). Twenty patients (39.2%) received a combination of vitamin K, fresh frozen plasma and human prothrombin complex. Warfarin was permanently ceased in thirteen patients. No bleeding complications occurred after initial presentation, regardless of the method of reversal.

**Conclusion**

A PT-INR > 9 can occur in patient groups on anticoagulation despite recent monitoring and/or a previous INR within target range. If critical bleeding develops there may be significant morbidity and mortality. Care needs to be taken with patients on warfarin, particularly those at high risk of bleeding, at the time of medication change and during an inter-current illness to avoid excessive anticoagulation. Haemorrhagic complications can be avoided with prompt reversal of warfarin therapy.

**P43**

## **Emerging Heterozygous HbS in Australia**

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A 24 year old African female presented to a GP at 8 weeks of pregnancy. A full blood count and other antenatal tests were performed. The full blood count revealed a microcytic anaemia. Iron studies and haemoglobin electrophoresis were recommended. These tests were performed at 16 weeks of pregnancy and the patient was found to be both iron deficient and heterozygous for HbS. It was advised that her partner also undergo screening for potential haemoglobinopathy/thalassaemia. He was also found to be heterozygous for HbS. There was a 25% chance that the unborn baby would have Sickle cell anaemia.

Patients with sickle cell anaemia suffer from a wide variety of complications and require life long medical care. Sickle cell anaemia is found in people with ethnic background from sub Sahara Africa, Caribbean, India, Saudi Arabia and Mediterranean countries. Prenatal screening of couples from these ethnic backgrounds is recommended. Across equatorial Africa the prevalence of heterozygous HbS ranges between 10- 40%. Significant migration from African countries, in particular Sudan, has resulted in increased prevalence of heterozygous HbS in Australia. Neonatal diagnosis of Sickle cell anaemia is of utmost importance as prophylactic penicillin is recommended, preferably within 2 months of age. Prophylactic penicillin is highly effective in reducing sepsis due to pneumococcus which is the leading cause of childhood death in those with Sickle cell anaemia.

This case highlights the potential impact of increased migration from Africa and the necessity for health professionals to be educated in regards to risk groups, diagnosis and clinical management.

**P44**

## **Haemoglobin Rothschild Presenting with Low Peripheral Oxygen Saturation by Pulse Oximetry: A Case Report**

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### **Introduction**

Genetic disorders involving haemoglobin are relatively common and often asymptomatic. We report the case of a man who was incidentally found to be hypoxaemic on oximetry performed prior to elective surgery. Investigation into the cause of his low oxygen saturation revealed the low oxygen affinity haemoglobinopathy, Haemoglobin Rothschild.

### **History**

A 46-year-old Caucasian male admitted for minor elective surgery was found on pre-operative oximetry to be hypoxaemic. Despite supplemental oxygen, his oximetry remained subnormal, however he had no clinical signs of hypoxia. Besides a mild restrictive ventilatory defect on pulmonary function tests, there was no history of lung or cardiac disease. Arterial gases on room air revealed a pH of 7.41, a pO<sub>2</sub> of 88mmHg, a pCO<sub>2</sub> of 34mmHg, bicarbonate of 21mmol/L and an oxygen saturation of 86%. These results led to the suspicion of a low oxygen affinity haemoglobin variant.

### **Results**

High Performance Liquid Chromatography showed an abnormal peak of 40.5%. The variant band moved between HbC and HbS on alkaline haemoglobin electrophoresis and with HbS on acid electrophoresis. DNA sequencing of the β-globin gene revealed the patient to be heterozygous for the codon 37 TGG→CGG mutation, causing an amino acid substitution of Tryptophan to Arginine in the β-globin chain, resulting in Hb Rothschild.

### **Discussion**

Hb Rothschild is an uncommon haemoglobinopathy characterised by decreased oxygen affinity. It is usually asymptomatic and does not show abnormalities in routine haematology parameters.

### **Conclusion**

This case demonstrates the limitation of oximetry in monitoring patients with haemoglobinopathies showing altered oxygen affinity. While these conditions are mostly asymptomatic, they raise the potential for unnecessary interventions if they are not recognised in settings where oximetry is used. However, once an altered oxygen affinity haemoglobin is discovered, oximetry may serve as a simple tool for screening patients' families, therefore preventing unnecessary investigation during future medical care.

**P45**

## **Refractory Immune Thrombocytopenia with Renal Cell Carcinoma Treated with Combined Left Nephrectomy and Splenectomy**

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*Gold Coast Hospital*

### **Summary**

We present a case of refractory ITP associated with left renal cell carcinoma treated successfully with combined nephrectomy and splenectomy.

### **Case History**

An 80-year-old male presented with epistaxis and purpura. Past history included a mechanical mitral valve replacement requiring warfarin. Examination was unremarkable. Investigations revealed platelets of  $5 \times 10^9/L$ . Other blood counts were normal. Bone marrow biopsy was normal with adequate megakaryopoiesis. A diagnosis of ITP was made. Due to bleeding and concurrent warfarin therapy, the patient received daily intravenous immunoglobulin (IVIG) and oral prednisone at 1 mg/kg. His platelet count normalised briefly but relapsed with platelets falling below  $50 \times 10^9$  on two attempts at steroid taper. Due to raised liver function tests and lactate dehydrogenase, a CT was performed. This revealed a 6.4cm left renal lesion consistent with renal cell carcinoma with involvement of left renal vein but without distant metastasis. The preferred urologic management was nephrectomy. We eventually proceeded with left nephrectomy and concomitant splenectomy and after pre-operative IVIG. Surgery was complicated by post-operative bleeding requiring repeat surgery and bradycardia requiring cardiac pacemaker. The histology revealed a clear cell renal cell carcinoma and normal spleen. The platelet count normalised and the prednisone weaned. At six months follow-up the patient is asymptomatic and platelets are normal. CT scan reveals no recurrence of carcinoma.

### **Discussion**

The association of ITP with solid tumours has been rarely reported with various solid tumours including breast, lung, oesophageal and cervical cancer. To our knowledge this is only the fourth reported case of an association between renal cell carcinoma and ITP. We also show the feasibility of a combined left nephrectomy and splenectomy.

P46

## Idiopathic Thrombocytopenic Purpura Masquerading as a Metastatic Ovarian Tumour

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### Background

Autoimmune idiopathic thrombocytopenic purpura (ITP) is a relatively common disorder which infrequently manifests with severe bleeding. Solid tumours, including ovarian malignancy and cystadenomas, have been associated. We present a case of ITP presenting with severe pelvic bleeding in the context of a previously undiagnosed ovarian tumour.

### Case report

A previously well 33 year old female presented with 24 hours of petechial rash, wet purpura and severe menorrhagia, associated with two days of increasing lower abdominal pain and swelling. On examination she was febrile, hypovolaemic and noted to have a firm, tender inferior abdominal mass. She was taking no medications or supplements.

Full blood examination revealed Hb 73g/L, WBC  $20 \times 10^9/L$  (mature neutrophilia without leucoerythroblastic features). CA-125 and CA19-9 were elevated (157.4 kU/L [normal <35kU/L] & 100 kU/L [normal <37kU/L] respectively); other tumour markers, viral serology (including HIV), an autoimmune screen and routine biochemistry were normal. Computed tomography scanning showed a complex, fluid filled 12×10×13cm pelvic mass, confirmed on ultrasound as a cystic, haemorrhagic lesion arising from the left ovary. No other structural abnormalities were detected in the chest, neck or abdomen.

A bone marrow biopsy demonstrated left-shifted megakaryocytic hyperplasia with no involvement by non-haematopoietic cells. The patient was treated with red cell transfusion, a single dose of pooled platelets, intravenous immunoglobulin (1g/kg) and prednisolone (50mg daily). She experienced a brisk response with platelets rising to  $215 \times 10^9/L$  by 72 hours. She awaits definitive surgery for a likely benign ovarian tumour complicated by haemorrhagic transformation.

### Conclusions

Although the perceived bleeding risk with ITP is low compared to thrombocytopenia associated with marrow aplasia, the presence of underlying structural abnormalities may result in dramatic haemorrhagic presentations. Bone marrow biopsy allows rapid diagnostic confirmation and remains the gold-standard, particularly where alternative differentials require exclusion. The observed association between ovarian pathology and ITP may be co-incidental, however putative mechanisms for a pathophysiological link have been proposed.

**P47**

## **Bone Marrow Examination in a Case of Childhood Cystinosis**

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### **Background**

Cystinosis is a rare autosomal recessive lysosomal storage disorder characterized by tissue deposition of intracellular cystine crystals. It is the most common cause of proximal renal tubular acidosis (Fanconi Syndrome) in children. Cystine crystals are often most easily clinically demonstrable in the cornea and bone marrow. The diagnostic test is a leukocyte cystine level. The assay is performed three times each year at one laboratory in Australia.

### **Case Presentation**

We present a 17 month old male, first child of non-consanguineous parents (Hungarian and Australian background) who presented with failure to thrive, vomiting and mild gross motor developmental delay. Investigations revealed hypophosphataemia, hypokalaemia, and acidosis with elevated urine and plasma amino acids. Proximal renal tubular acidosis was confirmed and Cystinosis suspected. To facilitate an early diagnosis and commencement of treatment, a general anaesthetic was arranged for a bone marrow and an eye examination, prior to the white cell cystine assay result being available.

### **Results**

Bone Marrow Aspirate revealed normal trilineage haemopoiesis with numerous cuboid and rectangular colourless crystals within and external to macrophages. These crystals exhibited birefringence under polarized light. Together with ophthalmological confirmation of crystals, these findings were used to confirm the diagnosis. Treatment was commenced with cysteamine, electrolyte and nutritional replacement.

### **Discussion**

The incidence of cystinosis is 0.5-1:100,000 live births and is most common in the Northern European population. An ovid-median literature search identified a number of case reports over 40 years in which bone marrow examination has assisted in the diagnosis of childhood cystinosis. Cystinosis commonly progresses to end stage renal failure in children, the age at which this occurs is delayed with early institution of treatment.

This is the first Australian case report describing the findings to our knowledge.

(Poster to include photos of bone marrow aspirate).

P48

## Dietary Folate Deficiency in Overweight Australians

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### Background

Clinical folate deficiency has previously been rare in the Australian population. We present two overweight patients who presented with megaloblastosis due to severe folate deficiency, as a result of an energy rich but micronutrient poor diet.

### Case Reports

A 30-year-old previously well man presented with syncope. He was overweight, with body mass index (BMI) 28kg/m<sup>2</sup>. Full blood examination (FBE) revealed pancytopenia: haemoglobin (Hb) 68g/l (normal 125-160), mean cell volume (MCV) 103fL (normal 80-95), white cell count (WCC) 3.1x10<sup>9</sup>/L (normal 4.0-12), neutrophil count 1.5x10<sup>9</sup>/L (normal 2-8), platelets (Plt) 87x10<sup>9</sup>/L (normal 150-400). Blood film: moderate anisopoikilocytosis with macrocytosis and nucleated red blood cells (RBC), and hypersegmented neutrophils. Bone marrow aspirate demonstrated grossly megaloblastic haematopoiesis. Serum folate was <1.8 (normal >6.0), and serum B12 115pmol/L (normal 130-857). The diagnosis of severe folate deficiency was made. The patient was screened for coeliac disease – anti-antigliadin IgG, IgA antibodies and anti-tissue transglutaminase IgA antibodies negative. Dietary history revealed an energy-rich diet comprised largely of meat and processed carbohydrates, with few folate-rich foods. Folate and B12 supplementation was commenced, and the patient commenced a dietary education program. His haematological indices rapidly normalised.

A 39-year-old woman presented with a two-month history of lethargy. Past medical history included hepatitis C infection and morbid obesity (BMI 65kg/m<sup>2</sup>). FBE revealed pancytopenia: Hb 61g/L, MCV 100fL, WCC 0.98x10<sup>9</sup>/L, neutrophil count 0.38x10<sup>9</sup>/L, and Plt 28x10<sup>9</sup>/L. Blood film: marked anisopoikilocytosis with macrocytosis, nucleated RBC, and left-shifted granulopoiesis with giant bands and hypersegmented neutrophils. Further investigation revealed severe folate deficiency, with undetectable serum and red cell folate, and serum B12 of 178pmol/L. Screening tests for coeliac disease were negative. Dietary history revealed an energy-rich diet composed almost entirely of jelly and condensed milk, with no folate-containing foods. With folate and B12 supplementation, her haematological indices normalised.

### Conclusions

The emergence of highly energy-dense but micronutrient poor foods may have contributed not only to an 'obesity epidemic' in the Australian population, but also to a resurgence of clinically significant nutritional deficiency. Assessment of nutrient intake is therefore an important component of overall health maintenance, even in apparently well-nourished overweight or obese individuals.

**P49**

## **Modeled Cost-Effectiveness Analysis of Autologous Haemopoietic Stem Cell Transplant Compared with Standard Dose Chemotherapy for Relapsed, Aggressive Non Hodgkins Lymphoma**

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In 1995 superior survival for High Dose Chemotherapy (HDC) vs standard dose chemotherapy (SDC) was demonstrated in a European randomized trial and HDC became treatment of choice for relapsed aggressive NHL.

### **Study Aim**

Calculate Incremental Cost-Effectiveness Ratio (ICER) for HDC compared to SDC using Australian costs and European trial data

### **Methods**

21 pts transplanted from 1995 to 2002 with characteristics similar to the European trial were identified from the Newcastle Mater Hospital (NMH). All drug, transfusion, inpatient and outpatient attendance and similar relevant data from start of salvage treatment up to 100 days following conditioning and AHSTC were obtained and costed. SDC costs required modeling as all suitable pts are planned to receive HDC if possible, therefore no concurrent SDC arms exist. SDC cost for 6 cycles of combination chemotherapy was modeled from data available from the cycles received prior to HDC. The European trial survival data were used due to small numbers and follow-up short in the NMH cohort. A lifetime estimate of patient-years gained by HDC versus SDC was calculated from the area under survival curves (AUC) of HDC and SDC from zero to infinity. Costs and benefits were discounted in advance at 5% per annum. The ICER was calculated according to formula: Incremental Cost / Incremental Benefit =  $(\text{Costs}_{\text{HDC}} - \text{Costs}_{\text{SDC}}) / (\text{AUC}_{\text{HDC}} - \text{AUC}_{\text{SDC}})$ .

### **Results and Conclusion**

Cost for HDC and SDC were \$AU37,491 and \$AU33,360 respectively, with an incremental cost of \$AU4,131. The  $\text{AUC}_{0-\text{infinity}}$  were 6.5 and 3.5 patient life years respectively, leading to an incremental benefit of 3.0 life years gained. This gives an ICER of AU\$1,377 per discounted life year gained. Compared to published studies of ICERs for HDC vs SDC in multiple myeloma and metastatic breast cancer these results support HDC as a cost-effective treatment in relapsed aggressive NHL

## P50

### A Bone of Contention: Best Approach for Harvesting Bone Marrow to Maximize TNC and CD34+ Cell Counts

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#### Background

Bone marrow (BM) has been utilized as a source of stem cells for transplantation for many years. Although the use of BM has decreased with the advent of mobilized stem cells, utilization is increasing once again due to the lower rate of chronic GVHD associated with BM as a stem cell source. There is no generally accepted technique for harvesting BM. Protocols vary both in relation to the volume of each aspirate and the number of aspirates performed at each puncture site.

#### Aim

To investigate whether differences in the volume and number of aspirates taken at the time of BM harvest affect the number of mononuclear cells (particularly CD34<sup>+</sup> and CD3<sup>+</sup>) collected.

#### Method

Consented adult donors were positioned in the prone position for collection of bone marrow from the posterior iliac crests. Triplicate samples of 5, 10 and 20ml aspirate volumes were taken at the start. Subsequent harvesting was performed using 10ml aspirate volumes. Triplicate samples were taken from the 10ml aspirates when harvest volumes of 250, 500, 750 and 1000mls were attained. Samples were analysed for total nucleated cell (TNC) count, CD34<sup>+</sup> and CD3<sup>+</sup> cell counts.

#### Results

The following table shows results from 4 BM harvests. Total cell counts ( $\pm$  SEM) taken from 5, 10 and 20 ml aspirate volumes at the beginning of harvest are shown in the third column. Cell counts from 10ml aspirates after 250, 500, 750 & 1000ml of BM have been taken are shown in the last 4 columns:

Aspirate vol (mls)		Start of harvest (Mean $\pm$ SEM)	250mls (Mean $\pm$ SEM)	500mls (Mean $\pm$ SEM)	750mls (Mean $\pm$ SEM)	1000mls (Mean $\pm$ SEM)
5	TNC x10 <sup>6</sup>	265 $\pm$ 57	Not Tested	Not Tested	Not Tested	Not Tested
	CD34 x10 <sup>6</sup>	4.2 $\pm$ 0.7				
	CD3 x10 <sup>6</sup>	22.8 $\pm$ 6.4				
10	TNC x10 <sup>6</sup>	423 $\pm$ 66	322 $\pm$ 45	220 $\pm$ 45	230 $\pm$ 81.5	147 $\pm$ 48
	CD34 x10 <sup>6</sup>	7.7 $\pm$ 3.2	5.2 $\pm$ 1.7	3.0 $\pm$ 0.5	4.4 $\pm$ 2.7	1.5 $\pm$ 0.9
	CD3 x10 <sup>6</sup>	27.6 $\pm$ 5.1	23.4 $\pm$ 7.9	17.6 $\pm$ 4.7	16.4 $\pm$ 9.3	12 $\pm$ 6.4
20	TNC x10 <sup>6</sup>	569 $\pm$ 64	Not Tested	Not Tested	Not Tested	Not Tested
	CD34 x10 <sup>6</sup>	7.8 $\pm$ 1.2				
	CD3 x10 <sup>6</sup>	47.9 $\pm$ 15.1				

#### Conclusion

The aspiration of increasing volumes of BM does not produce a proportional increase in total numbers of NC, CD34<sup>+</sup> or CD3<sup>+</sup> cells collected. The highest ratio of CD34<sup>+</sup> to CD3<sup>+</sup> cells is observed with 10ml aspirates. The data suggest that for any given volume of marrow, multiple aspirations of smaller volumes will result in collection of greater numbers of all cell types. This conclusion needs to be verified by appropriate study. The data also indicate that there is a sudden drop in yield of both CD34<sup>+</sup> and CD3<sup>+</sup> cells once 750ml of BM has been aspirated in 10 ml aliquots. Further harvesting beyond this point is unlikely to be of benefit and if insufficient cells have been obtained, a second harvest may be a better option than continued aspiration on the same occasion.

**P51**

## **Successful Treatment of Transplant Associated Microangiopathy with Rituximab**

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### **Aim**

Transplant associated microangiopathy (TAM) does not respond to plasma exchange. Rituximab has been reported as effective in treating this condition. In this case report, we describe resolution of severe TAM following rituximab infusion.

### **Method**

A 26 year old woman underwent a matched sibling allogeneic bone marrow transplant for very severe aplastic anaemia. She received conditioning with ATGAM and cyclophosphamide. GvHD prophylaxis comprised ciclosporin and methotrexate. The patient experienced significant post-transplant complications including drug-induced cardiomyopathy, acute GvHD requiring systemic steroid, and CMV viraemia. After day +34, she became thrombocytopenic (platelets  $< 10 \times 10^9/l$ ), LDH rose to 3500U/l and she had 5.4% red cell fragments in the peripheral blood. Ciclosporin was replaced with mycophenylate mofetil. The patient received 4 doses of rituximab at a dose of 375mg/ m<sup>2</sup> commencing on day +41. She did not receive plasma exchange treatment.

### **Results**

The patient suffered severe complications of TAM. At day +41 she developed seizures, and at day +52, she became aphasic, with right sided weakness. MRI brain confirmed a left frontal lobe haemorrhage. From this point, she was given regular platelet transfusions which had previously been avoided. Following treatment with rituximab, laboratory and clinical parameters steadily improved. LDH fell to 293U/l and red cell fragmentation to 0.3% at day +96. The patient recovered her speech and limb weakness, and was discharged from hospital at day +86.

### **Conclusion**

TAM is a grave complication of transplant, associated with a high mortality. Diagnostic criteria are becoming better defined but reports of its incidence vary widely. There is no accepted optimal treatment. Two recent reports have documented the successful use of rituximab for patients with TAM [1,2]. Rituximab seems to have been a life-saving manoeuvre in our patient, and further investigation into its efficacy is warranted.

### **References**

- 1 Au W, Ma ES, Lee T, Ha S, Fung AT, Lie AKW, Kwong Y. (2007) Successful treatment of thrombotic microangiopathy after haematopoietic stem cell transplantation with rituximab. *British Journal of Haematology* **137**, 475-478
- 2 Ostronoff M, Ostronoff F, Calixto R, Florencio R, Florencio M, Domingues MC, Souto Major AP, Sucupira A, Tagliari C. (2007) Life-threatening haemolytic-uraemic syndrome treated with rituximab in an allogeneic bone marrow transplant recipient. *Bone Marrow Transplantation* **39**, 649-651

**P53****LACE-conditioned Autologous Stem Cell Transplantation is an Effective Regimen for Patients with Relapsed or Refractory Hodgkin's Disease and Non-Hodgkin Lymphoma with a Favourable Toxicity Profile**

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**Aim**

To assess the efficacy, short and long term toxicity of LACE-conditioned autologous stem cell transplant (ASCT) for relapsed Non-Hodgkin lymphoma (NHL) and Hodgkin's disease (HD).

**Method**

Prospective data was collected between 1997 and 2000 on patients receiving LACE-conditioned ASCT for relapsed HD and NHL at the Alfred and Newcastle Mater hospitals. The initial 31 consecutive patients received standard supportive care. The remaining 58 patients received empirical GCSF post-ASCT. Pulmonary function tests and cardiac gated blood pool scans were done pre-ASCT and 4, 8, 12 and 24 months following ASCT.

**Results**

89 patients (HD-12, diffuse large B cell lymphoma-40, follicular lymphoma-20, T cell lymphoma-9, Burkitt lymphoma-2, other-6) received high dose chemotherapy (Lomustine, Cytarabine, Cyclophosphamide, Etoposide) and ASCT. At the time of transplant, 29 (32.6%) patients were in complete remission and 43 (48.3%) had active disease. Patients received a median of two prior treatment regimens (range 1-7).

Progression free survival (PFS) and overall survival (OS) at a median follow up of 50.3 months was 33.3% and 47.4% respectively. PFS and OS was significantly improved in patients with chemosensitive disease at the time of ASCT (53.1% vs 22.4%,  $p = 0.0036$ ; 67.5% vs 36%,  $p = 0.0002$ ).

Short term treatment related toxicity was low with WHO grade 3/4 toxicity as follows: hepatic 7%, nausea and vomiting 9%, mucositis 4%, diarrhoea 3%. There were significantly fewer infections and inpatient bed days in those receiving empirical GCSF ( $p < 0.05$ ) but no difference in antibiotic treatment duration. There was no significant long term pulmonary or cardiac toxicity and no treatment related mortality.

**Conclusion**

LACE-conditioned ASCT is an effective treatment for relapsed NHL and HD with results comparable to alternative conditioning approaches in the published literature. It has the advantage of minimal early toxicity and negligible long term pulmonary and cardiac complications. The empiric use of GCSF reduces the number of infections and inpatient stay.

**P54**

## **Cytomegalovirus Viraemia and Reactivation After Allogeneic Stem Cell Transplant**

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### **Aim**

Cytomegalovirus (CMV) is a significant problem after allogeneic bone marrow transplantation (BMT). This study aim to evaluate risk factors for CMV viraemia in a single transplant referral centre since the introduction of a uniform CMV prevention policy in the NSW BMT network.

### **Method**

This is a prospective study enrolling patients who underwent BMT at St. Vincent's Hospital, Sydney. All patients routinely received assays for CMV reactivation using CMV DNA PCR from day 0 to day +100 weekly (and thereafter as clinically indicated). Matched unrelated recipients received thrice weekly prophylactic ganciclovir; sibling matched marrow recipients received anti-CMV therapy on an expectant basis.

### **Results**

Between Oct 03 and Apr 07, 100 patients was eligible to be evaluated, 57 (57%) from an unrelated volunteer donor. Median age was 54. 70% of these patients were deemed poor risk; 45% had grade 2-4 acute GVHD. 31 (31%) patients were detected to have CMV viraemia within 100 days of BMT; 18 had unrelated donors and 13 had sibling donors, a reactivation rate of 33.3% and 27% respectively. The median time of onset and viral loads were similar. CMV sero-status in the recipient was an important predictor of subsequent reactivation. In sero-positive recipients, 15 reactivated out 36 sero-positive donors (42%), whereas 15 reactivated after having sero-negative donors (50%, RR 1.08 p=0.82). In seronegative recipients, 1 reactivated after have a BMT from seropositive donor (8.3%; RR 0.16 p=0.057); none reactivated with BMT from a seronegative donor. Overall acute GVHD grade, age, disease risk status conditioning and whether the donor was related were not significant factors contributing to CMV reactivation. Overall survival was similar regardless of reactivation status.

### **Conclusion**

Recipient CMV sero-positivity is the most significant risk factor for reactivation. Under the current arrangement, where Ganciclovir prophylaxis is given routinely to MUD recipients, CMV reactivation rates appears similar between sibling matched and unrelated matched groups.

**P55****Cytomegalovirus (CMV) Reactivation after Allogeneic Haemopoietic Stem Cell Transplantation (HSCT)**

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**Background**

The recent availability of rapid molecular diagnostics has led many HSCT units, including our own, to move to preemptive antiviral therapy for CMV re-activation.

**Methods**

We undertook a retrospective review of 42 consecutive allogeneic HSCT recipients over a 12 month period to define the characteristics and risk factors for CMV reactivation and disease.

**Results**

There were 26 myeloablative and 16 reduced intensity conditioning (RIC) HSCT. 26 donors were siblings (12 MA 14 RIC) and 16 (14 NA 2 RIC) were unrelated donors. CMV reactivation was monitored weekly by polymerase chain reaction (PCR) and occurred in 15/42 (36%) patients. All patients reactivated before day 100 (mean 39). In the at-risk groups (donor or recipient CMV seropositive), 15/32 (46.9%) had CMV reactivation. Reactivation was treated with either intravenous ganciclovir or oral valganciclovir until PCR negativity was achieved; 6 of 15 requiring at least 4 weeks of antiviral therapy. The only risk factor significantly associated with CMV reactivation was recipient CMV seropositivity ( $p=0.001$ , RR=11.65, CI 2.02-229.88). Myeloablative conditioning ( $p=0.45$  RR 1.56 CI 0.65-4.31), unrelated donor ( $p=0.52$  RR1.46 CI 0.59-3.05) and prophylactic corticosteroids used for GVHD prophylaxis ( $p=0.17$ , RR 1.91 CI 0.796- 3.816) were not significantly associated with CMV reactivation. Confirmed CMV disease occurred in 2/32 (6.3 %) of the at-risk group and 2/15 (13.3%) of all CMV reactivated cases. Both patients had CMV gastrointestinal disease, at days 46 and 169. The late onset case occurred in the absence of background CMV viremia. There were 2 deaths in the CMV reactivated cases due to non CMV-related cause.

**Conclusion**

We suggest that a preemptive approach to CMV reactivation should be used with caution in seropositive recipients and caution is required in using oral antivirals in patients with severe gut GVHD.

**P56**

**Recent Trends in Unrelated Donor Stem Cell Transplantation: A Report From the ABMTRR**

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**Aim**

To investigate and highlight recent trends among patients undergoing allogeneic haematopoietic stem cell transplant (HSCT) with unrelated donors (URD) in Australia and New Zealand.

**Methods**

Patients were selected from the database of the Australasian Bone Marrow Transplant Recipient Registry (ABMTRR). Patients selected for this study had undergone HSCT with URD between the years of 2001 and 2006.

**Results**

A total of 891 allogeneic HSCT with URD were performed in the years 2001 to 2006. The annual numbers have increased steadily, from 101 in 2001 to 172 in 2005 and 146 in 2006. A total of 243 HSCT (27%) involved patients aged up to 14 years, and 648 (73%) involved patients aged 15 or over. Among paediatric transplants, 125 (51%) utilised cord blood (including double cord), 89 (37%) utilised bone marrow and 29 (12%), peripheral blood. The major indication for transplant in paediatric HSCT was ALL (77, 32% of paediatric HSCT). Among adult transplants, the stem cell source was peripheral blood in 409 (63%), marrow in 195 (30%) and cord blood in 44 (7%). The major indication for adult transplant was AML (231, 36% of adult HSCT). The number of adult HSCT for patients aged 50 years or over increased from 20 in 2001 to 39 in 2006. The number of adult HSCT involving reduced intensity conditioning also increased, from 11 in 2001 to 48 in 2005 and 30 in 2006.

**Conclusions**

The annual numbers of URD HSCT in Australasia have increased steadily in recent years. Recent trends in practice include increases in numbers of older patients and HSCT using reduced intensity conditioning. The ABMTRR is a valuable national resource which provides accurate and timely information on HSCT activity and outcome in these two countries.

**P57****Case Report - Acute Myeloid Leukaemia of Donor Cell Origin Occurring Four Years after Allogeneic Haematopoietic Stem Cell Transplantation**

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This case report concerns a 38 year old man who received a myeloablative allogeneic haematopoietic stem cell transplant from his father in 2003 for Acute Myeloid Leukaemia while in first complete remission. He had been diagnosed with AML with trilineage dysplasia and trisomy 8 seven months previously, and had achieved cytogenetic remission after induction chemotherapy. Post transplant course was complicated by recurrent infections and chronic extensive Graft versus Host Disease affecting skin, oral mucosa, and lungs. Circulating myeloid blasts appeared roughly four years post transplant, and bone marrow examination on Day 1450 showed AML with monosomy 7 and t(3;3)(q21;q26). Chimerism analyses using microsatellite analysis for simple sequence length polymorphisms confirmed all circulating cells, including blasts, to be of donor cell origin. By the time this result was available the patient had received a second allogeneic transplant with reduced intensity conditioning from the same donor. The AML progressed rapidly and our patient died day 59 post second transplant.

**Conclusion**

Donor cell leukaemia is a rare and potentially fatal complication of allogeneic haematopoietic stem cell transplantation.

**P58**

**Immediate (<6 months) versus Delayed (≥6 months) Reinfusion of Cryopreserved Haemopoietic Stem Cells (HSCs) for Autologous Transplantation in Patients With Hodgkin's and non-Hodgkin's Lymphoma (HL & NHL): Rates of Engraftment are Comparable**

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**Aim**

For nearly two decades our Department has had a policy of offering 'rainy day' or insurance storage of HSCs to patients with lymphoma in remission, so the cells would be available for possible use in a transplant (HSCT) should the patient's disease undergo a late relapse. Our aim in this study was to compare the engraftment rates and other outcomes in two groups of patients undergoing HSCT, namely those whose cells were reinfused 'immediately' (after <6 months storage at -196°) and those whose reinfusion was delayed (≥6 months).

**Methods**

using our comprehensive BMT database we identified all patients in our institution with HL & NHL who had undergone HSCT between 1988 and 2006. We used simple statistics to compare days to neutrophil & platelet engraftment (counts  $\geq 0.5$  and  $20 \times 10^9$ /litre, respectively), survival rates and other outcomes.

**Results**

151 lymphoma patients were transplanted in the 18 year period, 88 in the immediate and 63 in the delayed group ((mean storage intervals 3.8 and 18.9 months respectively; maximum 9.3 years). Times to neutrophil (11 vs 11 days;  $p=0.23$ ) and platelet (13 vs 16 days;;  $p=0.83$ ) recovery were not significantly different. There were no differences between the groups in terms of 30-day, 100-day and long-term mortality.

**Conclusions**

long-term cryopreservation of HSCs is technically feasible; engraftment rates and survival after HSCT using such cells are similar to those achieved after short-term storage. 'Rainy day' HSC cryopreservation could reasonably be offered to lymphoma patients at risk of late relapse where there is concern about the ability to collect adequate HSCs at the time of relapse.

P59

## Creation of a Simple Workload Index for Stem Cell Processing Laboratories

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### Introduction

There is a general consensus amongst stem cell processing laboratories across Australia that workload is increasing. To examine, document and manage this increase effectively, it would be beneficial to have a system for determining workload in these specialised facilities. A Workload Index provides a standardised measure reflecting staff time commitment to individual procedures carried out under normal working conditions. We have developed a time-based value system that accounts for time spent in laboratory procedures (including complex stem cell manipulation) performed according to current best laboratory practice.

### Method

The index was created in three steps:

1. *Identifying Procedures*: Procedures were categorised into six clinical protocols as follows:

- Hemopoietic Progenitor Cell (HPC) Products Received
- HPC Product Manipulation
- HPC Products Infused
- HPC Product Release Testing
- Administration & Documentation
- Other

2. *Assigning Time-Based Values*: Three separate stages were identified in each procedure. Each was defined, validated and measured as pre-processing, processing and post-processing. Values were assigned as minutes. Stages of each procedure were timed and recorded.

3. *Data Analysis*: Using a simple template, procedures were counted and recorded on a monthly basis and the total number of workload units calculated.

### Results

Data values are expressed as number of hours or workload units per month. We found an average of 300 hours per month during 2006. Preliminary analysis reveals trends in laboratory work practices and increased demands for documentation by staff.

### Conclusion

A Workload Index allows tracking of total workload, individual performance, prediction of workforce requirements and identification of areas of high staff utilisation. Documenting and monitoring changes can avoid work-related stress and provide a means to justify the need for additional staff. Further work will require validation of the index and refinement by assigning time values to functions such as training and teaching, administration and liaison with clinicians.

**P60**

## **Risk Factors for Blood Product Usage Following Sibling Allogeneic Haemopoietic Stem Cell Transplantation (allo-HSCT)**

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### **Background**

Red cell and platelet transfusion requirements have been reported to be lower following Reduced Intensity Conditioning allo-HSCT (RIC) when compared to myeloablative (MA) allo-HSCT. Previous studies examined RIC regimens with lower haemopoietic toxicity than many of the regimens in use today.

### **Methods**

We investigated risk factors for red cell and platelet transfusion in patients enrolled prospectively in an Australian study investigating the non HLA immunogenetics of sibling allo-HSCT. The transfusion requirements in the first year post transplant were reviewed for 122 patients transplanted between 2002 and 2006 in 3 Australian transplant centres.

### **Results**

Seventy-one patients received MA and 51 RIC regimens. Using regression analysis, the outcome variables of total red cell and platelet units transfused were analysed. The factors age, transplant centre, disease, transplant type (RIC v MA), days of neutropenia, death within 12 months, disease risk (high risk (HR) v standard risk (SR)) and ABO mismatch underwent univariate analysis. Associated variables with  $p < 0.2$  were included in a multivariable analysis. Duration of neutropenia, disease risk, death within 12 months and transplant centre were significantly associated with higher red cell and platelet usage ( $p < 0.0001$ ). Transplant type was not associated with transfusion requirement. Each additional day of neutropenia resulted in a 9% increase in number of red cell units transfused and 11% of platelet units. HR MA patients used an estimated 17 units of red cells compared to 12 units for SR. HR RIC patients used an estimated 26 units compared to 10 for SR RIC patients. HR patients used an estimated 16.9 platelet units compared to 8.5 for low risk.

### **Conclusion**

These data highlight the importance of disease risk and degree of myelosuppression as key risk factors for blood product usage following allo-HSCT.

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## Allogeneic Bone Marrow Transplantation for Haematological Malignancies in Patients Infected with HIV

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### Background

Optimal management of intercurrent haematological malignancy in patients with HIV, in particular the feasibility of allogeneic bone marrow transplantation, remains to be defined. We present our experience of allogeneic transplantation in patients with HIV.

### Case series

*Patient 1:* 38yo man with secondary myelodysplasia without prior AIDS-defining illnesses, maintained on second-line antiretroviral therapy of stavudine, lamivudine and tenofovir. He received a HLA-matched sibling peripheral blood stem cell transplant (PSCT), conditioned with cyclophosphamide 60mg/kg and TBI 13.2Gy in 6 fractions, with standard GVHD and infective prophylaxis. Transplantation was complicated by BK-virus haemorrhagic cystitis and prolonged neutropenia with sepsis necessitating withdrawal of anti-retrovirals. Engraftment occurred day 48, and bone marrow aspirate (BMA) confirmed remission. He subsequently developed progressive renal impairment due to post-transplant glomerulopathy, but remains otherwise well and in remission 21 months later.

*Patient 2:* 41yo man with multiply-relapsed acute myeloid leukaemia in CR3, without AIDS-defining illnesses maintained on fifth-line anti-retroviral regimen of emtricitabine, tenofovir, and fosamprenavir with undetectable viral load and CD4 count 275cells/ $\mu$ L. He received an HLA-matched sibling PSCT, conditioned as above, with additional anti-microbial prophylaxis with entecavir for HBV; azithromycin for MAC and ciprofloxacin for previous rhodococcus pulmonary infection. Transplantation was complicated by neutropenic sepsis, pericarditis, and grade 2 cutaneous graft-versus host disease (GVHD). Engraftment occurred day 21; BMA confirmed remission. He remained well until day 67, when he presented with drug resistant pseudomonal sepsis, multiorgan failure, and succumbed day 78.

*Patient 3:* 24yo man with T-cell acute lymphoblastic leukaemia in CR2 without AIDS-defining illnesses, maintained on second-line anti-retroviral regimen of tenofovir, ritonavir, and fosamprenavir, with undetectable HIV viral load and CD4 count 204cells/ $\mu$ L. He received an HLA-matched sibling PSCT, conditioned with etoposide 60mg/kg and TBI 13.2Gy, with extended anti-microbial prophylaxis. This was complicated by grade 2 cutaneous GVHD. Engraftment occurred day 25; BMA confirmed remission. At day 101 he relapsed, was treated with palliative intent and died day 105.

### Conclusions

Allogeneic transplantation is a feasible therapeutic modality for high-grade haematological malignancies in carefully selected patients with well-controlled HIV infection. Preventive management of infective complications and drug toxicity are critical.

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## **Prediction of CD34+ PBSC Collection - G-CSF and Chemo Mobilisation Versus G-CSF Mobilisation Alone - Is the Peripheral Blood CD34 Always Reliable?**

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### **Introduction**

Despite its acceptance as an effective treatment option in a range of haematological and other settings, peripheral blood stem cell (PBSC) collection and cryopreservation for later reinfusion post-myeloablative therapy remains a highly resource consumptive procedure. The role of peripheral blood (PB) CD34 counts in predicting optimal timing of PBSC collection is well established and contributes to a more efficient means of obtaining adequate CD34 dose.

### **Methods**

Our aim was to investigate the affect that mobilisation regimen had on the ability of the PB CD34 count ( $\times 10^6/L$ ) to predict collection CD34 ( $\times 10^6/kg$ ). A retrospective analysis on 516 consecutive PBSC collections (179 patients) between January 2005 and December 2006 assessed overall predictive power and predictive power in patients mobilised using G-CSF alone compared to G-CSF/chemotherapy. Viable PB CD34 and collection CD34 counts were performed using a single platform method on a BD FACSCalibur™ with ISHAGE gating strategy.

### **Results**

Results show good overall prediction for all samples— median PB CD34 count was  $23.0 \times 10^6/L$  (range 0.2–1980.7) and median collection CD34 count was  $1.75 \times 10^6/kg$  (range 0.01–97.1),  $R^2$  value = 0.919(log transformed). For G-CSF/chemo mobilisation (n=371), median PB CD34 count was  $31.9 \times 10^6/L$  (range 0.2-1980.7) and median collection CD34 count was  $2.2 \times 10^6/kg$  (range 0.01–97.1),  $R^2$  value = 0.917. For G-CSF alone mobilization (n=148), median PB CD34 count was  $9.3 \times 10^6/L$  (range 1.4 – 338.2) and median collection CD34 count was  $0.7 \times 10^6/kg$  (range 0.1 – 16.7),  $R^2$  value = 0.895

### **Conclusions**

These results demonstrate good predictive power of the PB CD34 for the collection CD34 result and predictive power appears greater in the G-CSF/Chemo mobilization group compared to G-CSF group alone. Additionally, G-CSF/Chemo mobilisation generally result in higher CD34 yields compared to G-CSF group alone and a specific PB CD34 count in the G-CSF/Chemo mobilisation group is likely to result in a higher CD34 yield compared to the G-CSF alone group.

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## “Short Course” Methotrexate as GVHD Prophylaxis in Reduced Intensity Conditioning Allogeneic Stem Cell Transplantation

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Reduced intensity conditioning allogeneic haematopoietic stem cell transplantation (RIC-HSCT) is generally associated with low early transplant related mortality (TRM) but acute and chronic graft-versus-host-disease (GVHD) remain significant post transplant complications with a negative impact on survival and quality of life. Despite this, little data exists as to the most effective GVHD prophylactic regimen post RIC-HSCT. We examined the role of standard “short course” intravenous methotrexate (MTX), prednisone and cyclosporin as GVHD prophylaxis post RIC-HSCT. From 4/98 to 4/07, 45 consecutive patients (pts), median age 55 (25-71), received “short course” MTX (defined as 15 or 10mg/m<sup>2</sup> day +1, together with 10mg/m<sup>2</sup> on days +3, +6 and +11), cyclosporin and prednisone as per the Ruutu protocol as GVHD prophylaxis post RIC-HSCT. 84% received an HSCT from an HLA 6/6 matched related donor, the remainder from a 5/6 mismatched related donor. Most HSCT were performed for AML (14/45) or NHL (14/45) with 73% patients receiving a fludarabine based conditioning regimen. Median follow up was 436 (7-3305) days. Median time to neutrophil and platelet engraftment was 15 (9-29) and 20 (9-104) days respectively in evaluable pts. Primary graft failure occurred in 2/45 (5%) pts. Day 100 mortality was 18%. Rates of acute and chronic GVHD were 29% and 40% respectively with 24% of evaluable pts developing extensive chronic GVHD. Median disease free (DFS) and overall survival (OS) was 349 and 436 days respectively. In conclusion, GVHD prophylaxis using “short course” MTX is associated with acceptable engraftment and acute GVHD rates post RIC-HSCT.

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## **The Production of Normal Unrelated MHC-Mismatched Mesenchymal Stem Cells from Human Placenta for Use in Phase 1 Clinical Trials**

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### **Background**

Mesenchymal stem cells (MSC) exist in bone marrow, placenta and various other tissues. They are thought to play a key role in tissue repair and regeneration, and immune modulation. The therapeutic potential of MSC is currently being investigated by several groups, using mostly bone marrow-derived MSC. The placenta may offer an alternative, and more readily accessible, source of MSC for therapeutic use than bone marrow.

### **Aim**

To evaluate the feasibility of isolating mesenchymal stem cells from placental tissue for use in phase 1 clinical trials.

### **Method**

Human term placenta was obtained with human research ethics committee approval and informed consent. MSC were isolated from the placenta by dissection, tissue digestion and density gradient purification. Cells were then expanded *ex vivo* in tissue culture flasks for 22 days involving approximately 15 population doublings. MSC were quality controlled by checking for sterility, Gram stain appearance, endotoxin content, mycoplasma presence, viability, purity (using flow cytometry) and karyotype.

### **Results**

MSC were cryopreserved at passage 2 and 3 (days 15 and 22 respectively) yielding a total of  $2.4 \times 10^9$  cells from a single placenta. All quality control criteria were met: MSC were of a high purity (<1 % CD45 positive, >95 % double positive for CD73 and CD105). Viability was 96 % at passage 2 and 98 % at passage 3. Gram-stain was negative and bacterial and fungal cultures showed no growth at 14 days. Endotoxin content was <2EU/ml and mycoplasma testing negative. Karyotype analysis is ongoing but prior preclinical production runs have shown no major genomic changes using G-banding and single nucleotide polymorphism analysis.

### **Conclusion**

MSC of high purity and viability can be routinely isolated from human term placenta at low passage number. *Ex vivo* expansion of placenta-derived MSC yields large numbers of cells which can be used for therapeutic evaluation in clinical trials.

**P65****Chemokine Receptor Expression of Mesenchymal Stem Cells Derived from Murine Bone Marrow and Bone**

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**Background**

Mesenchymal stem cells (MSC) are adult stem cells most commonly derived from the bone marrow. They have multipotent lineage differentiation potential and an immuno-privileged status. This renders them good candidates for the repair of tissue injury and regeneration. Additionally, it is thought that bone marrow-derived MSC migrate to sites of acute inflammation in preference to bone marrow. However, the molecular mechanisms for this are not known, although in acute inflammation chemokines are released including stromal cell-derived factor (SDF-1), monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP-1 $\alpha/\beta$ ), interleukin (IL)-8, fractalkine and interferon-gamma-inducible 10-kDa protein (IP-10). These chemokines are important in cell migration.

**Aim**

To determine the chemokine receptor expression of murine MSC in order to determine the molecular homing mechanisms used by MSC to normal tissue and to inflamed tissue.

**Method**

Adult MSC were isolated from bone marrow and bone by type 1 collagenase digestion, percol density gradient fractionation and plastic adherence. After culture in  $\alpha$ -MEM, 20% FCS, cells were flow sorted for CD45<sup>-</sup>, CD31<sup>-</sup>, Sca1<sup>+</sup> and characterised as MSC by the induction of mesodermal differentiation. RNA was then extracted for chemokine receptor expression using RT-PCR.

**Result**

MSC expressed the chemokine receptors CXCR4, CXCR1, CXCR5, CXCR6, CCR1, CCR4, CCR7, CCR9 and CCR10 (important in binding to SDF-1), and MCP-1, MIP-1 $\alpha/\beta$ , IL-8 and IP-10 (utilised in migration).

**Conclusion**

MSC express a wide range of chemokine receptors. Future studies will determine which of these receptors are most important in MSC homing to normal and inflamed tissue.

**P66**

## **Comparison of Chemokine Receptor Expression on Bone Marrow and Placental MSC**

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Multipotent mesenchymal stromal/stem cells (MSCs) are good candidates for cellular therapy and have been shown to home to inflamed tissue after intravenous injection.

### **Aim**

In order to characterise possible homing mechanisms of MSC, we aimed to determine the chemokine receptor expression of *in vitro* cultured MSC from placenta (pMSC) and bone marrow (bmMSC).

### **Methods**

We used a panel of primers and mAb to determine which chemokine receptors and adhesion molecules are expressed by MSC. Placentas from normal term pregnancies and bone marrow (BM) from iliac crest of healthy volunteers were used in this study. Collagenase digested placental cell suspension or BM aspirates were fractionated by percoll density gradient and the interface cells cultured in DMEM/ 20 % FCS. After 2-7 passages, the cells were analysed by flow cytometry or RT-PCR.

### **Results**

By PCR, a broad range of chemokine receptors including CCR7, CCR8, CCR10, CCR11, CXCR3, CXCR4 and CXCR6 could be detected. Two chemokine receptors, CCR1 and CCR3 had variable expression between donors. Antibody staining for chemokine receptors showed that cell surface expression was restricted, however, both pMSC and bmMSC had cytoplasmic CCR1, CCR3, CXCR3, CXCR4 and CXCR6. No cell surface or cytoplasmic staining could be detected for CCR7 or CCR10.

### **Conclusions**

Both p- and bmMSC expressed a similar range of chemokine receptors. Therefore both are likely to use similar mechanisms for homing to inflamed tissue after intravenous delivery, thus facilitating their use in the repair of damaged organs and tissues.

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## Do Mesenchymal Stem Cells Derived from Bone and Bone Marrow Exhibit Neuronal Differentiation Potential?

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### Background

Bone marrow-derived mesenchymal stem cells (MSC) have been shown to improve cerebral function in animal models of stroke and spinal injury, suggesting that they may be cellular candidates for the repair and regeneration of neural tissues. The mechanism of action for such repair is unknown but includes the possibility that MSC can differentiate into neuronal cells.

### Aim

To investigate the neuronal differentiation potential of murine Sca-1<sup>+</sup>CD45<sup>-</sup>CD31<sup>-</sup> MSC (mMSC) isolated from bone marrow and bone.

### Methods

Murine MSC were obtained from the bone marrow and bone of GFP transgenic mice. Bone fragments were digested with type I collagenase and the mononuclear fraction isolated by percoll density-gradient. Cells were cultured in  $\alpha$ -MEM/20% FCS and non-adherent cells removed. Cells were then flow-sorted for CD45<sup>-</sup>, CD31<sup>-</sup>, Sca1<sup>+</sup> cells. Sorted cells were *ex vivo* expanded in  $\alpha$ -MEM/20% FCS. After further passages, cells were harvested and mesodermal differentiation to fat, cartilage and bone tissue was confirmed. MSC neural differentiation potential was then explored using a neural induction medium. Differentiated cells were analysed by immunofluorescence for the display of neural antigens and tested for the presence of sodium and potassium ion channels.

### Results

The phenotype of the adherent/flow sorted MSC was Sca-1<sup>+</sup>, CD90<sup>+</sup>, CD44<sup>+</sup>, CD31<sup>-</sup>, CD45<sup>-</sup> and CD11b<sup>-</sup>. These cells were able to differentiate into bone, fat and cartilage. Neurally-differentiated MSC showed morphology typical of neural cells and positive immunofluorescence staining for the neural specific antigen  $\beta$ III-tubulin. However, on electrophysiological testing we could not demonstrate the presence of voltage-gated sodium channels, although potassium channels were readily demonstratable.

### Conclusion

Murine MSC can be induced *in vitro* to demonstrate a neural morphology and expression of the neural specific marker  $\beta$ III-tubulin. However, electrophysiology studies indicated that these cells do not express sodium ion channels typical of neural cells. Thus, therapeutic efficacy of MSC in neural disease may be mediated by mechanisms other than neural differentiation.

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## Histological Evaluation of Mesenchymal Stromal Cells Homing to the Site of Acute Inflammation

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### Aim

The aim of this project was to determine if mesenchymal stem cells (MSC) home preferentially to the site of acute inflammation compared to normal, non-inflamed tissue. Acutely inflamed myocardium following acute myocardial infarction was used as a paradigm for acutely inflamed tissue.

### Methods

MSC isolation: the iliac crest, tibia and femur of C57BL/6 mice transgenic for green fluorescent protein (GFP) were isolated. The bones were crushed and fragments were digested with collagenase and subjected to Percoll density centrifugation. Adherent cells were cultured in  $\alpha$ -MEM/20 % FCS and flow-sorted to isolate a CD45<sup>-</sup> Sca1<sup>+</sup> population. GFP<sup>+</sup> MSCs were examined for mesodermal differentiation ability and cell surface phenotype (Sca1<sup>+</sup>, CD90<sup>+</sup>, CD44<sup>+</sup>, CD31<sup>-</sup>, CD45<sup>-</sup> and CD11b<sup>-</sup>).

GFP-MSC imaging: Bone marrow, lung and heart tissues were collected from normal and myocardially infarcted mice that were intravenously injected with GFP<sup>+</sup> MSC. Cryosections were cut and visualised with or without an anti-GFP antibody. Secondary antibodies conjugated with Alexa<sup>488</sup> and Alexa<sup>546</sup> fluochromes were evaluated for enhanced MSC detection within tissues.

### Results

The optimised adherence/FACS GFP<sup>+</sup> MSC isolation protocol resulted in a greatly enriched population of MSC. Furthermore, GFP<sup>+</sup> MSC expressed GFP at high levels as determined by fluorescence microscopy. However, anti-GFP antibody staining enhanced the signal detection in fixed tissues greatly. The Alexa<sup>546</sup> conjugated secondary antibody was found to be far superior to the GFP only or anti-GFP Alexa<sup>488</sup> due to high level of autofluorescence present in the tissues studied. At initial time points after GFP-MSC injection, cells were present in the lung but not in the infarct zone of the inflamed myocardium.

### Conclusions

A method to track GFP<sup>+</sup> MSCs *in vivo* was successfully optimised. This murine model will help to elucidate the molecular mechanisms of MSC homing to sites of acute inflammation, which we hypothesise may be due to increased local concentrations of inflammatory chemokines.

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## Optimising the Isolation of Haematopoietic Stem Cells, Proangiogenic Macrophages and Mesenchymal Stem Cells as Cell Therapy Candidates for Tissue Repair and Regeneration

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### Aim

To develop optimal purification techniques for the isolation of haematopoietic stem cells (HSC), proangiogenic macrophages (PM) and mesenchymal stem cells (MSC) for study in a model of acute inflammation.

### Method

Bone and bone-marrow cells were taken from adult C57BL/6 mouse tibias and femurs. Mononuclear cells were collected from the interface following percoll density centrifugation. HSC and PM populations were then fractionated using magnetic bead (MACS) and fluorescence-activated (FACS) cell sorting for positive and negative isolation. Additionally, MSC were isolated from the mononuclear cell population by plastic adherence in ex vivo expansion cultures and sorted for known MSC markers after the first passage.

### Results

All three cell populations were identified from bone and bone-marrow mononuclear cell preparations. To isolate HSC, mononuclear cells were magnetically depleted for mature lineage cell markers (CD5, CD11b, CD45R, Ly-6G, Ly-6C, 7/4 and Ter119) using MACS. The negative cell fraction was then further selected for CD45<sup>+</sup>, c-Kit<sup>+</sup> (CD117) and lineage<sup>dim</sup> using FACS. The purity of CD45<sup>+</sup>/c-kit<sup>bright</sup> HSC was >99%. PM were magnetically enriched for the angiogenic cell marker, Tie-2 using MACS. The positive cell fraction was then selected for CD45<sup>+</sup>, CD11b<sup>+</sup> and Tie-2<sup>+</sup> by FACS. The purity of the CD45<sup>+</sup>/CD11b<sup>+</sup>/Tie-2<sup>+</sup> PM was >99%. MACS depletion or enrichment for cells of interest greatly increased the efficiency of FACS in isolating these two cell populations. MSC were cultured in  $\alpha$ -MEM medium plus 10% foetal calf serum and fluorescently sorted for CD45<sup>-</sup> and Sca1<sup>+</sup> markers after the first passage. Purified MSC were then returned to culture for further expansion. The purity of CD45<sup>-</sup>/Sca-1<sup>+</sup> MSC was >99%.

### Conclusions

Three separate cell populations (HSC, MSC and PM), that may be involved in tissue inflammation and repair, have been isolated in the mouse. These pure cell populations will now undergo further characterisation using functional assays and their possible participation in an animal model of acute inflammation and tissue repair will be explored.

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## **Biophotonic Imaging of Luciferase-Transgenic Murine Mesenchymal Stem Cells (MSC) Is a Promising System For Tracking MSC Migration *In Vivo***

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### **Aim**

Mesenchymal stem cells (MSC) are most commonly derived from the bone marrow and are thought to be good candidates for cellular repair of injured tissues. After intravenous injection MSC have been shown to home to inflamed tissue. Studies of MSC function and migration would be greatly facilitated by a system that allowed multiple sequential *in vivo* imaging of injected MSC. Biophotonic imaging of luciferase-expressing MSC was explored for this purpose.

### **Method**

Luciferase-transgenic mice that express the luciferase reporter gene (based on pGL3, Promega) under the control of the murine *Atm* (ataxia-telangiectasia) promoter have been generated (FVB<sup>ATM-Luc<sup>+</sup>/+</sup>). To isolate MSC from FVB<sup>ATM-Luc<sup>+</sup>/+</sup> mice (MSC<sup>luc</sup>), bones were crushed, digested with type I collagenase and cells fractionated by percoll density-gradient centrifugation. At the first passage adherent cells were flow sorted for CD45<sup>-</sup>, CD31<sup>-</sup>, Sca1<sup>+</sup> and were cultured in  $\alpha$ -MEM with 20 % FCS for subsequent passages. The cells were further characterised by induction of mesodermal differentiation to fat, cartilage and bone. Live wildtype FVB mice given intravenous MSC<sup>luc</sup> were imaged using a Xenogen Imager.

### **Results**

Luciferase detection was initially detected *in vitro* using a microtitre plate and the lowest concentration at which luminescence could be detected *in vitro* was  $1 \times 10^3$  MSC<sup>luc</sup> /well. Luciferase activity in MSC<sup>luc</sup> was also sufficient for their *in vivo* detection after injection into wild type FVB (luciferase-negative) mice. After intravenous tail vein injection, the lowest dose at which luminescence could be detected was  $5.0 \times 10^5$  MSC<sup>luc</sup>/mouse and was predominantly in the chest and abdominal regions. Luciferase activity could be detected for up to 14 days after injection of higher doses of MSC<sup>luc</sup> ( $1.0 \times 10^6$  MSC<sup>luc</sup>/mouse).

### **Conclusion**

We conclude that biophotonic detection of MSC<sup>luc</sup> is a promising system for *in vivo* studies of MSC migration and engraftment in normal mice and in murine models of inflammation and tissue repair.

**P71****Development of a Murine Model of Acute Myocardial Infarction as a Paradigm for Acute Inflammation for the Assessment of Cell Therapies for Tissue Repair and Regeneration**

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**Background**

Under conditions of acute ischemia-derived myocardial inflammation, intravenously (i.v.)-administered mesenchymal stem cells (MSCs) have been shown to migrate to, and engraft into, damaged cardiac tissues, producing functionally restorative effects. The mechanisms underlying this *in vivo* homing have yet to be fully characterized, but are likely to critically depend upon the availability of large numbers of circulating MSCs, and the access that this introduced and potentially therapeutic cell population has to local microvasculature in the inflamed area.

**Aim**

This study compared recipient tolerance to escalating doses of intravenously delivered MSCs in two distinct murine models of acute myocardial infarction (AMI).

**Methods**

In a reperfused AMI model, the descending left coronary artery was transiently occluded for 45 minutes, while in a bland (non-reperfused) AMI model, the same vessel was permanently ligated. Doses of MSCs were delivered i.v. (tail or jugular vein) either 2 or 24 hours after surgery.

**Results**

A decrease in sensitivity to the systemic delivery of MSCs, evidenced as an increase in the maximum tolerable dose (MTD), was found to depend upon both the route of i.v. delivery and time elapsed since surgery. MTD was enhanced dramatically (6-8 fold) in both models when a highly viable (>90%) MSC dose was delivered in a vehicle of a relatively large volume that incorporated an anticoagulant.

**Conclusion**

These experiments optimized a clinically relevant animal model of acute inflammation for use with a potentially efficient and non-invasive cell therapy for tissue regeneration.

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**Can The Sysmex XE2100 IG% Count Replace Manual Blood Film Differentials For Enumerating Myelocytes?**

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**Aim**

To assess the accuracy of the XE2100 (IG Master software) IG% parameter, as a means of reporting myelocyte percentages.

**Materials and Methods**

Sample Selection: The study was carried out at Canterbury Health Laboratories. 503 samples were assessed.

Analyser Setup and Calibration: The Sysmex XE2100 analyser was set up, calibrated and controlled according to manufacturer's instructions.

Blood Film Review: Blood films were made on all samples. A manual 200-cell differential was performed on each sample, independently, by 2 experienced morphologists as per the NCCLS protocol. Band form neutrophils were included in the neutrophil count and metamyelocytes were included in the myelocyte count.

**Results**

The enumeration of Immature Granulocytes by the XE2100 in samples with a WBC count  $<1 \times 10^9/L$  did not correlate with the manual differentials. The IG% reported by the XE2100 correlated well with the myelocyte percentage from the manual cell differentials (2 x 200 cell differential) ( $r = 0.898$   $p < 0.001$ ,  $r^2 = 0.807$ ). The XE2100 IG% correlation with the manual 400 cell differential myelocyte percent count was superior to that achieved when the two morphologists were compared with each other ( $r = 0.870$ ,  $p < 0.001$ ,  $r^2 = 0.757$ ).

The XE2100 had a tendency to have slightly higher IG% counts than the manual differential myelocyte counts (slope = 0.656, y intercept = 0.225).

**Conclusion**

For patients with WBC counts  $> 1 \times 10^9/L$ , the XE2100 IG% produces more accurate myelocyte percentages than can be achieved with a 200-cell manual differential. This allows for extended differentials from the XE2100 to be reported without the requirement of blood film review.

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## A Method for *In-vivo* Erythrocyte and Platelet Labelling and Simultaneous Kinetic Studies

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### Aim

To establish a simple and reproducible method enabling the simultaneous measurement of erythrocyte and platelet kinetics using *in-vivo* CFSE-labelling.

### Method

C57Bl/6 mice (n=5) were injected intravenously with CFSE to label all erythrocytes and platelets. Blood samples were taken daily for 5 days then less frequently for a further 45 days. Samples were analysed using FACS and the reduction in percent fluorescent erythrocytes and platelets calculated over time. Additional mice (n=6) were depleted of platelets following CFSE injection using a monoclonal antibody to GpIIb/IIIa. Platelet and erythrocyte kinetics were also studied in these mice.

### Results

There was no apparent toxicity following the intravenous injection of CFSE. In the control mice the rates of erythrocyte and platelet production and loss were highly reproducible, and the mean erythrocyte and platelet life-spans obtained were comparable to those previously published. In the platelet-depleted mice, the fluorescent platelet population was eliminated following antibody administration and a rapid rate of platelet production observed. Varying levels of anaemia also occurred in these mice, the extent of which closely correlated with the rate of red cell production at Day 10.

### Conclusion

The use of CFSE to measure erythrocyte and/or platelet kinetics has several advantages including: the ability to label all cells *in-vivo* thus eliminating potential sampling bias and preventing *in-vitro* processing damage to cells; intracellular labelling therefore minimising immunogenicity and interference with cellular interactions; and immediate analysis post collection with no additional labelling steps being required. This method has the potential to be applied in a multitude of experimental situations such as the screening of mutant mice displaying abnormal erythrocyte and/or platelet counts, and to study the effects of cytokines, growth factors and pharmacological agents on erythrocyte and platelet production and destruction.

**P74**

**The Implementation of “The International Consensus Group for Hematology Review” Guidelines into a Large Private Pathology Laboratory**

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The first automated full blood count analyzer was introduced in the 1950's by Wallace Coulter. Prior to the advent of these technologies, morphological examination of the blood film was the mainstay of diagnosis. With improved technology, it is acknowledged that automated systems can provide superior results than manual counting techniques. In 2002 a group of twenty experts including Dr Berend Houwen (the founder of the International Society for Laboratory Haematology (ISLH)), published guidelines to standardize results obtained from automated analyzers and manual review of the blood film.

This presentation examines these criteria and their implementation into a private haematology laboratory which performs in excess of 3000 full blood counts per week day. The main laboratory of Sullivan Nicolaides is located at Taringa in Brisbane and the analysers utilised in this laboratory are the Sysmex XE-2100 series.

The project was designed in accordance with the International Consensus Group for Hematology Review guidelines and aimed to reduce the number of slides requiring review in a safe and extensively validated manner. This was achieved by careful planning, staff co-operation, rigorous examination of the results and lengthy discussion of the proposed changes.

Stage 1 assessed the flags related to blasts, atypical and abnormal lymphocytes, left shifted and immature granulocytes.

Stage 2 looked at the flags generated with respect to red blood cell flags.

Stage 3 aimed to reduce the number of films reviewed by assessing the impact of the adjusting the lower and upper limits of Hb and MCV and the upper limit of the eosinophil count.

The modifications to laboratory procedures as a result of this implementation and the corresponding reduction in the film review rate will be detailed.

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## The Art of Keeping Morphology Training Alive and Kicking

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The Alfred is a quaternary hospital servicing four campuses with an array of specialist units including oncology and stem cell transplantation. Servicing the complex clinical units with resource constraints makes timely reporting of blood film morphology a challenge. Approximately 600 Full Blood Examinations (FBE)/ day are performed by the Haematology Unit; 21% of which require blood film morphology.

Use of a computer rule base (CHESTER) and an automated verification system keeps the FBE morphology review rate relatively low. In 2002 the film review rate was 23.7%, with 0.9% requiring differentials from 146,016 FBEs, to the current review rate of 21.1% with 0.7% requiring differentials from 192,287 FBEs in 2006-2007. In that period no additional EFT were employed within the department.

Increasing workload, new infrastructure within the hospital, complex morphology, staff turnover and a 24/7 rotating roster have contributed to the need for extra staff to be skilled in morphology. Recently, strategies have been instituted to enhance training requirements including:

New staff now have a minimum of 4 weeks training in morphology prior to starting the 24/7 shift.

Staff were interchanged from blood bank to accommodate morphology training using ½ day shifts and a “buddy” system.

Delegation of morphology training to specific experienced staff

Extension of educational morphology tutorials

Introduction and extension of an internal Quality Assurance Proficiency (QAP) programme.

The outcome has seen a 23% increase in the number of trained morphology staff, providing greater flexibility to cover staff absences, enhance team work, improve morale and contribute to professional development.

These strategies need to be ongoing and other time efficiencies introduced., taking the form of digital morphology imaging and improved analyzer / computer technology, but the skill in and appreciation of human blood cell morphology will remain a mandatory requirement of haematology scientists working in a teaching hospital for a long time to come.

**P76**

## **Design of a Bone Marrow Examination Factsheet for Families**

Elisa Rough, Catherine Crock, Maria McCarthy  
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### **Aim**

To design and write a suitable information page on bone marrow aspiration and trephine biopsy suitable primarily for parents, children and adolescents. It was to be available both on our hospital website as well as in a hard copy print version.

### **Background**

The Royal Children's Hospital, Melbourne has a dedicated web area- Kids Health Info for Parents which aims to provide clear information in appropriate language on a range of medical conditions, procedures and treatments. In early 2007 we identified that many parents of children requiring bone marrow examinations were requesting formal written information, in addition to verbal explanations given at the time of consent. Enquiries made to other paediatric centres in Australia did not reveal an available written "factsheet" for provision of such information.

### **Method**

An information sheet was written by the Clinical Haematology Department in consultation with the Department of General Medicine and Children's Cancer Centre. Assistance with most appropriate wording and formatting was sought from the Kids Health Info for Parents co-ordinators. Illustrations of the procedure and needle were designed by a medical illustrator from the hospital Education Resource Centre.

### **Conclusion**

We plan to evaluate parent and child/adolescent, as well as health care provider response to the factsheet. The number of visits to the webpage, as well as clinical hospital usage will be evaluated. We hope our factsheet will provide a model which could be adopted by other Australian and overseas paediatric units.

(Poster to include copy of factsheet and illustrations.)

P77

## **An Equipment Management and Validation System to Meet Regulatory Requirements**

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*Queensland Cord Blood Bank at The Mater, Mater Health Services, South Brisbane, QLD Australia*

### **Aim**

To develop generic contracts, an equipment management system, validation master plan and template forms to facilitate training of external service providers, equipment management, validation and ongoing maintenance to meet the regulatory requirements of the Therapeutic Goods Administration (TGA) Code of Good Manufacturing Practice for Human Blood and Tissues and Netcord-FACT International Standards.

### **Method**

Standard operating procedures, generic service provider contracts, a validation master plan, equipment matrix, monthly maintenance schedule and template forms for the training of service providers, and recording of authorised signatures were modified or developed. In addition, templates for equipment validation plans, Installation Qualification (IQ), Operational Qualification (OQ) and Performance/Process Qualification (PQ), and specifications were implemented to customise the laboratory system. Meetings were held with equipment service providers to specify the regulatory requirements with respect to the use of appropriately certified equipment for calibration and the standard of required documentation, and to address facility Workplace Health and Safety practices.

### **Results**

As existing service contracts expired, these were replaced by generic contracts. The existing equipment matrix was modified to include an inventory and replacement schedule, details of validation, calibration and service schedules. The templates were used to record the formal retraining of service providers, and to develop individual equipment validation plans, specifications, IQ/OQ and PQ for all types of equipment and a monthly service/calibration schedule. Existing equipment was revalidated to the extent that this was possible. New equipment undergoes IQ, OQ and PQ. A monthly schedule details equipment due for service/calibration.

### **Conclusion**

This cost-effective system developed for management and validation of equipment simplifies the process, standardises documentation and validation processes, provides records of service provider training, timely scheduling of equipment service and calibration and ensures the suitability of equipment for its intended purpose and regulatory compliance.

**P78**

## **Myelodysplasia with Lymphoid Transformation Achieving Prolonged Partial Response with Vincristine/ Prednisone**

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<sup>2</sup> *Queensland Health Pathology Services- Central*

<sup>3</sup> *Queensland Health Pathology Services- PA Hospital*

### **Summary**

A patient presents with pancytopenia due to myelodysplasia with presumed lymphoid blast population. He achieves a partial response and transfusion independence with vincristine/ prednisone.

### **Case History**

A 67 year old male smoker with a background of significant ischaemic heart disease presents with 1 month history of exertional dyspnea. Investigation shows severe pancytopenia (Hb 57g/l; Plt  $12 \times 10^9$ ; Neut  $0.1 \times 10^6$ ). Blood film shows pelgerised neutrophils without blasts, folate normal and B12 123 pmol/l. Marrow reveals trilineage dysplasia with a blast population of 25%. Immunophenotyping shows blasts co-expressing surface CD38 and CD34, and intracytoplasmic  $\mu$  and Tdt. Myeloid and other lymphoid markers are negative. PCR reveals a monoclonal IgH gene rearrangement. Cytogenetic analysis failed. Echocardiogram is consistent with ischaemic cardiomyopathy. After declining induction chemotherapy he is commenced on palliative therapy with transfusions and prednisone (25mg/day) with vincristine (2mg monthly). After 6 weeks the FBC showed only mild persistent thrombocytopenia (Plt  $134 \times 10^6$ ) but was otherwise normal. He became asymptomatic and transfusion independent. Reassessment marrow at 1 month showed no blasts but persistent significant trilineage dysplasia despite adequate B12 replacement. Flow cytometry was unsuccessful. Karyotype confirmed 46XY. Treatment continued. After 6 months he developed mild recurrent thrombocytopenia with persistent marrow dysplasia and re-emergence of a blast population (5%) again expressing surface CD38 and CD34.

### **Discussion**

Lymphoid transformation of myelodysplasia is a rare but well described phenomenon. The response to palliative ALL therapy reaffirms the view that MDS may arise from a pluripotent progenitor.

P80

**The Tasmanian Familial Leukaemia and Lymphoma Research Study**

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The Tasmanian familial leukaemia and lymphoma research group was formed in early 2006. We propose to investigate the genetics of haematological malignancies (HM) using both genome wide association and familial linkage analyses. A study conducted in the 1970s and 1980s identified a large number of HM families with multiple cases of disease. From these records and those of the Tasmania Cancer Registry (TCR), we have performed extensive genealogical research and expanded these families, identifying a number of new cases. To date, 15 priority pedigrees have been selected for immediate recruitment, comprising numerous affected people. The initial part of the study has involved confirming and reclassifying cases according to the WHO classification of HM. A significant difficulty encountered has been that historically most hospitals and the TCR have shredded many of the deceased patients notes. However of the 135 cases in our 15 priority families, the diagnosis was able to be confirmed/reclassified and some clinical information obtained for 105 cases to date. This success has been due to the excellent records available from the study conducted in 1970s/80s. The LK0016 pedigree has ~970 descendants from the founder pair spread over 8 generations with 11 (10 confirmed) affected individuals, of which 5 are all siblings. The LK0124 family contains ~3948 descendants over 10 generations with 18 (17 confirmed) affected individuals. Within this family 3 of the affected had a rare site of involvement of their diffuse large B cell lymphoma (DLBCL). They all presented with primary central nervous system (CNS) lymphoma. This as a rare site for lymphoma (1% of DLBCL), and compared to data available from the Royal Hobart Hospital pathology database, there has only been 13 other patients diagnosed since October 1993 with primary CNS lymphoma. Therefore to find three in this one pedigree is interesting and genome wide linkage analysis using the Affymetrix 250K arrays are about to be performed for this pedigree. This and other interesting clinical facts about the affected patients in these pedigrees will be presented.

**P81**

**Myelodysplasia Due to Copper Deficiency: A Case Report and Review of the Literature**

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**Aim**

We present a case highlighting copper deficiency as a cause of reversible myelodysplasia.

**Method**

Case report.

**Results**

A 41-year-old man presented with a ten day history of severe fatigue. A full blood count showed that he was pancytopenic with the following counts: Hb: 77g/L, platelets:  $122 \times 10^9/L$ , MCV: 84fL, neutrophils:  $0.03 \times 10^9/L$ , LDH 135U/L).

Nine months previously he had been diagnosed with colon cancer. This was treated surgically by subtotal colectomy and subsequent hemihepatectomy for a solitary hepatic metastasis. No chemotherapy or radiotherapy was administered. He has continued in remission with the last normal blood count ten weeks previous to this presentation. Amongst other herbal medications, he was taking ammonium tetrathiomolybdate (a known copper chelator in phase I/II trials as an anti-angiogenic agent) and zinc supplements.

A bone marrow examination revealed markedly dysplastic erythropoiesis and granulopoiesis with prominent vacuolation of precursor cells. Perl's staining of the aspirate showed the presence of ringed sideroblasts (40%). Serum copper and ceruloplasmin levels were 3 micromol/L (normal range 12-24) and 55mg/L (normal range 200-500) respectively.

Upon cessation of all medications, his blood counts, copper and ceruloplasmin levels returned to normal.

In adults, copper deficiency is most likely to occur in the setting of total parenteral nutrition, post-gastric resection, short-bowel syndrome or excessive zinc ingestion.

**Conclusion**

This is the first reported case of myelodysplasia related to the copper chelator ammonium tetrathiomolybdate. Copper deficiency is an uncommon but often overlooked cause of reversible myelodysplasia and should be considered in the initial workup in patients who present with predominant neutropenia and anaemia of uncertain aetiology.

P82

## Pentasomy 21 in Childhood AML

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<sup>2</sup>*Centre for Children's Cancer and Blood Disorders, Sydney Children's Hospital, Randwick, NSW, Australia*

### Aim

To describe a case of spontaneously regressing acute myeloid leukaemia (AML) associated with pentasomy 21 in a child with normal constitutional chromosomes.

### Case report

A phenotypically normal two month old female was admitted with Hb 46g/L, Plts  $75 \times 10^9/L$  and WCC of  $12 \times 10^9/L$  with 12% circulating blasts. Morphology was suggestive of megakaryocytic leukaemia on peripheral blood film. Bone marrow aspirate showed 25-30% myeloblasts. Flow cytometry was negative for CD61, positive for myeloid markers and CD7. Cytogenetic analysis revealed the presence of pentasomy 21. Constitutional chromosomal analysis was normal. The patient was not treated and peripheral blasts regressed. Repeat bone marrow aspirate at six weeks showed morphological improvement but with significant dysplasia and persistence of the abnormal cytogenetic clone. Clinically the infant is well and has achieved normal developmental milestones.

### Discussion

Literature review revealed only one other single case report of pentasomy 21 in a phenotypically normal infant. One case in the adult literature has been reported in which the patient succumbed to AML. Traditionally spontaneously regressing AML also known as Transient Myeloproliferative Disease (TMD) is thought to be restricted to children with Down's Syndrome (DS), and is hypothesized to relate to a cytogenetic abnormality of chromosome 21. Possible candidate leukaemogenic locations have been theorized but not yet firmly identified. Up to 20% of TMD/DS patients go on to develop acute leukaemia. The patient described above has some presenting features not usually seen in TMD/DS patients however the haematologic abnormalities appear to be resolving in a similar manner.

### Conclusion

Pentasomy 21 is a rare cytogenetic abnormality that when identified in cases of childhood AML may indicate a similar clinical course to that observed in TMD of DS. Identification of this cytogenetic abnormality may aid the decision to avoid toxic chemotherapy in this population.

**P83**

**Treatment-Related Myelodysplasia and Secondary AML Following Fludarabine Combination Chemotherapy**

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<sup>2</sup> *University of Melbourne, Parkville, Australia*

**Background**

Fludarabine combination chemotherapy achieves high response rates in CLL and low grade lymphoma. Fludarabine inhibits DNA repair and augments the cytotoxic effect of DNA damaging agents such as cyclophosphamide and mitoxantrone. This mechanism may also affect marrow progenitor cells to increase the risk of myelodysplasia or secondary acute myeloid leukaemia (MDS/sAML). We have previously reported our experience with 137 patients treated with fludarabine combination chemotherapy with 10 patients developing MDS/sAML (crude rate 7.3% at a median follow-up of 40 months).

**Methods**

Review of the Peter MacCallum Cancer Centre Pharmacy database from 1996-2006 identified 158 patients treated with fludarabine combined with cyclophosphamide and/or mitoxantrone with or without rituximab who have at least 12 months follow-up since starting treatment.

**Results**

Sixteen cases of MDS/sAML have been identified for an overall rate of 10%. Patients included 10 with follicular lymphoma, 2 with CLL and 4 with other low grade lymphomas. Most patients had been pre-treated with one to six lines of chemotherapy and/or radiotherapy. One patient was untreated prior to fludarabine combination treatment. MDS/sAML developed at a median time of 47 months following commencement of fludarabine treatment. Four patients had multiple courses of fludarabine combinations. Karyotypic analysis was typically complex. Median overall survival post MDS/sAML diagnosis was 9 months.

**Conclusion**

Fludarabine combination chemotherapy is associated with an increased risk of MDS/sAML particularly in patients who have been previously treated. This complication needs to be considered when evaluating the potential benefit of this treatment in lymphoproliferative disorders.

## P84

### A Case Report of t(8:16)(p11;p13) Translocation Variant

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#### Background

The t(8:16)(p11;p13) translocation is a rare finding in acute myeloid leukaemia, accounting for 6.5% of M4/M5 FAB subtype. It has characteristic clinicopathological features of disseminated intravascular coagulation, extramedullary infiltration and erythrophagocytosis in blast cells, associated with a poor prognostic outcome with survival often less than 1 year.

The t(8:16) has been shown to fuse the MOZ (monocytic leukaemia zinc finger) gene, which encodes a histone acetyltransferase (HAT) at 8p11.2 to the CBP (CREB binding protein) on 16p13.3, which encodes an acetyltransferase and transcriptional co-activator.

Variant t(8:16) with complex karyotypes have been reported.

#### Case

A 64 year old male presented with a ten day history of shortness of breath, lethargy and bruising. Peripheral blood examination showed a haemoglobin of 129g/L, white cell count of  $48.5 \times 10^9/L$ , platelets  $62 \times 10^9/L$ , monocytes  $3.0 \times 10^9/L$  and blasts of  $13.9 \times 10^9/L$ . Coagulation profile was consistent with disseminated intravascular coagulopathy. Bone marrow examination showed acute monoblastic leukaemia with prominent erythrophagocytosis.

Cytogenetics analysis showed 46, XY,-8, t(8;16)(p11;p13), t(der8;21)(q11;q22.3), +mar[20].

The patient commenced induction chemotherapy on day 4 of admission, but subsequently suffered a fatal retroperitoneal haemorrhage on day 11 despite blood product support and reversal of coagulopathy.

#### Conclusion

To date, there have only been 66 reported cases of t(8;16) including variants with complex karyotypes. Reports of a second translocation of the derivative 8, with a breakpoint at q11 which is then inverted so that the 8qter is in telomere association with the telomere of chromosome 21q have not been published.

**P85**

### **9p Deletions in Paediatric Acute Lymphoblastic Leukaemia**

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We present 14 patients diagnosed with acute lymphoblastic leukaemia at the Royal Children's Hospital, Brisbane since 2000

All had either a homozygous or heterozygous deletion of 9p, detectable on karyotype or by FISH using p16. This abnormality was found as a sole abnormality (4/14) or as part of a complex karyotype (9/14).

All patients achieved complete remission. One patient relapsed 14 months post diagnosis and died. One patient had testicular relapses 21 and 32 months post diagnosis, proceeded to bone marrow transplant and is alive 30 months post bone marrow transplant. All other patients remain alive either on treatment or one to four years off treatment.

Of the patients with a complex karyotype, 4/9 were hyperdiploid and the remainder pseudodiploid. There was no significant association with any other cytogenetic abnormality.

Deletion of 9p is associated with an intermediate prognosis. This was borne out in this cohort of patients.

**P86****The Use of Paediatric Chemotherapy Protocols in the Treatment of Young Adults with Acute Lymphoblastic Leukaemia**

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**Aim**

Many recent studies suggest young adult patients with acute lymphoblastic leukaemia (ALL) have far superior outcomes when treated on more intensive Paediatric protocols compared with adult protocols. The difference is believed to lie in dose intensity and the choice of antileukaemic agents – in particular the addition of L-asparaginase. We examined the feasibility of giving a paediatric protocol to an older group of patients.

**Method**

Seventeen patients, age range 16-33 years, diagnosed with either T or B cell ALL were treated on the ANZCCSG (Australian and New Zealand Children's Cancer Study Group) Study VII multiagent chemotherapy protocol over the period November 2002 to 2007.

**Results**

The median age of the patients was 22 years (16-33), 65% were male and 65% were B-lineage. CR at the end of induction was 92%. There were 4 deaths, three regime related and one relapse. Asparaginase toxicity was noted in 50% - with abnormal liver function requiring treatment modification, pancreatitis and thrombosis being the most common. Two patients developed hyperglycaemia requiring insulin. Significant drug induced neuropathy was noted in three patients, again requiring dose and drug modification. The average number of admissions for febrile neutropenia was 5 (range 1-10).

**Conclusion**

Despite small numbers we found a significant rate of morbidity and mortality directly related to the dose intensification used in this protocol originally designed for the paediatric age group. Toxicity related to L-asparaginase was significant. With further follow up we will be able to determine the disease free survival and see whether this will offset the considerable toxicity seen to date.

**P87**

## **Myeloblast Infiltration of the Temporal Artery Preceding Transformation of RAEB-1 to Acute Myeloid Leukaemia by 15 Months in a 68 Year Old Man**

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We report a case of a patient with refractory anaemia with excess blasts (RAEB-1), diagnosed with a malignant myeloid cellular infiltrate of the temporal artery in the setting of complete cytogenetic remission (CCR). He subsequently developed LC and leukaemic pulmonary infiltrates in the context of transformation to acute myeloid leukaemia (AML) 15 months later. Temporal artery leukaemic infiltration in association with MDS has not been described.

### **Case**

A previously well 68-year-old man was diagnosed with poor risk MDS - RAEB-I (blasts 8%, trisomy 8) and enrolled in a Phase III multi-centre randomised trial of azacytidine therapy (25mg/m<sup>2</sup> s/c D1-7 per cycle).

Nine months/cycles into treatment, the patient presented with one month of frontal headache, without meningitic, infective or vasculitic symptoms. Clinical examination, CT brain and CSF assessment were unremarkable. Full blood examination (FBE): normochromic, normocytic anaemia (haemoglobin (Hb) 96 g/L [135-180]), normal white cell and platelet morphology, no circulating blasts. Vasculitic screen was negative. Bone marrow biopsy demonstrated blast reduction from 8→1%, despite persistent trilineage dyshaematopoiesis and trisomy 8. [Marrow assessment four months later (cycle 13) demonstrated CCR].

Due to the onset of jaw claudication over the following month, temporal arteritis was considered (ESR 101 mm/1hr). Biopsy of the right temporal artery demonstrated a periarteriolar collection of atypical myeloid cells: strongly immunoreactive with myeloperoxidase. Treatment with prednisolone (1mg/kg) resulted in rapid resolution of symptoms and inflammatory markers. The patient remained well and continued on trial for 23 cycles/months, until cytogenetic relapse.

One month following treatment cessation, the patient developed fevers and a widespread papular rash (no lymphadenopathy nor hepatosplenomegaly). A leukoerythroblastic FBE suggested transformation to AML – Hb 95 g/L, leukocytes 11.6 x 10<sup>9</sup>/L, neutrophils 2.32 x 10<sup>9</sup>/L, Platelets 98 x 10<sup>9</sup>/L, blasts 0.93 x 10<sup>9</sup>/L. Skin biopsy demonstrated LC with associated vasculitis. The patient became hypoxic – lung biopsy demonstrated leukaemic infiltrate with associated vasculitis. Significant leukocytosis developed [WCC: 41.8 x 10<sup>9</sup>/L (blasts: 12.54 x 10<sup>9</sup>/L)] and the patient expired one month later due to complications of his disease.

**P88****Impact of FLT3-internal Tandem Duplication (ITD) Status on Relapse Free Survival (RFS) and Overall Survival (OS) in Patients with Normal Karyotype (NK) Acute Myeloid Leukaemia (AML) Treated with Chemotherapy or Allogeneic Transplantation**

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FLT3-ITD mutations are associated with inferior outcomes in patients with AML. In adult AML, 35-50% have a normal karyotype, associated with an intermediate prognosis. FLT3-ITD mutation analysis is being used to stratify patients in this heterogeneous group.

**Aims**

To perform a single centre retrospective study evaluating OS of patients with NK AML according to FLT3-ITD status following chemotherapy or allogeneic transplantation.

**Methods**

Two groups of patients with NK AML were evaluated by Kaplan-Meier survival probabilities: Group 1: 28 patients treated with chemotherapy (7 allografted) 23 were < 60 years; Group 2: 31 patients treated with allogeneic transplantation [8 in first complete remission (CR1)].

**Results**

Group 1: FLT3-ITD mutations were detected in 39% (11/28) overall, and in 39% (9/23) of patients < 60. FLT3-ITD+ patients had a significantly worse RFS and OS compared with FLT3-wild type (WT), with a median OS of 227 versus 883 days ( $p = 0.02$ ) and RFS 147 versus 514 days ( $p = 0.007$ ). In the subgroup of patients aged < 60, RFS was also reduced (147 versus 595 days,  $p = 0.007$ ) with no difference in OS ( $p = 0.07$ ).

Group 2: The detection of FLT3-ITD mutations was varied between patients transplanted in CR1 (6/8) versus those allografted >CR1 (3/23). The median survival of all allografted FLT3-ITD+ patients compared to FLT3-WT irrespective of disease status was reduced: 212 versus 1283 days ( $p = 0.002$ ).

**Conclusion**

FLT3-ITD mutations confer reduced OS and RFS on patients with NK AML. The observation that the substantial majority of patients allografted beyond CR1 were FLT3-ITD negative suggests that these patients are less likely to proceed to transplant, perhaps because of early relapse. Conversely, the somewhat higher than expected frequency of FLT3-ITD mutations in patients transplanted in CR1 suggests that these patients may be preferentially referred due to other prognostic factors.

**P89**

## **Single Centre Experience with the Use of FLAG-amsacrine for the Treatment of Refractory/Relapsed AML**

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### **Aim**

Evaluate the efficacy and tolerability of FLAG (fludarabine, cytarabine, GCSF)-amsacrine as salvage chemotherapy for patients with relapsed or refractory Acute Myeloid Leukaemia (AML).

### **Methods**

Between January 2004 and July 2007, 15 patients with refractory or relapsed AML were treated at The Alfred Hospital with FLAG-amsacrine (Fludarabine 30 mg/m<sup>2</sup>/day days 1-5; Cytarabine 2000 mg/m<sup>2</sup>/day days 1-5 Amsacrine 100 mg/m<sup>2</sup>/day days 1-5; GCSF 300 mcg/day days 1-6;). Retrospective chart review was performed and data related to toxicity, response and survival obtained. Overall survival from the date of treatment until the date of death from any cause was estimated according to the Kaplan-Meier method.

### **Results**

Median age was 56 years (range 32-68) and median disease duration 12 months. Three patients were primary refractory, 9 patients had relapsed, including 3 patients after stem cell transplantation. Overall, eight (53%) patients achieved complete remission, two had a partial response, and five were refractory to treatment. Recovery of neutrophils and platelets occurred at a median of 32 and 17 days from the start of therapy, respectively. There were no treatment-related deaths. Two patients in complete remission had one cycle of consolidation therapy with FLAG-amsacrine and remain in continuous complete remission without further treatment. With a median follow up of 5 months (range, 1 to 25), the median overall survival was 8 months. Four patients (3 with relapsed disease; 1 with refractory disease) remain in continuous complete remission with a median duration of 14 months (5-25). Two patients in complete remission underwent stem cell transplantation.

### **Conclusion**

FLAG-amsacrine is an effective and well tolerated therapeutic option for advanced AML.

**P90****Evaluation of the *InvivoScribe* Technologies FLT3 Mutation Assay for Mutation Detection in AML Patients**

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**Aim**

To evaluate the FLT3 Mutation Assay kit (*InvivoScribe* Technologies) for identification of both the Internal Tandem Duplication (ITD) and D835 point mutations characterised in the FLT3 gene of patients with Acute Myeloid Leukemia (AML). FLT3 mutations are an informative prognostic marker in this disease.

**Method**

The FLT3 Mutation Assay is a DNA PCR-based test for detection of FLT3 ITD/D835 gene mutations. The assay contains separate ITD, D835 and sample quality PCR reactions with appropriate positive (D835 and ITD) and negative (normal) DNA controls. The study population comprised three groups: (1) patients with AML (confirmed by other pathology testing), (2) negative controls (patients with non-malignant disease), (3) a de-identified FLT3 10-DNA control panel consisting of confirmed positive and negative controls provided by John Hopkins Medical Institute (Baltimore, MD) for the purposes of test validation.

**Result**

The FLT3 mutation detection rate for AML patient samples was 27%. This agrees with the expected range 25-35%. No false positive results were obtained from the 'negative' patient group. The panel of 10 DNA quality control samples gave 100% concordance with the expected results as confirmed by John Hopkins Medical Institute. The stated sensitivity of 1% was confirmed for the assay.

**Conclusion**

The mutation detection rate of FLT3 in AML patients was in accord with published data of 25-35%. The quality control panel confirmed specificity and provided a rigorous test of the assay. A modification was made by inclusion of a sensitivity control to monitor the inter-experiment variation. The use of the commercial *InvivoScribe* Technologies FLT3 Mutation Assay offers an efficient approach for laboratory test validation as the assay has been pre-optimised and validated against different specimen types and simplifies meeting the regulatory requirements for *in vitro* diagnostic devices.

**P91**

**CD4+/CD56+ Haematodermic Tumour ( Blastic NK Cell Lymphoma)  
Terminating as Acute Myelomonocytic Leukaemia**

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Tumours expressing CD4 and CD56 involving skin, bone marrow and lymph nodes are known as CD4+/CD56+ Haematodermic Tumours or Blastic NK Cell Lymphomas.(1) The classification is under review by WHO-EORTC .The term plasmacytoid dendritic cell leukaemia/lymphoma is favoured.The tumour has a significant risk of leukaemic dissemination and progression to Acute Myelomonocytic Leukaemia.

The patient described presented with multiple subcutaneous nodules of the abdomen. Immunohistochemistry performed showed a CD4+/CD56+ phenotype . This profile may be seen with T/NK lineage and myelomonocytic leukaemia. It may also be seen in the CD4+/CD56+ Haematodermic neoplasm. The plasmacytoid dendritic cell precursor neoplasm is an aggressive disease with poor prognosis with recent studies suggesting patients are best treated with acute leukaemia regimens.

Bone marrow trephine showed the normal marrow architecture replaced by extensive hypercellular infiltrate. There was extensive collagen fibrosis and sheets of bland mononuclear cells.

The morphology, immunophenotype (CD4+/CD56+) and clinical features (skin infiltrate) were most suggestive of a diagnosis of Blastic NK cell Lymphoma.

The patient underwent 2 cycles of Hyper-CVAD and achieved haematologic remission and remained in remission after 6 cycles Hyper-CVAD.

6 months after diagnosis the patient had an autograft. Bone marrow examination performed 42 days post BEAM revealed an extensive marrow infiltrate by the aggressive lymphoma. On immunophenotyping, a population of abnormal monocytes expressed CD4/56/43/33/64/cMPO.This appeared to be a leukaemic relapse with a shift to (myelo)monocytic differentiation .

Over the next 10 days numerous blasts were present in peripheral blood with some blasts having a histiocytic appearance. The patient died 2 days later.

Haematodermic tumours occur more frequently in males than females and 90% have skin lesions on presentation. There is minimal blood and bone marrow involvement initially and terminates in fulminant leukaemia . A median survival of 12-14 months has been reported.

**P92**

## **Auer Rod Like Inclusions in Lymphoma Cells: A Case Presentation**

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The presence of Auer rods in blasts is considered pathognomonic of myeloid lineage. The case described is that of a 90 year old male with a previously diagnosed B-cell Lymphoproliferative disorder who was noted to have the rare finding of Auer rod-like inclusions (ARLI) in a small proportion of circulating lymphoid cells.

The patient originally presented with a moderate lymphocytosis, splenomegaly and a small IgG kappa paraprotein (which remains unexplained). The B-cell immunophenotype was CD19+, CD20+, CD79b+, FMC7+, CD23+, and lambda++ but CD5- and CD10-. Because of a rapidly enlarging spleen and evolving cytopenia, he underwent splenectomy. Histology and immunohistochemistry favoured a diagnosis of Splenic Marginal Zone Lymphoma.

The patient remained in reasonably good health with no further specific treatment until presenting with pneumonia approximately two years later. It was at this time that the ARLIs were noted.

The inclusions were presumed to be most likely immunoglobulin or partial immunoglobulin crystals. Investigations were performed to elucidate their nature, including cytochemistry, immunophenotyping, both surface and cytoplasmic, electron microscopy and immunofluorescent staining. Flow cytometry showed surface expression of IgM and lambda, but no definitive plasmacytoid differentiation (only weak CD38 and CD138-). Whilst the cytoplasmic analysis suggested the presence of intracytoplasmic IgM lambda, surface staining with the relevant antigens makes this inconclusive. Immunofluorescent staining of blood smears for heavy chains and light chains respectively suggested the presence of occasional rod-like cytoplasmic structures fluorescing positively when stained for IgM and lambda.

We concluded that the cytoplasmic rod-like inclusions were most likely deposits of immunoglobulin. A brief literature review is presented of similar cases and their findings with regard to the nature of the ARLI.

**P93**

## **Regression of Acquired C1 Esterase Inhibitor Deficiency Following Chemotherapy for Hodgkin Lymphoma**

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### **Background**

Acquired C1 esterase inhibitor (C1EI) deficiency is a rare condition which may complicate autoimmune conditions and B-cell non-Hodgkin lymphoma (NHL). Whether Hodgkin lymphoma (HL) can cause C1EI deficiency is uncertain, with few reported cases.

### **Case report**

A 63-year-old female presented with a four year history of recurrent unprovoked angioedema. Other history included facial swelling following treatment with several antibiotic preparations, dating back over 20 years. Her angioedema had been poorly responsive to corticosteroids, antihistamines, danazol and mast-cell stabilizers. She was otherwise well, reported no B-symptoms and had no clinically evident lymphadenopathy or organomegaly.

A diagnosis of acquired C1EI deficiency was supported by undetectable C1EI antigen, low C1EI function (10%, normal > 70%), suppressed C4 (< 0.03g/L, normal 0.13-0.43g/L) and normal C3 levels (0.84g/L, normal 0.81-1.68g/L). Tests for paraprotein, cryoprotein, antinuclear antibody and extractable nuclear antigens were negative. Computed tomography scanning revealed pathological lymphadenopathy in the left supraclavicular area and retroperitoneum, with mild splenomegaly. Excision biopsy of a node demonstrated classical HL, mixed cellularity type. There was no evidence of bone marrow involvement.

Treatment was commenced with ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) without administration of C1EI concentrate. Therapy was well tolerated, without exacerbation of angioedema. Following three cycles of ABVD she was in radiological and metabolic complete remission. After completion of six cycles of ABVD, she remains in complete remission 18 months following diagnosis. She reported no episodes of angioedema either during chemotherapy, or at subsequent review. Laboratory assessment following completion of therapy demonstrates ongoing regression of C1EI deficiency with normal C1EI function (131%).

### **Conclusions**

Our case demonstrates that acquired C1EI deficiency may be associated with HL, in addition to a well described association with NHL. Eradication of the underlying lymphoma resulted in remission of C1EI deficiency in this case, thus strengthening the clinicopathological association between the two diseases.

**P94****Rituximab for Recalcitrant Necrobiotic Xanthogranuloma – The First Case Report**

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**Background**

Necrobiotic Xanthogranuloma (NX) is a very rare disease of destructive xanthomatous granulomas classically involving the face, periorbital region and trunk which is commonly associated with paraproteinaemia. It is typically indolent and refractory to therapy [1]. Although the pathogenesis is unknown, CD20 staining has recently been demonstrated in NX lesions [2]. Rituximab, a monoclonal CD20 antibody, may therefore be of therapeutic benefit.

**Aim**

To document the first case report of Rituximab therapy in a patient with Necrobiotic Xanthogranuloma.

**Method**

Mrs CE, a 58 year old woman, presented with a background history of dyslipidaemia, hypothyroidism, hypertension and a 12g/L IgG kappa Monoclonal Gammopathy of Uncertain Significance (MGUS). She presented in December 2001 with an 18 month history of bilateral slowly enlarging, tender, yellow plaques on the upper and lower eyelids. This was associated with a 5 month history of indurated plaques on her right upper arm, left forearm and left thigh. Biopsy of the lesions confirmed the diagnosis of NX.

Over the following sixteen months she was treated with numerous chemotherapy regimes with no improvement. Numerous corneal and skin grafts have been required to treat progressive, disfiguring periorbital disease and exposure keratopathy.

In February 2007, Mrs CE received four 700mg doses of Rituximab at weekly intervals with no side effects.

**Results**

No improvement in skin lesions or blood parameters, including paraprotein, have been noted at time of report.

**References**

- 1 Flann, S et al Necrobiotic xanthogranuloma with paraproteinaemia. Clin Exp Derm, 31, 248-251
2. Ho, VH et al Receptor Expression in orbital inflammatory syndromes and implications for targeted therapies. Tissue Antigens 70. Issue 2 page 105

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## **Oxidative Haemolysis Secondary to Rasburicase Administration in Patient with Anaplastic Large Cell Lymphoma**

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### **Aim**

To report a case of oxidative haemolytic anaemia after administration of rasburicase in a patient with previously undiagnosed glucose-6-phosphate dehydrogenase (G6PD) deficiency.

### **Case Summary**

A 57-year old male from El Salvador, presented with spontaneous acute tumour-lysis syndrome, 10 days after a new diagnosis of clinical stage IIIA anaplastic large cell lymphoma (Alk-1 positive). He was hospitalised with abdominal pain, ascites, acute renal failure, hyperkalaemia, hyperuricaemia and raised serum lactate dehydrogenase. The acute renal failure secondary to elevated serum uric acid concentrations was treated with intravenous (IV) rasburicase 15mg. Imminent haemolysis was heralded by the appearance of extensive blister cells (cells of oxidative injury) on the peripheral blood film. Supravital staining of peripheral blood with methyl violet displayed Heinz bodies in 99% of erythrocytes. Haemolytic anaemia developed as evidenced by a marked decrement in haemoglobin from 113 to 70 g/L. Arterial blood gases revealed elevated methaemoglobin concentrations which peaked at 12.2%. G6PD deficiency was confirmed on laboratory testing (2.1U/gHb), although even on retrospective questioning there had been no known previous personal or family history. The patient was aggressively treated with IV fluid therapy, IV cyclophosphamide, blood transfusion and non-invasive ventilation. He subsequently developed inferior vena caval thrombosis, pulmonary embolism and hepatic dysfunction. He died 12 days after administration of rasburicase with multi-organ failure and progressive malignancy.

### **Conclusion**

G6PD deficiency is a recognised contraindication to the administration of rasburicase. With a limited number of prior reported cases of its complications, we seek to heighten awareness of this contraindication. Rasburicase causes oxidative stress due to the production of hydrogen peroxide in the conversion of uric acid to allantoin. Screening for G6PD deficiency should be performed whenever possible prior to rasburicase administration in patients at risk of tumour-lysis syndrome.

**P96****Successful Treatment of Primary Mediastinal B Cell Lymphoma in Pregnancy**

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**Summary**

A 28 year old pregnant female with primary mediastinal B cell lymphoma achieves PET negative remission with 6 cycles of R-CHOP21 given from 20 weeks gestation. A healthy female child was delivered at 39 weeks.

**Case History**

The patient presented at 17 weeks gestation with SCV obstruction and bilateral internal jugular vein thrombosis. MRI revealed a 5 X 5cm right sided mediastinal mass and no subdiaphragmatic disease. She is heterozygous for the Factor V Leiden mutation. Her full blood count was normal and a marrow biopsy was not performed. Mediastinal biopsy confirmed a CD 20 positive large B cell lymphoma. The patient was commenced on corticosteroids and anticoagulated with enoxaparin. The patient elected to continue the pregnancy. After literature review and careful discussion with the patient she was commenced on CHOP plus Rituximab at 20 weeks gestation. Therapy was given on 3 week basis without growth factor support. Therapy was well tolerated and after 3 cycles she had a partial response on MRI. Serial ultrasounds confirmed appropriate fetal growth along the 10<sup>th</sup> percentile. Elective caesarean section was planned for 3 weeks after the 6<sup>th</sup> cycle of R-CHOP. She delivered a healthy female weighing 2400g. Neonatal APGAR scores and full blood count were normal. 2 weeks later both patient and baby were well and CT confirms only small residual soft tissue mass which is PET negative. Ongoing paediatric review is planned.

**Discussion**

Mediastinal B cell lymphoma in pregnancy is uncommon and use of R-CHOP in this scenario rarely reported. Our case confirms the feasibility and tolerability of such an approach. This reinforces the need to consider alternatives to termination in these patients.

**P97**

## **Diagnosis of EBV-Associated B-Cell Lymphoproliferative Disorders in the Elderly Using EBER In-Situ Hybridization**

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### **Aim/Method**

Epstein-Barr virus-associated B-cell lymphoproliferative disorders in the elderly population are increasingly described however the disease can be difficult to diagnose. We describe two patients who developed EBV-driven lymphoproliferative disease diagnosed with the use of EBER *in-situ* hybridization.

### **Result**

An 80 year old woman presented with rash, fevers, sweats, weight loss and anaemia. Imaging, lymph node and bone marrow biopsies failed to identify the cause of her illness. Following progression of symptoms, an axillary lymph node excision biopsy revealed a polymorphous infiltrate of lymphocytes showing EBV positivity with EBER *in-situ* hybridization (Vision Biosystems). This patient resolved spontaneously with disappearance of lymphadenopathy and symptoms. A 56 year old male presented with a one-month history of generalized lymphadenopathy, anaemia and B-symptoms. He was diagnosed with an EBV-associated lymphoproliferative disorder based on histopathology of an excised cervical lymph node with numerous lymphoid cells positive for EBER-*ish*. His illness was complicated by pure red cell aplasia. The patient was treated with a single dose of Rituximab and showed dramatic improvement with resolution of lymphadenopathy, systemic symptoms, anaemia and decrease in EBV PCR quantitative titre.

### **Conclusion**

EBER-*ish* is a useful diagnostic tool to assist in the diagnosis of EBV-associated lymphoproliferative disorders. This may allow us to identify a subset of patients with lymphoma in whom therapeutic strategy may be different to EBV negative lymphoma. We describe two patients who achieved remission following minimal treatment.

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## Aggressive Lymphoma in Pregnancy - Successful Outcome Using CHOP-14 and Rituximab

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### Aim

The diagnosis of aggressive lymphoma during pregnancy is associated with unique management issues. We highlight the case of a 28-year-old primigravida presenting with ataxia in the second trimester of pregnancy secondary to spinal cord compression.

### Method

We review available literature of chemotherapy in pregnancy, and advances in lymphoma therapy. The patient had MRI imaging of the spinal cord at 22 weeks gestation, revealing cord compression at T6 by a posterior intraspinal extradural lesion, subsequently confirmed as diffuse large B-cell lymphoma. On limited staging investigations, no other sites of disease were identified. She received six cycles of CHOP-14 & rituximab chemotherapy with pegulated G-CSF support during late second and third trimesters. Due to her paraparesis, vinblastine was substituted for vincristine.

### Result

The treatment was well tolerated, with a single significant episode of respiratory infection requiring intravenous antibiotics. Serial obstetric ultrasounds were satisfactory. At 38 weeks gestation, she was induced and delivered via vaginal delivery a healthy son of low birthweight. Follow up MRI scan revealed no evidence of tumour mass, and PET scan was clear except for mildly increased activity in the soft tissues at the site of her previous surgery (and mass). She received consolidative radiotherapy to this region (36 Gy in 20 fractions), and has ongoing neurological improvement. The baby has very low B-cell numbers with reduced immunoglobulin levels, but is without infections and continues to thrive.

### Conclusion

Management of aggressive lymphoma arising during pregnancy is challenging, with limited available information. We report the first known case of a successful outcome in this setting using CHOP-14 and rituximab with pegulated G-CSF support, which resulted in a healthy mother and baby.

**P99****Hepatosplenic Gamma-delta T-cell Lymphoma. A Challenge to Diagnose and Treat**

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**Introduction**

Hepatosplenic gamma-delta T cell lymphoma is rare, accounting for less than 1% of lymphoma cases. The prognosis is poor with rare reports of long term remission, typically following aggressive therapy including stem cell transplant. A case highlighting the difficulty in diagnosis and treatment of this rare lymphoma is reported

**Case report**

A 28yr man had a 6 year history of SLE presenting with arthralgia and serositis, with positive ANA, anticardiolipin antibodies and lupus anticoagulant. He had been managed with prednisolone and NSAIDs and more recently with hydroxychloroquine. He now presented with several weeks of flu-like malaise with rigors, sweats, and mild headache. He had high spiking fevers and laboratory evidence of a brisk autoimmune haemolytic anaemia. A screen for an infective source of fever including echocardiogram was negative and his fever and haemolysis were attributed to active lupus. The hydroxychloroquine was ceased and he was managed with increased prednisolone at 40mg/d. However, the fevers and haemolysis persisted and he was readmitted for further investigation. CT scan revealed hepatosplenomegaly. There was no lymphocytosis but flow cytometry of peripheral blood and marrow revealed an expanded population of CD2+, CD5-, CD7+, CD3+, CD4-, CD8-, CD56+, gamma-delta+ T-cells. The BMAT did not reveal any definite morphologic evidence of lymphoproliferative disease even with specific T-cell immunostaining but TCR gene studies revealed a monoclonal rearrangement on a polyclonal background. These findings were suggestive of a gamma-delta T-cell lymphoma. However, the presence of expanded gamma-delta T-cell populations and dominant T-cell clones has been reported as a reactive finding in SLE in the absence of a lymphoproliferative disorder. A PET scan showed no evidence of FDG avid lymphoma. Ultimately, a liver biopsy was performed and revealed intrasinusoidal T-cell infiltration characteristic of hepatosplenic gamma-delta T-cell lymphoma. Cytogenetic analysis of peripheral blood revealed isochromosome 7q, Trisomy 8, loss of Y and also a 6:11 translocation. He was commenced on HyperCVAD chemotherapy, with incomplete and shortlived response, with progression of fevers, haemolysis and hepatosplenomegaly following cycle 1b. Treatment with pentostatin and alemtuzumab resulted in a further temporary response. He had no HLA compatible family members and was considered for volunteer donor allogeneic bone marrow transplant. Unfortunately, due to progressive disease and probable sepsis he died of multiorgan failure.

**Conclusion**

Hepatosplenic gamma-delta T-cell lymphoma is a rare entity that should be considered in patients with pyrexia of unknown origin and hepatosplenomegaly. Diagnosis depends on characteristic histological findings in conjunction with immunophenotyping and cytogenetic results. Prognosis is poor, so aggressive therapy including early stem cell transplantation is indicated.

**P100**

## **An Unusual Presentation of Cutaneous Intravascular Large B Cell Lymphoma as Recurrent Superficial Thrombophlebitis**

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### **Aim**

Intravascular large B cell lymphoma is a rare disorder which is often widely disseminated at diagnosis with a poor prognosis. Prognosis is improved in disease limited to the skin. We present the case of a 62 year old man who initially presented with recurrent superficial thrombophlebitis of his legs.

### **Method**

The patient initially presented with persistent skin and soft tissue changes, with erythema, swelling and induration of his lower legs. There was no clinical evidence of infection, lymphadenopathy or hepatosplenomegaly. CRP, ESR and IgG levels were mildly raised. Fever and night sweats were absent. The findings were perceived to be most consistent with superficial thrombophlebitis, with concerns of an underlying inflammatory cause. Antibiotics, anti-inflammatory preparations, and low molecular weight heparin did little to relieve his symptoms.

A right leg deep skin and muscle biopsy was performed which confirmed the presence of an intravascular B cell lymphoma. Staging investigations including CT chest, abdomen and pelvis, PET scan and bone marrow biopsy did not reveal disease elsewhere. Full blood count was normal, and LDH was mildly raised.

The patient was commenced on 6 cycles of R-CHOP (replacement of doxorubicin with mitoxantrone in view of a mild reduction in ejection fraction detected on echocardiogram), followed by two further doses of rituximab at monthly intervals.

At 12 months post chemotherapy, the skin changes have resolved, with normalisation of LDH and no evidence of relapse on imaging.

### **Conclusion**

This is an atypical presentation of the cutaneous variant of intravascular large B cell lymphoma as recurrent superficial thrombophlebitis, with lack of pyrexia and absence of significantly raised inflammatory markers at presentation. Cutaneous involvement appears to confer a better prognosis and improved response to treatment, and the patient remains well after systemic anthracycline based chemotherapy and rituximab.

**P101**

## **Radioimmunotherapy with <sup>131</sup>I-Rituximab for Relapsed Diffuse Large B Cell Lymphoma**

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### **Aim**

Radioimmunotherapy (RIT) with <sup>131</sup>I-rituximab is effective treatment in the management of relapsed and refractory non-Hodgkin Lymphoma with an overall response rate of 76% and complete remission in 53% with similar results using murine anti CD-20 radioimmunotherapy (Zevalin<sup>TM</sup> and Bexxar<sup>TM</sup>). The value of RIT in relapsed Diffuse Large B Cell Lymphoma (DLBCL) is less clear. This single institution phase II study was designed to assess the efficacy and safety of <sup>131</sup>I-rituximab in this patient group.

### **Methods**

Patients received induction with 4 once weekly unlabelled doses of rituximab 375mg/m<sup>2</sup> and individualized <sup>131</sup>I-rituximab doses administering 0.75Gy whole body exposure. Consolidation therapy was administered commencing day 56 and continued every 2 months with unlabelled rituximab 375mg/m<sup>2</sup> for 6 treatments. Patients were monitored with weekly FBE until 12 weeks post therapy or recovery from nadir. Severity and duration of cytopenia and assessment for the development of MDS and hypothyroidism were noted. Follow up PET/CT scans were performed at 6, 12, 24 and 52 weeks post treatment.

### **Results**

Eight patients have been enrolled to date and 6 have received treatment with <sup>131</sup>I-rituximab.

Two patients had a severe deterioration in performance status following enrolment and were unable to proceed with treatment. Three patients have had a complete response with PFS of 18, 12 and 2 months. Grade 4 neutropenia occurred in one patient. All 6 treated patients had had prior rituximab. Two of the responders had refractory disease.

### **Conclusions**

Treatment with <sup>131</sup>I-rituximab is effective and safe therapy in some patients with relapsed DLBCL who may not be suitable for intensive chemotherapy. Those with rapidly progressive and bulky disease are least likely to respond.

**P102****Burkitt Leukaemia in the Setting of Common Variable Immunodeficiency**

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**Background**

While common variable immunodeficiency (CVID) is associated with the development of lymphoproliferative disorders, and Burkitt lymphoma/leukaemia (BL) commonly arises in patients with acquired immune deficits, an association between CVID and BL has not previously been reported.

**Case Report**

A 51 year old man presented with left sided facial parasthesiae, fevers, night sweats, and weight loss. He had first come to medical attention aged 12 with recurrent sinus and respiratory tract infections. Investigation then revealed severe hypogammaglobulinaemia, consistent with common variable immunodeficiency. A regimen of prophylactic antibiotics and intravenous immunoglobulin (IVIg) was instituted.

Further investigation at presentation revealed haemoglobin 161g/L (normal 115–160), platelet count 79x10<sup>9</sup>/L (normal 150–396), and white cell count 25.9x10<sup>9</sup>/L (normal 3.9–12.7). The blood film showed lymphoid blasts with morphological characteristics of Burkitt lymphoma. A bone marrow aspirate and trephine confirmed effacement of normal marrow by these blasts. Immunohistochemistry demonstrated that expression of CD20, weak CD10, and nuclear BCL6 and Ki67. Cytogenetics were hypodiploid, with loss of Y and 3q and an 8:14 translocation, confirming the diagnosis of Burkitt leukaemia. Magnetic resonance imaging of the mandible demonstrated diffuse hypointensity consistent with malignant infiltration of bone.

After discussion regarding the risks of induction chemotherapy in view of his comorbidities, the patient decided to proceed with treatment. He received CODOX–M/IVAC, with intravenous hydration and prophylactic rasburicase. Despite this, he developed acute renal failure and multiple electrolyte disturbances resulting from tumour lysis, and bilateral nosocomial pneumonia. These problems responded to treatment including broad spectrum antimicrobial agents and invasive cardiorespiratory support.

A bone marrow aspirate and trephine performed 25 days after commencement of chemotherapy demonstrated remission. Unfortunately, within 10 days his disease relapsed in the peripheral circulation, and he was managed with palliative intent.

**Conclusions**

This case raises questions regarding possible immune and infectious etiologies of this novel disease association, and illustrates the challenges of managing induction chemotherapy for high-grade malignancies in patients with severe immunodeficiencies.

**P103**

## **A Pilot Study of Rituximab in Combination with Outpatient Based VGF/F-GIV Salvage Therapies for Relapsed/ Refractory CD20+ Lymphomas**

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### **Aim**

We have previously trialed an outpatient regimen of Vinorelbine, Gemcitabine and filgrastim (VGF), with or without Ifosfamide (F-GIV) for advanced lymphomas. We have now evaluated these regimens incorporating Rituximab for advanced CD20 positive non-Hodgkin's lymphoma (NHL).

### **Method**

Patients were stratified into Group 1 (G1-first relapse, follicular NHL >12 months, other NHL >6 months); Group 2 (G2-primary refractory, early relapse, or > first relapse); Group 3 (G3-relapse post-ASCT with PFS > 6 months). G1 and G3 received R-VGF (Rituximab 375mg/m<sup>2</sup> IV, Vinorelbine 25mg/m<sup>2</sup> IV, Gemcitabine 1000mg/m<sup>2</sup> IV Day 1 and 8, Pegfilgrastim Day 9) every 21 days. G2 received R-FGIV (same as R-VGF with addition Ifosfamide 3g/m<sup>2</sup> on Day 1). Patients failing to achieve >50% reduction in disease and PET negativity after 2 cycles were escalated (G1 and G3 to R-F-GIV; G2 to IVAC (inpatient Etoposide, Ifosfamide, Cytarabine, with Pegfilgrastim) for a further 2 cycles.

### **Result**

Twelve patients have been accrued. Diagnoses are DLCL 10; FL 1, Mantle Cell 1, Nodal Marginal Zone 1. Eleven patients have received > 2 cycles; 8 have completed 4 cycles. Median age for those beyond cycle 2 is 66y (range 38-84). Rates of grade 3 or 4 haematological toxicity with R-VGF (18 cycles) were: neutropenia 2/18; thrombocytopenia 3/18; with R-F-GIV (8 cycles): anaemia 1/8, neutropenia 6/8, thrombocytopenia 4/8. There were six grade 3 or 4 non-haematological toxicities. Response (Cheson BD, 1999) after two cycles was achieved in 6/7 in G1 (2CR, 4PR, 1PD); 1/2 in G2 (1CR, 1PD); 2 in G3 (1 in CR, 1 off study post cycle 1 due toxicity but in CR). At final assessment, response in 6/8 (5CR, 1PR, 2PD).

### **Conclusion**

R-VGF and R-F-GIV appear to be well tolerated outpatient salvage regimens for CD20 positive NHL. Early outcome data suggest the regimen has significant activity.

**P104****CHOP-R 14 Day and Pneumocystis jiroveci Pneumonia**Nick Murphy, Frank Hong, Goeffrey Chong, Daniela Zantomio*Department of Clinical Haematology and Oncology Austin Health Melbourne Australia*

We report three cases of Pneumocystis jiroveci pneumonia (PCP) over one month in patients with diffuse large B cell lymphoma (DLBCL) on CHOP-R 14 day and describe their clinical features. Raised clinical awareness of this association is important, as routine co-trimoxazole prophylaxis is uncommonly prescribed with this regimen. The presentations of the three patients were similar. All had advanced stage DLBCL and were under 50 years of age without co-morbidities. The presentation of PCP was abrupt with pyrexia, dyspnoea and hypoxia. The diagnosis was confirmed by immunofluorescence on sputum in two cases and presumed due to typical clinical course in the third. All patients presented after cycle 5 of CHOP-R 14 day and none were neutropenic. Imaging showed characteristic bilateral pulmonary infiltrates, although changes were subtle at presentation. Low immunoglobulin levels and a reduced CD4/8 ratio were noted in all patients. Prompt recovery with co-trimoxazole therapy was observed. The close clustering of our cases raised the possibility of nosocomial spread of the infection, though it was not possible to confirm this with molecular testing. Our report highlights the need to recognise the differing immunosuppressive effects of 14 day versus 21 day CHOP-R chemotherapy. The reasons for this may relate to shorter time between steroid pulses, the use of cytokines that facilitate neutrophil recovery to a greater degree than lymphocyte recovery. The addition of rituximab may play a role. Increased awareness of the association between CHOP-R 14 day and PCP is important to facilitate prompt recognition and institution of appropriate therapy. Routine prophylaxis should be considered in this patient group, though this has not been assessed in a randomised study. The possibility of nosocomial spread highlights the importance of respiratory isolation when PCP is suspected.

**P105**

## **Composite Lymphoma – A Case Report of Simultaneous Diffuse Large B-Cell (DLBCL) and Hodgkin Lymphoma (HL)**

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### **Background**

Composite lymphoma (CL) is the rare simultaneous occurrence of two or more morphologically and genetically distinct lymphomas at the same anatomic site. Most cases include combinations of non-Hodgkin's lymphoma (NHL), or rarely HL and B-cell NHL.

### **Case report**

We report a case of composite de novo DLBCL and HL, in the inguinal lymph node (LN) and bone marrow (BM) of an 83 year old male, presenting with several months of constitutional symptoms, normochromic normocytic anaemia, mild lymphocytosis, widespread non-tender lymphadenopathy and hepatosplenomegaly, on a two year background of stable, untreated chronic myelomonocytic leukaemia.

LN biopsy demonstrated effaced architecture comprised predominantly of HL (mixed cellularity) with the coexistence of a small confluent aggregate of DLBCL. Immunohistochemistry demonstrated CD30 and CD15 membrane and paranuclear dot positivity in a large portion of the node. However, the large cell aggregate (negative for CD30 and CD15) showed strong CD20 membrane and weak CD79a cytoplasmic staining. BM examination showed identical findings. Epstein Barr virus (EBV)-in-situ hybridisation on BM sections detected EBV encoded RNA transcripts in both tumour cells. Microdissection of the 2 tumours with both IgH PCR and sequencing results are awaited to confirm distinct clonal populations.

### **Discussion**

This case represents an unusual entity with two lymphoproliferative disorders presenting concurrently at the same site. Despite the unique clinicopathology, it is not clearly established whether they represent distinct clonal entities. The analysis of DNA/RNA within the different malignant cells can generate information about the molecular changes that distinguish Hodgkin cells from normal and neoplastic B cells. They may originate from the same precursor that has undergone initial malignant transformation, but what is more important, is the subsequent molecular events that determine which direction – Hodgkin or neoplastic mature B-cell. EBV is reported at high frequencies within HL. In this case, it would appear that EBV may be the common link and may represent an important oncogenic or genetic event in the pathogenesis of this entity.

**P106**

## **Improving Cytotoxic Safety**

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### **Aim**

Standardisation of practices relating to the safe handling and administration of cytotoxic drugs and related waste.

### **Method**

Publication of the Queensland Government Department of Industrial Relations: *Guide for Handling Cytotoxic Drugs and Related Waste, 2005*, provided an opportunity to revise current processes throughout Cancer Care Services.

A gap analysis performed in May 2005 identified the need to review, update and develop control measures such as:-

- Policies, Standard Operating Procedures (SOP),
- Staff Education,
- Staff Exposure Logs,
- Personal Protective Equipment (PPE),
- Clinical Supplies,
- Patient Checklists,
- Cytotoxic Risk Assessments and
- Cytotoxic Drug Registers.

Cancer Care Services Cytotoxic Risk Management Group facilitated the review, implementation and evaluation of systems and processes.

### **Results**

The development of a Standard Operating Procedure manual aligning practices and processes with legislative requirements and guidelines for the safe handling and administration of cytotoxic drugs and related waste. Initial cytotoxic risk assessments were completed in all clinical areas throughout Cancer Care Services and recommendations in place. Cytotoxic awareness sessions for staff have been rolled out across the organisation and induction awareness in district orientation is being implemented. Patient checklists have been developed and implemented to ensure specific criteria are met prior to the administration of cytotoxic drugs and patient education material reviewed to ensure patients are appropriately informed of their treatment. All staff handling cytotoxic drugs and related waste have access to exposure logs and the use of PPE is regularly reviewed to ensure care and maintenance procedures are adequate. Recent audit results have demonstrated improved compliance with practices and procedures and regular auditing schedules are in place to ensure the continuation of our quality program.

### **Conclusion**

The permanent appointment of a Safety and Quality Officer and Nurse Educator with Cytotoxic Risk as a portfolio has enabled Cancer Care Services to provide resources and ongoing support across the facility.

**A328**

**P107**

## **Sarcoidosis Causing False Positive FDG-PET Following Treatment for Sclerosing Mediastinal (thymic) Large B-cell Lymphoma**

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FDG-PET is integral in the staging, response evaluation and follow up of patients with lymphoma, however is not tumor-specific with uptake seen in a numerous benign conditions - the most frequent cause for false-positive results is inflammation (including granulomata and abscesses). We report a case of a 36-year-old woman who was diagnosed with sarcoidosis 14 months following treatment for Non-Hodgkin's Lymphoma (NHL), in the setting of persistently positive FDG-PET imaging.

### **Case**

The patient was initially diagnosed with bulky stage IIA sclerosing mediastinal (thymic) large B-cell lymphoma following a presentation with a mediastinal mass complicated by SVC obstruction. An FDG-PET scan could not be performed at diagnosis.

Treatment was initiated with R-CHOP chemotherapy, however upon ascertainment of the definitive diagnosis, was changed to MACOP-B chemotherapy. An FDG-PET scan performed shortly after 12 weeks of treatment demonstrated multi-focal uptake within mediastinal and abdominal nodes. Given the low clinical suspicion for progressive disease, favourable CT appearance and patient choice, the patient was observed with serial imaging. Three months later, the patient proceeded to further radiotherapy to the mediastinum (changes in the abdomen had resolved)

The patient remained well, however FDG-PET scanning continued to demonstrate metabolically active mediastinal disease. The patient again declined excisional biopsy, and continued to be followed with serial FDG-PET scans which remained positive despite apparent clinical remission.

Fourteen months after the completion of chemo-radiotherapy, the patient underwent lymph node biopsy by mediastinoscopy: widespread non-necrotising granulomata in keeping with sarcoidosis/sarcoidal reaction. Angiotensin converting enzyme levels performed at this time and at initial diagnosis were both normal: 42 U/L (12-68). Whilst a sarcoidal reaction to lymphoma cannot totally be excluded, the absence of overt relapse over many months of observation makes this unlikely.

### **Conclusion**

This case clearly demonstrates the limitations of FDG-PET imaging in the management of patients with lymphoid malignancies, the importance of considering an alternative diagnosis and highlights the need for excisional biopsy when FDG-PET imaging does not correlate with the clinical picture.

**P108**

**Diffuse Large B-Cell Lymphoma and Hydatid Disease: A Case Report**

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**Introduction**

Hydatid disease is a zoonotic illness caused by the larval form of *Echinococcus sp.* The occurrence of hydatid disease and malignancy is rare. Occasional cases of cyst rupture causing life-threatening complications during chemotherapy have been reported.

**Method**

A 61-year-old lady was diagnosed with Stage IIIA Diffuse Large B-cell Lymphoma (DLBCL) in January 2007. She had breast cancer in 1998, treated with surgery and chemotherapy - 4 cycles Epirubicin, Cyclophosphamide (EC), and 4 cycles Cyclophosphamide, Methotrexate, 5-Fluorouracil (CMF), followed by 5 years of Tamoxifen, then maintenance Arimidex therapy.

She had an old calcified cyst identified in 2006 measuring 6.6 x 5.8 x 4.6cm (segment 7/8) in the liver. Despite negative hydatid serology, the combination of the calcified cyst and a childhood history growing up on a rural property with livestock meant hydatid disease was most likely. Her brother also had hydatid disease 20 years ago.

She had 5 months of left upper abdominal discomfort, with back pain 6 weeks prior to diagnosis of lymphoma, 7 kg of weight loss and no night sweats. Imaging confirmed extensive para-aortic and left supraclavicular lymphadenopathy, splenomegaly, and the calcified cyst.

Biopsies confirmed DLBCL. She commenced chemotherapy with Rituximab, Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone (R-CHOP) x 6 cycles. As the hydatid cyst was heavily calcified, therefore likely to be nonviable, a decision was made to not treat it. Hydatid serology was monitored throughout therapy and remained undetectable. Imaging confirmed that the cyst size and contents remained unchanged.

**Discussion**

Serological and imaging studies monitoring hydatid disease during intensive chemotherapy for Diffuse Large B-cell Lymphoma allowed treatment completion without complications or disease reactivation.

**Reference**

- 1 Ali R, Ozkalemkas F, Ozkocaman V, Ozcelik T, Akalin H, Ozkan A, Altundal Y, Tunali A. Hydatid disease in acute leukaemia: effect of anticancer treatment on echinococcosis. *Microbes and Infection* 7 (2005); 1073-1076

P109

## In vivo Tracking of Dendritic Cells in Patients with Multiple Myeloma

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### Aim

Dendritic cell (DC) immunotherapy is being actively studied in multiple myeloma (MM). We aimed to use positron emission tomography (PET) or Single Positron Emission Tomography (SPECT) to determine the *in vivo* distribution of monocyte-derived non-matured DC (nmDC) or matured DC (mDC) administered to patients with MM.

### Method

Eligible patients had stable or slowly progressive MM and elevated serum MUC-1 or MUC-1 expression on marrow plasma cells. DC were derived from GM-CSF+IL-13 stimulated autologous monocytes and pulsed with Mannan-MUC1 fusion protein, and matured by FMKp and IFN- $\gamma$ . Prior to injection, DC were labelled with either <sup>18</sup>Fluorine-fluorodeoxyglucose (FDG), <sup>111</sup>Indium (In)-oxine or <sup>64</sup>Copper-pyruvaldehyde-bis-N-4-methylthiosemicarbazone (<sup>64</sup>Cu-PTSM). Labelled DC were given either as a single intravenous (iv) dose or by concurrent subcutaneous (sc), intradermal (id) and intranodal routes (in).

### Result

<sup>18</sup>FDG tracking was unsuccessful due to high radiolabel efflux. <sup>64</sup>Cu-PTSM-labeled mDC (n = 2 patients) demonstrated tracking to regional nodes but quantitation was also limited due to cellular efflux. <sup>111</sup>In-oxine however gave reproducible tracking of both nmDC and mDC (n = 6) to regional LN following either sc or id administration, with mDC revealing superior migration to regional LN. sc and id routes produced similar levels of DC migration. No definitive immunological or clinical responses were observed.

### Conclusion

Both mDC and nmDC may be successfully labelled with <sup>111</sup>In-oxine or <sup>64</sup>Cu-PTSM and their fate tracked *in vivo*. Mature DC demonstrated superior ability to migrate to regional LN; both subcutaneous and intradermal injection routes produced similar efficacy of DC migration suggesting that multiple simultaneous DC doses via either sc or id routes may be required to enhance the efficacy of DC based vaccines.

**P110****Chromosome Abnormalities Detected in Metaphase and/or Metaphase FISH in Multiple Myeloma Patients Correlated with Patient Survival**Ken Romeril<sup>1</sup>, Richard Parfitt<sup>1</sup>, Bart Baker<sup>2</sup>, Catherine Wood<sup>1</sup><sup>1</sup>Wellington Blood and Cancer Centre, Wellington Hospital, Wellington, New Zealand<sup>2</sup>Palmerston North Hospital, Palmerston North, New Zealand**Aim**

We have reviewed the cytogenetic abnormalities in 40 patients from Wellington and Palmerston North Hospitals during the period March 2005 to May 2007. Patients were all under the age of 70 and were being considered for auto-transplantation. Recent studies have shown that certain abnormalities detected by FISH, such as t(4:14), and P53 deletions have an adverse prognosis and also abnormalities such as t(11:14) confer a more favourable prognosis. We wanted to see if we could correlate these cytogenetic abnormalities with patient survival.

**Method**

A prospective analysis of transplant data was carried out from our database and laboratory results of karyotype and FISH analysis were collected. Patients were assigned to the International Staging System for myeloma. Cytogenetic analysis was performed at diagnosis using 24 hour unstimulated cultures. FISH studies were performed on fixed cells from unstimulated cultures using probes for 11q 22 (ATM); D1221 13q deletion, 14q 32 (IgH) and TP53. Also t(14:16) was used more recently.

**Result**

Data was available on 40 patients (males = 25, females = 15) and of these 25 patients proceeded to transplantation. The abnormality rate by conventional cytogenetic techniques was only 30%. In contrast FISH abnormalities were found in 65% of patients with 3 having a t(4:14) (7.5%). P53 deletions in 7.5%, t(11:14) in 5%, 6q- in 5%. The most common abnormality detected was hyperdiploidy which was found in 30%. The incidence of t(4:14) detected was lower than that commonly reported in the literature which is usually around 15%.

In terms of impact on survival the 5 patients with either P53 or 6q- abnormalities all had markedly reduced survival times of between 3 and 12 months. We were not able to confirm that a 13q deletion had an adverse prognosis in patients undergoing stem cell transplantation.

**Conclusion**

The FISH analysis rate of 65% was comparable with other studies and confirms that patients at our centre with findings of a P53 or 6q- deletion have an adverse clinical outcome. Two of our t(4:14) patients with normal marrow karyotype studies have survived over 24 months and recent data using gene expression array has indicated that there are several sub-types of t(4:14) with variable survival times. We feel it is important to continue to do both conventional metaphase cytogenetics and FISH as they do provide different prognostic information.

**P111**

**Autoimmune Haemolytic Anaemia and Neuropathy with IgA Osteosclerotic Myeloma**

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Osteosclerotic myeloma is frequently associated with extramedullary manifestations, such as peripheral neuropathy, organomegaly, endocrinopathy and skin changes (features of POEMS syndrome). Autoimmune haemolytic anaemia (AIHA) is rarely associated with multiple myeloma, but has not been previously described in osteosclerotic myeloma. We report on the first such case, with AIHA developing in an otherwise indolent osteosclerotic myeloma.

A 60 year old male presented with distal limb weakness and had a diagnosis of chronic inflammatory demyelinating polyneuropathy was made, supported by neurophysiological studies. An IgA lambda paraprotein (2g/L) was detected on serum immunoelectrophoresis. There was no immune paresis or Bence Jones proteinuria. Bone marrow biopsy and serum chemistry were normal. Skeletal survey demonstrated two markedly sclerotic lesions, reported as consistent with Paget's disease, and a vertebral crush fracture. Intravenous immunoglobulin infusions lead to stabilization of his neuropathy.

Approximately 18 months later he presented acutely with dyspnoea. Anaemia was demonstrated with numerous spherocytes on the blood film. There was biochemical evidence of haemolysis (increased bilirubin and lactate dehydrogenase and absent haptoglobin) and the direct antiglobulin test was positive (IgG). Bone marrow biopsy showed increased erythropoiesis and reactive changes, but no plasmacytosis. CT scan demonstrated mild splenomegaly, but no lymphadenopathy. Biopsy of a sclerotic rib lesion demonstrated complete effacement by malignant plasma cells. He was treated with pulsed cyclophosphamide and dexamethasone. The paraprotein became undetectable and the haemolytic anaemia improved.

This case extends the spectrum of extramedullary features seen in osteosclerotic myeloma to include AIHA. The autoantibody was IgG, whereas the paraprotein was IgA, indicating immune dysregulation, rather than a direct binding of the paraprotein to erythrocytes, as the cause for autoimmunity.

**P112**

**Plasma Cell Myeloma Immunophenotype Differs in Patients Receiving Biological Agents**

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**Aim**

To compare the plasma cell myeloma (PCM) immunophenotype in patients treated with either thalidomide or bortezomib (biological therapy) with a cohort of newly diagnosed patients, and those receiving standard combination chemotherapy regimens.

**Method**

Flow cytometry was performed using a BD FACScalibur on bone marrow aspirate samples from 47 consecutive patients at PMCC between January-July 2007. Plasma cells (PC) were defined as CD38+++ / CD138+++ events (minimum 50 required). Contamination of the 'PC-gate' was excluded by comparing un-gated CD138+++ , CD45, and untransformed forward scatter versus log-side-scatter respectively. Fluorescence in >20% PC defined CD19, CD20, CD27, CD28, CD45, CD56 and CD126 expression.

**Results**

43 patients (46 episodes; 27M:16F) were evaluated with median age 60 (42-81); median PC events, 455 (range 53–1356). Student's T-test excluded significant differences between diagnostic, conventional, and 'biologically' treated cohorts (N=9, 17, and 20 respectively) regarding disease status or duration, and twenty-nine known karyotypes [19 normal, 7 complex, two t(4;14), one t(11;14)]. Patients who had received biological agents were dual CD19-/CD56-, CD20+ more often than standard-treatment or diagnostic groups respectively [(35%, 13%, and 0%), (35%, 13%, and 17%)]. Remaining antigens were expressed with similar published frequency except for CD126 (<20%). Discordance of CD126 expression with published data especially within the diagnostic cohort probably suggests a low avidity immuno-conjugate rather than a unique finding.

**Conclusion**

Patients who have received biological treatment agents exhibit a different myeloma phenotype, more often demonstrating dual absence of CD19 and CD56 expression and increased CD20 expression. These findings have ramifications both for the monitoring of residual immunophenotypic disease, should the antibody repertoire be limited to CD38/CD138/CD19/CD56, as well as potentially broadening the opportunity for adjunctive CD20-targeted immunotherapy. Whilst currently representing a patient cohort inherently refractory to standard therapy, increased patient numbers and karyotype data are however required to confirm any specific drug related phenomena.

**P113**

**CID Chemotherapy in Multiple Myeloma. A Case Series**

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**Background**

Best initial chemotherapy for patients with multiple myeloma who may require autograft is uncertain. While studies with thalidomide are encouraging, in Australia it is only available for relapsed or refractory disease. Infusional regimens such as VAD often require inpatient administration and are associated with high rates of line related complications. Cyclophosphamide, oral idarubicin and dexamethasone (CID) chemotherapy allows out-patient based treatment and avoids line related complications.

**Aim**

To review the introduction of CID chemotherapy for the treatment of multiple myeloma at Prince of Wales Hospital.

**Methods**

The records of all patients who received CID chemotherapy for treatment of multiple myeloma from 2005 to 2007 were reviewed. Outcomes assessed included disease response (EBMT/IBMTR/ABMTR criteria), overall mortality, toxicities related to chemotherapeutic regimen, costs, and ability to provide treatment on an outpatient basis.

**Results**

Twelve patients (age range 45 -79, 8 males, 4 females), ten previously untreated, received CID. Ten patients were classified with stage III disease (Salmon and Durie criteria). A total of forty eight cycles of CID were administered (range 2-8 cycles per person). No grade III-IV haematological toxicity, infective complications, unplanned hospital admissions, or treatment related mortality occurred. Six patients achieved a partial response, one had stable disease and five had progressive disease (overall response (OR) 50%, compared to reported OR of 67-74% with VAD). Three responders subsequently required additional treatment (on average 4 months post CID), including one patient who underwent autologous transplant. Progression of disease on treatment was evident after two-three cycles of CID.

**Conclusions**

The oral CID regimen was administered entirely on an outpatient basis and was extremely well tolerated in this small cohort of patients with extensive disease. In our experience this regimen achieved similar response rates to VAD and will continue to be an option for the treatment of multiple myeloma in this institution.

**P114****An Unusual MRI Appearance of Hepatic Plasmacytomas. A Case Study and Review of the Literature**

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<sup>1</sup> *Wellington Blood & Cancer centre, Wellington Hospital*

<sup>2</sup> *Department of Radiology, Wellington Hospital*

We describe a case of a 53 year old man with IgG Myeloma who presented with a florid relapse following autograft. His relapse presentation consisted of fevers, weight loss, and sweats accompanied by a rapidly increase in his paraprotein, bone pain, hypercalcaemia and renal impairment. A routine ultrasound of his kidneys detected multiple hypoechoic lesions in his liver. A subsequent MRI scan revealed multiple lesions which were mildly hyperintense on T2-weighted images with slight ring enhancement. T1-weighted images were hypointense. The lesions were considered to be most consistent radiologically with metastatic carcinoma or multi-focal hepatocellular carcinoma. Fine needle aspiration of a lesion confirmed multiple plasmacytomas.

Myeloma infiltration of the liver is a rare occurrence. The MRI appearances of hepatic plasmacytomas are not well described and have previously been reported as being hyperintense on T1- weighted images. This case reveals an atypical MRI appearance of multiple plasmacytomas. The case highlights the importance of interpreting radiological results in the clinical context and obtaining cytology/histology to confirm the diagnosis in unusual clinical scenarios.

**P115**

**Evaluation of the GeneXpert BCR-ABL Monitor™ Assay for Routine Monitoring of CML Patients**

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**Aim**

The measurement of BCR-ABL mRNA fusion transcripts by quantitative RT-PCR provides a valuable assessment of minimal residual disease level in CML and can indicate prognosis following therapy with tyrosine kinase inhibitors. However a high level of standardisation and technical expertise is required to ensure the change in disease level is due to biological not experimental variation. We report our evaluation of GeneXpert BCR-ABL Monitor™, an automated real-time RT-PCR system that has the potential to greatly simplify routine testing.

**Methods**

Manual quantitative RT-PCR using Taqman technology on the Applied Biosystem 7700 Sequence Detection System and GeneXpert BCR-ABL Monitor™ system were used to measure BCRabl levels in EDTA whole blood from 12 CML patients over varying storage times and conditions. RNA was also trialled as an alternative sample type in the GeneXpert system.

**Results**

Automated and manual BCR-ABL results showed strong correlation over a 5 log range ( $R=0.99$ ) from blood and RNA samples. In addition GeneXpert results from samples stored at room temperature or 4°C for up to 72 hours. showed no significant change

**Conclusion**

The GeneXpert BCR-ABL Monitor™ system offers a highly automated alternative for BCR-abl monitoring in CML patients producing equivalent results to our manual quantitative PCR method but requiring far less staff time and training. The establishment of an ideal storage time for blood samples aids selection of the most appropriate throughput machine, currently available as a 1, 2 or 4 module system. The validation of the use of RNA in the system provides opportunity for participation in an international standardisation and RCPA QAP program to satisfy Medicare and NATA requirements respectively

P116

**Nilotinib Inhibits the Function of Normal Human T-Lymphocytes in a Similar Manner to Imatinib**Stephen Blake<sup>1,2</sup>, Timothy Hughes<sup>1,3</sup>, Graham Mayrhofer<sup>1,2</sup>, A Bruce Lyons<sup>1,3,4,5</sup><sup>1</sup>Hanson Institute for Cancer Research, IMVS Adelaide, Australia<sup>2</sup>School of Molecular and Biomedical Sciences Adelaide University, Australia<sup>3</sup>School of Health Sciences Adelaide University, Australia<sup>4</sup>Flinders Medical Center, Department of Endocrinology, Adelaide, Australia<sup>5</sup>School of Medicine, Flinders University, Adelaide, Australia

Imatinib has been shown to inhibit the function of T-cells *in vitro* and *in vivo*, predicted to be due to off target activity against the Src-family kinase LCK, prominently involved in T-cell activation. Nilotinib is a structural variant of imatinib with 15-30 times increased potency against BCR/ABL, the oncogenic target of both drugs. Nilotinib additionally has activity against other kinases that are also inhibited by imatinib, including c-kit and PDGFR. On this basis we investigated whether nilotinib also inhibited T-cell function. We found that nilotinib inhibited the proliferation of T-cells in response to a variety of mitogenic stimulus at IC50's of 1 - 3  $\mu$ M. Imatinib also inhibited mitogenic stimulation with slightly higher IC50's of 2-4.2  $\mu$ M. Antigen specific T-cell proliferation in response to influenza or tetanus proteins was inhibited almost completely at 2.5-5  $\mu$ M nilotinib and 5-10  $\mu$ M imatinib. Expression of the T-cell activation markers CD25 and CD69 in response to stimulus was inhibited by both drugs, with nilotinib again slightly more potent than imatinib. Additionally T-cell IFN- $\gamma$  production in response to stimulus was also inhibited by nilotinib at concentrations similar to those affecting T-cell proliferation. Inhibitory effects on T-cell function by nilotinib were not due to toxicity, as T-cell apoptosis was not increased in the presence of high concentrations of nilotinib. We propose that nilotinib, like imatinib has activity against the Src-family kinase LCK and initial experiments using the Jurkat T-cell line indicate LCK phosphorylation is blocked by 10  $\mu$ M nilotinib. Additionally nilotinib has been shown to inhibit LCK *in vitro* at an IC50 of 5.2  $\mu$ M. Overall we have shown that nilotinib inhibits multiple T-cell functions at concentrations able to be achieved clinically, indicating that nilotinib may inhibit patient T-cell function at a similar or slightly greater level to that observed with imatinib.

**P117**

## **Undiagnosed Myeloproliferative Disease in Cases of Intraabdominal Thrombosis – The Utility of the JAK2 617F Mutation**

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### **Background and Aims**

Extrahepatic portal vein thrombosis (EHPVT) and Budd Chiari Syndrome (BCS) frequently result from multiple concurrent factors such as cirrhosis, intraabdominal sepsis, procoagulant states and underlying myeloproliferative disorders (MPD). The JAK2 V617F mutation is a point mutation in the JAK2 tyrosine kinase that is variably present in MPD. The incidence depends upon the sub-classification of the MPD and the sensitivity of the assay utilised. This case series aims to illustrate the diagnostic utility of JAK2 V617F mutation in atypical cases of MPD that may otherwise not have met traditional diagnostic criteria.

### **Methods**

Granulocytic DNA was obtained and Real time-PCR was performed using allele-specific primer and probe to provide a quantitative expression of the V617F mutation.

### **Results**

The JAK2 V617F point mutation was found in three cases of EHPVT who had multiple thrombotic events but did not fulfil the traditional diagnostic criteria for MPD.

### **Conclusions**

A sensitive assay for the JAK2 V617F mutation has the potential to diagnose atypical MPD in multiple undiagnosed cases of intraabdominal thrombosis and therefore alter the management and prognosis of these cases.

**P118****Detection of BCR-ABL DNA Breakpoints in CML Patients by Short Range PCR**

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**Aim**

To develop a PCR-based method for determining the DNA sequence of the BCR-ABL translocation breakpoint in CML.

**Methods**

Tagged PCR primers were designed to span the common 3kb breakpoint region of BCR and corresponding 140kb breakpoint region of ABL. Primers were spaced to bind at approximately 500bp intervals in order to limit the size of the target sequence and ensure efficient amplification. PCR was performed with pools of 1-6 BCR forward primers and pools of 24-282 ABL reverse primers. The first round products were diluted and amplified in a second round using similarly sized pools of nested primers. The resulting products were sequenced and the sites of breakpoint identified.

**Results**

Samples from 23 patients are being studied and at the time of writing, the translocation breakpoint has been isolated and sequenced in 13. Study of the remaining patients is in progress. No favoured breakpoint site or sequence has thus far become evident, although the numbers are small. The DNA sequence results from 5 patients have been used to establish a DNA-based quantitative PCR system for measurement of MRD in CML and the results will be reported separately at this meeting by Latham *et al.*

**Conclusions**

The present approach using short-range multiplex PCR is a promising strategy for isolating and sequencing the BCR-ABL translocation breakpoint. The information so derived may shed light on the translocation mechanism and, importantly, may enable the use of a DNA-based method for measurement of MRD in many patients with CML. DNA-based measurement has several potential advantages over RNA-based measurement.

**P119**

## **MRD Measurement in CML Using DNA Rather Than RNA**

Sue Latham<sup>1</sup> Paul Bartley<sup>1</sup> Henry Martin-Harris,<sup>1</sup> Lixia Bai,<sup>1</sup> David Ross,<sup>2</sup> Timothy Hughes,<sup>2</sup> Alec Morley<sup>1</sup>

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<sup>2</sup>*Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia*

### **Aim**

To develop a DNA-based method for measurement of MRD in CML and determine its sensitivity and specificity.

### **Background**

Current RNA-based methods for quantification of the BCR-ABL translocation have a number of disadvantages, including the potential for RNA degradation, requirement for a reverse transcription step, uncertain sensitivity and specificity, and only an indirect relationship between assay result and cell number.

### **Methods**

The sequence of the BCR-ABL translocation breakpoint in 5 patients was used to design PCR primers spanning the breakpoint in order to develop a 3 round nested quantitative PCR. Dilutions of the diagnosis DNA were used to establish a standard curve. The dilutions between successive PCR rounds ensured that amplification remained exponential until the third round which was a quantitative real-time PCR using a Taqman probe. Performance of the assay and its sensitivity was measured by mixing different amounts of patient DNA in up to 25 mcg of normal peripheral blood DNA and assaying the MRD of the resulting material; specificity was determined by quantifying "MRD" in 25 mcg of normal peripheral blood DNA.

### **Results**

Assay of mixtures of DNA from 5 patients showed a virtual 1:1 linear relationship between input and measured BCR-ABL sequences down to a level of approximately  $10^{-6}$ . The level to which BCR-ABL sequences could be detected was essentially determined by the extent of the degradation of the DNA in the sample being assayed. Studies of specificity used samples from 20 normal individuals, each of which was tested against 3 different sets of BCR-ABL primers. No false-positive results were observed

### **Conclusion**

MRD can be quantified in CML in a sensitive and specific fashion using a DNA based assay. This type of assay overcomes many of the disadvantages of RNA-based measurement and may come to be widely used as methods for determining the sequence of the BCR-ABL translocation breakpoint improve.

**P120****Clinical Profile of JAK2 Positive Myeloproliferative Disorders**

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**Aim**

To describe the clinical profile of JAK 2 positive myeloproliferative disorders (MPD) at presentation and to assess the impact of mutation detection on diagnosis approach.

**Method**

In this retrospective observational study, 66 patients with a possible MPD were tested for JAK 2 mutation using an allele specific PCR method. The haematological and biochemical profile were evaluated at presentation for JAK 2 positive patients. Clinical complications at presentation and follow up were documented.

**Results**

31 of 66 patients (47%) of patients tested positive to having the JAK 2 mutation. Of this cohort, 16 were males and 15 were females. The average age of presentation was 65 years with a range from 33 to 89 years. The diagnosis was revised in 12 out of the 31 (39%) patients who were tested JAK 2 positive from being a secondary erythrocytosis or a reactive thrombocytosis to a definitive MPD. Five patients had splenomegaly and nil had hepatomegaly. Complications included TIA/stroke (n=7, 23%), venous thromboembolic events (n=4, 13%), bleeding (n=3, 10%) and gout (n=1, 3%).

Five (16%) patients had elevated haemoglobin, leucocytosis and thrombocytosis, 7 patients (23%) had erythrocytosis and thrombocytosis, 3 patients (10%) only erythrocytosis and leucocytosis, 5 patients (16%) only leucocytosis and thrombocytosis, and 3 (10%), 1 (3%), 11 (35%), had only erythrocytosis, leucocytosis or thrombocytosis in isolation respectively.

13 patients had bone marrow examination, all of which were cytogenetically normal.

**Conclusion**

The haematological profile of JAK 2 positive myeloproliferative disorders is variable and one should have a low clinical threshold for suspicion. JAK 2 testing resulted in revision of diagnosis in a significant proportion of our cohort. The incidence of thromboembolic events concurred with published literature.

**P121**

## **Evaluation of the IppoQuant Software System for Analysis and Monitoring of BCR-ABL by Real-time Quantitative PCR**

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### **Aim**

To evaluate the IppoQuant software package for testing, result analysis and trending of quantitative PCR (Q-PCR) bcr-abl analyses in monitoring disease burden and response to therapy in Chronic Myeloid Leukaemia (CML).

Early identification of adverse trends in bcr-abl level analyses is paramount to optimal patient management. Q-PCR generates a significant data management workload in the molecular pathology laboratory and most Laboratory Information Technology Systems (LIMS) fail to provide informative graphing and data trending for clinicians. The ability of the IppoQuant software data management package to address requirements for traceability, follow-up and flexibility in reporting was assessed in a routine diagnostic setting.

### **Methods**

Established quantitative bcr-abl methodology was linked to the IppoQuant Software to optimise experiment planning, reagent usage, individualise patient reporting and to monitor experimental quality.

### **Results**

The software was evaluated over a trial period for the routine monitoring of minimal residual disease in CML. It demonstrated improved flexibility over the established LIMS database and was simple to use. Scheduling of Q-PCR was easily optimised for minimising result turn around whilst maximising reagent management. Patient reports were generated that allowed trend analysis by combining present and historical data.

### **Conclusion**

The IppoQuant Software provides experiment scheduling, reagent management, on board storage of patient data, individualised patient reporting with analysis, trending and traceability. It has multi-instrument capability and has extended relevance to a range of quantitative fusion gene markers in leukaemia. The IppoQuant Software is a versatile and easy to use tool for Q-PCR data management and is time-saving and cost-effective for busy clinical laboratories.

**P123****Management of Elephantiasic Myxoedema with Plasmapheresis and Rituximab Therapy: A Case Report**Cindy Lee, Michael Leahy*Department of Haematology, Fremantle Hospital and Health Service, WA, Australia***Introduction**

Plasmapheresis can be performed to supplement immunosuppressive or cytotoxic therapy in the management of progressive autoimmune disorders, and more recently in combination with rituximab with variable efficacy.

We report a case of severe elephantiasic myxoedema managed with this entity.

**Case Report**

A 55 year old woman presented with Graves' hyperthyroidism in association with ophthalmopathy in 1995, and subsequent rapid development of pretibial myxoedema.

Initial therapy with a combination of radioactive iodine and multiple ophthalmic surgical procedures were undertaken in view of the relentless progression of her myxoedema, within months involving her upper limbs, her hands in particular, and lower limbs with marked impairment of function. Her face, lips and tongue were also involved with resultant speech impairment.

Subsequent treatment utilised in consultation with dermatology and immunology included steroid therapy (pulse methylprednisolone), azathioprine, cyclosporin, octreotide, intravenous immunoglobulins, thalidomide, acitretin, all with minimal response. Further antibody (TSH receptor antibody) reduction was attempted with cyclophosphamide, mycophenolate mofetil and eventual referral for rituximab and plasmapheresis. The use of plasmapheresis following rituximab, in conjunction with high dose cyclophosphamide resulted in significant reduction in TSH receptor antibody levels, correlating with marked clinical improvement. At present, imatinib has been commenced, with its potential antifibrotic effects as the theoretical basis for its use.

**Discussion**

This case highlights the complications and considerable difficulties in the management of rare autoimmune conditions such as elephantiasic myxoedema. It appears that the combination of plasmapheresis, rituximab and cyclophosphamide is a useful entity in this uncommon disorder.

P124

**Disease Heterogeneity Associated with Two Novel *JAK2* exon 12 Mutations in *JAK2V617F*-negative Polycythemia vera**

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The discovery of the *JAK2V617F* mutation in the Myeloproliferative Diseases (MPD) has greatly advanced the understanding of the pathogenesis of these diseases, led to more accurate diagnosis of MPD patients, and provided an avenue for development of new therapies. Janus kinase 2 (*JAK2*) is the primary signalling, non-receptor tyrosine kinase associated with multiple cytokine receptors and is essential for the activity of several key haemopoietic cytokines. Importantly, several groups have shown that the *JAK2V617F* mutation results in altered growth factor responses *in vitro*, and an MPD phenotype in murine bone marrow transplant models. The *JAK2V617F* lesion is found in >95% of Polycythemia vera (PV) patients and approximately 50% of Essential thrombocythemia (ET) and Idiopathic myelofibrosis (IMF) patients, however a key question relates to the nature of alternate lesions in *JAK2V617F*-negative MPD patients. Mutations have now been reported in the receptor for thrombopoietin (*MPL*) in approximately 10% of *JAK2V617F*-negative IMF and 2% *JAK2V617F*-negative ET patients, and alternative mutations in exon 12 of *JAK2* have recently been identified in *JAK2V617F*-negative PV patients.

Through a network of clinical haematologists in South Australia we have enrolled and characterised a cohort of 62 PV patients and screened for *JAK2* mutations using several approaches. We report the detection of the *JAK2V617F* mutation in 59/62 patients, consistent with reported frequencies in PV where similar sensitive methods have been employed. We have also identified two novel exon 12 mutations in *JAK2V617F*-negative patients from this cohort and we show that these are associated with distinct clinical profiles. Alignment with recently reported *JAK2* exon 12 mutations is consistent with the existence of a highly localised cluster of activating mutations in the majority of PV patients lacking the *JAK2V617F* mutation.

**P125****Comparison of Automated And Manual Collection Procedures Using the Cobe Spectra Apheresis Machine**

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**Aim**

The stem cell harvest yield obtained per leukapheresis procedure is partially determined by the collection efficiency. The aim of this study is to compare automated and manual collection procedures, particularly with respect to the effect on the efficiency of peripheral blood stem cell (PBSC) collection.

**Method**

One hundred and thirty-two leukapheresis procedures were performed using Cobe Spectra machines. Fifty-seven patients had undergone mobilisation with granulocyte colony stimulating growth factor (GCSF) alone (10 patients) or chemotherapy followed by GCSF +/- stem cell factor (SCF) (47 patients) from April 2005- April 2007. Automated collection procedures were performed in 12 patients for a total of 15 leukaphereses when the white cell count (WCC) was  $<10 \times 10^9/L$ . Otherwise the manual method for peripheral blood stem cell collection was performed and included 7 procedures in 7 patients with white cell count  $<10 \times 10^9/L$ . Collection efficiency (CE) of automated and manual methods of collection was analysed on the basis of peripheral blood white cell and CD34 counts for all patients and the CE of automated and manual methods were compared in patients with peripheral WCC  $<10 \times 10^9/L$ .

**Results**

In patients with WCC  $<10 \times 10^9/L$ , the median peripheral blood white cell count was significantly lower in the automated collection group ( $2.3 \times 10^9/L$ ) as compared to the manual collection group ( $7.3 \times 10^9/L$ ) ( $p=0.002$ ). However there was no difference in peripheral blood CD34 count or number of CD34 cells harvested. Collection efficiency using the automated method was 58.7% which is significantly higher than the CE of 39.5% for the manual collection procedure ( $p=0.047$ ). The final collection volume was lower using automated collection with median of 65.4ml versus 277ml in the manual group. The CE was similar for lower peripheral blood CD34 counts (15-50/ $\square$ ) and higher CD34 counts ( $>50/\square$ ) with the automated collection efficiency again being significantly higher than for the manual group. The collection efficiency was reduced with increasing white cell counts in the manual collection group.

**Conclusion**

The automated collection procedure using the Cobe Spectra machine is more efficient than manual collection when the peripheral white cell count is  $<10 \times 10^9/L$  and results in lower volume product for processing and storage.

## P126

### Occurrence of JAK2 Positive Polycythaemia Rubra Vera, Ph+ Chronic Myeloid Leukaemia and Gastrointestinal Stromal Tumour in a Patient: A Case Report

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#### Background

Several tyrosine kinase abnormalities have been identified linked to specific disease processes including Philadelphia chromosome positive (Ph+) chronic myeloid leukaemia and Polycythaemia Rubra Vera (PRV), Essential Thrombocythaemia (ET) and Idiopathic Myelofibrosis (IMF) associated with a novel somatic mutation in the Janus kinase 2 (JAK2) protein.

Gastrointestinal stromal tumours (GIST) are rare mesenchymal tumours with specific histologic features and expression of c-Kit, a transmembrane tyrosine kinase which binds stem cell factor (SCF).

Up until recently, the t(9;22) and JAK2 (V617F) mutations appeared mutually exclusive. We are unaware of any reported cases with the t(9;22), JAK2 mutation and GIST occurring concurrently.

#### Case report

Our case is of a man presenting with an erythrocytosis at age 67 years. His haemoglobin at presentation was 201 g/L with a red cell count of  $8.2 \times 10^{12}/L$ . Further investigations confirmed an absolute erythrocytosis and a diagnosis of PRV was made. He had a normal karyotype on bone marrow cytogenetic analysis but a JAK2 V617F mutation was subsequently identified in peripheral blood.

A GIST tumour was incidentally identified and confirmed histologically. Subsequent sequencing of this mass has identified a base substitution (D842V) mutation in the PDGFR protein but no mutations in the more common exon 9 and 11 regions.

After a 2 year period of stability requiring only intermittent venesection, the patient developed a leucocytosis. Reassessment of his bone marrow was undertaken and CML was diagnosed with a t(9;22) identified by cytogenetic analysis and fluorescence insitu hybridisation (FISH).

The patient was placed on imatinib therapy (400mg daily) and has achieved haematologic remission, but his transcript levels have a <2 log reduction at nearly 12 months of treatment.

#### Discussion

This appears to be the first reported case of occurrence of a t(9;22), JAK2 (V617F) mutation and GIST tumour in the one patient. We are aware of one report of t(9;22) and the JAK2 mutation co-existing following long term therapy for CML.

Whilst the current case may simply be a chance finding, the presence of 3 distinct pathologic entities with well characterized tyrosine kinase associations, poses interesting questions regarding the possible underlying pathophysiologic mechanisms.

P127

## Mouth Care - Does It Matter? Chlorhexidine Mouthwashes - Does It Work?

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### Aim

Review randomised control trials for the Effectiveness of Chlorhexidine Mouthwashes for Mucositis in Patients Receiving Chemotherapy or Bone Marrow Transplant

### Method

Meta-analysis of published literature

### Results

Seven randomised controlled studies with 338 patients receiving chemotherapy or bone marrow transplantation were reviewed. Data was reported separately for the chemotherapy and bone marrow transplantation (BMT) groups as mucositis severity differs in these two groups.

Three studies in the chemotherapy group analysed severity of mucositis on a four point scale in patients using chlorhexidine compared to placebo WMD 0.69 (95% CI -0.91 to -0.48). This result slightly favours use of chlorhexidine however the total sample size of these three studies was small with 59 participants (Ferretti 1990; McGaw 1985; Rutkauskas 1993).

One study reported the presence of oral candidiasis with chlorhexidine compared to placebo. This study supported the use of chlorhexidine however the total sample size was 16 (Ferretti 1998; Rutkauskas 1993).

Two of the studies in the BMT group analysed mucositis severity on a three point scale in patients using chlorhexidine compared to placebo WMD 0.37 (95%CI -0.70 to -0.04). This result slightly favours treatment with chlorhexidine however the total sample size was 65 participants. One study with a sample size of 51 participants reported on the presence of oral candidiasis with chlorhexidine compared to placebo. This study supported the use of chlorhexidine (Ferretti 1988).

Four of the included studies reported on colony forming units of bacteria, however this data was not reported as an increased colony forming unit count does not necessarily represent presence of infection.

### Conclusion

There has been insufficient high quality evidence from this review to support or refute the use of chlorhexidine mouthwashes. As a result there has been no change to current clinical practice.

P128

## Dealing with Vancomycin Resistant Enterococci in a Haematology/Oncology Unit – A Practice Review

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### Aim

Reduce and control the prevalence of Vancomycin Resistant Enterococci (VRE) in Haematology/Oncology patients during their admission as an inpatient.

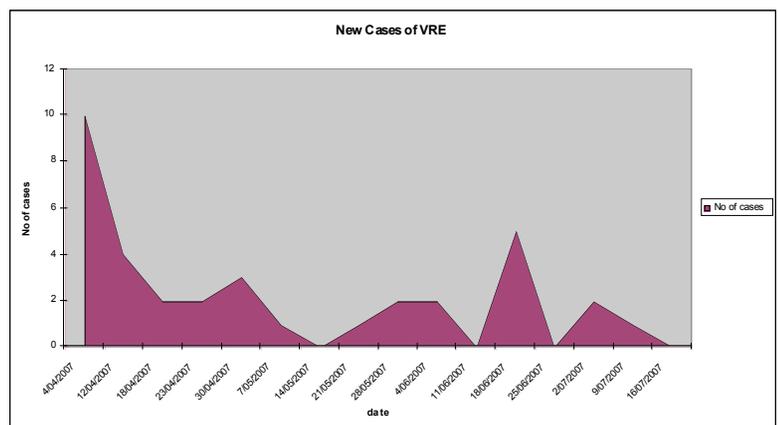
### Method

At the end of March 2007, 2 clinical specimens confirmed VRE bacteraemias in 2 patients. Commencing in April 2007 VRE screening of all inpatients in the Unit was conducted. 10 patients were found to be positive. A multi-pronged Ring Fencing strategy was implemented which involved decanting of patients, education, cleaning and screening. Stricter infection control measures for hand hygiene and personal protective equipment were implemented throughout Cancer Services. Education and guidelines about VRE was made available to all hospital staff. Rectal swabs were obtained from all Haematology/Oncology inpatients. Swabbing of negative patients and patients with an unknown status was undertaken before a patient was transferred to the Haematology/Oncology Unit, every Monday, upon arrival to the unit and on discharge. Patients were educated about VRE, the need for a result and how their status would not affect the treatment that they required or received.

The ward was appointed an afternoon cleaner.

### Results

There was a total of 516 patients' swabbed, with 35 patients' becoming VRE positive. The range 0-10, Mean 2.18, median 2, mode 2.



**Conclusion:** There was a significant impact on the hospital as there was a bed block of patients' admitted from emergency department. The screening process will continue to be monitored and evaluated to ensure the effectiveness of these interventions.

**P129**

## **A Novel Approach to Prevent Blood Wastage**

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### **Background**

Trauma patients may require transfusion in the emergency department (ED) prior to the completion of pretransfusion testing. Emergency O negative red cells may be used in this situation. The Royal Children's Hospital in Melbourne is the major paediatric trauma centre for the state of Victoria. Without a remote blood fridge in the emergency department the supply of O negative red cells for emergencies was leading to blood wastage. It is difficult in an emergency situation to always administer or return the product to blood bank within 30 minutes of issue.

### **Aim**

To provide a transfusion service that met the needs of the emergency department and reduced wastage of the emergency O negative red cells. To improve patient safety, as staff in emergency were becoming wary of ordering the O negative red cells, due to the high percentage of wastage.

### **Method**

"Blood in Motion" (BIM) was purchased to be used specifically to transport emergency O negative red cells to the ED for trauma patients. BIM was introduced after the identification of multiple episodes of blood wastage identified by the laboratory incident reporting system. Wastage and usage data for the ED was extracted from the laboratory information system and incident reporting system for six months prior to the introduction of BIM and for the same 6 month period for each year since the introduction of BIM.

### **Results**

Prior to the introduction of the BIM for the emergency department the Royal Children's Hospital reported a 20% wastage of emergency O negative blood. The reported wastage of emergency O negative blood has decreased significantly since the introduction of BIM. Despite increased requirements of emergency O negative red cells of nearly 100%, wastage has decreased to 6%.

### **Conclusion**

The introduction of BIM for the transportation of emergency O negative red cells for trauma patients at the Royal Children's Hospital has significantly reduced the wastage of this precious resource. Temporary safe storage solutions for red cells such as BIM allow for patient's to receive blood in a timely manner, whilst reducing blood wastage. The introduction of "blood in motion" also improved relations between the emergency department and blood bank and improved emergency staff confidence in requesting O negative red cells as is evidenced by the marked increase in usage.

**P130**

**The Possible Benefits of Nasogastric Feeding Versus No Nasogastric Feeding in Patients Undergoing Allogeneic Peripheral Blood Stem Cell Transplants:  
A Pilot Study**

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Patients undergoing allogeneic peripheral blood stem cell transplantation (PBSCT) are often malnourished resulting in increased morbidity and mortality. PBSCT recipients are prone to varying degrees of nutritional insult related to chemotherapy and radiotherapy, nausea, vomiting, mucositis and poor appetite. Current evidence suggests that enteral feeding may be beneficial to maintain patients weight, reduce the duration of antibiotic therapy whilst neutropenic, and decrease the severity of acute graft versus host disease (aGVHD).

We have hypothesized that nasogastric (NG) feeding improves selected patient outcomes compared to non NG feeding during hospital admission in patients undergoing allogeneic PBSCT. Patients will be allocated a particular trial arm on an alternate basis. Patients will be fed from day -1 to day +14. If a patient still requires ongoing feeding they will be brought off study and continue to be fed. Selected patient outcomes will then be measured day-7, day-1, day +14, day +21, and day +84. Selected patient outcomes to compare include percentage of weight loss, blood pre albumin levels, duration of intravenous antibiotics whilst neutropenic, length of hospital stay, presence and grade of aGVHD, and patients perception of overall health status. Once patients have been discharged they will be followed up in our haematology outpatients unit.

The poster will discuss the previous findings of similar studies, aims, inclusion / exclusion criteria, study design and outline, as well as the indication of use for various NG feeds.

This nursing research is currently being reviewed by St Vincent's Hospital Human Research Ethics Committee. Once the study has been approved recruitment of participants will begin. We anticipate this to be in early July 2007. The study will close once we have 20 participants or after two years, depending which comes first.

**P131**

## Using Collection Efficiency Data as a Quality Monitoring Tool

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### **Aim**

To monitor quality parameters in the assessment of Peripheral Blood Stem Cell (PBSC) Collections.

### **Methods/Results**

PBSC collections were performed using the Cobe Spectra™ Machine (Gambro BCT).

Retrospective audit of the records of PBSC collection procedures performed in our institution since January 2007 to June 2007-07-16

28 procedures were performed on 24 patients, equalling 1.16 procedures per patient. Mean collection efficiency on procedures was 47%.

5 out of 28 (17.8%) collections failed to reach a collection efficiency of 25%. This was due to a variety of factors. 60% were poor mobilisations.

Bench marking was performed with a similar unit within Australia.

From this report recommendations were made looking to improve collection efficiency within the unit.

### **Conclusion**

Collection of efficiency data is a vital tool as a quality monitor to indicate effective and efficient standards of PBSC collections with in the hospital's Bone Marrow Transplant unit.